Food Industry

Edited by Innocenzo Muzzalupo

FOOD INDUSTRY

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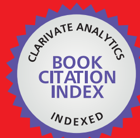

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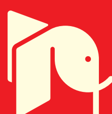

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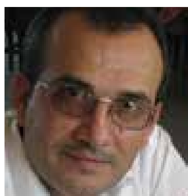

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Preface

The Food and Agriculture Organization of the United Nations and the World Health Organization have a strong interest in promoting national food control systems that are based upon scientific principles and guidelines, and which address all sectors of the food chain. This is particularly important for developing countries as they seek to achieve improved food safety, quality and nutrition, but will also require a high level of political and policy commitment.

Because of the increase in world population, the global food industry has the largest number of demanding and knowledgeable consumers. This population requires food products that fulfill the high quality standards established by food industry organizations. Food shortages threaten human health, and the disastrous extreme climatic events make food shortages even worse.

This collection of articles is a timely contribution to issues relating to the food industry; they were selected for use as a primer, an investigation guide and documentation based on modern, scientific and technical references. This volume is therefore appropriate for use by university researchers and practicing food developers and producers.

The contributions of chapters in the book are divided into six sections:

- Types of food
- Food quality
- Food safety
- Food processing
- Economic and social aspects
- Products management of food industry

All chapters have been written by renowned professionals working in food industry and related disciplines.

I acknowledge the authors for willingly contributing their chapters without which we would not have been able to publish this book. I am equally grateful to Ms Viktorija Zgela, the Publishing Process Manager for the able assistance she provided and to the Information Technology Department for providing the requisite framework that greatly enhanced the work of putting together the chapters in the book.

The Technical Editors deserve commendation for preparing the online publication and print versions of the book.

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Section 1

Types of Food

Chapter 1

An Overview on Cagaita (*Eugenia dysenterica* DC) Macro and Micro Components and a Technological Approach

Ediane Maria Gomes Ribeiro,
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Gisela Maria Dellamora Ortiz,
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Additional information is available at the end of the chapter

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1. Introduction

Many fruit species native to the Brazilian Cerrado region have great economic and ecological potential, as well as social importance to the native population (Bezerra, Silva, Ferreira, Ferri, & Santos, 2002). These fruits often supplement the diet and are a source of medicine, textile fibers, building materials and fuel. The development of new technologies may result in these fruits becoming potential sources of economic exploitation (Martinotto, Soares, Santos, & Nogueira, 2008).

The Cerrado region has an abundance of species of fruit, still underused by local communities for scientific unknown and lack of incentive for marketing (Veira, Costa, Silva, Ferreira & Sano, 2006). The sustainable use of these species can be an excellent alternative to add value to raw materials available in the Cerrado region and improve the health of the population, thereby contributing to the income of rural communities and encouraging the conservation of native species.

The cagaita tree, belongs to the Myrtaceae family of plants, consisting of 14 genera and represented by 211 species that naturally occur in the Cerrado. Myrtaceae is one of 10 plant

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families found in this biome or ecosystem that together contribute to more than 51% of its richness. The cagaita is found in the Brazilian states of Goias, Minas Gerais, São Paulo, Tocantins and Bahia (Silva, Chaves, &Naves, & 2001). It occurs at highest densities in latosoil and is observed in areas with mean annual temperatures between 21.1°C and 25.5°C and at altitudes of 380 m to 1100 m (Souza, Naves, Carneiro, Leandro & Borges, 2002).

The cagaiteira, is a medium-sized tree, is 30 m tall, and has a cylindrical and twisted trunk, ranging from 20 cm to 40 cm in diameter. Its suberous bark and crevices are very unique. Its crown is long and dense, with square hairless branches, and except for the buttons, the pedicels, leaves and young branches are puberula. It is a deciduous plant and is selectively heliophytic and xerophilous (Donadio, Môro, & Servidone, 2002).

Flowering occurs in the middle of the dry season, from mid-July to early August, with the simultaneous emergence of new leaves of the cuprea (Fig.1) (Brito, Pereira, Pereira, & Ribeiro, 2003). The cagaiteira's flowers are always axillary and are either singular or clustered in arrays of three. They are hermaphrodites, and complete, are from 1.5 to 2 cm in diameter, actinomorphic, dialipetalous, dialisepalous, tetramerous, and are endowed with white petals (Lorenzi, 2000).

Figure 1. Cagaiteira flower and branches with flowers Source: www.plantasonya.com.br.

The cagaita tree can be used almost entirely, bringing its economic value, and the great potential for sustained exploration (Table 1).

Feature	Utility	References
Tree	Ornamental landescape	Martinottoet al., 2008
Flowers	Apiculture	Lorenzi, 2002
Stalk	Construction, furniture, pallets, firewood and charcoal	Chaves & Telles, 2006; Martinotto et al., 2008
Shell	Tannery, antidiarrheal	Lorenzi, 2002; Chaves & Telles, 2006; Martinotto et al., 2008
Leaves	Lawn trees, antidiarrhoeal, antifungal, moluscocida and treatment of diabetes and jaundice	Chaves & Telles, 2006; Martinotto et al., 2008

Table 1. Forms of exploitation and use of *Eugenia dysenterica* DC.

The cagaiteira has a great potential for use in agricultural production systems, because it has high production and relatively stable over the years, the potential of the fruit to processed products, good living with pasture, high tolerance to drought, edaphic and biotic stress, fire resistance and ease of production by seed and seedling establishment in the field among other factors (Veira, Costa, Silva, Ferreira & Sano, 2006).

According to Zucchi, Brondani and Pinheiro (2003), the cagaita fruit is a flattened and globular pale yellow berry, 2 to 3 cm in diameter, containing from 1 to 3 white seeds that are encased in a slightly acidic pulp (Fig. 2). These seeds are attached to the fruit by a dry, membranous mesocarp, although the endocarp is juicy. The seeds are globular in shape, pale yellow when ripe, with an acidic flavor and weigh between 14 to 20 g (Silva, Chaves, & Naves, 2001).

Figure 2. Cagaita fruit (*Eugenia dysenterica* DC): A – unripe; B – imature; C – ripefruit; D – Fruit with the seed (Source: Tatagiba, 2012; Stolfi, 2012).

2. Cagaita pulp process

The mature fruits of cagaita (*Eugenia dysenterica*) are harvested by hand. After cleaning (immersion in sodium hypochlorite 200 ppm) and selection, the fruits are depulped, packed in polyethylene bags, and freezing and stored at -18 ºC (Fig. 3).

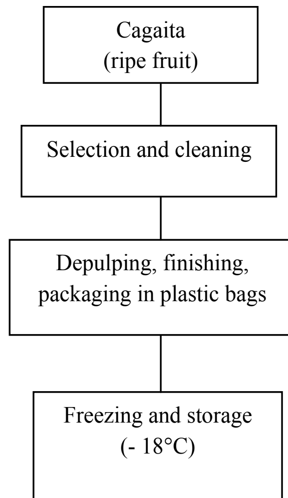

Figure 3. Whole cagaita pulp process (Cardoso et, 2011)

3. Nutritional and proximal composition

Studies have shown that the cagaita fruit's nutritional composition indicates a high water content (95.01%). It has the highest percentage of polyunsaturated fatty acids (such as linoleic (10.5%) and linolenic acids (11.86%)), surpassing corn, sunflower, peanut, soybean, olive and palm oils. Fatty acids play an important role in the human body, forming the basis of substances that are critical for developing cell membranes found in the brain, the retina and the reproductive system (Almeida, 1998).

Carvalho et al. (2010) found the moisture content of cagaita pulp to measure 94.12%, the titratable acidity at 13.78 g m^{-1} and a pH of 3.05. These values are higher than other fruits of the same genera, such as the pitanga and jambo. Conversely, the ash and protein contents are lower than the jambo and pitanga (Oliveira, Figueiredo, & Queiroz, 2006). Similarly, Ribeiro (2011) evaluated the proximal composition of the cagaita pulp that was extracted with and without peels. The test results for moisture, ash, protein, lipids and carbohydrates (by difference – NIFEXT) for this pulp (with and without peels) varied from 90.08 to 88.55 g•100g^{-1}; 0.25 to 0.33 g•100g^{-1}; 1.85 to 2.03 g•100g^{-1}; 0.20 to 0.36 g 100g^{-1} and 7.62 to 8.73 g 100g^{-1}, respectively. The titratable acidity, pH and soluble solids ranged from 13.78 to 14.63

g.100ml^{-1}; 2.90 to 2.69 and 8.20 to 8.70°Brix, respectively, leading to the conclusion that removing the peel results in a reduction of carbohydrate content such that some remains in it after extracting the juice. Silva, Lacerda, Santos, and Martins (2008) found 20.01 TEV (Energy Total Value), 94.34 (moisture); protein 0.82; lipids 0.44; carbohydrates 3.08 and ash 0.28 g m^{-1} in the cagaita pulp. The authors did not mention whether the pulp was obtained with or without peels.

In cagaita pulp extracted with the peels, Roesler et al., (2007) found 2.09 (proteins); 0.32 (lipids); 0.23 (ash); 89.71 (moisture); 20.47 (total sugars) pH of 2.8 and 26.4 (total acidity). Cardoso et al., (2011) found 0.73 g of citric acid 100g^{-1}, pH of 3.3 and soluble solids of 9.12°Brix in cagaita pulp from the Cerrado region of the state of Minas Gerais. Moisture content was 91.56 g 100g^{-1}, with similar results found by Roesler et al. (2007) in cagaita pulp from the Cerrado region within the Goias state.

Silva, Santos-Junior and Ferreira (2008) investigated the cagaita fruit at different stages of maturation; however, the results for the moisture did not differ significantly, ranging from 92.77 to 93.21 g 100g^{-1}.

From the results obtained by Ribeiro (2011), one can conclude that the cagaita fruit, with or without peels, is basically made up of carbohydrates and water. As expected, the moisture content in pulp extracted without peels was higher than the moisture content in pulp with peels. This was because the latter contained peels and the former was essentially pulp with a high water content. The value for cagaita pulp without peels was 90.08 g 100g-1 and the pulp with peels was 88.55 g 100g-1. However, no significant difference ($P < 0.05$) was found. Removing the peels yields a reduction in carbohydrate content, although some remains in it after extracting the juice.

Other researchers studying the cagaita fruit obtained similar results. Roesler et al., (2007) evaluated only the pulp, obtaining 89.71%. Silva, Santos-Junior & Ferreira (2008) investigated the fruit at different stages of maturation, however, the results for the moisture content did not differ significantly, ranging from 92.77 to 93.21 g 100 g^{-1}.

Martins (2006) found a carbohydrate content of 5.4 g 100 g^{-1}, which was lower than that recorded by Ribeiro (2011), at 7.62 and 8.73 g 100 g^{-1}. These results may be related to the geographic location of the analyzed fruits. For example, the temperature, sun exposure and maturity, among other factors, may have had an effect on the results.

Due to its low lipid content, the cagaita fruit is recommended as part of a low calorie diet. The values found by Ribeiro (2011) varied from 0.20 to 0.36 g 100 g^{-1} for pulp extracted with and without peels. Those values were similar to those reported by Martins (2006) and Roesler et al., (2007), being 0.20 and 0.32 g 100 g^{-1}, respectively. It is worth noting that this was the only parameter that did not yield a significant difference in the 5% level of significance, showing a higher content of lipids in the peels of the fruit.

Vallilo, Garbelotti, Oliveira, and Lamardo (2005) evaluated other Myrtaceae fruits and found similar low values of lipids: 0.23 g 100 g^{-1} in Surinam cherry (*Eugenia uniflora* L), 1.53 g 100 g^{-1} in cambuci (*Campomanesia phaea* Berg), 0.80 g 100 g^{-1} pears in the field (*Eugenia klotzchiana* Berg) and 0.54 g 100 g^{-1} in guava (*Psidium guajava*).

The protein levels were low; although, as expected, they were higher in the fruit with their peels (2.03 g 100 g^{-1}) than in peeled fruits (1.85 g 100 g^{-1}). Some studies with the same result found similar values: 2.09 g 100 g^{-1} for the whole pulp (Roesler et al., 2007) and 0.99 g 100 g^{-1} in cagaita pulp (Martins, 2006).

In another study of guava, cited later in this paper, Gutiérrez, Mitchell, and Solis (2008), reviewed the fruit in relation to its protein content of 0.88%.

It can be concluded that the cagaita fruit is not high-caloric due to its low levels of protein, carbohydrates, and especially lipids.

4. Glucose, fructose and sucrose

Carvalho et al. (2009) and Ribeiro (2011) found a high concentration of fructose (2.54 g 100 mL^{-1}), followed by glucose (1.75 g 100 mL^{-1}) and the lowest concentration of sucrose (0.59 g 100 mL^{-1}) (P >0.05) (Fig. 4). The high fructose content can be explained by the fact that the cagaita fruit used in their study was fully ripened.

Many different factors could have contributed to the low soluble sugar content in the cagaita pulp. One factor is mineral fertilization, where potassium is the primary mineral element causing starch accumulation in Citrus leaves (Lavon, Goldschmidt, Salomon, & Frank, 1995). On the other hand, the shortage of free sugars may trigger ethylene synthesis because defoliation, which drastically reduces sucrose transport to the fruit, increases ethylene synthesis (Ortolá, Monerri, & Guardiola, 2007) and 1-aminocyclopropane-1-carboxylic acid (ACC) accumulation (Gómez-Cadenas, Mehouachi, Tadeo, Primo-Millo, & Talón, 2000).

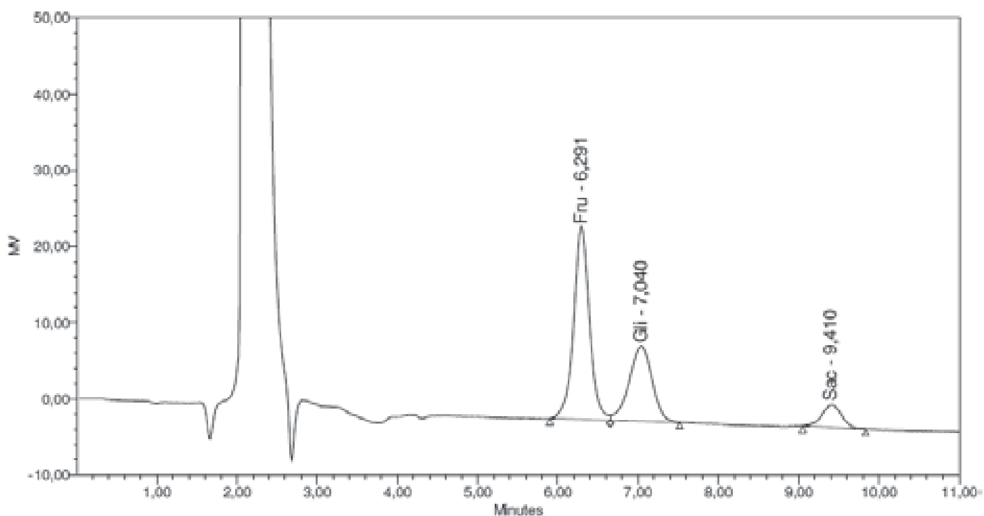

Figure 4. HPLC chromatogram of glucose, frutose and sucrose in whole cagaita pulp.

The low glucose, fructose and sucrose values in the cagaita indicate that this fruit is less sweet and contains less sugar than the guava, for example. This comparison has been verified by Lee & Kader (2000). Analyzing the fruits by High Performance Liquid Chromatography (HPLC), their study found higher values in the ripe guava pulp (11.52 g.100 ml^{-1} of sucrose, fructose 11.37 g.100 ml^{-1} and, glucose 5.12 g.100 ml^{-1}.

5. Ascorbic acid (vitamin C)

According to Andrade, Diniz, Neves & Nóbrega (2002), the sources of ascorbic acid are classified by different levels: high sources, such as strawberry, guava and pineapple, contain 100 to 300 mg•100 g^{-1}; medium sources, such as orange, lemon and papaya contain an average of 50 to 100 mg•100 g^{-1}; and low sources, such as lime, pear and mango, contain 25 to 50 mg•100 g^{-1}. The vitamin C content in cagaita pulp as reported by Ribeiro (2011) was 56.66 mg 100 g^{-}1 (Fig. 5) and by Cardoso et al. (2011), it was 34.11 mg 100 g^{-1}, with 30.03 mg 100 g^{-1} of ascorbic acid and 4.08 mg 100 g^{-1} of de-hydro ascorbic acid. Therefore, the cagaita can be classified as a medium source of ascorbic acid. The pulp of the cagaita fruit has shown considerable promise for its vitamin C content and is considered a source of that nutrient when compared to other fruit. Silva, Santos-Junior, and Ferreira (2008) found the level of vitamin C to be 27.46 mg 100 g^{-1} in cagaita pulp from the Cerrado region in the state of Goias.

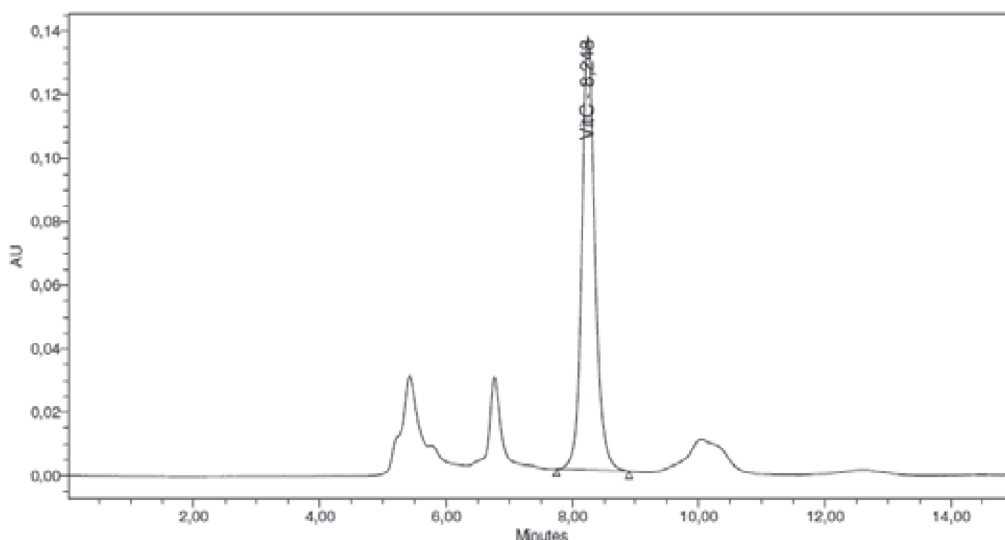

Figure 5. HPLC chromatogram of ascorbic acid in whole cagaita pulp.

On the other hand, the National Sanitary Surveillance Agency (ANVISA) legislation (Brazil, 1998) recommends that for a food to be considered a "source" of a certain vitamin, it should contain, at least, 15% of the Recommended Daily Intake (RDI) per 100 g of reference. To be

considered "rich" in a vitamin, it should contain at least 30% of the RDI. Therefore, the cagaita can be categorized as rich in vitamin C because it exceeds 30% of the RDI (US National Academy of Sciences, 2000).

The ascorbic acid content of 26 kinds of exotic fruits from a variety of species and families were evaluated by Valente, Albuquerque, Sanches-Silva and Costa (2011). The results ranged from 1.42 to 117 mg 100 g^{-1}, and those fruits that had values similar to cagaita were guava (*Psidium guajava*) with 65.8 mg 100 g^{-1}, kiwi (*Actinidia chinensis* Planch), cv. Hayward with 55.2 mg 100 g^{-1}, papaya (*Carica papaya*), cv. Taiwan with 64.2 mg 100 g^{-1} and mango (*Mangifera indica* L), cv. Palmer, with 40.9 mg 100 g^{-1}, among others.

6. Polyphenols compounds

In general, phenolic compounds behaving as antioxidants are multifunctional, achieving bioactivity in several ways: fighting free radicals by donating a hydrogen atom from a hydroxyl group (OH) of their aromatic structure; chelating transition metals, such as the Fe^{2+} and Cu^{+}; interrupting the propagation reaction of free radicals in lipid oxidation; modifying the redox potential of the medium and repairing the damage in molecules attacked by free radicals (Podsedek, 2007; Kyungmi & Ebel, 2008). These same phenolic compounds also block the action of specific enzymes that cause inflammation, modify the metabolic pathways of prostaglandins, permit platelet clumping and inhibit activation of carcinogens (Liu, 2005; Valko et al., 2007).

Historically, like tannins, phenolic compounds were classified as anti-nutrients, which have demonstrated adverse effects on human metabolism. However, identifying the specific properties of these phenolic compounds has stimulated the development of research aimed at identifying their potential health benefits (Kaur & Kapoor, 2001).

It is worth noting that a substance can be defined as polyphenolic antioxidant if it meets two conditions: (1) presence at a low concentration on the substrate to be oxidized (and this may delay or prevent oxidation), and (2) high stability of radicals formed after the reaction (Kaur & Kapoor, 2001).

Several spectrophotometric methods have been developed for the quantification of phenolic compounds in foods. The most commonly used by the scientific community is the Folin-Ciocalteau method, which involves the oxidation of phenol with a reagent and yellow phosphomolybdate heteropolyacid phosphotungsten (Folin-Ciocalteau) and colorimetric measurement of W-Mo blue complex formed in reaction in an alkaline medium (Singleton, Orthof, & Lamuel-Raventos, 1999). The results are expressed in gallic acid equivalents.

Some results of the polyphenols content in ethanolic (18.38 g GAE kg^{-1}) and aqueous (16.23 g GAE kg^{-1}) extracts of cagaita pulp were reported by Roesler et al. (2007). The content of the total phenolics in cagaita pulp was evaluated by Ribeiro et al. (2011) who found 10.51 mg GA g-1 in pulp with peels and, in the pulp without peels, found 9.01 mg gallic acid g-1.

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Therefore, no significant difference was found at a 5% level between them. Thus, the cagaita fruit was found to have high total phenolic compounds.

7. Antioxidant capacity

Determining the antioxidant activity of foods, in addition to recognizing its antioxidant potential before being consumed, is important to assess the defense against oxidation and degradation reactions that can lead to the degradation of its quality and nutritional value (Lima, 2008). Currently, there are no approved or standard methods for the determination of antioxidant activity. However, several *in vitro* methods have been and are being tested to evaluate the total antioxidant activity of substances and foods, especially in complex matrices such as wine, fruits and other vegetables. These methods are necessary because of the difficulty in comparing and measuring each compound separately and also because of the potential interactions between different antioxidants in the system. (Cao & Prior, 1999; Kulkarni, Aradhya, & Divakar, 2004; Scherer & Godoy, 2009).

The methods most often cited in the literature include the antioxidant power in the reduction of iron (FRAP), DPPH (radical 2,2-diphenyl-1-picrihidrazil) Activity of Oxygen Radical Absorption (ORAC), ABTS [acid 2,2 - Azin-bis (3-ethylbenzothiazoline) – 6 - sulfonic acid Spectrometry and Electron Spin Resonance (ESR) (Kulkarni, Aradhya and Divakar, 2004; Lima, 2008).

While evaluating the efficiency of using methanol and ethanol as solvents to determine the antioxidant activity in cagaita pulp (Ribeiro et al., 2011) found that the amount of ethanol ranged between 6.6% and 96.82% and that of methanol ranged between 11.20% and 92.60%, in different concentrations. It was also shown that the cagaita pulp reached its maximum value at a concentration of 500 μg ml^{-1}, in both cases.

Roesler et al. (2007) found the antioxidant activity (IC_{50}) in cagaita pulp extracted with peels to measure 387.47 mg ml^{-1} in the ethanolic extract and 879.33 mg ml^{-1} in the aqueous extract.

8. Carotenoids

Gomes et al. (2011) measured the total carotenoid content in the whole cagaita pulp and also in the freeze-dried pulp and found 0.87 and 9.29 mg 100 g^{-1}, respectively (Table 2 and Fig. 6). Lutein was the most abundant carotenoid in the whole and freeze-dried pulps (0.21 and 2.22 mg 100 g^{-1}, respectively), followed by zeaxanthin (0.19 and 2.05 mg 100 g^{-1}, respectively) and β-carotene (0.11 and 1.33 mg 100 g^{-1}, respectively).

According to these results, cagaita may be a source of lutein and zeaxanthin (which are natural antioxidants), particularly in freeze-dried pulp. By microencapsulating the freeze-dried pulp, it can become a beneficial food additive because cagaita pulp is widely consumed in the Brazilian Cerrado.

Samples	Total Carotenoids	β-carotene	9-cis-β-carotene	13-cis-β-carotene	β-criptoxantin	α-carotene	Lutein	Zeaxanthin
Whole Pulp	8.22 ± 0.06	0.97 ± 0.08	Nd	Nd	0.35 ± 0.01	Nd	1.81 ± 0.12	1.99 ± 0.05
Saponified Whole Pulp	5.83 ± 0.52	1.70 ± 0.18	0.20 ± 0.01	0.09 ± 0.01	1.49 ± 0.11	0.18 ± 0.16	0.85 ± 0.01	0.79 ± 0.02

Source: Gomes, 2012

Table 2. Carotenoids (μg/g) and isomers of saponified and not saponified cagaita pulp

Lutein can be found in a variety of vegetables and is especially plentiful in cabbage (15 mg 100 g^{-1}), parsley (10.82 mg 100 g^{-1}), spinach (9.20 mg 100 g^{-1}) and pumpkin (2.40 mg 100 g^{-1}). However, it is found in lower concentrations in fruits such as peach and orange (0.02 and 0.35 mg 100 g^{-1}, respectively).

Gomes (2012) identified α-carotene, β-carotene isomers and 9:13-cis β-carotene, β-cryptoxanthin, lutein and zeaxanthin in the pulp produced in cagaita Damianópolis, Goias, Brazil (Fig. 6).

The β-carotene and β-cryptoxanthin the most abundant carotenoids, lutein and zeaxanthin and the carotenoids intermediate, and the α-carotene carotenoid the minority (Table 2).

There were significant differences in levels of total carotenoids according to the saponification step. The hydrolysis step was necessary to facilitate the identification of different carotenoids. The average concentration of total carotenoids found in the extracted pulp without the saponification step was 8.22 mg / g. There was a 29% decrease in total carotenoid content of the pulp subjected to saponification step (5.83 μ / g ± 0.18). This drop was expected and may occur as a function of temperature application of tests, and also by the exposure time of the pigment to the alkali (Mercadante, 1999; Penteado, 2003).

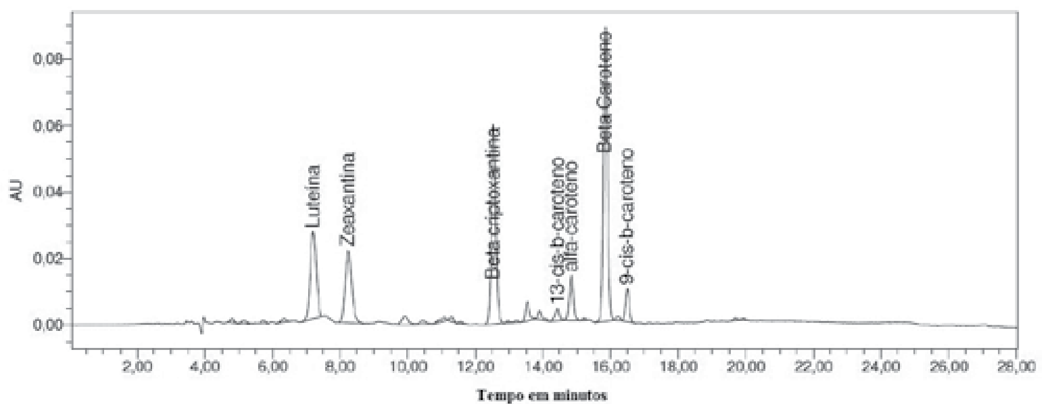

Figure 6. HPLC chromatogram of saponified cagaita pulp. Source: Gomes, 2012

Cardoso et al. (2011) found a lower total carotenoid content (0.77 mg100 g^{-1}) in the cagaita pulp from the Cerrado in the state of Minas Gerais. The major carotenoids were the α--carotene (0.31 mg 100 g^{-1}) and β-carotene (0.39 mg 100 g^{-1}) provitamin A carotenoids. They still found a small quantity of lycopene (0.06 mg100 g^{-1}), however lutein and zeaxanthin were not found.

9. Minerals

According to Carvalho et al. (2009), the most abundant mineral found in the cagaita pulp was potassium (75.83 mg 100 g^{-1}), followed by sodium (6.80 mg 100 g^{-1}), phosphorus (6.68 mg 100 g^{-1}) and magnesium (5.92 mg 100 g^{-1}). The levels of zinc were lower (0.23 mg 100 g^{-1}), as were the levels of iron (0.06 mg 100 g^{-1}) and calcium (0.65 mg 100 g^{-1}) (Table 1). Higher values of calcium (0.8 mg 100 g^{-1}) and, similarly, iron (0.04 mg 100 g^{-1}) were found by Silva, Santos-Junior Junior, and Ferreira (2008) in the cagaita pulp, but zinc was not found at higher levels. Leterme, Buldgen, Estrada, and Londoño (2006), in analyzing the fruits of araçá-boi (belonging to the same family and genus as the cagaita), found similar values: 78 mg 100 g^{-1} (potassium), 7 mg 100 g^{-1} (phosphorus), 2 mg 100 g^{-1} (sodium) and 9 mg 100 g^{-1} (magnesium), respectively.

Mineral	mg/100g	Mineral	mg/100g
Potassium	75.83 (± 0.43)	**Aluminum**	0.23 (± 0.06)
Phosphorus	6.68 (± 0.14)	**Zinc**	0.23 (± 001)
Sodium	6.80 (± 0.13)	**Manganese**	0.13 (± 0.01)
Magnesium	5.92 (± 0.08)	**Iron**	0.06 (± 0.01)
Calcium	0.65 (± 0.08)	**Copper**	0.01 (± 0.01)

Mean Value (± Standard deviation (n = 3)). Source: Carvalho et al., 2009

Table 3. Minerals in the unpeeled cagaita pulp (*Eugenia dysenterica* DC).

Comparing the cagaita (*Eugenia dysenterica* DC) to the results of the study by Dembitsky et al. (2011), in which different fruits were analyzed, confirms that the acerola (*Malpighia punicifolia* Linn) contains lower amounts of potassium (41 mg/100 g), zinc (0.09 mg/100 g) and manganese (0.7 mg 100 g^{-1}) and much higher amounts of calcium (4 mg 100 g^{-1}), iron (37 mg 100 g^{-1}) and magnesium (22 mg 100 g^{-1}).

While analyzing the fruits of guava-boi (*Eugenia stipit*ata Mark Vaughn) that belong to the same family and genus as the cagaita, Leterme, Buldgen, Estrada, and Londoño (2006) found similar amounts: 78 mg 100 g^{-1} of potassium, phosphorus 7mg 100 g^{-1}, mg 100 g^{-1}, 2 mg 100 g^{-1} and 9 mg 100 g^{-1} of sodium and magnesium. These variations could be due to climatic conditions, soil type and the addition of fertilizers, for example.

10. Volatile compounds

Volatile compounds are responsible for the aroma and flavor of foods. The same fruit, even if native to Brazil, can vary greatly from region to region, with different varieties having a dissimilar volatile composition (Alves & Franco, 2003). The methods used for the extraction of volatile substances are time-consuming, requiring large amounts of sample (Sánchez-Palomo, Díaz-Maroto, & Pérez-Coello, 2005). Solid-phase Microextraction (SPME) is a fast, low-cost technique that allows the extraction of volatile substances that can then be analyzed by gas chromatography coupled to mass spectrophotometry (GC/MS). This technique replaces traditional extraction methods, avoiding the formation of artifacts without the need for solvents, thereby minimizing artifact formation (Pawliszyn, 1997; Riu-Aumatell, Castellari, & López-Tamames, 2004).

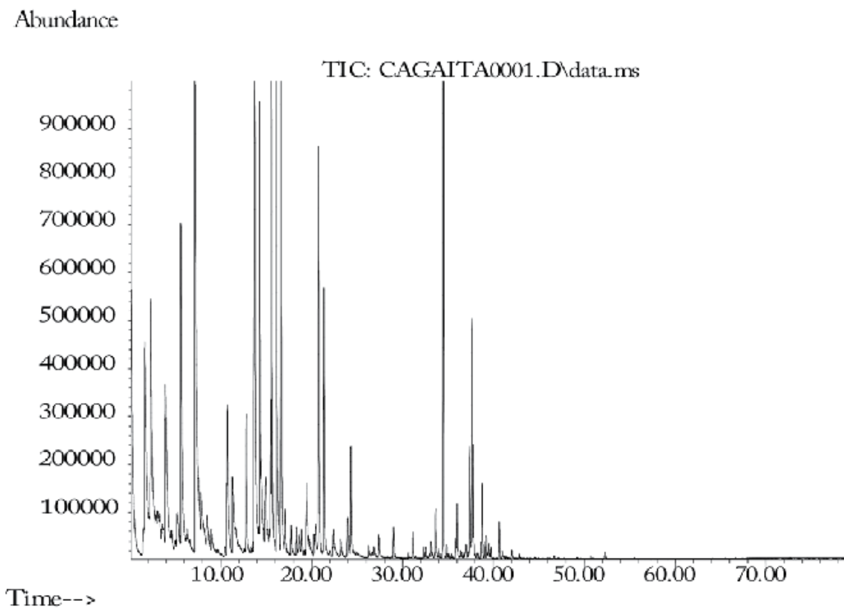

Figure 7. Chromatogram of the cagaita pulp volatile compounds. Source: Cardoso et al, (2011).

Fifty six volatile compounds were found in cagaita pulp extracted by solid phase micro-extraction and were analyzed by GC/MS. Among them, 19 could not be identified by Carvalho et al. (2009). Ethyl hexanoate was the most abundant compound in the cagaita pulp (51.4%), followed by the ethyl butanoate (14.7%), which also imparts the fruity aroma of the fruit juices and pulp. The results revealed that a greater concentration of esters, mainly methyl, ethyl hexanoate (6.5%) and butanoate, are responsible for the fruity aroma. Alcohols and terpenes were present at low concentrations, with ethanol being the most abundant (3.0%). These volatile compounds were also found in pineapple, apple and papaya, among other fruits (Van Den Dool, & Kratz, 1963; Adams, 1972). Alves and Franco (2003) also identified some major com-

pounds in murici, finding esters and alcohols. Ethanol (28.1%), ethyl hexanoate (25.1%) and methyl hexanoate (5.2%) were the major components. However, they reported that the high ethanol levels could be due to fermentation following maturation. Because no other authors reported these compounds, it is not possible to compare the reported results. A typical total ion chromatogram obtained from the cagaita pulp analysis is presented in Figure 7.

It is noteworthy that this is the first time that volatile compounds have been found in cagaita fruit from the Cerrado region in Goias.

11. Membrane processes applied to cagaita pulp

The consumption of fruit juice in Brazil and in the industrialized world has increased significantly in recent decades. Using fruit juice or pulp that has been clarified by the membrane processes of microfiltration is already a reality in the international market. The cagaita pulp can be introduced as a new product used in the formulation of carbonated beverages, energy and isotonic drinks. The demand for products with less nutritional and sensory changes led to the development of non-thermal preservation techniques such as the process of membrane separation. The membrane separation process is based on the selective permeability of one or more components through a membrane. The determination of the hydraulic permeability is an important tool in evaluating the permeate flux and the integrity of the membrane. Cardoso et al., (2011) evaluated the cagaita pulp clarified by microfiltration with a tubular polyethersulfone membrane (0.3 μm) at 2 Bar (Fig. 8). A mean flux after 2 hours process was 20 L./m^2 h. and the clarified juice yield was 43%. The results for the flux of the juice permeate were acceptable and the permeate was clear and translucent.

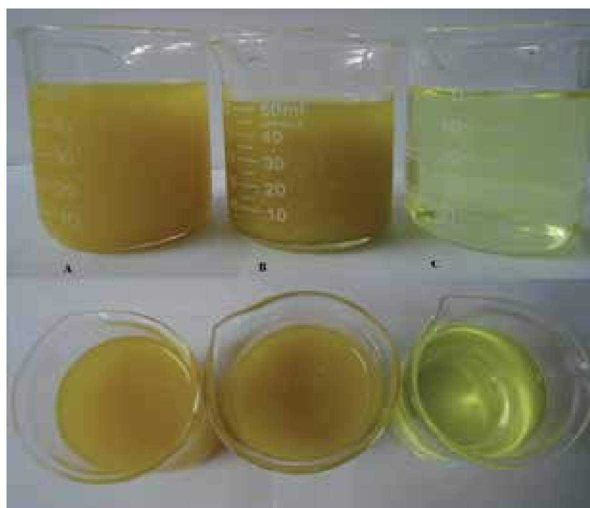

Figure 8. Cagaita pulps (*Eugenia dysenterica* DC): A – Whole, B – Concentrated e C – Clarified. (Cardoso et al., 2011).

12. Microbiological quality

Microbiological studies of cagaita pulp revealed no growth of microorganisms. Coliforms at 45°C, were indicative of its tolerance to sample 10^2 CFU (colony forming units) as was the absence of salmonella in 25 g of the sample (Carvalho et al., 2009). Therefore, the analyzed pulps were found fit for human consumption because they were in accordance with standards established by ANVISA (Brasil, 1998).

Samples	Total Coliforms (UFC/mL)	Thermotolerant Coliforms (UFC/mL)	Yeast and Mold (UFC/mL)	*Salmonella* sp. (Absence 25 g or mL)
WCP	< 10	< 10	< 10	Absence
RCP	< 10	< 10	< 10	Absence
CCP	< 10	< 10	< 10	Absence

WCP: Whole Cagaita Pulp; RCP: Retentate Cagaita Pulp; CCP: Clarified Cagaita Pulp

Table 4. Microbiological analysis of whole, retentate and clarified cagaita pulp.

13. Particle size of the cagaita pulp

Particle size analysis is an important tool to observe the enzymatic hydrolysis and the particle size reduction in order to optimize the membrane pore size before clarification processes.

Particle size analysis can be an useful tool to observe particle size reduction during enzymatic hydrolysis optimization to reduce juice viscosity. Few studies are found in the literature reporting the use of particle size analysis to observe viscosity decrease in fruit juices.

Laser diffraction analysis was used to evaluate the effects of cloud particle characteristics such as shape, volume fraction, and soluble pectin on the viscosity of cloudy apple juice. Cloudy apple juice results in a suspension of irregular-shaped particles ranging from 0.25 to 0.5 μm in size. Data indicate that the effect of nonspherical particles on cloudy apple juice viscosity can be neglected and soluble pectin can significantly increase the viscosity (Genovese & Lozano, 2000).

The distribution of the average particle diameter, i.e., its frequency as measure by Carvalho et al. (2009 and 2011), was 12.11%, and the average particle diameter within cagaita pulp was 68.17 μm (Fig. 9). The presence of nanoparticles of less than 1 micrometers was still observed, but in low frequency (0.1%).

After enzymatic hydrolysis of lemon juice at different incubation times, Carvalho et al., (2006) evaluated the particle size reduction in prior membrane microfiltration processes in order to obtain better permeate fluxes. The whole lemon juice showed a wide distribution of

particle size ranging from 5 to 900 μm, and the greatest particle size reduction after hydrolysis ranged from 5 to 200 m. There were few particles above this size.

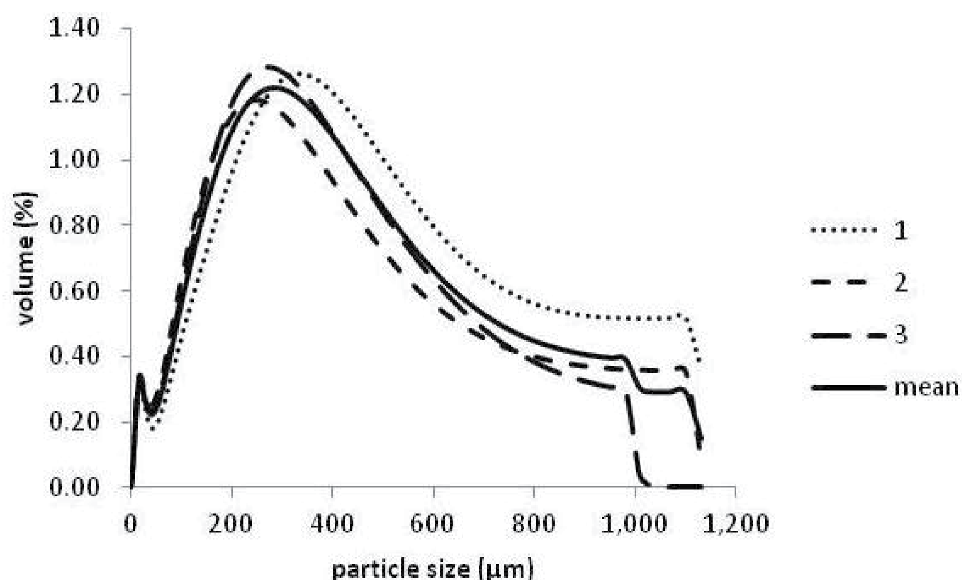

Figure 9. Particle size and frequency of cagaita pulp (*Eugenia dysenterica* DC).

14. Conclusions and future trends

Based on the results reported by several authors cited in this paper regarding the physical and chemical characteristics of the antioxidant action of the cagaita fruit, one can conclude that there is potential for therapeutic and medicinal applications. Additionally, a variety of new products with beneficial properties, such as jams, juices and energy beverages, can be made from the fruit of the cagaita. Using an established technology such as membrane processing, to acquire clarified juice, and then adding nutrients, offers the potential for another profitable business venture. Because the population of the Brazilian Cerrado region consumes the fruit both, whole or processed by hand, the industrial manufacture of cagaita fruit products is a viable business opportunity, especially considering that most of the production fails to be fully utilized at this time.

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Chapter 2

The Bean – Naturally Bridging Agriculture and Human Wellbeing

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Additional information is available at the end of the chapter

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1. Introduction

Human existence requires a steady supply of food containing a multitude of vitamins, minerals, trace elements, amino acids, essential fatty acids and obviously starch. Advances in crop production have mostly occurred in cereals like rice, wheat and maize, whereas grain legumes like bean and lentils only have experienced a quarter of these advances [1]. The shift have had consequences on the human wellbeing [2] as cereals after polishing or dehusking only contain small amounts of protein and micronutrients.

The plant family *Leguminosae* is particular interesting as it is protein rich and possesses the capability to fix atmospheric N_2, which makes it independent off fuel-driven supplies of nitrogen fertilizers. Common bean (*Phaseolus vulgaris* L.) is without comparison eaten more than any other grain legume [3]. Because of its importance it is often considered the 'poor man's meat' although this comparison may not give full justice to the bean. Beans are rich in the amino acids lysine and methionine, making beans complementary to cereals. In addition, they are rich in dietary fibre and low in oil content. Beans are genetically very diverse, adapted to local conditions and dietary preferences. An evaluation of the various collections by in particular CIAT and USDA Plant Germ System for useful traits has started but sophisticated plant breeding of the bean is sparse [e.g. 4, 5].

Beans are consumed as mature grain and immature seeds as well as green pods and leaves taken as vegetables [6]. As early as 1958, the UN organisation FAO organised a conference where the production and consumption of bean were discussed. In this context, [7] noted that data on production and consumption on grain legumes generally were incomplete. It seems plausible that this condition prevails till today given that a large proportion of the

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bean crops are produced for home consumption in backyards and small gardens and frequently it is also intercropped with maize by smallholders as a secondary crop. Consequently, reliable statistics may be difficult to obtain regarding production.

Bridging agriculture and human wellbeing is the answer to major challenges like world hunger, diminishing natural resources, and climate changes. The bridging can be done in two ways, either by enhancing the content of nutrients in the starch-rich stable food or by enhancing the accessibility of nutrient-dense food in the diet. Acknowledging beans importance in the diet of large segments of the world population, we will in this chapter explore possibilities to bridge the production side with the consumption side. This we will do by focussing on enhancing the amounts of important nutrients in our dominant diets.

Enhancing the content of nutrient in the available food can be done via traditional fortification through the processing of diet elements. Or it can be done via the so-called 'biofortification', which aims at improving the genetic basis for making plant foods more nutritious as the plants are.

Improving our access to nutrient dense food elements requires a different look as such food elements already may be part of the traditional diet. Such a look requires that local production and productivity is our vantage point and that peoples' specific preferences and cultures may influence their preferences for cultivating particular cultivars. Such a vantage point requires that people are involved in the process [8] and this chapter will pursue this using the *Phaseolus* bean as a model for one nutrient-dense element of the diet.

2. The bean

Improving the content in the starch-rich food elements like wheat, rice and maize is obviously possibly but the starting point is very low (Table 1). The grain legumes, on the contrary, have a high starting point from where to seek improvements. Beans are superior to cereals in their macro- and micronutrient content as demonstrated in Table 1, in agreement with [9] although trials with other pulses under farmers' conditions have demonstrated that genetic potential are not always expressed under more marginal conditions. Furthermore, the legumes holds a potential for entering the diet in a diversity of ways, ranging from the dry mature seeds, to green seeds and pods as well as leaves used as vegetables, see also [10]. An efficient bridge to human wellbeing can thus be established by enhancing the access to and intake of the beans with their high nutrient density.

The production and the uses of legumes decrease in some regions while it increases in others. Brazil and Argentina have become major producers and exporters of soya bean due to its value in the feed industry, while the production of grain legumes for home consumption decreases steadily in a country like Bangladesh [12]. A historical view since 1970 show however a consistent decline in the average annual consumption of grain legumes per capita from 9 to 7 kg per person [6].

	Protein (%)	Mg (%)	P (%)	S (%)	K (%)	Ca (%)	B (ppm)	Na (ppm)	Cr (ppm)	Mn (ppm)	Fe (ppm)	Ni (ppm)	Cu (ppm)	Zn (ppm)	Mo (ppm)
Bean	25.0	0.171	0.396	0.178	1.450	0.177	11.0	23.0	0.00	18.0	65.0	1.00	3.00	38.0	28.0
Pigeonpea	23.6	0.157	0.370	0.126	1.710	0.110	11.4	10.1	0.14	14.0	29.9	3.69	11.8	23.2	1.22
Maize	8.4	0.122	0.380	0.206	0.430	0.005	-	-	-	7.9	33.2	0.43	2.84	29.0	0.34
Maize dehusked	1.1	0.002	0.024	0.113	0.015	0.011	0	96.3	0.40	0.15	3.58	0.29	0.18	0.9	0.02
Potato flour	0.7	0.005	0.009	0.097	0.121	0.017	0	41.0	0.37	0.5	7.5	0.10	0.09	0.6	0.01
Wheat flour	15.1	0.010	0.030	0.162	0.398	0.030	0	11.6	0.31	14.7	29.6	0.12	3.37	21.7	0.81
Basmati ris	4.2	0.030	0.012	0.162	0.132	0.043	0	9.0	0.36	12.9	5.2	0.25	2.05	23.5	0.55

Table 1. Nutrient content of two grain legumes (Cajanus cajan: pigeonpea and Phaseolus vulgaris L.: bean) and maize (with or without husk) cultivated under farmers' conditions in eastern and southern Africa. Included is also the content of rice, wheat and potato flour sampled from various shops. After [11] and Høgh-Jensen, unpublished data).

In a trial with approx. 100 bean genotypes grown under relatively fertile one-site conditions in Malawi, an unexpected small variation was observed in terms of iron and zinc content of the grain. Mean contents of iron in the bean grains were 67.7 (± a SE of 0.95) and zinc were 33.6 (± a SE of 0.54) ppm (Høgh-Jensen and Chirwa, unpublished data). This demonstrated that genetic diversity may not be fully expressed when conditions are the same. However, seven of the best performing varieties were selected for subsequent trialling under varying local conditions in Malawi and Tanzania in the dry season of 2005 utilizing residual moisture. This trialling expressed on average over 230 plots selected for variation a content of 90 and 37 ppm iron and zinc, respectively (Table 2). The promising varieties consequently performed above expectations and certainly above average - even under fairly harsh conditions and less welcoming soils.

What varied the most was actually the yield between farmers. Consequently, the low hanging fruit is to focus on trialling and selecting the highest yielding varieties and to work with farmers to optimize the cultivation of beans (Table 3). Breeders have had some success by simply selecting for yields under conditions with semi-controlled drought periods [13,14] or across environments [15]. This approach does not disregard the more sophisticated breeding efforts like marker-assisted selection [e.g. 5]. However, the diversity seems yet only partly tapped, which means that local conditions to a large extent can be accommodated in a simpler trialling approach. The effect of this localness is expressed in the yield differences shown in a trialling of 6-8 bean varieties in Tanzania and Malawi, ranging from 100 kg grain per hectare to almost 3 tonnes (Table 3).

Variety per country	Grain yield (kg DM ha^{-1})	Grain weight (g 1000 $grains^{-1}$)	Iron (ppm)	Zinc (ppm)
Malawi				
101	1671	516	88	39
102	1410	444	88	39
103	1131	442	114	46
104	1470	478	88	38
108	1262	419	110	41
109	1749	503	91	39
Napilira	1280	408	104	42
Tanzania				
Jesca	782	324	61	33
Lyamungo85	860	358	81	32
Lyamungo90	1015	393	77	31
Selian94	1010	314	88	36
Selian97	1121	346	82	34
Uyole84	710	259	70	33
Uyole96	746	404	87	40
Wanja	924	385	78	34

Table 2. Mean grain yield, individual grain weight, and iron and zinc content in dry matter for tested varieties in Malawi and Tanzania in the dry season of 2005.

Farmer	Country	Grain yield (kg ha^{-1})	Country	Grain yield (kg ha^{-1})
1	Tanzania	296	Malawi	1129
2		740		1146
3		146		2006
4		634		1508
5		432		1696
6		155		1602
7		189		1600
8		99		1415
9		1924		844
10		1475		1068
11		2829		648
12		704		1518
13		1534		1241
14		1336		876
15		716		1573

Table 3. Average bean grain yield per farmer, who tested 6-8 varieties.

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3. Innovation in a value chain that also accommodate human well-being

Documented trialling efforts have so far been dominated by the researchers and only including the farmers, processers, traders, etc. to a limited extend. This does not mean that actors of change like innovative farmers, NGO, etc., have not had such activities. Our experiences tell us however that many of these data are difficult to get access to as they appear in reports, notebooks, newsletters, and similar documents that are found on shelves and stores. It appears logical that such trialling efforts must be linked to a learning process. The localness must however not hinder that the conclusions from such learning processes to be made available to others. The increasing using of open online repositories of research documents, which often is termed "grey literature", is an important step to share knowledge. The increasing publication rate in open access literature is another that will bring actors of change into the knowledge stream and to our common building of joint research capacity [see e.g. 16].

Since the Second World War, the innovation model in science has been linear, although a new model – less linear – emerged in the 1990s, called the 'Triple-Helix model', based on interactions between policy, science and society [17]. Increasingly, this model is being seen as also having a fourth leg, namely that of business. The fairly sequential linear innovation approach where production >> processing >> retailing may be adequate when talking about industrialized agricultural commodities. However, when quality requirements are less standard, the development of the requested traits at the commodities at various steps along the chain may require quite different orchestrated processes [16].

Such a process have been depicted by [18], drawing on experiences from working with small and market-inexperienced farmers, small processers with limited financial and processing capacity, and more fragmented retailers where market requirements are only partly known. Due to the limited experiences and capacities along the chain, a number of learning loops are included where the various stakeholders interact. These interactions are centred on value chain forums and actions related to each transforming step in the chain. Such value chain forums are found very important to enable the adjustment and enabling of mutual learning. Included in the model are also the feedback loops and the transformation of the intelligence regarding market requirements (Figure 1).

The value chain forums can be regarded as the places that prototyping is taking place. Prototyping is an important step in the innovation process as this is where ideas are being presented, discussed and validated – or maybe even more importantly discharged. Prototyping is a very important mode of action to avoid mistakes that will be very expensive in the longer run, if the solutions are allowed to travel further up the value chain. Clearly such learning processes are a challenge to researchers as management is becoming management of the process and not management of the variables.

Prototypes are designed to answer questions. The prototypes need not to be sophisticated but should best be as simple as possible. Simplicity is important to keep costs down and to enable the question-solution discussion. At a moment where management wisdom insists that speed to market is the key to competitiveness, the maintenance of the learning loop is

important – the cycle should be kept running to produce different ideas. Simplicity and differentiation is the two carrying principles here! But the circle MUST be stimulated by feeding in intelligence from the other actors along the chain, e.g. retailers and sellers, among others, to maintain chain agility. Consequently, innovation is not solely about technology. Innovation in this context is more about means to obtain, consolidate, translate and manage knowledge, means to transform knowledge, and organisational learning. In that sense, innovation becomes a culture of prototyping [see e.g. 20,21]. The possibilities of including dietary requirements in the first learning loop (Figure 1) are good as long as these requirements can be quantified and described and as long as they are causal.

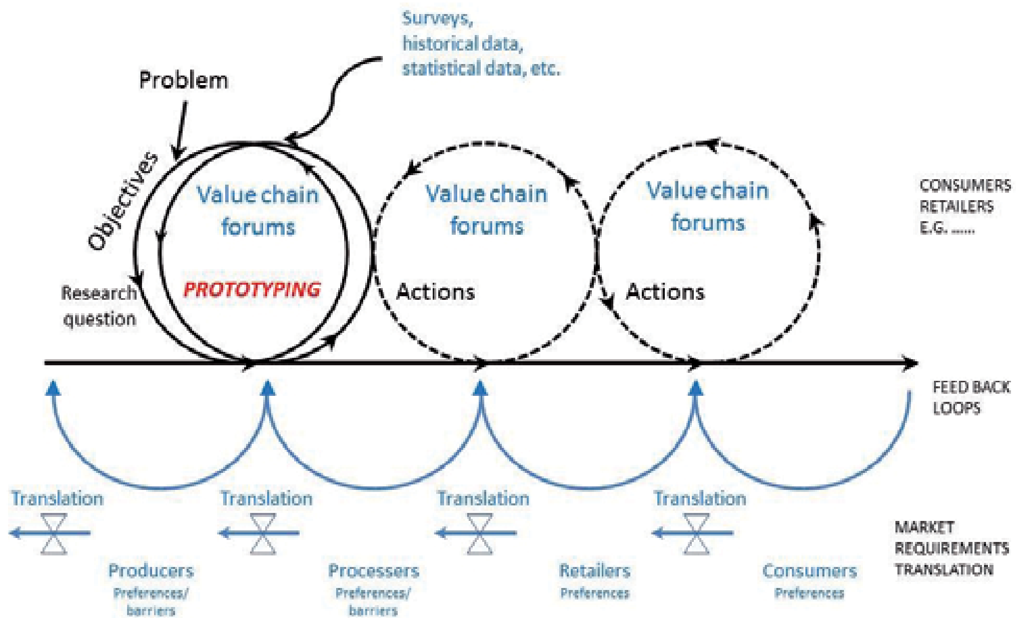

Figure 1. Prototyping in value chains innovation and development [modified after 19].

Nutrient dense diets can be sought in two ways. One is to find variation within one element of the diet that can form the basis for selecting the most promising in order to enhance the content. Or to seek a better production and/or access to the part of the diet that is particularly contributing with the nutrients. The later may be done by enhancing the production potential of bean varieties. It may however also be by promoting the use of the leaves for vegetable stews. [22] documented that the iron contents of the leaves compared to the mature grains could be 5-10 times higher on a dry matter basis. Leafy vegetables are indeed good sources of iron but they are mostly eaten for their vitamin-A and vitamin-C content. On a volume basis, the leafy vegetable and the boiled beans may provide similar amounts of iron. The boiled mature grain may however be a much better source of zinc [22].

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4. Naturally bridging agriculture and human wellbeing

To maintain productivity in agroecosystems, before the era of the fertilizer industry, humans traditionally have included animals in the systems and used their manures as fertilizers to drive the cereal production [23] in combination with grassland legumes to enhance the supply of nitrogen via symbiotic fixation [24]. Depending on locality, up to an average of 4-5 tonnes of manure could be applied per hectare in the UK [23] or as low as 1.5 tonnes per hectare in extensive mid-USA or north Spain [25].

The tropics have few examples where livestock is integrated in the agroecosystems in the same manner as frequently found under Northern temperate conditions [26]. As fertilizer use in Africa is still a very modest proportion of worlds fertilizer use [27], the cereal yields per area unit has remained low (Figure 2).

The response of data like those of low yield levels (depicted in Figure 2) follows a paradigm development [26], which also is referred by [8]. During the 1960s and 1970s, an external input paradigm was driving the research and development agenda which later has been known as the 'Green Revolution'. In the early 1980s, the balance shifted from mineral inputs only, to low external input sustainable agriculture (LEISA) where organic resources were believed to enable sustainable agricultural production. During the 1990s, the Integrated Natural Resource Management research approach and ultimately the Integrated Soil Fertility Management paradigm emerged. Still it was however argued that Sub-Saharan African farmers must use more fertilizer, improved germplasm, etc. to achieve a so-called "Second Green Revolution" [see e.g. 29].

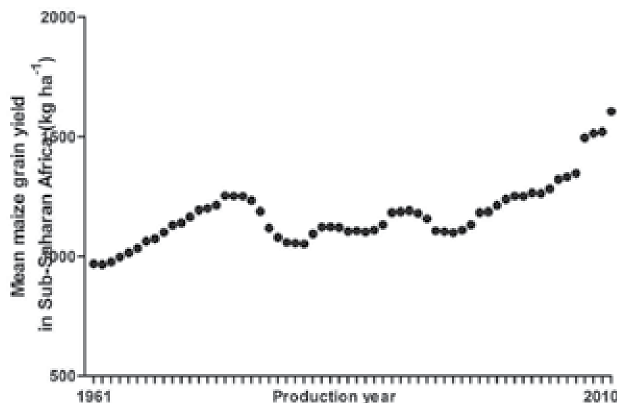

Figure 2. Official UN 5-year running average maize yield in the Sub Saharan African region between 1961 and 2010 [30].

A critical lesson from all this work is that a highly context-specific approach is required which takes into account the fertility status of the soil, the availability of organic inputs and the ability to access and pay for mineral fertilizers [28,31].

The response further assumes that the markets are perfect and that all agricultural commodities are entering a market. On one hand, large proportions of the diet of Africans are produced and consumed locally and may not enter the market. The part that enters the market may ignore the markets needs and preferences as it is sold as surplus on a local market. One commonly used model of innovation is the so-called value-chain model developed by Kline and Rosenberg, which emerged from studies of technological innovation. Modern innovation models must thus see many reverse processes and feedback loops in the incremental changes along the value chain, which further often has to include local conditions, cultural preferences, etc.

Elements in the bridge between agriculture and human wellbeing would thus be to trial for locally adapted bean varieties and to form a network among researchers that can promote a legume-based agriculture in these regions, in their particular social context. This approach would also recognize that a large proportion of the bean production occurs under conditions of significant drought stress [32], where agricultural inputs may not be an economically viable option. To overcome these particular stress conditions in combination with a vulnerable crop establishment phase, [10]) suggested investing in semi-perennial leguminous crops that has capacity to cope with short term weather variations. However, given the dominant role that beans have in nutrition in Africa and Latin America, robustness to environmental stress must be sought (Table 3) and combined with proper seed availability programmes [33].

The traditional plant-based diet is quite voluminous, i.e. it has high moisture content, with a limited protein and fat content. This is a particular challenge to children who require a diet of higher nutrient density than adults [34,35]. Some studies suggest that supplementary intake of animal protein, especially milk and fish, may stimulate childhood growth [e.g. 36]. However, some population segments may not have access to animal protein or cultural reasons limit their use of animal protein. Furthermore, dietary compositions vary over season in rural Africa and there may be temporal windows with surplus, adequate or lack of particular nutrients. Such windows may be influenced by reproduction cycles, health issues, harvest time and storage capacity, climate variability, household composition, among others [e.g. 37,38,39]. There is thus every reason to seek a higher density of nutrients in plant-based diets.

Cereals typical have a positive correlation between the nitrogen supply of the crop, thus the nitrogen content of the grain and the iron and zinc content [40]. Legumes are self-reliant on nitrogen through the biological fixation process. Consequently, correlations between nitrogen, iron and/or zinc content cannot be expected.

5. Seeking the nutrient dense diet – An adaptability analysis

It has frequently been assumed that farmers management and local growth conditions are fairly homogeneous and recommendations based on information generated on experimental stations dominate the extension services [e.g. 22]. However, homogeneity may be an illusion [e.g. 11]. Methods must thus be applied that allows for evaluation of performance under varying conditions.

Differentiating farmers are thus the approach in the so-called adaptability analysis [41]. This is an analysis that depicts the performance of the individual genotype across a wide range of environments versus the mean performance of the tested varieties can indicate if some varieties perform better or worse.

In terms of dry matter grain yield (Figure 3), there were no significant difference between the regression lines fitted to the observations in Malawi whereas the lines differed significantly (p<0.05) in Tanzania. In Tanzania, the slopes of the lines (Figure 3, right) had the following order in decreasing order: Selian97 > Selian94 > Lyamungo90 > Wanja > Lyamungo85 > Uyole96 > Jesca > Uyole84.

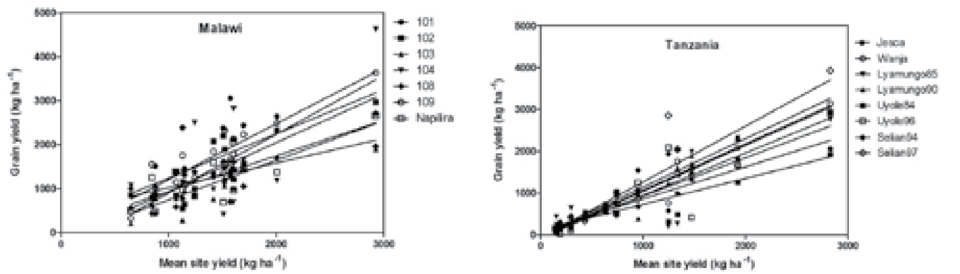

Figure 3. Individual observations and regression lines of grain dry matter yield of individual genotypes versus the mean site yield.

Phosphorus content in the grain follows pretty much a 1:1 ratio – so there is no effect of environment here as the slopes of the fitted regression lines did not differ (p>0.05). This is surprizing as the environment generally is considered P-scarce. The two environments clearly gave different proportions of phosphorus in the grain (Figure 4). And most observations from Malawi indicate that phosphorus in no way could be viewed as a limiting factor for beans at the current site with a mean site phosphorus proportion of 0.5% in the grain. Further, there seems no reason to believe that the individual genotypes could maintain a higher proportion of phosphorus in the gain across a phosphorus limiting environments as it appears to be the case in Tanzania.

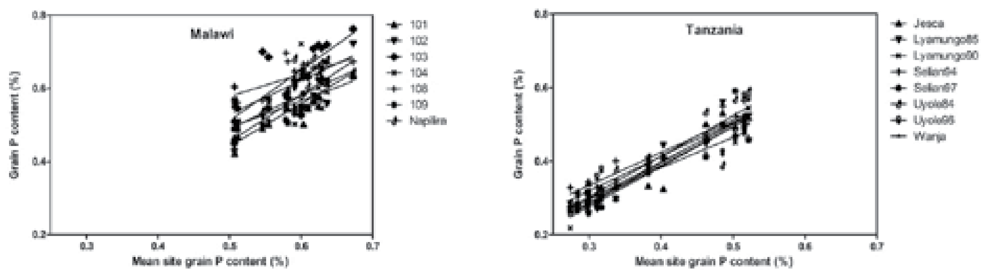

Figure 4. Individual observations and regression lines of the proportion (%) of phosphorus in the grain dry matter of individual genotypes versus the mean site yield.

A picture similar to phosphorus emerge (Figure 4) when plotting the proportions of iron in the grain (Figure 5). Obviously the two sites gave a different proportion of iron in the grain and there were tendencies to believe that some genotypes could be richer or poorer in iron than others. The 3 varieties with the highest proportion of grain iron content in Malawi were 103, 108 and Napilira while they in Tanzania were Selian94, Uyole96 and Selian97. In the Tanzanian case, the righest in iron thus seems to be the highest yielding across environments. An almost identical picture emerged regarding the proportion of zinc in the grain (Figure 6). The 3 varieties with the highest proportion of grain zinc content in Malawi were 103, 108 and Napilira while the 2 varieties with the richest zinc content in Tanzania were Selian94 and Uyole96.

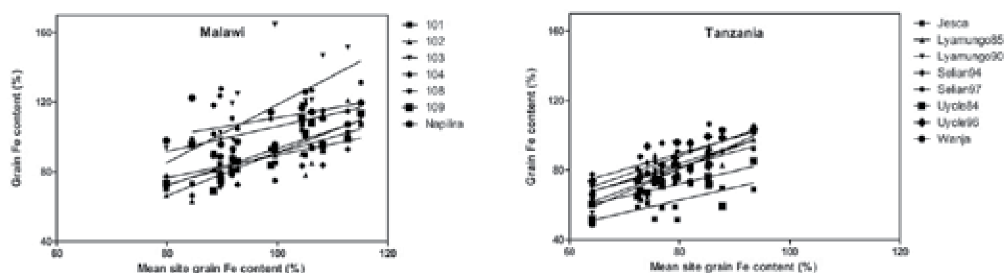

Figure 5. Individual observations and regression lines of the proportion (%) of iron in the grain dry matter of individual genotypes versus the mean site yield.

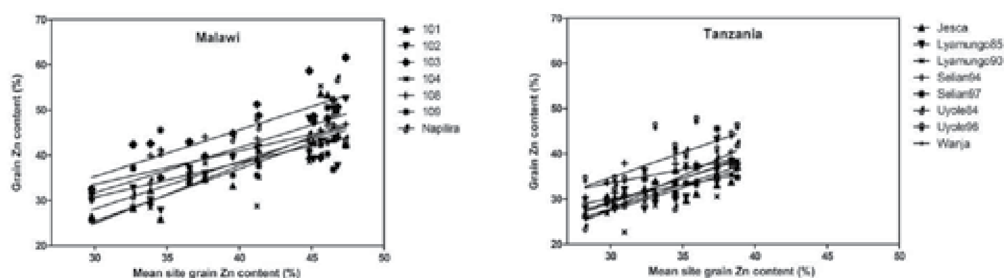

Figure 6. Individual observations and regression lines of the proportion (%) of zinc in the grain dry matter of individual genotypes versus the mean site yield.

Interestingly, however, is the fact that grain size did not appear to explain the differences between the element concentration as Malawi tended to have varieties that had individually larger grains (Table 2) and the grains with the highest proportion of phosphorus, iron and zinc in the grain dry matter. That eliminates a theory of element dilution at the end of the grain filling period which is often observed in bread wheat [42] but not always in other

crops [40]. In other words, bean appears to continue to fill in elements in to the grain together with carbon while maturing.

The current data (Figures 4-5) demonstrate that efforts to find the genetic material that tend to accumulate elements, which are important for human wellbeing, in higher concentrations in the grains are justified. Naturally we may - from an evolutionary point of view - wonder what benefit the plant gets from this. But it should not stop us from utilizing this variation in modern plant breeding efforts.

However, we are in a situation where we rely on small scale farmers to increase their production substantially. This production is both for home or local consumption but even more also for industrial purposes because of the rapid urbanisation of Africa and Asia. Building on farmers' capability and knowledge of their own environments may be the best way to enhance output from agriculture. That requires innovative approaches at farm level to test and select the best suited genetic material (Figure 2) to that particular environment. This will further require new approaches to seed supply systems as "one type fits all" approach will not do the job. On the contrary, seed supply systems must build on an approach of "multiple types to fit any environment", which obviously is a major challenge to extension and research.

6. A bowl of beans

The complementarity in the amino acid composition among beans and maize has been recognized for long [7, and references herein]. Grain legumes are characterised by being markedly deficient in the essential amino acids of methionine and tryptophan but rich on lysine. Cereals normally hold more methionine than the grain legumes so a high complementarity and higher combined nutritional value could be expected. Indian scientists were front runners in documenting such efforts [e.g. 43,44]. In recent years there has been a change in the consumption of grain legumes in developed countries were they increasingly are viewed as "health foods".

The traditional plant-based diet in part of Africa and Asia can be quite voluminous, i.e. it have a high moisture content, and the protein and fat content may also be limited [35,44]. This pose a particular challenges to population segments that cannot ingest sufficient food to cover their needs, in shorter or longer periods of their lives [e.g. 35,36,37].

Dietary diversity is important for the wellbeing of humans [45,46]. An inexpensive bowl of beans or other grain legumes would benefit many people. Agriculture has the potential to supply this bowl. Here we argue that by accepting that conditions vary much locally, we will have to adapt a learning approach to selecting bean varieties based on local productivity of the various genotypes given the local pest and disease pressures, soil fertilities and soil fertility management practices, on local preferences for processing and eating the beans, on the beans role in the local cropping systems, on differentiated population and resource groups.

From the industry's point of view, improved yields will be favourable as intensification will support a profitable production. This is clearly illustrated with the case of soybean production in South America [47]. Such cases highlight the expected situation in the future where the industrial focus on particular functional traits [48] will enhance the focus on the combination of yields and particular quality requirements. In a future, where production must be increased to meet the needs of additional 2 billion world inhabitants, quality traits of importance for human health and wellbeing may come into focus. Such traits must include iron and zinc.

Beans are to a large extent multiplied and reseeded from previous crops. Thus, the localness is already expressed in communities' planting preferences. To distribute new improved seed types are by experience very difficult when these types of crops are in question. The best the food industry can do to secure abundant supplies of beans when working with a multiple of smallholders are thus to contract on particular quality traits. Such outlet and market preferences have previously been found to have strong impacts on farmers' behaviours.

In the time of writing these lines, the food prices seem permanently to have left the relatively low levels of post-2007-2008 price peak [49]. Bean is a crop that is largely controlled by smallholders and the crop thus has a potential to contribute to the food security of the households. We have in this paper argued that bean holds the potential to bridge agriculture and human wellbeing because of its nutritional value, because it's genetic diversity and because it is controlled by local communities. The presented data suggest that farmers and change actors may improve the quality of the diet by simply going for the varieties that performs the best.

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SAKE Alcoholic Beverage Production in Japanese Food Industry

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Additional information is available at the end of the chapter

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1. Introduction

SAKE brewing is an important sector of the Japanese food industry. It has maintained a strong relation with the culture in areas producing it, as have other alcoholic beverages such as wine, beer, and tequila in other countries. *SAKE* has a history extending back 1000 years into antiquity, and brewers' skills and techniques have been cultivated scientifically for longer than the discipline of chemistry has even existed. Particularly, low-temperature sterilization of *SAKE* was conducted in the 16th century, before Louis Pasteur invented pasteurization. The method is carefully described in old Japanese literature.

The significance of *SAKE* culture and its old techniques of brewing has been investigated using modern scientific analysis and brewing research methods. Furthermore, in *SAKE* brewing, unique techniques have been examined, such as fermenting under low temperature, achieving more than 18% high alcohol concentrations without distillation, open fermentation systems without sterilization, and creation of a fruity aroma in *SAKE*. Furthermore, yeast, mold, and the raw material—rice—have bred to be suitable *SAKE* brewing. Preferences for *SAKE* among young (20–30s) consumers have been elucidated recently, and the potential for new *SAKE* development has been reported. This report describes the history of *SAKE*, propagation methods of *SAKE*, its production materials, and recent research related to it.

2. History of *SAKE*

Cultivation of rice, the raw material for *SAKE* brewing, originated in China. Seed rice harvested more than 10 millennia ago have been found in Kiangsi province and Hunan province in China. Probably, Japanese rice was introduced from China, where rice was cultivated

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in dry fields using dry rice cultivation methods. Introduced from China early, rice was cultivated in dry fields in Japan also. However, the method of rice cultivation in paddy fields boosted yields to higher levels than those achieved in dry fields. The wet method brought social changes: a reliable labor force is necessary for cultivation by planting rice in paddy fields, harvesting it, and maintaining paddy fields, equipment, and irrigation. The labor force resources from families were limited. More labor was required from settlements. The settlements formed communities. Later communities formed ancient Japan. At that time, rice was of particular value: it was a divine food. *SAKE*, made from that divine food of rice, was also revered as blessed. It was used as a sacrifice to the gods. Moreover, people believed in a divine spirit indwelt in rice. Extending that belief, people believed that intoxication by alcohol beverages as *SAKE* made from rice brought gods into the human body. Furthermore, they solidified the community by sharing divine foods as rice and beverage as *SAKE* among members as they cooperated in rice cultivation [1].

Alcoholic beverages can be made from cereals as beer, *SAKE*, or whisky. Saccharification processing is extremely important. Generally, it is an important feature of Asian alcoholic beverage production that mold cultivate in cereal, so-called '*KOJI*', is used for production. However, according to ancient literature, Osumi-no-Kuni-Fudoki, which recorded the culture and geography of Kagoshima in ancient times before production of *SAKE* using *KOJI*, alcoholic beverages were made with saliva as a saccharifying agent with a method of chewing rice in the mouth. It was produced by that method until the eighth century [1].

In China, ancient Chinese *KOJI* had been used from ancient times, as described in Chinese ancient texts such as the Chi-Min-Yao-Shu. Chinese *KOJI* is made from barley or wheat. It is kneaded cereal flour with water and hardened as a brick or cake. Modern *KOJI* is made from non-heated cereal flour or wheat as material. However, it is described that ancient *KOJI* made from a mixture of heat-treating material with mixed non-heated wheat flour, roasted wheat flour, and steamed wheat flour, and the mixture cultivated *KOJI* mold after kneading with water or extraction without inoculation of seed mold. Kanauchi and co-authors [2, 3] reported their features. Results show that *Aspergillus* spp. was grown on and within steamed cereal cake as the dominant *KOJI* mold, *Rhizopus* spp. was grown on and within a non-heating cereal cake as the dominant *KOJI* mold. Furthermore, both *Aspergillus* spp. and *Rhizopus* spp. as dominant *KOJI* molds were grown on and within a roasted cereal cake and a cereal cake mixed with heat-treating of cereal materials of three kinds [2,3]. Mold strains were dominant selectively in cereal cake because denatured protein was impossible to decompose by *Rhizopus* spp., but *Aspergillus oryzae* was impossible to assimilate non-heated starch in wheat flour [2,3]. In modern China, non-heated cereals such as barley or peas are used, whereby *Rhizopus spp.* or similar physiological features have *Mucor* spp., which can grow on non-cereals, predominant in it. It is difficult to decompose denatured cereal protein to enhance their uptake for nutrition of micro-organisms, *Rhizopus* spp. has a weak protease or peptidase to grow on steamed cereals [4].

It remains unclear whether Chinese type *KOJI* was used for *SAKE* production or not by ancient Japanese. However, steamed rice is used for *SAKE* production where *Aspergillus* spp. has been used since ancient times.

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Between the Asuka-Nara Era and Heian Era (5th – 8th century), imperial families and courtiers established huge craft factories of which wide areas were dedicated to crafts. Many technicians and workers were employed in them, monopolizing practical technologies of all areas. Brewery also continued in such factories, and *SAKE* was brewed using advanced technologies during those eras.

During the Heian period (8th century and thereafter), *SAKE* rose to importance for use in regional ceremonies or banquets. *SAKE* brewing by SAKE-NO-TSUKASA was both a *SAKE* brewery and a supervisory office of the imperial court. Some kinds of *SAKE* were brewed for emperor, imperial family, and the aristocracy for use at ceremonies or banquets. Brewing methods were described in *ENGISHIKI*, an ancient book of codes and procedures related to national rites and prayers. For example, *GOSYU* was specially brewed for the Emperor using steamed rice, *KOJI*, and mother water. The mash was fermented using wild fermentative yeast for ten days; then the mash was filtrated. The resultant *SAKE* was used for subsequent brewing as mother water. The *SAKE* brewed steamed rice, *KOJI*, and strained *SAKE* brewing were repeated four times to produce *SAKE* with a very sweet taste [1]. The literature in this period described *SAKE* of more than two kinds. The minor aristocracy and many people were not able to drink *SAKE* because it was extremely expensive.

Between the later Heian Era and Muromachi Era via the Kamakura Era (12th–16th century) *SAKE* was produced and sold at Buddhist temples and private breweries. During that period, it was a popular alcoholic beverage. In the 12th century, the feudal government issued alcohol prohibition laws many times to maintain security. Officers destroyed *SAKE* containers throughout cities.

At the beginning of the Muromachi Era, according to the '*GOSHU-NO-NIKKI*', *SAKE* was brewed already using a modern process in which rice-*KOJI* and steamed rice and water were mashed successively step-by-step. Moreover, the techniques applied lactic-acid fermentation, which demonstrates protection of the mash from bacterial contamination and dominant growth of yeast during *SAKE* production [1, 5].

During the 16th century, the *TAMON-IN* Diary was written for 100 years. *TAMON-IN* were small temples belonging to the *KOFUKUJI* temple in Nara. The diary described heating methods used to kill contaminated germs already in this century. In Europe, Louis Pasteur announced low-temperature pasteurization of wine and milk in 1865. However, Japanese brewers had acquired experimentally pasteurized *SAKE* during the 16th century [5].

During the Edo Era, the brewing season extended from the autumnal equinox to the vernal. However, results show good tasting *SAKE* brewing conducted in midwinter using a method called '*KANZUKURI*'. The brewing techniques of those brewers in the Ikeda, Itami, and Nada districts (Osaka City and Hyogo Prefecture) held the leadership in *SAKE* brewing at that time. During the Genroku period (end of the 17th Century), the total number of breweries was reported as greater than 27,000 [5].

After the Meiji Era, *SAKE* brewing methods changed drastically based on European science. Many improvements of *SAKE* brewing were accomplished by applying beer-brewing methods directly. However, many special techniques are used in *SAKE* production. For example,

mold culture is not required in beer brewing. Japanese brewers built the technology of *SAKE* production which mixed European beer brewing and old Japanese traditional techniques during the Meiji Era [5].

3. *SAKE* materials

3.1. Water

Water is an important material used in *SAKE* brewing, accounting for about 80% (v/v) of *SAKE*. It is used not only as the material but also in many other procedures such as washing and steeping of rice, washing of bottles or *SAKE* tanks, and for boiling. Generally, approx. 20–30 kl of water is necessary to process one ton of rice for *SAKE* brewing [5, 6]. The water for *SAKE* brewing must be colorless, tasteless and odorless; it must also be neutral or weakly alkaline, containing only traces of iron, ammonia, nitrate, organic substances, and micro-organisms. In particularly, iron ions are injurious to *SAKE*, giving it a color and engendering deterioration [5, 6]. Therefore, iron in brewing water is removed using appropriate treatments such as aeration, successive filtration, adsorption (with activated carbon or ion-exchange resins) and flocculation (with a reagent of alum) [7, 8].

3.2. Rice

The quality of rice, the principal raw material of *SAKE*, strongly affects the *SAKE* taste, but details of its effects are not clearly elucidated. Contrary to the other Asian alcohol production, Japonica short-grain varieties are used for *SAKE* production. In Korea and Taiwan, other short-grain varieties might also be used for alcoholic beverages.

3.2.1. Grain size

Large grains are suitable for *SAKE* production. Figure 1 shows rice grain size. The grain size is generally reported as the weight of 1,000 kernels. A weight of more than 25.0 g has been quoted as a mean value of 101 selected varieties by scholars [5, 9]. The selected varieties have a white spot in the center known as *SHINPAKU*, which contains high levels of starch.

3.2.2. Chemical constituents

Rice contains 70–75% carbohydrates, 7–9% crude protein, 1.3–2.0% crude fat, and 1.0 ash, with 12–15% water. Other components such as proteins or lipids in rice, excepting starch, are unnecessary for *SAKE* production. In fact, *SAKE* produced with rice having excessive proteins or lipids does not have good flavor or taste. Their compounds exist on the endosperm surface, mainly around the aleurone layer. Therefore they are removed by rice polishing. Moreover, the following have close correlations among the weight of 1,000 kernels: crude protein contents, speed of adsorption of water during steeping, and formation of sugars by saccharification of rice with amylases [5, 10].

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Figure 1. Rice grain size. Left side shows YAMADANISHI for variety of *SAKE* brewing rice. Right side shows HITOMEBORE for variety of diverting rice.

4. Microorganisms

4.1. *KOJI* mold (*Aspergillus oryzae*)

The scientific name of Japanese *KOJI* mold is *Aspergillus oryzae*. It grows on and within steamed rice grains. The mold accumulates various enzymes for *SAKE* production. Enzymes of about 50 kinds have been found in *KOJI*, the most important of which are amylases. α-Amylase (Endo- α- amylase, EC.3.2.1.1) and saccharifying amylase (Exo-α-glucosidase; E.C. 3.2.1.20) play important roles in amylolytic action [5, 11]. Furthermore, proteases of some kinds are also important enzymes: acid-proteases and alkaline-proteases are found in *KOJI*. In *SAKE* mash, the enzymes decompose protein to form amino acids and peptides (oligo-amino acid) at low pH values such as pH 3–4 [5]. Furthermore, amino acids or peptide-supported yeast grow with food or nutrition. The enzyme acts indirectly, decomposing rice protein while combining to an active site of the α-amylase [12].

The taxonomy of mold was studied for *Aspergillus oryzae* by Ahlburg and Matsubara (1878) and Cohn (1883). A report by Wehmer (1895) was published, describing *KOJI* mold class *A. oryzae* in detailed mycological studies as an *A. flavus-oryzae* group. They are slight graded on variations in morphological and physiological properties [5]. Murakami et al. identified and reported that *KOJI* mold strains used for *SAKE* brewing belonged to *A. oryzae* and not *A. flavus*. Two species were distinguished based on mycological characteristics of each authentic type culture of the two species [13, 14]. It is noteworthy that no Japanese industrial strain of *KOJI* mold is capable of aflatoxin production.

In *SAKE* brewing, conidiospores produced over bran rice, so-called TANE-*KOJI*, are sprayed and inoculated on steamed rice. *KOJI* is prepared in an incubation room, a so-called *KOJI*-MURO.

4.2. Yeast

4.2.1. Physiology of SAKE yeast

Fermentative multi-budding yeast, *Saccharomyces cerevisiae*, which has been used not only in *SAKE* brewery, but also in beer brewry, winery and bakery, was discovered in ca. 1830 by J. Meyen; it was named by E.C. Hansen in 1882 [5]. *SAKE* yeast is classified taxonomically in the *Saccharomyces cerevisiae* group [15]. However, the yeast was distinguished from other strains of *S. cerevisiae* by additional properties such as vitamin requirements [16, 17], acid tolerance, sugar osmophilic character, and adaptability to anaerobic conditions. Additionally, *SAKE* yeast has advantageous features that enable its growth under high sugar contents and low pH conditions, to produce *SAKE* under open system fermentation.

SAKE yeast formed a large amount of foam during main mash fermentation. Because one-third of the capacity of the fermentation vessel is occupied by foam during usual main fermentation, preventing foam formation would be greatly advantageous to breweries to save space occupied by the foam and scaling up the amount of *MOROMI* produced. Some large-molecular-weight compounds that arise from steamed rice grains are also regarded as taking part in foam formation. Recently, foam formation has involved existing proteins, with foam formation on the yeast surface.

Ouchi and Akiyama obtained foam-less mutants that have the same characteristics as the parent yeast except for foam-formation [18, 19]. A foam-less mutant of *SAKE* yeast, a favorite strain of *Saccharomyces cerevisiae* (The Brewing Society of JAPAN is distributing it as *SAKE* yeast), has become available for *SAKE* brewing. Recently, foam protein in SAKE yeast, AWA 1, was cloned. TAKA-AWA foam has been obvious molecular biologically [20].

Figure 2. TAKA-AWA foam. (Photograph by Shiraki Tunesuke Co., Ltd.)

4.2.2. Aroma production by SAKE yeast

The *SAKE* aroma is produced by yeast mainly because rice, as a *SAKE* material, has weaker aroma than materials used for wine or beer. Furthermore, *SAKE* contains ethanol, higher concentrations of alcohol, and many aroma-producing compounds. Aromatic compounds are an important factor used to characterize *SAKE*. Recently, a flavor wheel for *SAKE* was produced similar to existing ones used for wine and beer [21,22]. According to this wheel, the aromas can be categorized as floral aroma, fruit-like nutty, caramel-like, and lipid-like. A

fruit-like flavor is imparted to *SAKE* from yeast production because many Japanese consumers favor *SAKE* that has a fruit-like aroma. A yeast mutant producing fruity aromas was isolated for *SAKE* brewing. Their typical chemical components are ethyl caproate, which gives an apple-like aroma, and iso-amyl acetate or iso-amyl alcohol, which give a banana-like aroma. Before development of methods of breeding yeast to produce aromas, it was not easy for aromatic *SAKE* to be brewed and supplied stably for customers. Some competent *SAKE* brewers had controlled temperature severely to adjust enzymes that produced *KOJI* mold as amylase. Controlling the amounts of sugars as nutrient elements produced by amylase in mash adjusts the metabolisms of yeast growth and production of *SAKE* aromas as ethyl caproate and iso-amyl acetate. However, it is readily apparent that *SAKE* aroma synthesis by metabolic pathways or control mechanisms. The yeast producing fruity aroma was bred for use in commercial brewing [1, 5].

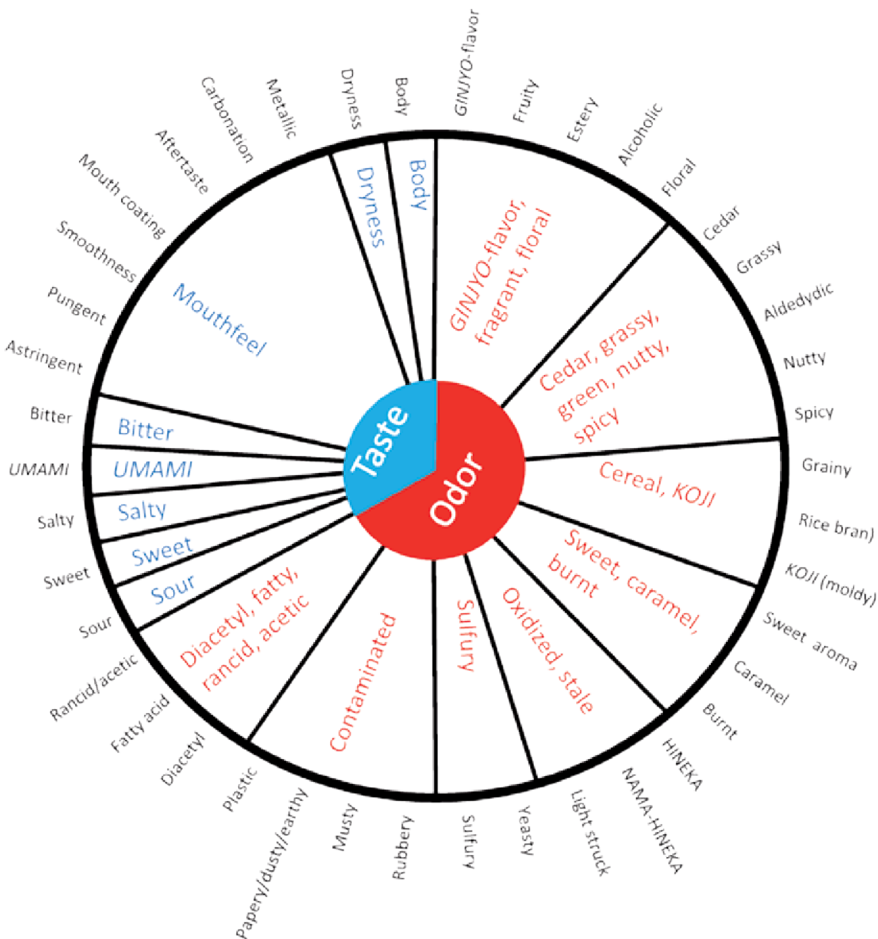

Figure 3. Flavor wheel of *SAKE.*

Typical yeast metabolic processes producing aromatic compounds are shown in Fig. 3.

- Higher alcohol metabolism pathway [1]

The higher alcohols as aromatic compounds are iso-amyl alcohol and iso-butyl alcohol. The alcohols are produced by two pathways by yeast as shown below.

1. $RCHNH_2COOH \Leftrightarrow RCOCOOH \rightarrow RCHO \rightarrow RCH_2OH$

2. $C_6H_{12}O_6 \rightarrow RCOCOOH \rightarrow RCHO \rightarrow RCH_2OH$

In these two pathways, 2-oxo acid is produced as a precursor. In the 1 pathway, 2-oxo acid is produced by deamination reaction between Ehrlich pathways. In the 2 pathway presented above, 2-oxo acid is produced between production of amino acid pathway. Oxo acid is produced by decarbonylation reaction and reduction reaction between both pathways, similarly as ethanol is produced from acetoaldehyde via pyruvic acid as oxo acid of one kind. For example, lacking amino acids as leucine and valine in *SAKE* mash, the yeast produces leucine and valine in *SAKE* mash. Furthermore, 2-oxo acid was transaminated from other amino acids. It is controlled by the amount of amino-acid-based amino bonds. Therefore, lacking extremely amino acid in mash, 2-oxo acid is converted to higher alcohol as iso-butyl alcohol and iso-amyl alcohol. Sufficing amino acid as leucine and valine in *SAKE* mash, the reaction of the 2 pathway inhibited by native feedback control and uptaken amino acid are converted by the 1 pathway.

- Fatty acid ethyl ester [1]

Ethyl caproate is a favorite flavor providing an apple-like aroma for Japanese consumers. This compound is produced by esterification from caproic acid as a precursor. Caproic acid is synthesized by fatty acid synthase between fatty acid synthesis pathway from acetyl-CoA and malonyl-CoA in *SAKE* yeast. Their synthase composes FAS 1 (Fas1p; β-subunit) and FAS2 (Fas2p:α-subunit), which are hexamer proteins (α6β6 subunit) [23]. Ichikawa reported a yeast breeding method that produces high levels of ethyl caproate that high levels of precursor of ethyl caproate were producing in yeast cells [24]. Cerulenin, an antifungal antibiotic produced by *Cephalosporium caerulens*, inhibits beta-ketoacyl-ACP synthase as *fatty acid* synthetase. A mutant of cerulenin-resistant yeast strain decreases synthesis of long-chain fatty acids by mutating Gly1250 Ser in the gene. The strain can produce high levels of caproic acid [25].

- Ethyl acetate group

Higher alcohol and esterified fatty acid produce a fruity aroma in *SAKE*. Usually, *SAKE* has 0.1 ppm or higher concentrations of ester compounds. That slight amount of ester produces a fruity aroma and intensifies the *SAKE* flavor. Excessive esters destroy the balance of the *SAKE* flavor. Many ester compounds produced mainly by yeast are acetate ester groups that react and which are produced by an alcohol–acetyl transferase reaction that transfers an acetyl bond from acetyl CoA to alcohol. Alcohol acetyl transferase (AATFase; E.C. 23.1.84) catalyzes the following reaction.

Acetyl CoA + Alcohol →Acetyl ester + CoA-SH

This enzyme, a microsomal enzyme, is an endogenous membrane protein dissolving by surfactant. Furthermore, more than 70% of the activity exists in it. AATFase has two isozymes of molecular weight 56 k Da. Isozyme P1 is reacted mainly in the yeast cell. Its activity has ca. 70–80% overall activity. Its optimum temperature is 25°C (Isozyme P2 is 40°C), and the optimum pH is 8.0. The pH range of its reaction is pH 7.5–8.5 (Isozyme P2 is pH 7.0–8.5). Their enzyme inhibited phosphatidylserine and phosphatidylinositol, having interfacial activity, and oleic acid and linoleic acid. Accordingly, this phenomenon showed that this enzyme has a hydrophobic active site in it [26].

4.3. Lactic acid bacteria *(Lactobacillus sakei)* [5, 27]

Lactic acid bacteria are the most important bacteria in *SAKE* brewing. Lactic acid bacteria are defined as listed below.

1. Bacteria ferment glucose and producing more than 50% lactic acid per 1 molar of glucose.
2. Bacteria is Gram positive. Their shapes are cocci or bacci.
3. They are facultative anaerobic bacteria.
4. They have no mobility.
5. They produce no spores.

Their fermentation types are two. One is homo type, 2 molar of lactic acid fermenting from 1 molar of glucose. The other is hetero type, 1 molar of lactic acid, 1 molar of ethanol and 1 molar carbon dioxide from 1 molar of glucose. Typical lactic acid bacteria for food processing are shown as the following: *Leuconostoc* spp. is a hetero-type cocci lactic acid bacteria, and *Pediococcus spp.* is a homo type cocci lactic acid bacteria. *Lactobacillus* spp. belongs to both types of bacci lactic acid bacteria.

In *SAKE* brewing, lactic acid bacteria are used in traditional seed mash, *KIMOTO* production for without sterilization safety open fermentation system without sterilization. In traditional seed mash, *MOTO*, production, it is known that *Leuconostoc mesenteroides* as hetero-lactic acid fermentation grows the *MOTO* preparation earlier under extremely low temperatures of less than 5°C. *L. sakei* as a hetero-lactic acid fermentation grows in it.

It is rarely that lactic acid bacteria spoil commercial *SAKE*. The bacteria are called *HIOCHI* bacteria, and have resistance to ethanol concentrations higher than 18% in *SAKE*. *SAKE*-grown *HIOCHI* bacteria have turbidity and an uncomfortable cheese-like smell from diacetyl [28]. Two types of lactic acid bacteria might be involved: one is *L. homohiochi*i (homo lactic acid fermentation type); the other is *L. heterohiochi* (hetero-lactic acid fermentation type). Both bacteria have resistance to ethanol. The coefficient for growth of two bacteria in *SAKE* is mevalonic acid, which is produced by *KOJI* mold. Recently, mevalonic acid nonproductive mutants have been bred for *SAKE-KOJI* production [1, 17, 29].

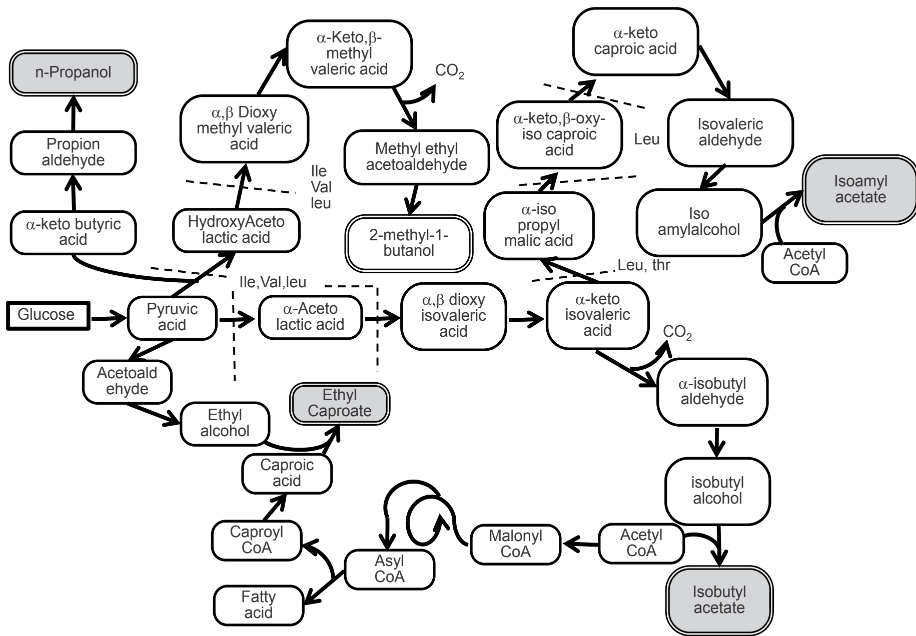

Figure 4. Production of aroma compound mainly by yeast. Broken line shows inhibition of the reaction by amino acid

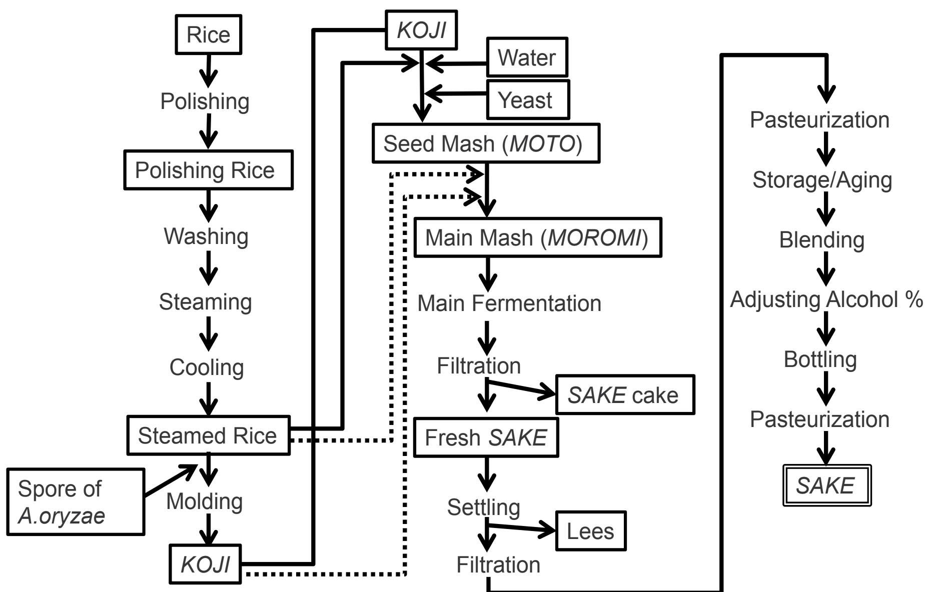

Figure 5. *SAKE* production.

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5. *SAKE* Production

5.1. Rice treatment (polishing, washing, and steeping)

In contrast to the use of malt in brewing beer or producing spirits, in *SAKE* brewing, polished rice is used. The main purpose of polishing is to remove unnecessary substances in rice aside from the starch, which are regarded as undesirable in *SAKE* brewing. Polishing removes surface layers of the rice grains, which contain proteins, lipids, and minerals. The ratio of percentages by weight of polished rice to the original brown rice is defined as the polishing ratio. Changes in the amounts of some constituents of the processed grain with various polishing ratios are presented in Table 1 (Research Institute of Brewing, Japan, 1964). Crude fat and ash contents decrease most rapidly, whereas the protein content decreases gradually until the polishing ratio reaches 50%, after which it remains practically constant. In contrast to changes in the crude fat content, the lipid content (by hydrolysis) does not change with increase of the polishing ratio [30].

	Polishing ratio (%)			
	100	80	60	50
Moisture	13.5	13.3	11.0	10.5
Crude protein	6.55	5.12	4.06	3.8
Crude fat	2.28	0.11	0.07	0.05
Ash	1.00	0.25	0.20	0.15
Starch	70.9	74.3	76.3	77.6

Table 1. Changes in the contents of some rice grain components after polishing [5]

The lowest polishing ratio is strictly regulated under the Liquor Tax Law. In general, polished rice of 75–70% ratio is used for reasonably priced *SAKE* brewing. In contrast, polished rice of a 60% ratio is used for special brewing brands such as GINJYO-SHU, and rice of less than 50% polishing ratio is used for Grand grade *SAKE*, DAIGINJYO-SHU. The latter is a prestige class of *SAKE*. Sometimes, the *SAKE* is brewed using rice of a 30% polishing ratio.

The rice polisher depicted in Fig. 6 is used for *SAKE* brewing. The roller made of carborundum and feldspar rotates around a vertical axis, and scrapes the surface of grains. Rice grains supplied from the hopper are polished and fall to the bottom of the basket conveyer. The grains go through the sieve to remove the rice bran. The rice is carried by the basket conveyer to the hopper. The operation continues until the grains are polished to the required ratio [5].

Generally, with a mill having a roller that is 40 cm in diameter, average times for polishing are 6–8 h for 89%, 7–10 h for 75%, 10–13 h for 70%, and 16–20 h for 60% polishing ratio [5].

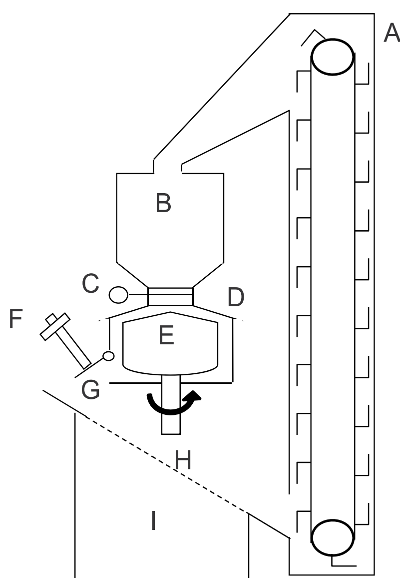

Figure 6. Diagram of a vertical type rice mill used in SAKE brewing [5]: A, the basket conveyor; B, rice hopper; C, the rice flow adjusting bulb; D, the polishing chamber; E, the roller; F, a resistance; G, exit; H, sieve and I, bran reservoir.

5.2. Washing and steeping

Rice is washed and steeped in water before steaming. During washing, the grains are polished further by collision of rice grains in water. During processing, the surface parts of the grains are removed, eliminating approx. 1–3% of the total grain weight [5]. Washed rice grains are passed into a vat and are steeped immediately in water. In washing and steeping procedures, the grains absorb water to about 25–30% of their original weight. The moisture promotes penetration of heat into the grain center during steaming and accelerates gelatinization of starch in the grains. Absorption of water is extremely important for preparing properly steamed rice, and controlling *KOJI* making and fermentation. The water absorption into grains differs according to the variety of rice and the polishing ratio [5, 10, 30]. Generally, rice grains are steeped in water for 1–20 h, and soft rice absorbs water within 1–3 h. Highly polished rice grains absorb water more rapidly. During washing and steeping, potassium ions and sugars are eluted from the grains [1, 31], whereas calcium and iron ions are absorbed onto the grains [5]. After steeping, excess water is drained off from the grains for about 4–8 h before steaming.

5.3. Steaming

Starch is changed to the α-form, and protein is denatured by the steaming process. Moreover, the grains are sterilized by steaming. The grains are usually steamed for 30–60 min, although previous reports show that steaming for as little as 15–20 min is sufficient to modify the starch and protein of rice produced in Japan [1]. During steaming, the grain moisture

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is absorbed to the extent of 7–12% of the weight of the starting rice grains, namely total water gain of about 35–40%. Historically, at many breweries, steaming processes usually generated steam from water in a large pot. Today, boilers are often used in many breweries for steaming. A steamer is a shallow and wooden tub in which is bored a hole (1/20 diameter of bottom) at bottom. The steamer is put above the 1.5–2.0 kl caldron, and rice is permeated by large amounts of steam from the caldron. Recently, a modern apparatus for steaming rice as belt conveyor type apparatus is used in automated modern breweries. The steamed rice is cooled to nearly 40°C for *KOJI* production, and the rice used for preparing *MOTO* and *MOROMI*-mash is cooled to less than 10°C. Breweries usually use machines to cool the steamed rice with a draft of air as it moves on the screened belt. A pneumatic conveyer system is often used to transfer steamed rice [1].

5.4. *KOJI* preparation [5]

A *KOJI* cultivates the *KOJI* mold, *Aspergillus oryzae* on and in steamed rice grains, and which accumulates various enzymes for *SAKE* production. For the preparation of *KOJI*, seed-molds are used at all breweries. The *Aspergillus oryzae* strains are cultivated in steamed bran rice dredging wood ash at 34–36°C for 5–6 days. This process results in abundant spore formation. Cultivation conditions influence the enzyme production. In general, higher cultivation temperatures (approx. 42°C) develop the activities of amylases. Lower temperatures (approx. 30°C) activate protease activities.

As cultivation times lengthen, more enzymic activities appear in the *KOJI* [32]. Nitrogenous substances and acids are accumulated more in *KOJI* that has been prepared from steamed rice of higher moisture contents [33]. They are regarded as related to the flavors and tastes of *SAKE*. After the steamed rice has been cooled to about 35°C by going through a cooling apparatus, it is transferred into the *KOJI-MURO*, a large incubating room, where temperature (26–28°C) and humidity are controlled at suitable levels to grow *KOJI* mold.

After inoculating or spraying *TANE-KOJI* as seed mold in the proportion of 60–100 g/1,000 kg of rice, then the mixture is heaped in the center of a table for *KOJI* preparation. At this stage, the temperature of the material is 31–32°C. As the spores germinate and mycelia develop, the rice begins to smell moldy like sweet chestnut. After incubation for 10–12 h, the heap of rice grains is mixed to maintain uniformity of growth, temperature, and moisture contents. After another 10–12 h, with growth of the mold, mold mycelia can be observed distinctly as small white spots on the grains. Furthermore, the material temperature has risen to 32–34°C. It is dispensed into wooden boxes, each with 15–45 kg of the grain. To control the rise in temperature and the moisture in the grain mass, the bottom of the box is made of wooden lattice or wire mesh. Temperature and moisture contents are also controlled by the thickness of the heaped grain layer in the box: 8 cm at the beginning, 6 cm at the first mixing, and 4 cm at the second mixing. Thereafter, at intervals of 6–8 h, the material is mixed and heaped again in the box. After incubation for about 40 h, the temperature of the material rises to 40–42°C. The mycelium develops to cover and penetrate the grains which have sufficient enzymes, vitamins and various nutritive substances for mashing and growth of *SAKE* yeast. Then the *KOJI* is taken out of the room and spread on a clean cloth to be cooled

until it is used for mashing. α-amylase and acid-protease activities increase during *KOJI* making. Carbohydrates are decomposed finally to water and carbon dioxide, which engenders the production of energy for growth of the mold.

5.5. *SAKE* mash fermentation

5.5.1. 'MOTO' as yeast starter

In *SAKE* brewing, *MOTO* is important as a yeast starter for the fermentation of *MOROMI*. *MOTO* is necessary to provide a pure and abundant yeast crop, and to supply sufficient lactic acid to prevent contamination of harmful wild yeast or bacteria during *MOTO* production or in the early stages of main fermentation.

In traditional *MOTO* preparation, lactic acid is produced by lactic-acid bacteria in the mash. In the modern method, pure lactic acid is added to the mash at the beginning of *MOTO* preparation. Lately, compression yeast cultivated using a method similar to that for baker's yeast used to ferment main mash safely with this yeast instead of *MOTO.* The amount of rice used for *MOTO* preparation is usually 7% of the total rice used for the entire *SAKE* mash.

- Traditional Seed Mash

KIMOTO is a traditional *MOTO.* Actually, *MOTO* has been handed down from early times, and the *MOTO* was modified to be simple and convenience by Kagi et al. [34]. The modified *MOTO* is called *KIMOTO.* The *YAMAHAIMOTO* is based on the same microbiological principle as that of *KIMOTO*, and has practically replaced *KIMOTO* because the related procedure is simpler [5].

Steamed rice (120 kg) is mixed with 60 kg of *KOJI* and 200 L of water in a vessel at an initial temperature of 13–14°C. It is then kept for 3–4 days with intermittent stirring and agitation. During this period, the rice grains are partially degraded and saccharified, and the temperature falls gradually to 7–8°C. The mash is then warmed at a rate of 0.5–1.0°C/day by placing a wooden or metal cask filled with hot water in the mash after warming for an additional 10–15 days, after which the temperature reaches 14–15°C. In *KIMOTO* mash, some micro-organisms grow successively to each other as Fig. 7, and mash brings acid condition to grow *SAKE* yeast easily without contamination [5].

In early stages, contaminating wild yeast or germs disappears within the first two weeks as a result of the toxic effect of nitrite, produced by nitrate-reducing bacteria from nitrate contained in or added to the water. Slight nitrate contained in the mother water is converted to nitrite, which has toxicity for micro-organisms by nitrate-reducing bacteria such as *Achromobacter* spp., *Flavobacterium* spp., *Pseudomonas* spp., and *Micrococcus* spp. (derived from *KOJI* and water). A toxic substance, nitrite, yeast of one kind from *KOJI* as *Pichia angusta* [35] was assimilated after oxidating nitrite during *MOTO* mash. Nitrite is toxic for lactic acid bacteria and fermentative yeast in traditional *MOTO.* Their utility micro-organisms are able to grow under the *MOTO* mash containing nitrite. After removing nitrite, lactic-acid bacteria including *Leuconostoc mesenteroides* and *Lactobacillus sakei* (derived from *KOJI*) can grow in the *MO-*

TO mash. These bacteria multiply to reach a maximum count of about 10^7–10^8/g. However, other gram disappear before fermentation by *SAKE* yeast begins because of the accumulation of high concentrations of sugar and because of acidification resulting from the growth of lactic-acid bacteria [5].

The mixing process helps to dissolve the nutrients contained in the *KOJI* and steamed rice, and mash promotes the growth of lactic-acid bacteria in the early stages [36].

- Convenient *MOTO* preparation as *SOKUJYO-MOTO*

Recently, *SOKUJYO-MOTO* is popular for use in *SAKE* brewing. It was devised by Eda [37]. It is based on the principle that addition of pure lactic acid to *MOTO* can prevent contamination by wild microorganisms. It takes a short time (7–15 days) to produce *MOTO* because of the time-saving lactic-acid formation by naturally occurring lactic acid bacteria, and saccharification of the mash proceeds quickly with the high initial mashing temperature (18–22°C). In this production, commercial lactic acid (75%, 650–700 m1/100 L of water) is added to the mash to adjust the pH value to 3.6–3.8. Although pure culture yeast is used as the inoculums, yeast grows more advantageously than do wild yeasts from *KOJI*. Furthermore, the latter eventually predominate during the *MOTO* process [5].

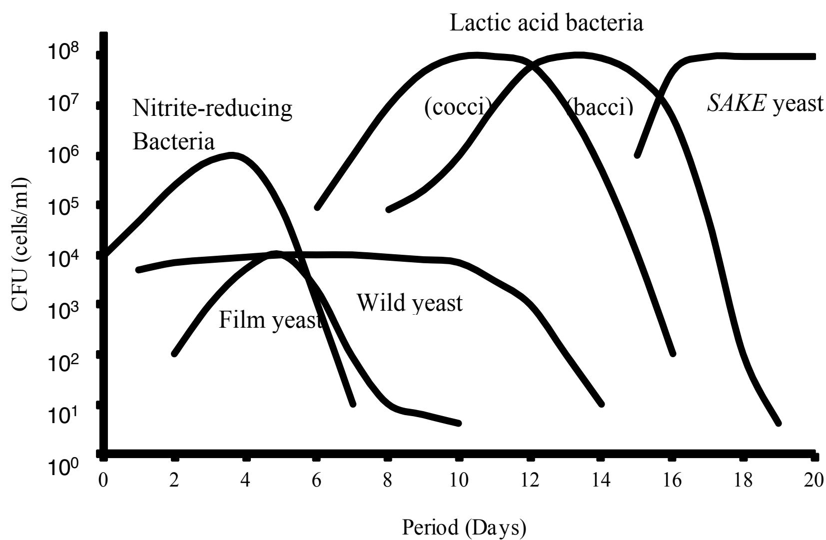

Figure 7. Changing numbers of micro-organisms in *KIMOTO* mash.

This predominance might be ascribed to the fact that the high mashing temperature and acidic conditions are close to the optimum for multiplication of both culture and wild yeasts. In addition, as opposed to the behavior in the classical process, no natural selection of wild yeasts by the toxic effect of nitrite occurs because the presence of lactic acid inhibits nitrate-reducing bacteria.

An example of the preparation of *SOKUJYO-MOTO* is the following: *KOJI* (60 kg) is added with 200 L of water and 140 ml of lactic acid (75%). A pure culture of *SAKE* yeast is inoculated to the mash (10^5 – 10^6/g). Its temperature is about 12°C. Steamed rice (140 kg) is added to the mixture, cooling it sufficiently to give a temperature of about 18–20°C. After keeping the mash for 1–2 days with intermittent stirring and agitation, it is warmed gradually in the same way as *YAMAHAI-MOTO* by increasing the temperature at a rate of approx. 1.0–1.5°C/day. As the temperature rises to about 15°C, *SAKE* yeast reaches its peak and fermentation begins.

The cultivation period can be shortened further by starting the mashing at 25°C and by keeping the temperature of *MOTO* over 18°C. Moreover, the variety of *SOKUJYO-MOTO* as *KOONTOKA-MOTO* (hot-mashed *MOTO*) is used by Japanese brewers. This mashing method is conducted at 56–60°C during several hours with subsequent inoculation of pure cultured *SAKE* yeast. To prevent excessive accumulation of sugars and the development of a high viscosity, the ratio of water to rice used is raised to 150–160 L/100 kg [5].

5.5.2. Main fermentation [5]

MOROMI, as main mash, is fermented in a large open vessel with a capacity ranging from 6–20 kl without special sterilization, in an open fermentation system. The weight of polished rice (1.5 t) was used for mashing one lot as standard. However, recently, larger vessels as 3–7 tons or sometimes over 10 tons have been used for mashing one lot. The *MOROMI* mash is brewed steamed rice, *KOJI* and water. Table 2 shows proportions of various raw materials used for a typical *MOROMI* mash. The preparation of stepwise mashing as three steps is one characteristic of *MOROMI* mash production. First, steamed rice, *KOJI* and water are added to the *MOTO*. Consequently, the total acid and yeast population in *MOTO* are diluted to about one-half. The temperature of the first mash is about 12°C, and the yeast propagates gradually. After two days, the yeast grows until 10^8/g, which reaches the same order as that in *MOTO*. As a second addition, the materials are added in an amount that is nearly twice as much as the first addition. The yeast population and total acids are diluted by about half too. The temperature of the second addition is lowered to 9–10°C. In a third addition, materials are added in a larger amount.

	1st addition	2nd addition	3rd addition	4th addition	Total
Total rice (kg)	140	280	890	160	2000
Steamed rice (kg)	95	200	720	160	1580
KOJI rice (kg)	45	80	170		420
Water (liter)	155	250	1260	160	2460

Table 2. Proportions of raw materials used in a typical *SAKEMOROMI* [1]

The amount of *MOROMI* bring 14 folds as same as *MOTO* mash. Whereby yeast cells are diluted. This stepwise addition of material plays an important role in suppressing the invasion of wild micro-organisms together with lowering the mashing temperature in each addi-

tion. In *SAKE* brewing, temperature control is also extremely important to balance saccharification and fermentation, both of which occur simultaneously in *MOROMI*. Therefore, we call it 'Parallel Fermentation'. Small quantities of sugars released from steamed rice and *KOJI* are fermented gradually by *SAKE* yeast until the alcohol content reaches nearly 20% (v/v). Accumulated alcohol of 20% v/v in the mash from 40% (w/v) of sugars. If such a high concentration of sugars is supplied at once, then *SAKE* yeast would not ferment alcohol in the mash. Instead, the mash fermentation at a low temperature (below 10–18°C) is also a characteristic of *SAKE* brewing which gives the mash a balanced flavor and taste as well as a high alcohol concentration. After the third addition of materials, the mash is agitated, usually twice a day. The mash density then reaches maximum levels 3–4 days later.

A foam resembles soap suds. Furthermore, it spreads gradually over the surface, and subsequently increases to form a thick layer. A fresh fruit-like aroma at this stage indicates healthy fermentation. The fermentation gradually becomes more vigorous with a rise in mash temperature, and a rather viscous foam rises to form *TAKA-AWA* (a deep layer of foam, shown in Fig. 2), which reaches to the brim of the vessel. In some breweries, it is broken down with a small electric agitator. At this stage, the yeast cell count reaches a maximum of about 2.5×10^8/g [38]. Because the alcohol concentrations increase, the foam becomes less dense, and is easily dispersed. The fermentation finishes usually during 20–25 days. In some breweries, pure alcohol (30–40%) is added to the mash to adjust the final concentration to about 20–22% (v/v).

Quite often, to sweeten the mash, 7–10% of the total amount of steamed rice is added during the final stage of the *MOROMI* process to produce glucose from starch by the saccharifying action of *KOJI* that accumulates in the mash.

5.5.3. Filtration [1,5]

After alcohol fermentation, the mash is divided into *SAKE* and solids by filtration. The mash is poured into bags of about 5 L capacity made of synthetic fiber, which are laid in a rectangular box. *SAKE* is squeezed out under hydraulic pressure. After complete filtration, the solids pressed in a sheet are stripped out of the bags. Recently several automatic filter presses for filtering *MOROMI* mash have been used. The *SAKE* lees or *SAKE* cake, residue squeezing *SAKE* as cake was called '*SAKE*-KASU', contains starch, protein, yeast cells and various enzymes, *SAKE* lees is used traditionally for making foodstuffs such as pickles and soup. In general, regarding 3 kl of *SAKE* containing 20% ethanol and 200–250 kg of KASU are obtained from one ton of polished rice. The slightly turbid *SAKE* is clarified to separate lees by standing in a vessel for 5–10 days at a low temperature.

5.5.4. Storage (aging) and bottling [5]

After settling the clarified *SAKE* for a further 30–40 days, The *SAKE* is pasteurized, killing yeasts, harmful lactic acid bacteria, and enzymes. The *SAKE* is heated to 60–65°C, passing it through a helical tube type heat exchanger for a short time. Recently plate-type heat exchangers with high efficiency of heat transfer have become available.

Figure 8. SAKE squeezer (Photograph by Hamada Co. Ltd)

As described in this chapter, the history of *SAKE* pasteurization began in the 16th century, before Pasteur's discoveries. After pasteurization, *SAKE* is transferred to sealed vessels for storage with or without addition of activated carbon. Pasteurization and the high content of alcohol in *SAKE* (usually 20%) prevent microbial infection. The blended *SAKE* is diluted with water to the appropriate alcohol content, usually 15.0–16.5% (v/v), and is filtered through activated carbon to improve the flavor and taste and to adjust the color and clarity. In modern procedures, filtration through activated carbon is followed by filtration through membranes or sheets having numerous pores of micrometer size, thereby removing minute particles including micro-organisms if any are present. This procedure enables the *SAKE* producer to omit pasteurization in the bottling procedure and therefore to prevent deterioration of quality caused by heating *SAKE*. The spoilage of *SAKE* is sometimes encountered, off-flavors and tastes are attributed mainly to the formation of diacetyl and acetic acid by *HIOCHI* bacteria.

SAKE is usually sold in a pale blue bottle of 1.8 l capacity, which is pervious to short and medium wavelengths in sunlight, as are beer, wine, and other alcoholic beverages. Coloring is spoilage of *SAKE* by sunlight, deferriferrichrysin precipitates, and tyrosine or tryptophan, kynurenic acid or flavin precipitates as precursors of colorants. Usually *SAKE* is aged and stored for a short time. It does not age for a long time of several years or longer. Vintage wine is aged much longer than *SAKE*. During storage, *SAKE* matures gradually. The maturation process is probably the result of oxidation reactions and physicochemical changes. *SAKE* changes and adopts a smoother taste. The storage temperature should be maintained carefully at 13–18°C, with consideration being devoted to the rate of maturation and the time of bottling.

SAKE is browned not only by amino-carbonyl reactions but also by still unknown reactions during aging. Long-aged *SAKE* has a sherry wine-like aroma that is attributable to furfurals

produced in *SAKE* during aging. Furthermore, the *SAKE* taste is smooth and less stimulated by ethanol because of molecules of ethanol and water flocculate in the *SAKE* during aging. However, research of *SAKE* aging has been conducted by many researchers [1].

Recently, aging of *SAKE* to add value has been attempted by some breweries with so-called *KOSYU* as old vintage *SAKE*. *KOSYU-SAKE* has rich and complex flavors and tastes like those of cherry wine and a brown color by amino-carbonyl reaction as shown in Fig. 9. Aged *SAKE* can even have a chocolate color.

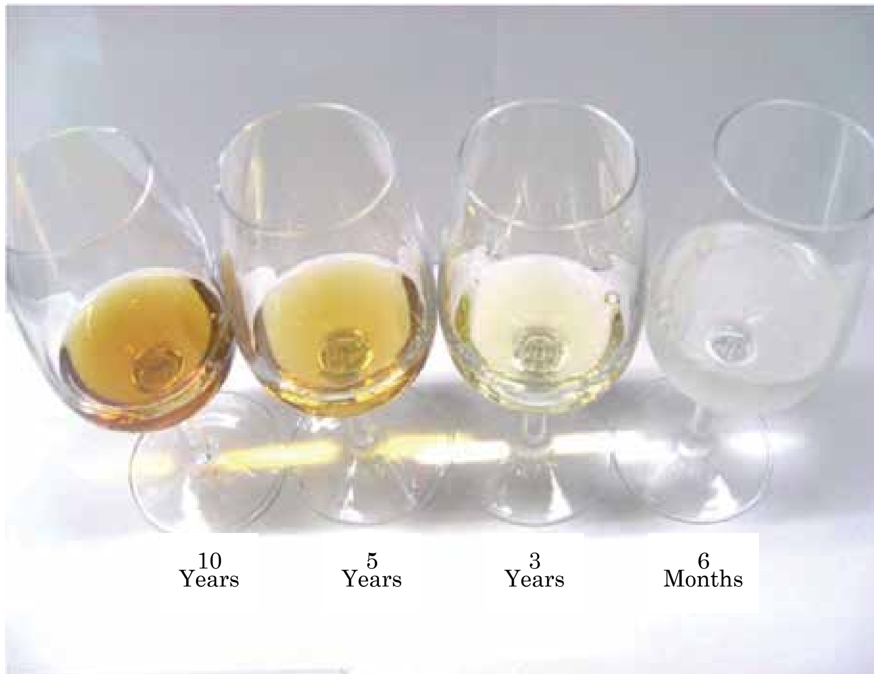

Figure 9. Changes of color of sake during aging.

6. Varieties of *SAKE* [39]

In Japan, *SAKE* production and labeling are regulated strictly by the Liquor Tax Law. According to this law, *SAKE* is made from defined raw materials and methods of production as follows: 1, *SAKE* is an alcoholic beverage produced by fermenting materials such as rice, rice-*KOJI*, and water, with subsequent filtering of the material mixture. 2, *SAKE* is an alcoholic beverage fermenting a material such as rice, water, *SAKE* lees, rice-*KOJI*, and other material as authorized by government ordinance and filtering the material mixture. 3, *SAKE* is an alcoholic beverage filtrate of a mixture of *SAKE* and *SAKE* lees. Moreover, *SAKE* has been categorized as grand, first and second class, by alcohol concentration, and sensory

evaluation by officers until 1992. However, labels and names of *SAKE* have not been regulated by law. For various reasons, many commercial products, *SAKE* which labels producing method or excessive name, was sold in the market and low-quality *SAKE* also was sold. Furthermore, many consumers were confused and purchased it mistakenly. Whereby, they were regulated by law in 1992.

***SAKE* type**	**Used material**	**Used rice Polishing ratio**	**Requirement**
GINJYO -SYU	Rice, rice *KOJI* and pure distilled alcohol	Less than 60%	Fermentation at low temperature; fruity- flavor; good and clear appearance
DAI-GINJYO-SYU	Rice, rice *KOJI* and pure distilled alcohol	Less than 50%	Fermentation at low temperature; fruity- flavor; good and clear appearance
JYUNMAI-SYU	Rice, rice *KOJI*	-	Good flavor; good and clear appearance
JYUNMAI-GINJYO-SYU	Rice, rice *KOJI*	Less than 60%	Fermentation at low temperature; fruity- flavor; good and clear appearance
JYUNMAI-DAI-GINJYO-SYU	Rice, rice *KOJI*	Less than 50%	Fermentation at low temperature; fruity- flavor; good and clear appearance
TOKUBETSU-JYUNMAI	Rice, rice *KOJI*	Less than 60%	Especially good flavor; good and clear appearance
HONJYOZO-SYU	Rice, rice *KOJI* and pure distilled alcohol	Less than 70%	Good flavor; good and clear appearance
TOKUBETSU-JYUNMAI	Rice, rice *KOJI* and pure distilled alcohol	Less than 60%	Especially good flavor; good and clear appearance

Table 3. Classification of *SAKE* types by law [39]

Instead of *SAKE* grades such as grand grade, fiesta grade and second grade that had been used until 1992, *SAKE* is categorized as *DAIGINJYO-SHU*, *GINJYO-SHU*, *JYUNMAI-SHU*, *JYUNMAI-DAIGINJYOU-SHU*, *JYUNMAI-GINJYOU-SYU*, *TOKUBETSU-JYUNMAI-SYU*, *HONJYOZO-SYU*, and *TOKUBETSU-HONJYOZO-SHU*, and the labeling *SAKE* is regulated by the law as shown in Table 3.

The polishing rice ratio and using *KOJI* ratio regulated by the law to sell their categorized *SAKE*. Then they must be shown on the label. JYUNMAI means that *SAKE* is brewed using only rice and rice-*KOJI* and mother water, and *GINJYO* means special brewing. *DAI-GINJYO* means special brewing and prestige class in the *SAKE* brewery. Consequently, *DAI-GINJYO* tends to be expensive, but the price of *SAKE* is decided by the policy of the brewery. Additionally, *KOSYU* as aged vintage *SAKE* or *NAMAZAKE* as non-pasteurized *SAKE* is displayed on the *SAKE* label. It is necessary that some method or public organization manage other *SAKE* label items.

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7. *SAKE* tastes

As explained in this chapter, *SAKE* is a favorite food and beverages and individual favored *SAKE* and components of *SAKE* are important factors for purchasing *SAKE*. Over 500 chemical compounds exist in *SAKE*, producing a complex flavor and taste in *SAKE*.

SAKE consumption has decreased since the 1970s, it is 1.7 million kL. Recently, in 2009, *SAKE* consumption is about one-third that of the 1970s. According to a survey of household spending conducted by the Public Management Ministry in Japan [40], consumers in their 20s spend 1100 yen per month for *SAKE*, those in their 30s spend 2500 yen per month, and those in their 60s spend 3800 yen per month. Elder consumers spend three times as much as young consumers. To examine favorite tastes of young consumers (20s–30s) play a role to development of new *SAKE* for them and to increase *SAKE* consumption in Japan.

	Appearance	**Intensity of aroma**	**Appeal of aroma**	**Intensity of sourness**	**Intensity of bitterness**	**Intensity of sweetness**	**Appeal (Balance) of tastes**	**Preference of consumer**
Appearance	1.000							
Intensity of aroma	0.450	1.000						
Appeal of aroma	-0.280	0.000	1.000					
Intensity of sourness	-0.590**	-0.300	0.100	1.000				
Intensity of bitterness	-0.200	0.160	-0.430*	0.120	1.000			
Intensity of sweetness	0.030	-0.380 **	0.210	0.030	-0.570	1.000		
Appeal (Balance) of tastes	-0.050	-0.190	0.550 *	0.070	-0.860 **	0.660**	1.000	
Preference of consumer	-0.110	-0.450 *	0.650 **	0.120	-0.800 **	0.670**	0.880**	1.000

(Correlations are significant; **, $P < 0.01$, *, $P < 0.05$)

Table 4. Correlations for Evaluating *SAKE* using Sensory Evaluation Methods

Suzuki and co-authors [41] investigated the opinions and preferences of panelists (22 persons, 20s–30s) to conduct a *SAKE* sensory evaluation for research into favorite tastes and consumer preferences. The correlation of sensory evaluations of *SAKE* are presented in Table 4. Correla-

tion was found between 'Intensity of aroma' and 'Balance of taste', for which the correlation factor is 0.55, and the relation between 'Appetite of consumers', with a correlation factor of 0.65. However, it showed a negative relation with 'bitterness', and the correlation factor was -0.430. Young consumers hope to buy and drink *SAKE* having a favorite flavor. Furthermore, correlation was found between 'Preferences of consumers', and 'Balance of taste', with a correlation factor is 0.88. 'Preferences of consumers', showed a relation with 'Intensity of bitterness', with close correlation factor of -0.80. Furthermore, a negative and close statistical correlation was found between 'Balance of *SAKE* tastes' and 'Intensity of bitterness', for which the correlation factor was -0.86. These data show that consumers hope to buy or drink *SAKE* with no bitter taste. 'Bitter' is a decreased balance of *SAKE* test and consumer appetite. Although bitter taste has played an important role in giving richness-taste to *SAKE* for a long time, young consumers are sensitive to bitter tastes in *SAKE*. It is therefore considered that the control of bitter taste must be undertaken in brewing processes.

8. Conclusions

SAKE brewing necessitates the use of high-quality techniques that have been developed experimentally without acquaintance with scientific method. Furthermore, unique techniques have been researched, as fermenting under low temperature, more than 18% of high alcohol concentration without distillation, open fermentation system without sterilization, and having a fruity aroma in *SAKE*. *SAKE* brewing using only rice as a material can yet produce fruity aromas such as those of apple, melon, or banana. Specially brewed *SAKE* for Japanese *SAKE* contests includes 6–7 ppm of ethyl caproate [42], which is a very high amount for alcoholic beverages, which is one reason that producing ethyl caproate yeast has been developed and fostered at public institutes in many Japanese prefectures. However, strong doubts persist that their *SAKE* has been adequately adapted to favor consumers. In questionnaire investigation, young consumers (20–30s) bring up the image that *SAKE* is a beverage for elderly people [41]. This is one reason for their image that *SAKE* is a cheap alcoholic beverage also. It is expected that *SAKE* consumption will decrease because the Japanese population is decreasing as result of the nation's low birthrate and high longevity.

All brewers and researchers of the *SAKE* field must make efforts to brew high-quality *SAKE* and suitable *SAKE* for consumers or for *SAKE* not only in Japan but also in foreign countries exporting it. Furthermore, *SAKE* can be highly appreciated by connoisseurs, just as 'Chateaux' wines, Grand cru, are in European countries.

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Chapter 4

Structuring Fat Foods

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Additional information is available at the end of the chapter

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1. Introduction

1.1. Fat roles

Food fat provides taste, consistency, and helps us feel full. Fat is a major source of energy for the body, and aids in the absorption of lipid soluble substances including vitamins A, D, E, and K. Dietary fat is essential for normal growth, development, and maintenance, and serves a number of important functions. Increasing evidence indicates that fatty acids and their derived substances may mediate critical cellular events, including activation and expression of genes, and regulation of cellular signaling [1].

When and how humans learned to use fats and oils is unknown, but it is known that primitive people in all climates used them for food, medicine, cosmetics, lighting, preservatives, lubricants, and other purposes. The use of fats as food was probably instinctive, whereas the other applications most likely resulted from observations of their properties and behavior under various environmental conditions. More than likely, the first fats used by humans were of animal origin and were separated from the tissue by heating or boiling. Recovery of oil from small seeds or nuts required the development of more advanced methods of processing, such as cooking, grinding, and pressing processes [2].

The total global oil and fat market is a huge economic factor. The rise of affluence in developing countries, this market is increasing and can be expected to increase further. The main fats commonly consumed are vegetable oils and fats, dairy fat and fats derived from animals, e.g. lard, tallow and fish oil [3].

Refining edible oils such as neutralization, bleaching, and deodorization, has been practiced for just over a century, but it has had a great impact on eating habits. Whereas the refining processes have increased the availability of sufficiently palatable oils, the oil modification

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processes (hydrogenation, interesterification, and fractionation) have increased the usefulness of edible oils by increasing their interchangeability [4].

2. Fats and oils

Fats and oils are water insoluble substances that are a combination of glycerin and fatty acids called triacylglycerols. Fats appear solid at ambient temperatures and oils appear liquid. Seeds, fruits, animal, and marine sources provide oils and/or fats; however, only a few of these sources are of economic importance. Fats and oils are the most concentrated source of energy of the three basic foods (carbohydrates, proteins, and fats), and many contain fatty acids essential for health that are not manufactured by the human body. Fats and oils are commonly referred to as triacylglycerols because the glycerin molecule has three hydroxyl groups where a fatty acid can be attached. The triacylglycerol structure is affected by the present and the position of attachment (alpha, sn-1; middle, sn-2; outer, sn-3) of each fatty acid to the glycerin. The chemical and physical properties of fats and oils are largely determined by the fatty acids that they contain and their position within the triacylglycerol molecule [2].

2.1. Fatty acids

Fatty acids consist of elements, such as carbon, hydrogen, and oxygen, which are arranged as a linear carbon chain skeleton of variable length with a carboxyl group at one end. Fatty acids can be saturated (no double bond), monounsaturated (one double bond), or polyunsaturated (two or more double bonds), and are essential for energetic, metabolic, and structural activities. An unsaturated fatty acid with a double bond can have two possible configurations, either *cis* or *trans*, depending on the relative positions of the alkyl groups.

2.1.1. Fatty acids occurrence

The fatty-acid carbon-chain lengths vary between 4 and 24 carbon atoms with up to three double bonds, with C18 the most common. Over 1000 fatty acids are known with different chain lengths, positions, configurations and types of unsaturation, and a range of additional substituents along the aliphatic chain. However, only around 20 fatty acids occur widely in nature; of these, palmitic, oleic, and linoleic acids make up ~80% of commodity oils and fats[4].

The most prevalent saturated fatty acids are lauric (C-12:0), myristic (C-14:0), palmitic (C-16:0), stearic (C-18:0), arachidic (C-20:0), behenic (C-22:0), and lignoceric (C-24:0). The most important monounsaturated fatty acids are oleic (C-18:1) and erucic (C-22:1). The essential polyunsaturated fatty acids are linoleic (C-18:2) and linolenic (C-18:3) [2].

2.1.2. Saturated fatty acids

Saturated fatty acids contain only single carbon-to-carbon bonds and are the least reactive chemically [2]. Saturated acids with 10 or more carbons are solids, and melting points increase with chain length. Melting points alternate between odd and even chain length, with odd chain

lengths having a lower melting point than the preceding even chain acid [4]. Most of the saturated fatty acids occurring in nature have unbranched structures with an even number of carbon atoms. These acids range from short-chain-length volatile liquids to waxy solids having chain lengths of ten or more carbon atoms. Fatty acids from 2 to 30 carbons (or longer) do occur, but the most common and important acids contain between 12 and 22 carbons and are found in many different plant and animal fats.Saturated fatty acids are also functionally divided into short- and long-chain acids and are most widely known by their trivial names. The short-chain saturated acids (4:0–10:0) are known to occur in milk fats and in a few seed fats [1]. Medium chain fatty acids (8:0, 10:0, 12:0, and 14:0) occur together in coconut and palm kernel oils, both tropical commodity oils. In both of these oils, lauric acid (12:0) predominates (45 to 55%). Palmitic acid (16:0) is the most abundant and widespread natural saturated acid, present in plants, animals, and microorganisms. Palm oil is a rich commodity oil source and contains over 40% of palmitic acid. Stearic acid (18:0) is also ubiquitous, usually at low levels, but is abundant in cocoa butter (~34%) and some animal fats, e.g., lard (5 to 24%) and beef tallow (6 to 40%). A few tropical plant species contain around 50 to 60% of 18:0 [4]. The long-chain saturated acids (19:0 and greater) are major components in only a few uncommon seed oils.

2.1.3. Unsaturated fatty acids

Unsaturated fatty acids contain one or more carbon-to-carbon double bonds and are liquid at room temperature with substantially lower melting points than their saturated fatty acid counterparts. Monounsaturated fatty acids have only one double bond in the carbon chain and polyunsaturated fatty acids have two or more double bonds in the carbon chain [2]. Polyunsaturated fatty acids, sometimes referred to as PUFAs or polyalkenoic acids, can be divided into conjugated (double-bonded carbon atoms alternate with single bonds) and unconjugated (double bonds are separated by one or more carbon atoms with only single bonds) [1].

The most common monounsaturated is oleic acid (18:1 9c). Oleic acid is found in most plant and animal lipids and is the major fatty acid in olive oil (70 to 75%) and several nut oils, e.g., macadamia, pistachio, pecan, almond, and hazelnut (filbert) contain 50 to over 70%. High oleic varieties of sunflower and safflower contain 75 to 80% oleic acid. *Cis*-monounsaturated with 18 or less carbons are liquids at room temperature or low-melting solids; higher homologues are low-melting solids. *Trans*-monounsaturated are higher melting, closer to the corresponding saturated acids. Double bond position also influences the melting point; both *cis*- and *trans*-C18 monounsaturated are higher melting when the double bond is at even positions than at odd positions [4]. Saturated fatty acids are very stable, but unsaturated acids are susceptible to oxidation; the more double bonds the greater the susceptibility. Unsaturated fatty acids, therefore, have to be handled under an atmosphere of inert gas (e.g. nitrogen) and kept away from oxidants or substances giving rise to free radicals [5].

2.1.4. Trans fatty acids

Monosaturated and methylene-interrupted polyunsaturated fatty acids are predominantly *cis*. *Trans* isomers, mainly monosaturated, are produced during catalytic partial hydrogenation,

and can be present in substantial amounts in hardened fats, generally as a mixture of positional isomers. Heat treatment during deodorization of commodity oils may result in low levels of *trans* isomers, particularly of polyunsaturated. The undesirable nutritional properties of *trans* fatty acids have led to alternative ways of producing hardened fats, such as interesterification or blending with fully saturated fats, and to the use of milder deodorization procedures [4]. It is important to note that *trans* double bonds do occur in natural fats, as well as in industrially processed fats, but generally much less abundantly than *cis* bonds. Thus some seed oils have a significant content of fatty acids with *trans* unsaturation [5]. Saturated and *trans* fatty acids have a higher melting point than unsaturated and *cis* fatty acids [1].

2.1.5. Health problems

Concerns about possible toxic effects of fatty acids with *trans*-unsaturation began with the publication of results of experiments with pigs given diets containing hydrogenated vegetable fat for 8 months. They had more extensive arterial disease than those given otherwise equivalent diets devoid of *trans*-unsaturated fatty acids. Subsequently, numerous animal feeding trials, epidemiological studies of human populations and controlled dietary experiments with human subjects have been reported [5]. In January 2003 the US Food and Drug Administration (FDA) instituted a requirement to list *trans* fat content as a separate item on the Nutrition Facts label on packaged foods from 2006. This change in labeling requirements has served as a catalyst to accelerate food product reformulation. On a voluntary basis, many food manufacturers and restaurants have reformulated their products and modified their operations to reduce *trans* fats in their offerings [6, 7].

Consumption of *trans* fatty acids raise the level of low density lipoprotein (LDL) cholesterol and decrease the level of high density lipoprotein (HDL) cholesterol. Based on results of epidemiological and intervention studies it is clear that these changes in blood profiles increase the risk of coronary heart diseases. The main food sources for *trans* fatty acids are cookies and confectionary, snacks, and frying fats [8]. Consumption of significant amounts of *trans* fatty acids has been a major health concern for consumers and regulatory agencies over the past decade. The major dietary sources of *trans* fatty acids are products formulated with partially hydrogenated fats. Examples include margarines, shortenings, bakery products, and fast foods. The regulatory mandate from FDA and consumer concerns have led to the development of alternative processes to produce foods with zero or reduced *trans* fatty acids contents [7, 9].

Fats and oils can be formulated as *trans*-acid-free products, but saturates are required for the solids contents that provide the functionality for plastic and liquid products. Reduced saturates may be an option in some cases, but a saturate-free product is probably impossible if functionality is to be maintained [2]. Obviously, if the fat is completely hydrogenated there will be no double bonds and hence no problem; however, partially hydrogenated fats have *trans* double bonds. *Trans* double bonds are rare in naturally occurring fats, the major natural source is milk fat because they are formed by bacterial action in the rumen. So, most naturally occurring oils and fats have *cis* double bonds; however, some *trans* double bonds are found in milk fat and some marine oils.

2.1.6. Low-trans

The new rules about *trans* fatty acids promise to strongly affect what is acceptable to consumers and food manufacturers. It will be difficult to meet all the demands for low-*trans* fats and other traits that are important to consumers with the current technology, especially for frying fats and oils. Seed suppliers are busy trying to furnish seeds with compositions that will meet these needs and find farmers to grow these crops. Contracts with oilseed processors have been made to process the harvest. Some food companies have pledged to use only *trans*-free fats and oils in their products [1].

Trans-free fat blends can be constructed by blending oils with fully hardened oils, or indeed where the entire blend has been randomized through interesterification. Blending vegetable oil types from different sources is an efficient alternative to hydrogenated vegetable oils, and still provides the appropriate physicochemical properties and nutritional requirements demanded. Such fat blends can also be rich in polyunsaturated fatty acids as well as being *trans*- free. *Trans*-free options are commercially available in the form of a blend of tailored emulsifiers and oil blends where they meet demands for shelf-life, processing and distribution requirements. These *trans*-free options are available for a wide range of products covering, snacks, cakes, breads, tortillas, nutrition bars, cookies and breakfast cereals. *Trans*-free oil blends are also routinely designed for margarines, where they impart structure and texture, and shortenings where they provide firmness and contribute to crumb structure [10]. The combination of *trans*-free modification techniques (full hydrogenation, interesterification and fractionation) and the availability of a variety of different feedstocks can be used to produce virtually *trans*-free hardstocks with a range of physical properties such as solid phase lines determining melting performances. Liquid seed oils, low in solids, are first fully hydrogenated to generate solids combined with a very low *trans* level (< 1.25%). These fully hydrogenated oils may subsequently be interesterified with non-hydrogenated liquid oil to reduce the solid fat content at high temperature (> 40°C). This solid fat content can be further reduced by fractionation [11].

2.2. Structural characteristics

Fats are the main structural components in many food products such as chocolate, confectionery coatings, dairy products, butter, cream shortenings, margarine, and spreads. The sensory characteristics of fat-structured materials such as spreadability, hardness, and mouth feel are highly dependent on the structure of the underlying fat crystal network. This fat crystal network is built by the interaction of polycrystalline fat particles. The amount, geometry, and spatial distribution of solid fat crystals as well as their interactions at different levels within the network all affect the rheological properties of fats and fat-structured food products. Fat crystallization largely determines consistency, physical stability, visual appearance, and eating properties [4, 12, 13].

2.2.1. Crystals

A crystal consists of a material in a solid state in which the building entities—molecules, atoms or ions—are closely packed so that the free energy of the material is at minimum. As

a result the entities are arranged in a regularly repeating pattern or lattice and are affected by the following points [14]: the molecules, or atoms, or ions are subject to heat motion; only the average positions will be fixed; diffusion can occur in a crystalline material, but the time scales involved are centuries rather than seconds; incorporation of a foreign molecule leading to a dislocation in the crystal lattice (Figure 1b); some solid materials are ''polycrystalline'', i.e., they are composites of many small crystalline domains of various orientations (Figure 1c).

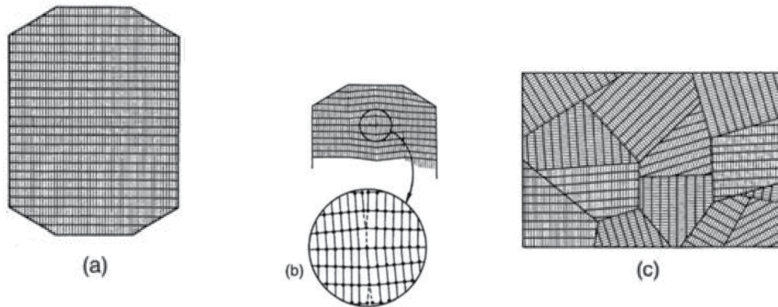

Figure 1. Two-dimensional illustration of crystalline order: (a) crystal lattice with perfect order, (b) a defect in the crystal leading to a dislocation in the lattice, (c) a polycrystalline material [14].

Different lattice arrangements and unit cells (Figure 2) can be constructed in terms of the lattice parameters, also known as Bravais lattices: three spatial dimensions - a, b, and c; and three angles - α, β, and γ. For example, cubic systems all must have equal lengths (a=b=c) and angles equal to 90° [15].

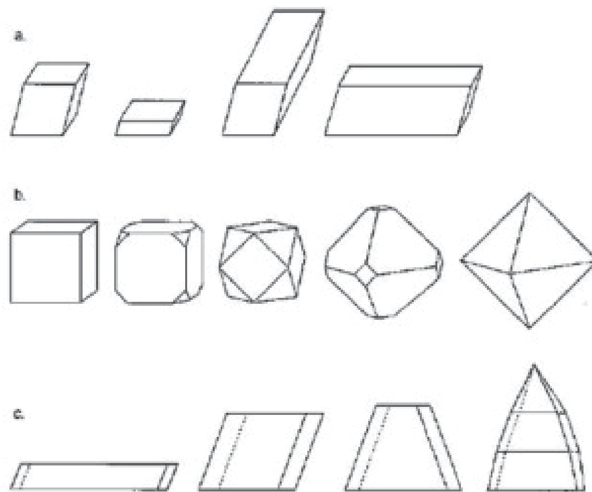

Figure 2. Variation in crystal morphology for identical unit cells: (a) rhombohedral, (b) cubic, and (c) monoclinic [15].

Crystals show enormous variation in external shape or habit caused by variation in the growth rate of the various faces of a crystal, which rates often depend on the composition of the solution. Corners and edges are rounded; curved faces can appear in large crystals; some needlelike crystals have a slight twist; some faces can grow faster than others (Figure 4). A noncrystalline solid is often referred to as an amorphous solid. Whether a material is crystalline or not can be established by x-ray diffraction. X-rays have a very small wavelength, of the order of 0.1 nm, which implies that individual atoms may cause scattering. If the atoms (or small molecules) occur at regular distances, sharp diffraction maxima occur, and the crystal structure can be derived from the diffraction pattern [14].

2.2.2. Crystallization

Many of the sensory attributes such as spreadability, mouthfeel, snap of chocolate, texture, etc., are dependent on the mechanical strength of the underlying fat crystal network. In addition to this obvious industrial importance, fat crystal networks form a particular class of soft materials, which demonstrate a yield stress and viscoelastic properties, rendering these plastic materials. The levels of structure in a typical fat network are defined as the fat crystallizes from the melt. The growth of a fat crystal network can be visualized thus: the triacylglycerols present in the sample crystallize from the melt into particular polymorphic/ polytypic states. These crystals grow into larger microstructural elements (≈ 6 mm) which then aggregate via a mass- and heat-transfer limited process into larger microstructures (≈ 100 mm). The aggregation process continues until a continuous three-dimensional network is formed by the collection of microstructures. Trapped within this solid network structure is the liquid phase of the fat [12].

The crystallization process consists of two steps: nucleation and crystal growth. Nucleation can be described as a process in which molecules come into contact, orient and interact to form highly ordered structures, called nuclei. Crystal growth is the enlargement of these nuclei. Nucleation and crystal growth are not mutually exclusive: nucleation may take place while crystals grow on existing nuclei [16].

Nucleation can only be achieved via supersaturation or supercooling. A solution is supersaturated if it contains more of a component than can be theoretically dissolved within it at a particular temperature. Supercooling refers to the degree to which the solution is cooled with respect to the melting temperature of the crystallized solution. It is very difficult to determine the parameters of supersaturation and supercooling for a crystallizing system, and therefore, as a good approximation, in practice only supercooling is usually considered for crystallization of triacylglycerol molecules from the melt [17].

When the temperature of a fat melt is decreased below its maximum melting temperature, it becomes supersaturated in the higher-melting triacylglycerol species present in the mixture. This so-called undercooling or supercooling represents the thermodynamic driving force for the change in state from liquid to solid. Fats usually have to be undercooled by at least 5-10ºC before they begin to crystallize. For a few degrees below the melting point, the melt exists in a so-called metastable region. In this region, molecules begin to aggregate into tiny clusters called embryos. At these low degrees of undercooling, embryos continuously form

and breakdown, but do not persist to form stable nuclei. The energy of interaction between triacylglycerol molecules has to be greater than the kinetic energy of the molecules in the melt so as to overcome Brownian effects. For these flexible molecules, it is not sufficient to simply interact; molecules have to adopt a specific conformation in order to form a stable nucleus. The adoption of this more stable conformation is relatively slow, thus explaining the existence of a metastable region. As the undercooling is increased (i.e., at lower temperatures) stable nuclei of a specific critical size are formed [18].

2.2.3. Polymorphism

An important way to characterize fats and oils is through the predominant crystalline phase, or polymorph, that tend to form upon crystallization. When the same ensemble of molecules can pack in different arrangements on crystallization, depending on the processing conditions, the substance is said to demonstrate polymorphism. The different polymorphic states of a particular substance often demonstrate quite different physical properties (such as melting behavior and hardness), but on melting yield identical liquids [17].

Polymorphism is the ability of long-chain compounds such as fatty acids to exist in more than one crystal form, and this results from different patterns of molecular packing in the crystals. Triacylglycerols may occur in three main forms, namely, α, β′, and β in order of increasing stability and melting point. When fats are cooled, crystals of a lower melting form may be produced. These may change slowly or rapidly into a more stable form. The change is monotropic, that is, it always proceeds from lower to higher stability. Polymorphism results in the phenomenon of multiple melting points. When a fat is crystallized in an unstable form and heated to a temperature slightly above its melting point, it may resolidify into a more stable form [1]. The polymorphs differ in stability, melting point, melting enthalpy, and density. The α-polymorph is the least stable and has the lowest melting point, melting enthalpy, and density. The β-polymorph is the most stable and has the highest melting point, melting enthalpy, and density. The β′-polymorph has intermediate properties [4].

Under rapid cooling conditions, triacylglycerol molecules usually crystallize in metastable polymorphic forms, which subsequently transform into polymorphs of higher stability. On the other hand, at slow cooling rates, triacylglycerol molecules of similar chain lengths have time to associate with each other in more stable geometrical arrangements, resulting in the formation of a more stable polymorphic form. Due to the dependence of fat crystallization on the degree of undercooling and the cooling rate used, different results will be observed when using different cooling rates [18].

2.2.4. Tempering

Before its solid fat content can be determined, the fat must be exposed to a prescribed temperature profile: first it has to be melted completely to destroy all traces of crystals, and then cooled to achieve virtually complete crystallization, and finally it has to be held at the measuring temperature to come to equilibrium at that temperature. Sometimes, depending on the fat used, an extra step is introduced where the fat is held at a particular temperature, which

is not the measuring temperature. This step is referred to as a tempering step. For confectionery fats, a tempering step of 40 hours at 26°C is mentioned in the standard methods to ensure that cocoa butter and similar fats like cocoa butter equivalents (CBEs) are converted to their β-polymorph before the SFC is measured [4].

Tempering is a technique of controlled pre-crystallization employed to induce the most stable solid form of cocoa butter, a polymorphic fat in finished chocolates. The process consists of shearing chocolate mass at controlled temperatures to promote crystallization of triacylglycerols in cocoa butter to effect good setting characteristics, foam stability, demoulding properties, product snap, contraction, gloss and shelf-life characteristics. Time–temperature protocols and shearing are employed to induce nucleation of stable polymorphs with the formation of three-dimensional crystal network structure influencing the microstructure, mechanical properties and appearance of products. The crystal network organization and the polymorphic state of the triacylglycerols crystals as affected by the crystallization conditions are major factors determining rheological and textural properties of crystallized triacylglycerols systems [19].

2.2.5. Solid fat content

The solid fat content (SFC) is a measure of the percentage of solid, crystalline fat in a sample at a selected temperature. Often, the SFC is measured at selected points within a temperature range. A measure of the SFC can be determined by a variety of methods: dilatometry, pulsed nuclear magnetic resonance (p-NMR), or differential scanning calorimetry (DSC). The method used and differences in the way it is executed can seriously affect the final result [4].

2.3. Fat design

Each application area requires its proper fat. The specifications of the fat depend on: recipe, equipment, procedure, temperature of fat and other ingredients, ambient temperature, storage and distribution temperature of the final product. Some conditions to attend a satisfatory fat design must be the compatibility among the components of the mixture: equivalent thermal properties (solid fat content, melting point and range); similar molecular size, shape and packing (to allow isomorphous replacement or formation of a single lattice unit in mixtures); similar polymorphism (transformation from stable to unstable forms should occur as readily for binary mixtures as with individual components) (Figure 3).

2.3.1. Processing

Edible fats and oils have been separated from animal tissues, oilseeds, and oil-bearing fruits for thousands of years. The combined largest source of vegetable oils is the seeds of annual plants grown in relatively temperate climates. The oilseeds are processed by expeller or screw press extraction, by prepress solvent extraction, or bay expander–solvent extraction. A second source of vegetable oil is the oil-bearing tree fruits and kernels. Oil-bearing fruits are pressed to obtain oil, sometimes after drying or sterilizing, or are cold pressed to preserve flavor and odor. Animal tissues may be wet- or dry-rendered (cooking processes) to sepa-

rate the fats. Edible meat fats are supplied by lard from pigs, tallow from cattle and sheep, and milk fat or butter from cows. After recovering, fats and oils can be physically and/or chemically refined. Chemical refining removes most impurities with an alkaline solution, whereas physical refining removes them by distillation [2].

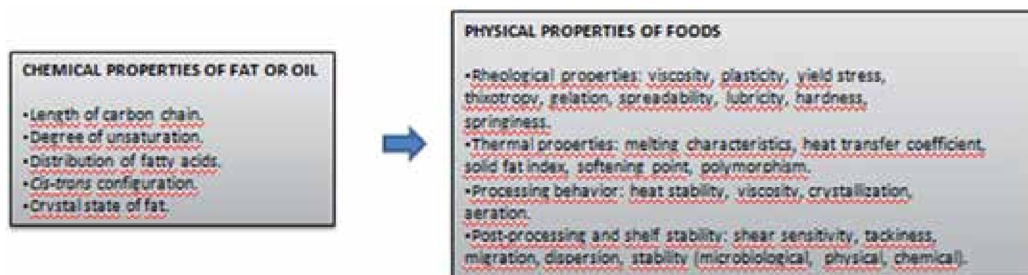

Figure 3. Physical and chemical functions of fats.

2.3.1.1. Industrialization

Searching for fat substitutes started in France during the Industrial Revolution. Large population shifts from farms to factories and, in France, a depression and an imminent war with Prussia, created a demand for butter that the milk supply could not meet, escalating butter prices. The first acceptable butter substitute, named "margarine", was produced by the French chemist Mege Mouries in 1869, on commission from Emperor Napolean. Soon after the introduction of the first butter substitute on the market, several inventors patented various modifications of Mouries' process [2, 4, 17]. Before 1900, animal fats were used as sources of fat with a high content of solids in margarine production. This led to a shortage of animal fats since they were also the main feedstock for soap making [3]. The best known modification processes applied today in the edible oil industry are hydrogenation, interesterification (chemical or enzymatic) and fractionation. The main purpose of these processes is to change the physicochemical properties of the oil or fat, by reducing the degree of unsaturation of the acyl groups (hydrogenation), by redistributing the fatty acids chains (interesterification) or by a physical separation of the triacylglycerols through selective crystallization and filtration (fractionation) [20].

2.3.1.2. Hydrogenation

Based on work done by the French chemist Paul Sabatier on the metal-catalyzed hydrogenation of unsaturated organic compounds, German chemist Wilhelm Normann developed the method for hydrogenation of edible oils in 1903. Chemically, the hydrogenation of oils is the reduction of the double bonds in unsaturated fatty acids to single saturated bonds, by the reaction of hydrogen gas in the presence of a metal catalyst. The metal catalyst used at the

time was nickel, and it has practically remained the same in the current hydrogenation procedures. Complete reduction of all double bonds in the oil would yield 100% saturated fatty acids, whereas reduction of only a fraction of the double bonds results in partially hydrogenated fats. During the process of hydrogenation the *cis* double bond can open up and reform into a *trans* double bond, as well as shift positions along the fatty acid carbon chain. Structurally, *cis* double bonds in unsaturated fatty acids produce a bend in the chain that prevents unsaturated fatty acids from packing as tightly as saturated fatty acids. As a consequence, a *cis* unsaturated fatty acid has a lower melting point than a saturated fatty acids with the same molecular weight. Conversely, the *trans* double bonds do not create a bend on the fatty acid chain. Therefore, *trans* unsaturated fatty acid chains are virtually straight, resembling saturated fatty acids, and display higher melting points than the corresponding *cis* isomers [21].

The aim of the hydrogenation process is the total or partial saturation of the double bonds of unsaturated fats to obtain hard or plastic fats or to improve the stability to oxidation of an oil. The obtained product depends on the nature of the starting oil, the type and concentration of the catalyst used, the concentration of hydrogen, and the experimental conditions under which the reaction takes place. Nickel catalyst was reported to catalyze undesirable side reactions such as *cis*, *trans* isomerization and positional isomerization of double bonds. The position of the double bonds affects the melting point of the fatty acid to a limited extent. The presence of different geometric isomers of fatty acids influences the physical characteristics of the fat to a greater extent [22].

2.3.1.3. Interesterification

Interesterification has been developed as an alternative to hydrogenation, with the specific aim of eliminating the formation of *trans* fatty acids. The process rearranges the distribution of the fatty acids either chemically or enzymatically, within and between the triacylglycerols, thus the fatty acid distribution is altered, but the fatty acid composition remains unchanged – this rearrangement can be done either in a random or controlled manner. The technique is effective and can be used to produce fat products for spreads that are soft and spreadable and also *trans*-free. Interesterification is nothing new, having been around for some time, and the basic principles were first documented in 1969 [10].

2.3.1.4. Fractionation

Fractionation is a fully reversible modification process; it is basically a thermo-mechanical separation process in which a multi-component mixture is physically separated into two or more fractions with distinct physical and chemical properties. The separation can be based on differences in solidification, solubility, or volatility of the different compounds: fractional crystallization, fractional distillation, short-path distillation, supercritical extraction, liquid-liquid extraction, adsorption, complexation, membrane separation, etc. are the main techniques practiced. Fractional crystallization refers to a separation process in which the fatty material is crystallized, after which the liquid phase is separated from the solid. It is based on differences in solubility of the solid triacylglycerol in the liquid phase, depending on

their molecular weight and degree of unsaturation; this is a consequence of the ability of fats to produce crystals. On an industrial scale, crystals can be obtained according to three main technologies: detergent fractionation, solvent fractionation and dry fractionation [20].

2.3.2. Fat replacers

Fat replacers are called by many synonyms with various nuances in their usage: fat *replacers* can provide some or all of the functions of fat; fat *substitutes* resemble conventional fats and oils and provide all food functions of fat; fat *analogs* provide food with many of the characteristics of fat; fat *extenders* optimize the functionality of fat; fat *mimetics* mimic one or more of the sensory and physical functions of fat in the food.

Fat replacers are most frequently used to replace fat in products with a high fat content and are used in a variety of food products, including frozen desserts, processed meats, cheese, sour cream, salad dressings, snack chips and baked goods. At the height of the interest in low-fat foods, more than 1000 fat-modified foods were introduced, with fat modified snacks being the fastest growing category of products in supermarkets at the time [11]. Normal fat contains nine calories per gram compared with five calories per gram for the sugar and protein components. If the proportion of fat is reduced the calorific value will fall. Corn starch, maltodextrin, pectin, gelatin, xanthan gum, guar gum, carrageenan, and soy protein were all commonly used ingredients in reduced fat products launched in the period 2008–10. Low in saturated fatty acids, sunflower oil was commonly used in new reduced fat foods. Fat replacers of the future will need to meet some important criteria, including reducing or replacing the target fat effectively, being available at a cost appropriate to the benefits provided, and being safe and legal with no appreciable side effects.

2.3.3. Shortening

Shortening was the term used to describe the function performed by naturally occurring solid fats such as lard and butter in baked products. These fats contributed a “short” (or tenderizing) quality to baked products by preventing the cohesion of the flour gluten during mixing and baking. Shortening later became the term used by all-vegetable oil processors when they abandoned the lard-substitute concept. Shortening has become virtually synonymous with fat and includes many other types of edible fats designed for purposes other than baking. Currently, a description for shortening would be processed fats and oils products that affect the stability, flavor, storage quality, eating characteristics, and eye appeal of prepared foods by providing emulsification, lubricity, structure, aeration, a moisture barrier, a flavor medium, or heat transfer [2].

Fats and oils added to breads, cakes and similar baked goods are often referred to as shortenings that contribute to tenderness, improve volume gain of bread dough, enhance texture, crumb structure and shelf-life of the products. In order to produce a satisfactory shortening, one has to pay specific attention to the crystal structure, and similarly the consistency of the shortening will depend on the ratio of solid to liquid fat present at different temperatures [10].

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Plastic shortening describes fats that are readily spread, mixed or worked. The property of plasticity is highly important in fats used as shortening agents in baked products. Commercially, these are prepared by hydrogenation of oils, during which, some of the double bonds are isomerised into *trans* fatty acids from their *cis* configuration. *Trans* fatty acids have higher melting points and greater stability against oxidative rancidity than their *cis*-isomers and are important contributors to the functional properties of hydrogenated products. To meet the requirements of health-conscious consumers fats having a wide melting range which crystallize in the b′ polymorphic form without the formation of *trans* fatty acids are needed [23].

Palm oil, because of its naturally β′ tending nature, is favoured for shortening applications, such that it can impart stability to the emulsion, smooth consistency and provide good aeration properties [10].

3. Mechanical properties

When triacylglycerols are cooled from the melt to a temperature below their melting point, i.e., when they are supercooled, they undergo a liquid–solid transformation to form primary crystals with characteristic polymorphism. These primary crystals aggregate, or grow into each other, to form clusters, which further interact, resulting in the formation of a continuous three-dimensional network. The mechanical properties of a fat, can be influenced by all these levels of structure; however, most directly by the level of structure closest to the macroscopic world, namely the microstructure [24].

It is during crystallization that the template for the final physical properties of the resulting fat crystal network is created. Hence, the mechanical properties of a fat crystal network are determined by the different levels of structure, such as chemical composition, solid fat content (SFC), and crystal habit (polymorphism and microstructure). To study the mechanical properties of fat crystal networks, rheologic tests are used, which measure how the crystallized material responds to applied forces (stress) and deformations (strain) [18].

Foods are edible structures created as a result of the responses of proteins, polysaccharides, and lipids in aqueous media to different processing methods, such as thermal processing, homogenization, and other physical treatments. The processing operations to which foods are subjected affect their structure and microstructure. Most, if not all, of the responses are physical in nature. By definition, rheology is the study of deformation and flow of materials. In foods, measured rheological responses are those at the macroscopic level. However, they are directly affected by the changes and properties at the microscopic level. Fractal dimension has been used to characterize food particles in addition to microscopic and size distribution data. The fractal dimension can be estimated by several techniques such as viscoelastic behavior [25].

3.1. Rheology and texture

Rheology has been defined as the study of the flow and deformation of materials, with special emphasis being usually placed on the former. In flow, elements of the liquid are deforming resisted by viscosity. Solids when stressed creep, i.e. continue to deform very slowly over a very long time scale. In structured liquids there is a natural rest condition of the microstructure that represents a minimum-energy state. When these liquids are deformed, thermodynamic forces immediately begin to operate to restore this rest state. This kind of energy is the origin of elasticity in structured liquids. Alongside these elastic forces are the ever-present viscous forces that produce viscoelastic effects [26].

Rheological methods can be divided into small and large deformation rheology. *Small deformation rheology* does not cause structural damage to the sample. They are performed in the linear viscoelastic region (LVR), in which the stress is directly proportional to the strain. *Large deformation rheology* is based on the deformation of a sample at a constant rate to the point where the force exceeds the structural capacity of the sample, causing it to permanently deform and break [18]. Sometimes, oscillatory testing is referred to as small amplitude oscillatory testing because small deformations must be employed to maintain linear viscoelastic behavior. Many processes, such as mastication and swallowing, are only accomplished with very large deformations. Collecting viscoelastic data relevant to this type of problem involves testing in the non-linear range behavior [27].

A frequently used method of measuring linear viscoelastic response is oscillatory testing, i.e. applying an oscillating stress or strain as an input to the liquid and monitoring the resulting oscillatory strain or stress output. Oscillatory tests are performed over a range of frequency. Short times correspond to high frequencies, and long times relate to low frequencies. In a sine-wave-shaped input of either stress or strain the resulting sinusoidal strain or stress output is separated into solid-like response, which is in phase with the input, and a corresponding liquid-like response which is $\pi/2$ (i.e. 90°) out of phase with the input. The solid-like component at any particular frequency is characterized by the storage modulus, G', and the liquid-like response is described by the complementary loss modulus, G''. The behavior normally seen for typical viscoelastic liquids is an initial elastic response, thereafter, a delayed elastic response where the deformation rate becomes slower and slower, ending up as a very slow but steady-state deformation at the longest times, i.e. the material is in steady flow. The overall G', G'' response of structured liquids is shown in Figure 4 [26].

G' expresses the magnitude of the energy that is stored in the material or recoverable per cycle of deformation, while G'' is a measure of the energy which is lost as viscous dissipation per cycle of deformation. For a viscoelastic material the resultant stress is also sinusoidal but shows a phase lag of δ radians when compared with the strain. The phase angle covers the range of 0 to $\pi/2$ as the viscous component increases. If G' is much greater than G'', the material will behave more like a solid, i.e., the deformations will be essentially elastic or recoverable. The loss tangent, $\tan \delta$, is the ratio of the energy dissipated to that stored per cycle of deformation. When G'' is much greater than G', the energy used to deform the material is dissipated viscously and the materials behavior is liquid like [25].

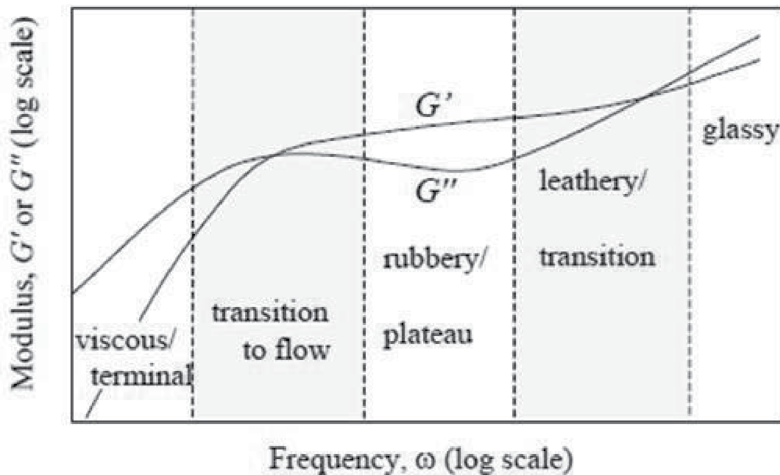

Figure 4. The various regions of an oscillatory test of structured liquids [26].

The penetrometry method and the two-plate compression method are large-deformation tests and are widely used to determine the yield stress or the firmness of a plastic fat. The large-deformation method has been widely used to study the physical properties of fat products, such as the spreadability of shortenings and the hardness of chocolate and milk fat, and the results have been found to correlate well with sensory tests [28].

Texture has been defined as the way in which various constituents and structural elements are arranged and combined into a micro- and macrostructure and this structure is externally manifested in terms of flow and deformation [29]. The structural elements of fats consist of solid fat crystals. They are suspended in liquid oil and when present in sufficient quantity form a three-dimensional network that imparts plastic properties to the fat. The external manifestations of this network structure include a number of physical and mechanical properties such as hardness, softness, spreadability, brittleness, shortening power, and aeration properties. The texture of fats is influenced by a number of factors, including the solids content, the fatty acid and triacylglycerol composition of the solids, the polymorphic behavior of the fat crystals, the size and shape of the crystals, the nature of the crystal network, mechanical treatment, and temperature history. Many of these factors are interrelated, making it difficult to establish the effect of each independently [30]. Crystallization usually results in harder materials with higher solid fat contents. In addition, microstructural differences must be taken into account when evaluating the functional properties of lipids. The possibility of different polymorphic forms must not be neglected either because it can influence the texture and sensory profile. The texture of plastic fats can be determined by three main methods such as: cone penetrometry, penetration by a probe, compression between parallel plates. The analyses and the evaluation of food texture are very important in food processing. Some of the attributes such as hardness and adhesiveness can be evaluated by texture analysis.

3.2. Fractal

Once the attraction forces have become larger than the repulsion, and also larger than Brownian motion, particles can remain together when they collide. The resulting aggregates or flocs have a very complex structure and most of the flocs do not have homogeneous internal structures. The center is usually denser than the outer regions; hence the mass does not change with the third power of the radius as in normal objects with constant density [31].

Many patterns in nature such as the geometry of coastlines, mountains, trees, and vegetables, for instance, cannot always be defined adequately by using the familiar straight lines, circles, conic sections, polygons, spheres, quadratic surfaces, etc. Fractal geometry was born out of this lack of geometrical tools. A geometric shape belongs to standard geometry when smaller and smaller portions of it become increasingly smooth. For example, a generic curve becomes a straight line, and a generic surface becomes a plane. Fractals are shapes whose roughness and fragmentation neither tend to vanish, nor fluctuate up and down, but remain essentially unchanged as one zooms in continually and examination is refined. Hence, the structure of every piece holds the key to the whole structure. Fractals are characterized by two types of symmetries: self-similar and self-affine. In self-similar each part is a linear geometric reduction of the whole, with the same reduction ratios in all directions. In self-affine, the reductions are still linear but the reduction ratios in different directions are different [32].

A fractal dimension is a powerful means of quantifying the structure of non-Euclidean objects by capturing the complexity of a structure's geometry in a single number. The challenge, however, is to give physical meaning to the number obtained [24].

The macroscopic rheological properties of the network are influenced by all levels of structure defined during the formation of the network, i.e. the structure of the individual triacylglycerols, the structure of the individual crystalline units formed, or the polymorphic nature of the network, and the microstructural level of structure. The microstructural aggregate or microstructural network present in fat crystal networks scale in a fractal manner in the range between the size of the individual particles composing the sample (microstructural elements) and the size of the microstructures. For colloidal aggregates and other fractal systems (such as fat crystal networks), fractal concept quantifies the way in which the mass of the sample/system increases with its size, according to the fractal dimension [33].

3.3. Fat crystal network

Early nucleation and crystal growth events lead to the formation of submicron primary crystallites from the melt. These crystallites associate into micron-range particles, which further aggregate into clusters, until a continuous three-dimensional network with voids filled with liquid fat is formed [18].

A structural hierarchy exists within fat crystal networks. Polymorphism has to do with different molecular packing arrangements of tryacylglicerol molecules, at the nanostructural range, within the primary fat crystals. Once the primary fat crystals are formed, they aggregate, or grow into each other, to form fat crystal clusters (or aggregate), which in turn cross-

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link to build up a 3-D fat crystal network. The shape of the fat crystal clusters can be spherulitic, feather-like, blade or needle-shaped. The size of fat crystal clusters can vary from several micrometers to more than 200 μm. Processing conditions can affect the size of the fat crystal clusters [28].

Fats are the main structural constituents of many food products including margarine, chocolate, butter and spreads. The sensory textural characteristics (i.e., spreadability, hardness, snap) of fat structured foods are dependent on their macroscopic rheological properties, which are a consequence of the structure of their underlying fat crystal network. This network arises from the interactions between polycrystalline fat particles, and provides the elastic component, or the solid-like behavior, of a plastic fat. The sensory properties of the fat-structured foods are dependent not only on the amount of solid fat crystals present and their polymorphism, but also their geometry and the spatial distribution of crystalline material [34].

The microstructure of fat crystal networks can be quantified by fractal dimensions, which can describes the combined effects of morphology and spatial distribution patterns of the crystal clusters in fat crystal networks. The usefulness in the quantification of the microstructure of fats using the concept of fractal dimension arises from the possibility of relating structure to physical properties [28]. The fractal model of fat crystal networks explains the scaling behavior of rheological properties of semi-solid fat products to their solid fat content by their microstructure, which can be quantified using fractal dimensions. In general, different microscopy fractal dimensions reflect different aspects of the micro- structure and thus have different physical meanings. An unambiguous agreement between physical fractal dimensions and microscopy fractal dimensions is required [25].

Fat crystal networks are statically self-similar, which means that the microstructure in a fat crystal network looks similar at different magnifications. Fractal structures are created by agglomeration, or clustering, of small particles to form a larger object in a random, iterative fashion under some constraint. In a similar fashion, fat crystal networks are built from clusters of polycrystalline particles (crystallites) that aggregate in a diffusionally limited, fractal fashion. Fractal mathematics have been used to relate the elastic properties of fat crystal networks to the spatial distribution of the network mass and to link crystallization kinetics and phase behavior to microstructure. The fractal dimension defines the cluster size and has been evaluated by rheology techniques. Rheology is the most common technique for the quantification of microstructure in fat crystal networks and utilizes the relationship of the shear storage modulus (G') to the volume fraction of network solid mass via the mass fractal dimension of the network [18].

The shape, size, and the strength of the fat crystal flocs making up the fat crystal network are always different. The weakest floc will become a flaw and acts as a stress concentrator. The elastic properties of the network depend on the number of connections between neighboring structural clusters, rather than on the amount of apparent solids. This implies that the connectivity of the networks increases with an increasing volume fraction of solids. An idealized view of the structure of a fat crystal network showing the one dimensional deformation of the links between crystal clusters [35]. In this same work, by using a modified

fractal model, which describes the increase of G′ with SFC well, show the idea that the stress-carrying mechanism in fat crystal networks is heterogeneous, i.e. since real networks are not fully connected and that connectivity of networks increases with the volume fraction of solids, the load-bearing volume fraction of solids in real networks increases in an exponential fashion with the apparent volume fraction of solids.

4. Fat foods

Fats and oils are the raw materials for liquid oils, shortenings, margarines, and other specialty or tailored products that are functional ingredients in food products prepared by food processors and restaurants and in the home. Humans have used fats and oils for food and a variety of other applications since prehistoric times, as they were easily isolated from their source. Fats and oils found utility because of their unique properties. These ingredients were found to add flavor, lubricity, texture, and satiety to foods. They have also been found to have a major role in human nutrition. Fats and oils are the highest energy source of the three basic foods (carbohydrates, proteins, and fats), and many contain fatty acids essential for health that are not manufactured by the human body [2].

While vegetable fats were used originally as a cheaper substitute for milk fat the ability to specify the properties of vegetable fat has considerable advantages. This ability arises because of the science and technology available to the fat processing industry. Some vegetable fats used in foods are not tailor-made but are simply a vegetable fat of known origin and treatment. The commonest example is palm kernel oil (HPKO), which is often used in foods.

4.1. Chocolate products

Chocolate can be described as a suspension consisting of nonfat particles (sugar and cocoa solids and, eventually, milk powder particles) dispersed in cocoa butter as a continuous phase. Molten chocolates represent a dense blend of phospholipid-coated sucrose and cocoa particles in liquid fat. Milk chocolate usually contains about 12 g of cocoa mass, 19 g whole milk powder, 48.5 g sugar and, additionally, 20 g added cocoa butter per 100 g chocolate [36].

The characteristic flavor of chocolate has to be developed in several processing steps. During processing, the components are mixed, refined, and conched to attain desired rheological properties for a final defined product texture and melting characteristics. A conche is a scraped-surface mixer that optimizes flavor development and turns chocolate mass into a flowable liquid. Through shear and longitudinal mixing, acidic flavors and moisture in the cocoa mass are reduced. Upon entering the conche, not all sugar and cocoa particles will be coated with cocoa butter. Fat in the chocolate will be released from the agglomerated chocolate mass and spread to cover these particles so that they can flow easily. The final chocolate mass viscosity should be deemed optimal for the ensuing tempering [37].

Cocoa butter, which amounts to 25-36% in finished chocolate, is responsible for the smooth texture, contractability, flavor release, and gloss of the product. The fat phase is

the only continuous phase in chocolate, thus responsible for melting behavior and the dispersion of all other constituents. A careful tempering of the chocolate is necessary in order to obtain the fine crystals in the correct form (β-modification). Cupuassu fat, a similar cocoa butter fat, shows polymorphic behavior like cocoa butter (β form) and needs to be tempered like cocoa butter; at 24-25ºC an α (alpha) form is present. The melting profiles of cocoa butter and cupuassu fat are similar as shown. At all temperatures, cocoa butter has a higher solid fat content than cupuassu fat. This suggests that cupuassu fat would be useful in filled chocolate manufacture as a softer filling fat compatible with cocoa butter. The fatty acid and triacylglycerol compositions of cupuassu fat in comparison with cocoa butter show that palmitic acid in cupuassu fat is present in much smaller amount (7.8%) than in cocoa butter (26.1%); stearic acid is about the same; oleic acid is higher in cupuassu. Particularly notable is the high amount of arachidic acid (20:0) in cupuassu fat. The triacylglycerol compositions reflect the fatty acid compositions, but give more useful information. Although cupuassu has a higher SOS content than cocoa butter, its contents of POP and POS are much lower reflecting its low level of palmitic acid. Total SOS-type triacylglycerols, i.e. POP+POS+SOS+SOA, is 57% in cupuassu and 83% in cocoa butter. Fractionation, as applied to fats such as shea and sal, would be needed to bring the total SOS-type content to the same as in cocoa butter. Fractionation could be used to modify cupuassu fat to make it more similar to cocoa butter for use as a CBE (cocoa butter equivalent), with 65% minimum of total SOS-type triacylglycerols [38].

Modified lipids are used in the majority of chocolate and confectionery applications, such as chocolate compounds, filling fats in pralines, aerated products and cold products such as ice cream toppings. Production economics is often related to price, speed of production and equipment requirements, which in turn are related to the raw materials and their ability to crystallize rapidly.

The quality is related to the capacity of the fat to remain stable in terms of appearance, texture and taste; and the sensory properties can briefly be described as appearance, smell, taste and the role that fat plays in mouth feel with regard to texture and melt off properties. In chocolate industry fat bloom is still a problem. It modifies (shortens) the shelf life of the end products and makes life difficult for product development. Fat migration is one of the causes of bloom, but it will also soften the products during storage [39].

In chocolate industry, for processing and texture reasons, however, it is not possible to reduce the level too much below 25%. This is insufficient to make a low calorie claim on the product, so two manufacturers have produced fats that melt like cocoa butter but have a lower calorific value. Like lauric fats they are incompatible with cocoa butter and so the products have to be made with cocoa powder [36]. Some fats go into confectionery as a component of other ingredients. The common example is nuts, which contain fats, often of types such as lauric or unsaturated fats. These fats are sometimes the origin of spoilage problems.

Studies correlating chocolate composition and textural or rheological properties are commonly found due to the source of new fat or cocoa butter replacers which strongly affect rheological parameters on chocolate manufacture and final product texture. According to that, adaptations on manufacturing scale have to be done in order to keep the desirable sen-

sory characteristics in the final product. Rheology is a useful feature on setting those issues. Several works have been conducted to study and understand rheological properties of chocolates. The various fats used in chocolate can contain different levels of trisaturated triacylglycerols. Since these can crystallize out early in the tempering process, they can, in some instances, have an effect on the rheology of the chocolate. Six basic source oils are permitted as non-cocoa vegetable fats (CBE - Cocoa Butter Equivalent) in chocolate throughout European Union - palm oil, shea oil, illipe butter, sal oil, kokum gurgi, and mango kernel oil. Among these six oils, four (palm, shea, sal, and mango kernel) usually have to undergo some form of fractionation process to concentrate the SOS type of triacylglycerol necessary for equivalence to cocoa butter. Palm oil is even more complicated since it contains a significant quantity of trisaturated triacylglycerols which also have to be removed [40].

4.2. Ice cream

Ice cream has been identified as three component foam made up of a network of fat globules and ice crystals dispersed in a high viscosity aqueous phase. The composition of ice cream varies depending on the market requirements and processing conditions. Although the quality of the final product depends largely on the processing and freezing parameters, the ingredients also play an important role. The physical structure of ice cream affects its melting rate and hardness, although the specific relationships have not all been worked out. Structure development in ice cream often is attributed to the macromolecules present in the ice cream mix – milk fat, protein, and complex carbohydrates. Milk fat interacts with other ingredients to develop the texture, mouthfeel, creaminess, and overall sensations of lubricity. Typically, ice cream contains 10 to 16% fat and its type and amount influence the characteristics of the resultant products by affecting their rheological properties. The fat content can influence the size of the ice crystals. Fat globules could mechanically impede the ice crystal growth. Since each type of fat exhibits a specific polymorphism function of its triacylglycerol composition, the thermal behavior of fats during ice cream processing should influence the physicochemical properties of the intermediate and final products [41].

A typical ice cream formulation has fat (7-15%), lactose (5-7%), other sugars (12-16%), stabilizers, emulsifiers and flavours (0.5%), total solids (28-40%), water (60-72%), milk protein (4-5%). Fat performs several functions in ice cream: it helps to stabilize the foam, it is largely responsible for the creamy texture, it slows down the rate at which ice cream melts and it is necessary to deliver flavour molecules that are soluble in fat but not water. The major sources of fat used in industrial ice cream production are butterfat, cream and vegetable fat [42].

Ice creams are metastable systems created from an emulsion o/w employing several unit operations: mixing, heating, cooling, freezing, aerating and packaging. While the ingredients combination is responsible by chemical characteristics, a sophisticated microstructural arrangement constituted by fat globules, ice crystals and air bubbles supported in a highly viscous matrix dictates mechanical, thermal and sensorial properties.

There are many factors within the microstructure of products, which determine the rheological properties, such as colloidal interactions between disperses components, the junctions between structural elements, the properties of this elements, the interfacial behavior be-

tween phases, the rheology, and structure of individual component phase. In order to improve the quality of this very appreciated foodstuff, ingredients research and their impact on formulations are very desirable. In [43] was investigated the potential of a chemically modified polysaccharide (N-succinil chitosan hydrogel) when applied as structuring agent in colloidal systems. It was found that the mixes resulting by combination among chitosan and palm fat presented good characteristics; the enormous structuring power presented by this biomolecule can be very useful to elaborate low-fat formulations with good textural properties. Moreover, taking in account the physiological activity, it can be employed in order to promote best nutritional quality in foods; this biopolymer and their derivatives, can be extensively explored, since appear do not has limitations in its potentialities.

In [41] was found in study that the replacement of hydrogenated vegetable fat by palm fat caused changes in melting ranges of formulations. Higher melting rate was observed by combination between palm fat and fructose syrup. In addition to effects expected on melting behavior and solids content, sugar blends employed in this study affected the air incorporation. There is consense that greater air content increase the melting resistance. However, despite to lower overrun, ice creams made with fructose syrup melted more slowly. Thus, the levels of air added into the products not allow safe conclusions about the influence of this parameter on physical behavior of assessed ice creams in this study. In [44] was evaluated also the influence of the substitution of hydrogenated fat in the manufacture of ice cream formulation with palm fat through rheology analysis, and compare the results obtained with the melting test. The rheological and the melting tests showed a better response from the ageing process, and a better formed structure with the formulation produced with hydrogenated fat. It was suggested that formulations produced with palm fat suffers a poorer partial coalescence by its crystallization profile and less membrane destabilization by the emulsifiers.

In another study was evaluated the influence of the substitution of hydrogenated fat in the manufacture of ice cream formulation with cupuassu fat through rheology analysis, and compare the results obtained with the melting test. The rheological tests showed similar response from the ageing process to both formulations, and the melting tests showed a slower meltdown of the structure with the ice cream produced with cupuassu fat. The results obtained demonstrated that cupuassu fat is a good substitute for hydrogenated vegetable fat for using in ice cream formulations [45].

4.3. Bakery

The functionality of fats in bakery products can be explained as: development of the structure; lubrication; aeration; heat transfer; moisture retention; improved shelf-life, volume, texture and flavor. In some cases the function of a fat can be either partially or completely replaced by some other ingredient, typically an emulsifier.

Fats shorten the texture of baked products by preventing cohesion of gluten strands during mixing, hence the term shortening. All-purpose shortenings are used primarily for cookies but are also common ingredients in cakes, breads, and icings and are also used for frying applications. The quality of cakes and icings is highly dependent upon aeration; therefore, a

variety of very specialized shortenings has been developed over the years to satisfy that demand. High ratio shortenings (containing mono and diglycerides), designed primarily for cakes, began to appear in the '30s. Fluid cake shortenings were commercialized in the '60s and offer many advantages including pumpability, ease of handling and the option of bulk delivery and storage [46].

Cake is a baked batter made from wheat flour, sugar, eggs, shortening, leavening agents, salt, nonfat dry milk, flavors, and water. Cake batters are essentially a 'foam', that is a system in which air bubbles are trapped and held in an aqueous phase. The main function of fat in cake making is to assist with the incorporation of air into the batter during mixing, and the air bubble size and stability. High-ratio cakes, rich in sugar and fat, are extensively used in the baking industry [47].

Margarine has always had the advantage over butter in that the properties of the product can be tailored to give the best performance in a particular system. For puff pastry, i.e., specialized margarines are easier to work with than butter. Various bakery margarines are manufactured to meet the technical requirements of particular uses.

The effect of different fats and margarines on the physical properties of cakes was investigated. The low *trans* fat suggested: greater volume and firmness; resilience comparable to hydrogenated fat; elasticity and chewiness were comparable to other formulations, as well the color parameters of the crumbs [48].

Textural properties are important quality parameters for this type of product. Physical and structure changes during aerated batters processing may alter their performance during baking or the quality of the final product. It is possible to test materials with particles and fiber in suspensions, since flushing effects may reduce sedimentation problems. In [49] was examined the influence of different types of fats (hydrogenated fat, margarine and vegetable oil) in formulation of cake batter, evaluating textural properties by Herschel-Bulkley equation using back extrusion analysis; and it were observed values close in all parameters to samples prepared with margarine and hydrogenated fat. It can be mentioned the break point values could be consider by the industry as an important parameter, pointing the need of less energy in their processes of pumping, i.e.

4.4. Food emulsions

4.4.1. Mayonnaise and salad dressing

Mayonnaise and salad dressing are emulsified, semi-solid fatty foods that by federal regulation must contain not less than 65% and 30% vegetable oil, respectively, and dried whole eggs or egg yolks. Salt, sugar, spices, seasoning, vinegar, lemon juice, and other ingredients complete these products. Pourable and spoonable dressings may be two phase (e.g., vinegar and oil) or the emulsified viscous type (e.g., French). There is a great variety of products available of varying compositions with a wide range in their oil content. Salad oils exclusively are used for dressing products; typical choices include soybean, canola and olive oils [46].

Emulsion is a thermodynamically unstable system due to flocculation, creaming, coalescence, phase inversion and Ostwald ripping. Emulsifier is a surfactant which can stabilize the emulsion by absorption at the interface, thereby lowering the interfacial tension. It is usually used to improve the emulsion stability. Proteins and polysaccharides are often applied in emulsion as emulsifier. Proteins are usually used for their surfactant and gelling properties to improve the textural characteristics and stability of emulsion, while polysaccharides are usually added to increase the viscosity or to obtain a gel-like product. It was studied the impact of the use of a biomaterial (N-succinil chitosan hydrogel) in elaboration and structuration of food emulsion, and in substitution of a part of the oil phase. Chitosan showed to be a versatile ingredient since that was capable to modify the rheological properties, acting as emulsifying agent, besides its already known antimicrobial and nutritional qualities [50].

4.4.2. Margarine

Margarine and spreads are prepared by blending fats and/or oils with other ingredients such as water and/or milk products, suitable edible proteins, salt, flavoring and coloring materials and Vitamins A and D. Margarine must contain at least 80% fat by federal regulation, however, "diet" margarines and spreads may contain 0-80% fat. These products may be formulated from vegetable oils and/or animal fats, however, the vast preponderance are all vegetable. Non hydrogenated oils typically represent the majority of the fat phase. Lesser amounts of partially hydrogenated fats, that are naturally semisolid at room temperature, and/or hard fractions of certain fats are added to the blend as required to deliver the desired structure and melting properties [46]. At the moment, interesterification technics have been employed to produce tailor fats. Margarine originated as a substitute for butter. The big advantage of margarine is that as a manufactured product the properties can be tailored to suit a particular use.

An acceptable margarine must be a soft plastic at room temperature; the ratio of solid or crystalline fat to liquid oil in the mixture must be such that when the fat crystals are of the proper size and well dispersed, the mass will offer some resistance to deformation and separation of solid and liquid fats will be negligible; all the fat crystals must melt completely at body temperature and leave a pasty sensation in the mouth; the fat crystals must not melt abruptely [51].

5. Prospective

The major brands are today sold all over the world. The economic impact of any bad image on these super brands has led the major companies to focus on brand image. The increase in obesity has focused on health aspects of foods in general but also on chocolate and confectionery products. Fat replacers of the future will need to meet some important criteria, including reducing or replacing the target fat effectively, being available at a cost appropriate to the benefits provided, and being safe and legal with no appreciable side effects [39, 52].

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Chapter 5

Mineral Composition of Blood Sausages – A Two-Case Study

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Additional information is available at the end of the chapter

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1. Introduction

1.1. Relevance of the assessment of mineral content in food

It is well known that a balanced diet is essential in maintaining a good health; hence, the nutritional value of foods is an important aspect of food quality [1]. In this context, more and more people are becoming very concerned about the chemistry of what they eat. Consequently, food industry is interested in maintaining a high standard of quality of their manufactured products which could meet the demands of an increasingly sophisticated consumer. Therefore, an important issue of food industry is the determination of food composition and the establishment of analytical controls [2].

Food scientists and food industry have long since been paying great attention to minerals in food, which has been mainly devoted to its essential role in human nutrition, i.e., physiological functions, humans' nutritional requirements, and mineral implication on safeness issues, i.e., mineral toxicity. There are more than 60 minerals in the human body, but only a few are considered to be essential, namely, iron, calcium, zinc, magnesium, phosphorus, sodium, potassium, manganese, selenium, copper. These minerals are absolutely essential to a host of vital processes, from bone and tooth formation, to the functioning of neurological, circulatory, renal and digestive systems, and some of them are necessary for regulation of enzyme systems [2,3].

Minerals deficiencies in human are common world-wide and there are evidences which suggest that deficiencies may play a main negative role in children's development, pregnancy

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and elderly health [3]. In this context, Ca, K, Mg and Fe are the most commonly under-consumed minerals in humans' diet [4]. Fe deficiency is the most common and widespread nutritional disorder in the world affecting both developing and industrialized nations [5]. Insufficient intakes of Fe cause anemia, fatigue, poor growth, rickets and impaired cognitive performance in humans [3]. On the other hand, the concentration of non-desired minerals in food can be increased by the persistent release of hazardous pollutants to the environment mainly derived from human industrial activity. This contamination of food supply can result in an increase of exposure of consumers to toxic metals such as lead, cadmium, arsenic and mercury, to levels higher than the tolerable daily intake [6].

The assessment of the mineral content in food is not only interesting from the nutritional and toxicological points of view. Since a few decades ago, instrumental analytical techniques based on atomic absorption or emission spectrometry applied to the determination of the mineral content coupled to multivariate statistical analysis have been proved to produce suitable methods to characterise food products, discriminate between food quality categories and control food authenticity, i.e., determination of the geographical origin of food, discrimination between cultivation methods (e.g. organic vs convenience crops), varieties of fruits and vegetables, or food processing practices [7-10].

The analysis of minerals in foods is challenging due to the wide range of concentrations present, which may vary from ppb to percent levels. The situation is further complicated by naturally occurring seasonal and varietal differences in concentrations within the same food [11]. Official methods by de AOAC offers many single element methods based on colorimetric techniques: UV/Visible spectrophotometry, and flame and graphite furnace atomic absorption spectrophotometry. However, although no AOAC food methods currently employ Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES), it is a well-established multi-element technique that no requires the use of dangerous solvents from the environmental point of view [11]. Its high specificity, multi-element detection capability and good detection limits result in the use of this technique in a large variety of applications. Detection limits typically range from parts per million (ppm) to parts per billion (ppb), although depending on the element and instrument, it can sometimes achieve even less than ppb detection [12]. ICP-AES provides higher reproducibility and quantitative linear range compared to conventional AES, and reduces molecular interferences due to a higher temperature (7000-8000 K) in the excitation source (plasma). On the other hand, ICP-AES is more expensive than conventional AES, and in complex samples, emission patters can be of difficult interpretation [13].

1.2. Blood sausages, making process and chemical composition

Meat products are generally made from various raw materials (from different origins and suppliers), which are combined at the formulation stage in obedience to criteria of composition, technological factors, sensory characteristics, legal regulations and also economic efficiency and profit [14].

Among meat and meat products, muscle foods are the most commonly consumed. However several edible meat by-products and their derivatives are also importantly consumed in a

number of countries, where meat by-products are usually linked to traditional or ethnic foods. Meat by-products are traditionally sold to the lower income market however, by different reasons – one of them could be the increase in tourism – their consumption seems to be increasing and some of the by-products are becoming delicacies in niche markets. Advantageously, meat by-products consumption contributes to increase the edible portion of slaughter animals, Furthermore, meat edible by-products constitute an excellent source of nutrients like essentials amino acids, minerals and vitamins [15,16]. Due to the great variety and specificity of edible meat by-products and their peculiar consumption patterns and their relative low economic value, there is relatively scarce information on their making process and chemical composition.

In some areas of the world, and to different degrees, blood is utilized as an edible meat by-product. For example, for several ethnic groups of Africa and India, blood is the primary source of animal protein, where it holds ritualistic importance. However, in some cultures (Islamic and Jews), blood consumption is seen as a taboo [17,18]. In Europe and Asia, animal blood has been traditionally used in making a variety of foods such as blood sausages, blood pudding, biscuits and bread, as well as blood soups and crackers [19,20].

From the nutritional point of view, blood is a good source of dietary protein, lysine and iron [19,21]. The high iron content of blood (approximately between 400-500 mg of iron per liter), coupled with the high absorption of heme iron compared to non-heme iron, is particularly useful for food based strategies designed to combat iron deficiency anemia. Furthermore, the environmental concern associated with blood disposal at slaughterhouses, together with blood nutritive value, has fostered research and industrial efforts to recover blood or blood components, to be used into a wide range of food products or as dietary supplements [22]. For example, blood or blood proteins (plasma or cellular fractions) are being used in meat products, primarily to increase protein levels and enhance water binding and emulsifying capacity.

Blood sausages are very popular traditional meat products in many parts of the world such as Europe, Latin America or Asia [23-26]. In Europe, blood sausages are normally called morcilla and morcella in Spain and Portugal, black pudding in Great Britain, blutwurst and Thuringer blood sausage in Germany, *blodpϕlse* in Denmark, *boudin noir* in France, *bloed worst* in Belgium, blood-tongue sausage and black pudding in Austria, *caltabosi cu singe* in Hungary, *vaerevorst i*n Estonia, *kaszanka* in Poland, *biroldo* in Italy. In Latin American countries, blood sausages are also produced and are named as *relleno, prieta, moronga, mocillón* in Mexico, Colombia, Peru or Argentina, and Morcela in Brazil; these sausages from Latin America show characteristics similar to those from Europe, especially to those of Iberian Peninsula [25]. In this sense, blood sausages from Latin America can be included into the group of creole meat products, which means that they were originated from the adaptation of former Iberian meat products (brought to America by immigrants) to local condition and circumstances, thus, involving an innovation process at that time.

Nowadays, blood sausages are currently receiving worldwide increasing attention because they have become gourmet products in several countries, thus leading to an increase in their production and potential markets [27]. Furthermore, increasing consumer demand for eth-

nic specialties has renewed interest in such products, leading to a consequent need to assure safety and longer shelf-lives in an expanding market. Moreover, the Governmental Institutions, e.g., European Union, are getting more involved in the protection of high-quality traditional foods from specific regions or areas, which reflects a policy of supporting the inhabitants of rural areas and promoting regional products [23,27].

Blood sausages are basically made with blood, fat and a variety of vegetable origin food; Moreover the use of meat, pork skin or offal (e.g., liver, intestine) is common, mainly in German blood sausages [28-31]. The vegetable-origin food used is enormously diverse so that, apart from spices and condiments, blood sausages can contain as main ingredients onion, leeks, cereals (rice, oat, flour, bread, etc.), sugar, fruits (apple, plum, etc.), nuts, etc. [32-34]. Other ingredients such as eggs, cream, milk are used in some types of blood sausages in France [32]. Moreover, as any meat product, blood sausages are added with common salt. The NaCl used in blood sausages from Mediterranean Europe tend to be between 1.2 and 1.5 % [35-37], and in blood sausages from Germany [38] and USA [16] tend to be higher, close to 2%. NaCl has a direct effect on the flavour and also increases the shelf-life, decreasing the water activity. Finally, several additives such as curing agents (nitrate and nitrite salts), pH modifiers (such as lactate or acetate salts) or emulsifiers can be also used [30,39].

The making process of blood sausages differs as a result of type, region and manufacturer. However, a common flow chart of the process of most of the blood sausages is depicted in Table 1 [21]. The initial mixture of blood sausages is complex by the number of ingredients used and pre-treatments to which those ingredients have been undergone. For example, meat can be cured previously to the mixture preparation, or pork rind can be cooked and emulsified. Similarly, several ingredients, such as fat, onion or rice, can be cooked before the mixture is prepared. Once prepared, the initial mixture is normally stuffed into natural or artificial casings and the sausage is cooked in hot water until a temperature of 65-75 ºC is reached in the inner part of it [31], and then the sausage is chilled before refrigeration storage. Some varieties of blood sausages are dried and/or smoked after cooking. Once cooked and chilled, most of blood sausages present a dark-red to black colour and a rather firm and sliceable texture [30] due to the formation of a gel structure from the interaction of collagen, starch, blood proteins, etc.; nonetheless, some blood sausages are soft and spreadable.

1. Raw matter selection
2. Preliminary preparation of raw materials (weighting, size reduction, premixing, precooking, curing, etc.)
3. Mixing
4. Stuffing
5. Cooking
6. Chilling

Table 1. General flow chart of blood sausage making process

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In general, meat and meat products are generally recognized as good sources of high biological-value proteins, group B vitamins, minerals as well as some other bioactive compounds [15]. The composition of meat products depends on their formulation. Thus, the chemical composition of blood sausages is diverse and would depend on the ingredients and manufacturing process used. As a matter of reference, Table 2 shows the proximate composition of several blood sausages from Europe and Latin America. Moisture is expressed as percentage of fresh weight, and values of protein, fat, available carbohydrate, fibre and ash are expressed as percentage of dry matter. The literature sources for the data are the following (see Table for superscripts correspondence): [a][36], [b][40], [c][38], [d][41], [e][42], [f][35], [g][37], [h][16], [i][43], [j][44].

Location and blood sausage name	Moisture	Protein	Fat	Available carbohydrate	Fibre	Ash
Europe						
De Burgos, Spain[a]	62.2	13.1	28.7	51.1	1.7	4.3
Asturiana, Spain[b]	38.5	7.0	69.1	8.0	-	2.9
With onion, Spain[b]	46.0	20.9	59.4	23.2	0.0	-
Blutwurst, Germany[c]	55.9	27.4	65.8	0.0	-	-
Thueringer, Germany[c]	66.2	58.9	32.3	0.0	-	-
Verivanukas, Finland[d]	61.1	19.3	22.6	43.9	9.8	-
Verimakkara, Finland[d]	54.7	28.7	42.0	21.2	6.2	-
Blodpølse, Denmark[e]	43.7	19.0	36.9	32.0	8.9	3.2
With rice, Portugal[f]	62.0	28.9	38.9	24.6	-	-
Boudin noir, France[g]	62.0	26.8	58.1	10.8	-	-
America						
Blood Sausage, USA[h]	47.3	27.7	65.5	2.5	0.0	4.4
Traditional, Chile[i]	77.8	47.3	38.3	0.0	5.9	8.6
Traditional, Bolivia[j]	44.5	31.7	57.3	10.8	-	1.8
With tongue, Bolivia[j]	48.8	41.2	55.5	0.0	-	3.3
Stege, Bolivia[j]	41.2	31.2	56.8	6.5	-	5.4

Table 2. Proximate composition of several blood sausages from Europe and America

Moisture content of blood sausages would depend inversely on the fat content and directly on the amount of moisture evaporated during an eventual drying/smoking stage. As can be seen in Table 2, the ranges of fat, available carbohydrate and protein in dry matter vary from 22.6 to 69.1, 0 to 51.1 and 7 to 58.9, respectively. There are great variations in dry matter composition between sausage types, which can be attributable to differences in the quanti-

ties of the main ingredients used, i.e., pork fat, cereals, vegetables, meat or blood. Thus, the presence and levels of fibre are the result of the use of vegetables, namely onion, leek, fruits, etc. Finally, ash content is related to the amount of common salt used in the making process. Regarding to the mineral content of blood sausages, the Fe content is the most reported in literature. Fe content of blood sausages is high due to the use of blood, and amounts reported vary from 6 to 16 mg per 100 g [16,36,42,45].

1.3. Aim of the study

In spite of their popularity and increasing interest, literature on the composition and quality of blood sausages is to our knowledge scarce. The knowledge of the chemical composition of blood sausages presents potential usefulness regarding nutritional, product characterization and quality control aspects. Among the chemical composition, the mineral content of blood sausages seems to be a key point in those aspects.Therefore, the main aim of the present study is to describe and determine, as case studies, the manufacturing process and the chemical composition with particular interest on the mineral content, of two typical blood sausages produced in two different parts of the world: a typical blood sausage with white onion (*Allium cepa*), from the region of Leon (north-western Spain), known as Morcilla de Leon; and typical blood sausage with white cabbage (*Brassica Oleracea* var. *capitata*), from the region of Tumbes (north-western Peru), known as Relleno de Tumbes.

2. Material and methods

2.1. Making process of the blood sausages

In order to collect information about the making process of the blood sausage Morcilla de Leon, four interviews were conducted with the correspondent production managers at the four main local companies producing this sausage in Leon city. The two-member interview panel asked a set of questions regarding general company characteristics, raw materials used, making process and storage conditions. Moreover, collecting data on the making process of Relleno de Tumbes was carried out by standardized open-ended interviews conducted with 15 homemade manufacturers at the region of Tumbes (Tumbes city and small villages at Zarumilla province). The questions asked were to know information on the raw materials used and the making process followed. In both cases, the interviews were followed by the observation of the sausage making process.

2.2. Chemical analysis

A total of 8 samples of Morcilla de Leon were manufactured by local producers (city of Leon, north-western Spain) and were purchased from local markets. The sample weights were approximately 250 g. Once taken, the sausages were transported under refrigeration (<4 °C) to the laboratory of Department of Food Hygiene and Technology (University of Leon). On the other hand, a total of 12 samples of Relleno de Tumbes were obtained from

small local producers and retail stores in Tumbes City (north-western Peru) and small villages around the city. For each sausage sampled, a 300 g sample was packaged individually in a bag and transported in refrigerated containers to the laboratory in Tumbes. Subsequently, samples were frozen at -40° C and were transported to the laboratory at University of Leon where upon arrival at laboratory the samples were kept frozen at -40 °C until the analysis was performed.

Determinations of moisture, fat, protein and ash contents in the sausage samples were performed in duplicate according to methods recommended by the AOAC International [46] – Official Methods nos. 950.46, 991.36, 981.10 and 920.153, respectively. Total dietary fibre was analysed following the AOAC 991.43 standard method [46], using the K-ACHDF 11/06 enzymatic kit (Megazyme, Wicklow, Ireland). Finally, the percentage of available carbohydrates was calculated by difference (100 – the percentage of the rest of components).

The analysis of mineral composition of sausages was performed by ICP-AES on wet digested samples. Duplicate aliquots of approximately 1 g (±0.01) of the previously homogenised samples were digested with 10 ml of concentrated HNO_3 in tightly closed screw cap glass tubes for 18 h at room temperature, and then for a further 4 h at 90 °C. For the analysis of sodium, potassium, sulphur and phosphorus, 1 ml of the mineralized solution was added with 8 ml of deionized water and 1 ml of scandium solution as internal standard. In order to determine the levels of calcium, copper, iron, magnesium, manganese and zinc, 3 ml of the digested solution was added with 6 ml of deionized water and 1 ml of Sc solution.

The instrumental analysis was performed with an Optima 2000 DV ICP optical emission spectrometer (PerkinElmer, Waltham, MA, USA). Instrument operating conditions were: radiofrequency power, 1400 W; plasma gas flow, 15.0 l/min; auxiliary gas flow, 0.2 l/min; nebulizer gas flow 0.75 l/min, crossed flow; standard axial torch with 2.0 mm i.d. injector of silica; peristaltic pump flow, 1 ml/min; no. of replicates, 2. The spectrometer was calibrated for Cu, Mn, Zn, Fe, Ca and Mg determinations (at 224.7, 257.61, 213.9, 238.2, 393.4 and 279.6 nm, respectively) with nitric acid/water (1:1, v/v) standard solutions of 2, 5 and 10 ppm of each element, and for Na, P, S and K (at 589.6, 213.6, 182.0 and 766.5, respectively) with nitric acid/water (1:9, v/v) standard solutions of 30, 50 and 100 ppm, respectively.

2.3. Statistical analysis

The software STATISTICA for Windows [47] was used for the statistical treatment of data. Furthermore, a principal component (PC) analysis, unrotated method, using the mineral composition as expressed as non-fat dry matter, was also performed.

3. Results and discussion

3.1. Making process

The Morcilla de Leon (Figure 1), typically produced in the region of Leon (north-western Spain), is made from a mixture of chopped onion (used at amounts between 65 and 75 % of

total weight), animal fat (lard and/or tallow; 10-20 %), blood (normally from pigs, 10-20 %), rice or breadcrumbs (2-10 %), salt (1-1.5 %), dry powdered paprika (1-2 %; including hot and sweet paprika), garlic and a mixture of spices (usually up to 1 g/kg) composed of several of the following: oregano, cumin, anis, cinnamon or pepper. Normally, onion and rice are precooked with the lard or tallow for 1-2 hours (until the onion becomes soft and tender). At the end of cooking, the condiments, spices and blood (liquid) are added and the mix is stirred from some minutes. Nevertheless, one manufacturer did not precook the onion and fat, and thus all ingredients (raw) were cold-mixed. The mixture, (hot if it was precooked or cold if not precooked) is stuffed in natural pork or beef casings of around 45 mm of diameter, tied or clipped forming 20-cm pieces. After the stuffing of the mix, the sausages are cooked in hot water at 80-90 °C for 20-45 min. After this step, sausages are drained hung at room temperature for a few hours and then chill-stored. This product is usually stored without packaging, and the shelf–life is around 12 days at refrigeration temperatures.

Figure 1. Spanish blood sausage Morcilla de León.

Relleno de Tumbes (Figure 2) is a typical blood sausage from Northern Peru, which consists of a mixture of blood (approximately 30%), pork lard fat (10%), chopped cabbage (40%), chopped red and Chinese onion (5%), chopped fresh paprika (2%; including sweet and hot paprika local varieties), common salt (1.5%) and a number of herbs and spices at low quantities (spearmint, coriander, garlic, cumin, pepper) and a in-situ-prepared annatto oil extract; furthermore, the addition of glutamate is common. The amounts indicated above are roughly estimated because the manufacturers did not use scales and the interviewers did not carry a scale in order to weight the ingredients used in the making process. The blood (liquid or

coagulated, sometimes precooked and shredded) is manually mixed with the lard, chopped vegetables and salt. Then, the mix is manually stuffed into natural pork casings (large intestine). The blood sausages are cooked in boiling water for approximately half an hour. After cooking, the blood sausages are cooled and then drained hung.

Figure 2. Peruvian blood sausage Relleno de Tumbes.

3.2. Chemical composition

3.2.1. Proximate composition

The proximate composition of Morcilla de Leon and Relleno de Tumbes are shown in Tables 3 and 4, respectively. Moisture is expressed as percentage of fresh weight, and values of protein, fat, available carbohydrate, fibre and ash as percentage of fresh and dry matter weights. Moisture content variability would mainly depend on fat content and the degree of drying loss during cooling and storage. Furthermore, the presence and variability of protein, fat, available carbohydrates and total dietary fibre would be respectively explained mainly by the amounts of blood, lard or tallow, rice or breadcrumbs, onion or cabbage (plus other vegetal condiments and species) used in the formulation. In fresh weight basis, both types of blood sausages have a similar percentage of moisture. However, Morcilla de Leon shows lower amount of protein and higher of fat, fibre and available carbohydrates, than Relleno de Tumbes, both in fresh and dry weight basis. This is explained by a higher amount of

blood and lower of vegetables and fat, being used in the Relleno de Tumbes making process, with respect to those being used for Morcilla de Leon.

	Mean ± SD (% of fresh weight)	Mean ± SD (% of dry weight)
Moisture	67.1 ± 5.8	-
Protein	5.2 ± 0.9	16.3 ± 3.6
Fat	14.2 ± 3.9	42.9 ± 7.6
Ash	1.9 ± 0.1	5.9 ± 1.2
Total dietary fibre	3.4 ± 1.5	10.1 ± 3.1
Available carbohydrate	8.2 ± 3.4	25.0 ± 6.3

Table 3. Proximate composition of the Spanish blood sausage Morcilla de Leon (n = 8).

	Mean ± SD (% of fresh weight)	Mean ± SD (% of dry weight)
Moisture	71.8 ± 6.9	-
Protein	11.9 ± 2.8	42.4 ± 10.2
Fat	9.4 ± 4.0	33.3 ± 13.9
Ash	2.1 ± 0.9	7.6 ± 3.2
Total dietary fibre	1.1 ± 0.4	3.9 ± 1.3
Available carbohydrate	3.6 ± 1.6	13.8 ± 6.3

Table 4. Proximate composition of the Peruvian blood sausage Relleno de Tumbes (n = 12).

3.2.2. Mineral composition

The mineral contents of Morcilla de Leon and Relleno de Tumbes are shown in Tables 5 and 6, respectively. Values (expressed as mg/100 g) are given in all fresh, dry and nonfat dry weight basis. Na is the mineral with the highest concentration, and the mean value seems slightly lower in Morcilla de León than in Relleno de Tumbes, where Na concentration shows a great variability between samples (high standard deviation). In average, Relleno de Tumbes contained higher amounts (approximately twice as much) of Ca and S macroelements and of Fe, Zn and Cu microelements.

The mineral content of blood sausages is the result of the sum of the contributions from all the ingredients used. In order to better ascertain the eventual contribution of ingredients to the mineral content of blood sausages, the mineral composition of the main ingredients used in Morcilla de Leon and/or Relleno de Tumbes is shown Table 7 [16,41,48-50]. From Table 7 and taking into account the quantities of the ingredients used in the making processes of the

blood sausages, it can be notice that blood appears to be the main source of Fe and Cu to blood sausages. On the other hand, onion and specially cabbage would be the main sources of K, Ca and Mn. Furthermore, S, P, Mg and Zn are importantly provided by both blood and vegetables, with the the high S content of cabbage being remarkable. Finally, lard seems not to be a good source of minerals and common salt, added at amounts of 1-2% to the sausage mixture, is the major source of Na in sausages (not shown in tables). In this context, the higher content of Fe and Cu in Relleno de Tumbes can be associated to the higher quantity of blood used. Similarly, the high content of Ca and S in cabbage together with the high quantity used in Relleno de Tumbes would account for the higher levels of those minerals with respect to Morcilla de Leon.

	Fresh weight	Dry matter	Non-fat dry matter
Macroelements			
Na	623 ± 131	1900 ± 615	3315 ± 1038
K	149 ± 27	452 ± 83	795 ± 121
S	76 ± 9	240 ± 61	402 ± 101
P	45 ± 13	136 ± 21	229 ± 35
Ca	29 ± 8	86 ± 21	146 ± 36
Mg	15 ± 2	48 ± 9	78 ± 16
Microelements			
Fe	10.96 ± 3.30	33.24 ± 10.96	58.71 ± 23.33
Zn	0.37 ± 0.12	1.14 ± 0.35	1.88 ± 0.41
Mn	0.20 ± 0.05	0.59 ± 0.10	1.00 ± 0.18
Cu	0.08 ± 0.02	0.26 ± 0.05	0.42 ± 0.12

Table 5. Essential mineral content (mg/100 g) of the Spanish blood sausage Morcilla de Leon (n = 8).

From the nutritional point of view, comparing the mineral content of blood sausages (fresh weight basis) with that of pork meat or muscle meat products, such as frankfurters or chorizos [16-51], the blood sausages had considerably higher levels of Ca (more than three times), and Mn, Fe and Cu (more than ten times). On the contrary, amounts of K, P, S and Zn are slightly lower in blood sausages (up to 60% lower than those in meat). The levels of Mg were roughly comparable in meat and blood sausages, and those of Na depends on the quantities of common salt added. Having into account the Reference Labelling Values (RLVs) reported by the Scientific Committee of Food from the European Union [52], which are the following: K (2000 mg), Ca (1000 mg), P (700 mg), Na (600 mg), Mg (375 mg), Fe (14 mg), Zn (10 mg), Mn (2 mg) and Cu (1 mg); interestingly, a portion of 100 g of blood sausage equals or exceeds the recommended daily intake of Fe and contributes with 10-15% the recommended daily intake of Mn and Cu. Thus, the high iron content of blood, coupled with

the high absorption of heme iron compared to non-heme iron, is particularly useful for food based strategies designed to combat iron deficiency anemia a major global malnutrition problem.

	Fresh weight	Dry matter	Non-fat dry matter
Macroelements			
Na	706 ± 335	2821 ± 1343	3951 ± 1640
K	142 ± 56	565 ± 223	798 ± 254
S	116 ± 22	411 ± 102	590 ± 108
P	48 ± 19	190 ± 67	271 ± 83
Ca	50 ± 18	180 ± 65	257 ± 83
Mg	14 ± 6	50 ± 21	71 ± 28
Microelements			
Fe	29.01 ± 8.55	101.03 ± 25.39	146.50 ± 36.81
Zn	0.70 ± 0.10	2.44 ± 0.51	3.51 ± 0.52
Mn	0.14 ± 0.07	0.52 ± 0.25	0.74 ± 0.36
Cu	0.13 ± 0.06	0.49 ± 0.25	0.69 ± 0.32

Table 6. Essential mineral content (mg/100 g) of the Peruvian blood sausage Relleno de Tumbes (n = 12)

Results of principal component analysis are shown in Figures 3 and 4. Principal component analysis was carried out with the mineral content expressed as mg per 100 g of nonfat dry matter for all the blood sausages analysed in this study. The first principal component (factor 1) accounted for a variance of 42.77% and the second of 19.89%. Figure 3 shows that samples from each type of blood sausage are located in two defined sets of results, which corroborate the differences in mineral contents found between both blood sausages. Figure 4 shows the projection of the variables (mineral contents) on the plane formed by the two principal components. The minerals with higher influence (factor loadings higher than 0.8) on factor 1 are Mn, Zn, and Ca. The mineral with higher influence on factor 2 was K (factor loading > 0.8).

Moreover, in Figure 4 it can be seen that the most correlated mineral contents, as indicated by the highest proximity of points in the plain, were S with Ca and Mn with Zn. The first relation could be explained by the significant contribution of cabbage and onion to the S and Ca content in the sausage mixture. However, the second relation is difficult to explain from the contribution of ingredients. Other remarkable correlation is that of Fe with Cu, with blood being the main source of both of them. This correlation could be not as strong as expected due to the feasible migration of Fe ions to ingredients and sausage mixture from the surfaces of cast iron equipment, i.e., pans, knives, etc. [53], which are frequently present at

small homemade sausage producing facilities in small villages. This reason could be responsible for part of the distance between the Fe and Cu points.

	Blood, pork	Pork fat	Onion	Cabbage
Macroelements				
Na	300	11	3	41
K	50	65	166	161
S	140	-	51	300
P	100	38	35	32
Ca	7	2	22	53
Mg	6	2	11	15
Microelements				
Fe	50	0.2	0.2	0.6
Zn	0.5	0.4	0.2	0.2
Mn	0.0	0.0	0.2	0.2
Cu	0.7	0.0	0.1	0.0

Table 7. Essential mineral content (mg/100 g) of the main ingredients used in Morcilla de Leon and/or Relleno de Tumbes

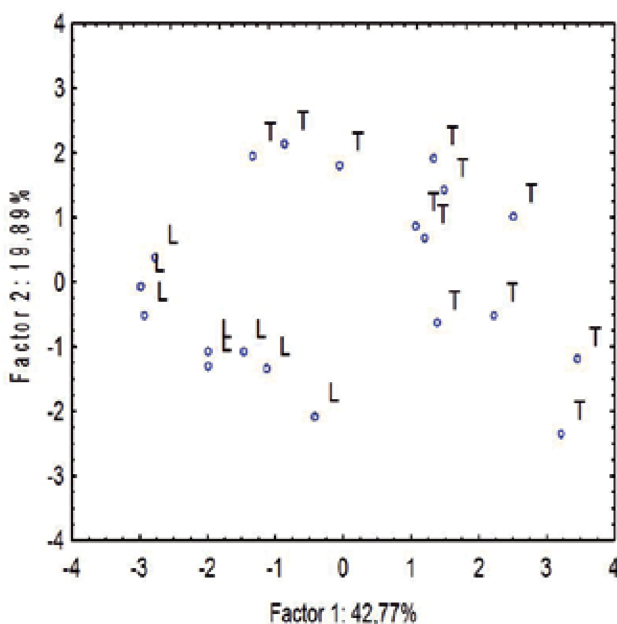

Figure 3. Principal component score plot (two first principal components or factors), considering mineral composition on non-fat dry matter basis, and showing samples according to sausage type: L, Morcilla de Leon; T, Relleno de Tumbes

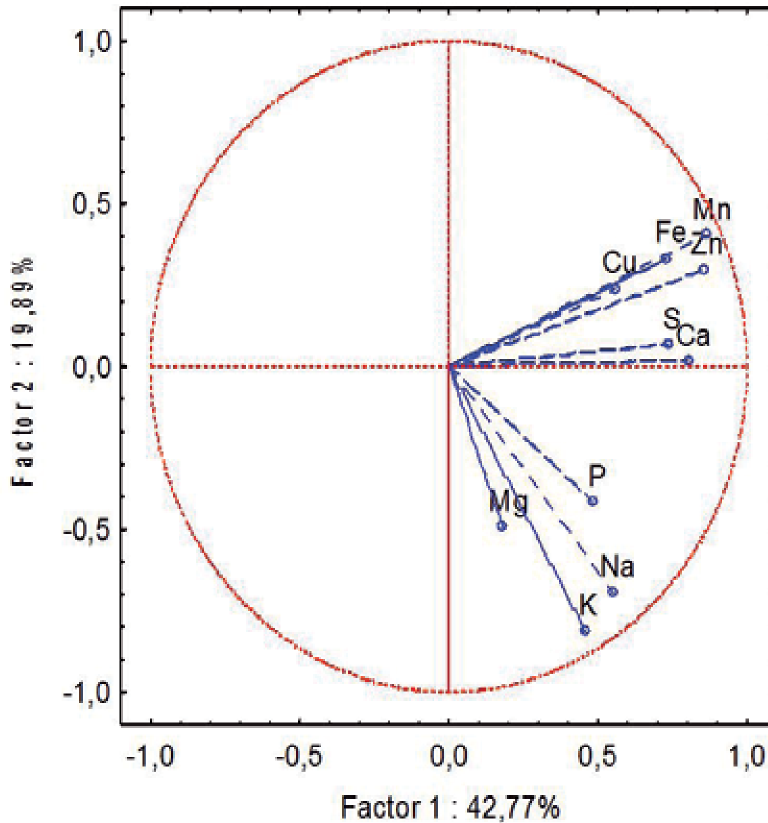

Figure 4. Projection of the normalised factor coordinates of variables (mineral contents) in the 1 x 2 factor plane obtained by the principal component analysis

4. Conclusion

The mineral content of two traditional blood sausages from different parts of the world: Morcilla de Leon and Relleno de Tumbes, as well as the proximate composition and general guidelines of the making process have been described in this study, which thus contribute to the chemical characterisation, diffusion and protection of these two traditional meat products.

The variety and quantities of ingredients used for blood sausage production have a significant relevance on their mineral content. Blood provides important quantities of Fe, Cu and Mn to the blood sausages from the nutritional point of view. The content of Fe of 100 g of Morcilla de Leon practically equals the daily requirements for adults and that of Relleno de Tumbes exceeds those requirements.

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Section 2

Food Quality

Chapter 6

Oxidation and Antioxidants in Fish and Meat from Farm to Fork

Sabine Sampels

Additional information is available at the end of the chapter

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1. Introduction

Both in meat and especially in fish there is a high risk of quality loss due to oxidation [1, 2]. Lipid oxidation in meat and fish-products leads to rancid taste and off flavor and development of many different substances from which some have even adverse effects to human health e.g. [3]. Oxidation limits storage time and thereby also affects marketing and distribution of both fish and meat products. Especially fish, being rich in n-3 polyunsaturated fatty acids (PUFA) is susceptible to peroxidation of PUFA resulting in restriction of storage and processing possibilities [4]. Furthermore, peroxidative products, particularly aldehydes, can react with specific amino acids to form carbonyls [5] and protein aggregates [6], causing additional nutritional losses. In red meat and also in red fish like salmon oxidation will not only deteriorate the lipids, but also the color [7, 8] and thereby affect visual consumer acceptability.

The addition of antioxidants is therefore necessary to increase storage stability, sensory quality and nutritional value of animal products [9, 10]. Due to the positive health effects of long chain n-3 PUFA, there is an increased interest to produce fish and meat products rich in n-3 PUFA [11]. Increasing the amount of easily oxidized PUFA in animal products however will also require a higher content of antioxidants in the end-product to protect the nutritional valuable fatty acids (FA). The importance of a well-balanced combination of PUFA and antioxidants, both for product stability and human nutrition, was also emphasized by [12]. Beside the traditionally used antioxidants in meat and fish also a wide variation of herbs, spices and fruits are used more and more as additives with antioxidative capacity [13-17]. In the recent years a lot of research has been carried out evaluating these natural substances as antioxidative additives in food products leading to novel combinations of antioxidants and the development of novel food products [17-20]. The high antioxidant capacity of these plant parts is particularly due to their content of different phenols, anthocyanins and ascorbic acid, which can act as radical scavengers [21].

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In addition to their antioxidative capacity, many of this natural substances have positive effects in the human body and documented health benefits and are therefore highly appreciated food additives [22-27]. So a combination of foods rich in omega 3 PUFA and plant substances rich in phenols and anthocyanins might result in nutritionally very valuable novel food products. These products could play and important role in the prevention of specific chronic-health problems beside dietary supplements where PUFA, probiotics and superfruits are achieving particular interest in the recent time [23, 28]. Finally nutritionally dense meals may be of interest and importance for people with particularly high nutritional demands, e.g. suffering from malnutrition [29].

For animal foods there are always two possible ways to include antioxidants: Via the feed or post mortem during the processing. Depending on the type of antioxidant, the one or the other way will be more effective. In general fat soluble antioxidants like tocopherol are more effective when present in the feed, while water soluble ones like vitamin C are more effective when added during processing [30, 31]. In addition there are synergistic effects between different antioxidants as for example shown for tocopherol and ascorbic acid [32] so a good combination of all available tools might be able to boost antioxidative protection for certain products.

The present chapter will give an overview of the main used and tested antioxidants, synergistic effects and the possible increased nutritional value. Feeding effects as well as a variation of processing and preserving methods for animal products from both very traditional and most recent techniques will be presented and their influence on oxidative stability will be elucidated.

2. General effects of lipid oxidation in meat and fish

Lipid oxidation is omnipresent in meat and fish and their products. Especially in products with a high amount of unsaturated FA, oxidation leads to rancidity, off-flavour and taste and to the formulation of toxic substances [2, 33, 34]. In the food industry a great deal of research and attention is spend on the on-going oxidative processes. The main aim is always to protect the raw material and the products as good as possible from oxidation through the whole process and during storage.

2.1. Short introduction to lipids

In order to get a whole picture about lipid oxidation, it is important to know some basics about lipids and FA. FA consist of carbon chains with a methyl (CH_3) group at one end and a carboxyl (COOH) group at the other. The C atoms in the chain can either be saturated or unsaturated meaning they form double bonds between each other. The FA which do not have double bonds are called saturated FA (SFA), those having one double bond are called monounsaturated FA (MUFA) and those with two or more double bonds are called polyunsaturated FA (PUFA) (Fig. 1). The FA are generally named in the scheme X:Y n-z where X is

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the number of carbon atoms in the chain, Y the number of double bonds and z the number of the last carbon atom with a double bond counted from the methyl end (see Fig. 1).

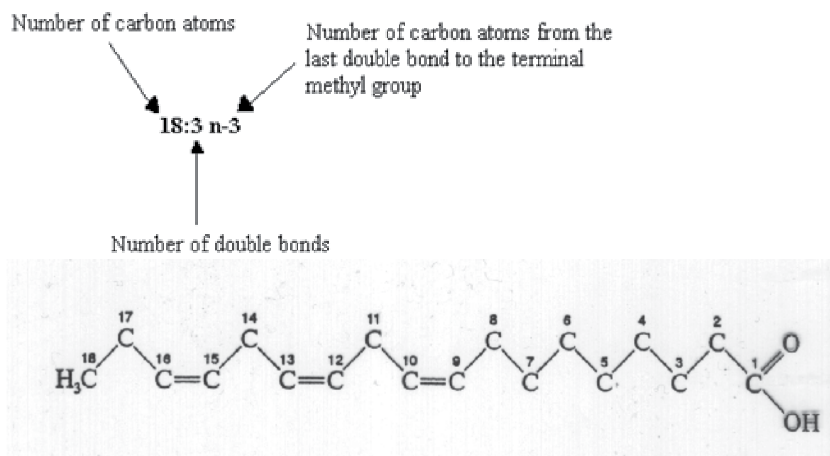

Figure 1. Linolenic acid, 18:3 n-3

The n stands in spoken language for omega so a FA with the last double bond at the third carbon atom from the methyl end is an omega 3 FA while the one with the last double bond at the sixth carbon atom from the methyl group is an omega 6 FA and so on. A very good in depth review about the classification and chemistry of FA and also about their biological functions has been done by [35, 36].

2.2. Reactivity of lipids to oxidation

The reactivity of unsaturated FA increases with their chain length and number of double bounds [37, 38]. Beside the number of double bonds also the placing of the double bonds and the form of the FA determine their oxidative reactivity. In general the n-3 FA are more prone to oxidation than the n-6 and those are more prone to oxidation than the n-9 FA [38].

In animal tissues the lipids are usually divided into two main classes: polar lipids (PL) and neutral lipids (NL). NL consist mainly of triacylglycerols (TAG) which are three FA bound to a glycerol molecule, and minor amounts of mono- and diacylglycerols, whereas PL include mainly phospholipids which are diacylglycerols including a phosphatic acid derivate [39]. TAG serve mainly as an energy source, whereas phospholipids are mainly constituents of the cell and organelle membranes being essential for their functionality and fluidity [39-41]. Phospholipids are in general more unsaturated due to their functionality and therefore also more prone to oxidation. In addition free FA (FFA) can occur in raw or processed tissues due to enzymatic breakdown of acylglycerols or phospholipids. The reactivity to oxidation is in general TAG>phospholipids>FFA.

The complicated thing about oxidation is that once it started a cascade of reactions will occur with each new molecule increasing the reaction speed and variability (Fig. 2). The kinet-

ics of oxidation in meat and meat products are described by [38] and [42]. Oxidation leads to the formation of lipid radicals (L.) that react further to lipid peroxides (LOO·) and hydroperoxides (LOOH). Auto oxidation in meat and fish can be initiated by light, heat, presence of metal ions and radicals. Very low concentrations of radicals are needed to start the reaction. Once initiated, oxidation propagates in a chain reaction (steps 2-6). In the termination reactions, lipid peroxides (LOO·) will react freely, forming a wide range of more stable products including aldehydes, alkanes and conjugated diens.

$$\text{Initiation} \quad LH + \text{Initiator} \rightarrow L \tag{1}$$

$$\text{Propagation} \quad L^{\cdot} + O_2 \rightarrow LOO \tag{2}$$

$$. + LH \rightarrow LOOH + L^{\cdot} \tag{3}$$

$$\text{Termination} \quad LOO^{\cdot} + LOO^{\cdot} \text{non-radical products} \tag{4}$$

$$. + LO^{\cdot} \text{non-radical products} \tag{5}$$

$$LOO^{\cdot\, + L\cdot} \text{ products} \tag{6}$$

In meat and muscle there are different possibilities to measure the degree of oxidation. The most used ones are listed very briefly here to facilitate the understanding of oxidation parameters used in this chapter:

- The peroxide value: determines the amount of hydroperoxides, which are among the primary products. However, as the peroxides are not stable and react further the results have to be evaluated carefully as, with on-going oxidation the peroxides first increase and reach a maximum but after a while the reaction speed towards secondary oxidation products is faster and the peroxide value decreases again [43].
- TBARS: Another very frequently method is the measurement of thiobarbituric reactive substances (TBARS). Thiobarbituricacid (TBA) reacts with malondialdehyd a secondary oxidation product from PUFA with 3 or more double bonds to a pink complex that can be measured at 532 nm. However the problem with that method is, that other substances also form coloured complexes with TBA and might result in wrong estimation of the oxidation status [44].
- Iodine value: A very traditional method which is still used sometimes to measure the iodine value as a number for the amount of lipid double bonds and the decrease of that number over time as a sign for oxidation.

- Volatile lipid oxidation products by Headspace GC-MS: During the last decade also more advanced methods have been used more and more for evaluation of oxidation. Content of Hexanal and other volatiles has been shown to give a quite good picture of oxidation status and mechanisms [45, 46]. However as these measurement are quite time consuming and expensive they are still not used routinely.
- Free fatty acids: The amount of free FA (FFA) is actually a value for lipolysis. But as the FFA are oxidised faster than bound FA, they can be regarded as a measurement for increased oxidative reactivity of the muscle or product.

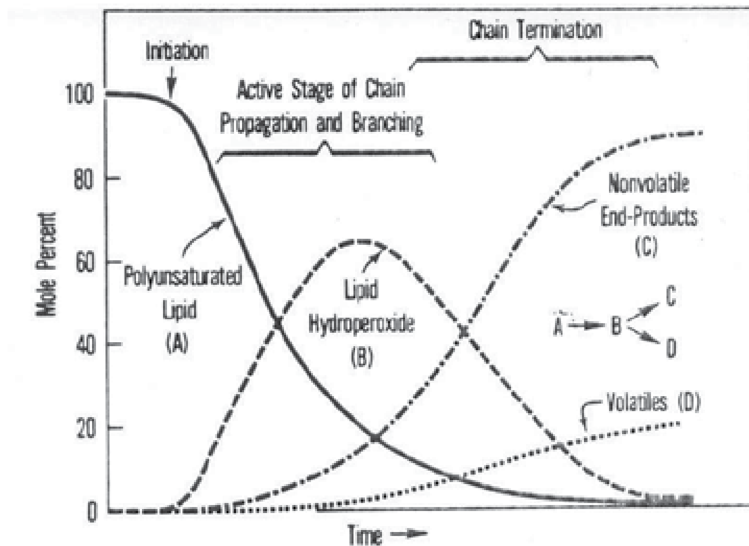

Figure 2. Hypothetical autoxidation of a polyunsaturated lipid as a function of time [47]

3. Antioxidants in feeds

Antioxidants can be introduced into the muscle by different means. Coming first in the natural chain from farm to fork would be to add the antioxidants via the feed. Also in the feed antioxidants are needed, to stabilize the lipids in the feed during storage, especially true is that for fish feed with high contents of PUFA.

The main used antioxidant in feeds is the fat soluble Vitamin E, normally added in the form of tocopherol acetate. Vitamin E is a generic name for all substances that have the biological function of α-tocopherol. These include the tocopherols with a saturated phytyl side-chain, (Fig. 3) and tocotrienols with an unsaturated isoprenoid side-chain, substituted to a chroman head. The different forms of tocopherols and tocotrienols are specified by the use of the Greek letters α, β, γ and δ, to denote the number and position of methyl groups linked to the chroman head [48].

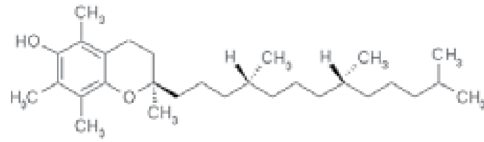

Figure 3. Structure of α-tocopherol

The water soluble vitamin C, ascorbic acid is another antioxidant used in feeds (Fig. 4). However studies have shown that in the live animal tocopherol shows a greater effect, while ascorbic acid works better added post mortem [30, 31].

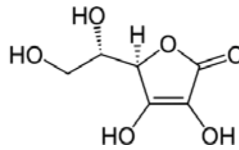

Figure 4. Structure of ascorbic acid

A third group of natural occurring antioxidants are the also fat soluble carotenoids, the precursors of retinol (vitamin A). They are for example found in corn. Carotenoids are hydrocarbons built from eight isoprene bodies (40 C atoms) (Fig. 5). Due to their structure and the conjugated double bonds, both vitamin E and the carotenoids, are radical scavengers that can build relatively stable radicals. In addition, carotenoids, tocopherols and tocotrienols are quenchers for singlet oxygen.

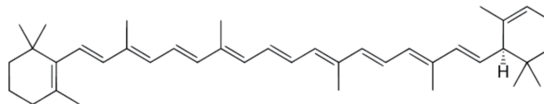

Figure 5. Structure of α-carotene

Carnosine (Fig. 6), a dipeptide, occurring in skeletal muscle which has also been tested as potential antioxidant, however added post mortem [49]. For example [30] suggested using a combination of feed additives and post mortem added antioxidants as for example a feed supplementation with a-tocopheryl acetate and post mortem applied carnosine.

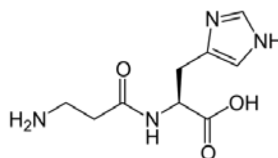

Figure 6. Structure of carnosine

Squalene is triterpene (30 C atoms) (Fig.7) that is present in plants and animal tissues as a key intermediate in the biosynthetic pathway to steroids. It has similar to the carotenoids conjugated double bonds and can hence build stable radicals and has been investigated as possible antioxidant [50]. Significant amounts of squalene in plant sources are detected in e.g. olive oil, wheat germ oil, bran oil and yeast [51] as well as in *Amaranthus* grain and *Ecchium* plants [52].

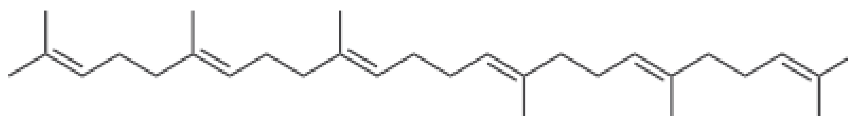

Figure 7. Structure of squalene

As mentioned before, there is also a growing interest to use novel sources of natural antioxidants for feeds, as for example from diverse vegetables [53, 54] or spices [55] or from more exotic sources as algae and lichen [56, 57]. [56] showed for example that feeding chicken with microalgae did not only increase muscle content of the long chain omega 3 FA docosahexaenoic acid, but also increased content of carotenoids and squalene. [58] supplemented pigs with cranberry powder and found both pro- and anti-oxidative effects, most possibly depending on muscle origin and later processing.

Moreover, there are also always interactions between different nutrients [59-61], which have to be taken into consideration when planning how to achieve antioxidative protection of animal foods. For example did high dietary lipid increase also muscle astaxanthin accumulation in salmon (*Salmo salar*) [60]. Astaxanthin is a carotenoid that gives the pink colour to salmon muscle but can also act as an antioxidant. In another study [48] showed that dietary intake of sesamin increased uptake of α-tocopherol in rats, which suggests that it is possible to increase the bioavailability of antioxidants through feed composition. However, if this mechanism is also valid for fish and other mammals, remains to be investigated.

Concerning the oxidation occurring in the feeds during storage [62] showed that ascorbic acid could protect vitamin E from oxidation in the diet for hybrid tilapia.

Also for the nutritional status of the animals the dietary added antioxidants are of importance. [63] was able to increase the survival of juvenile angelfish (*Pterophylum scalare*) with a combination of supplemented tocopherol and ascorbic acid in comparison to only tocopherol in the feed. Low dietary vitamin C content has shown to increase requirement of vitamin E in juvenile salmon [64], suggesting that the deficiency of one antioxidant will lead to the increased use of the available ones. However, bioavailability, efficiency and interactions with other substances might vary between different species as summarized by [63].

4. Antioxidants added during processing/effect of processing techniques

4.1. Oxidation factors in muscle and product

4.1.1. Metals

Oils and animal foods always contain a small amount of metals which are too difficult to remove, as for example iron from myoglobin, hemoglobin and the iron storage protein ferritin, or copper, zinc and heavy metals that are present in enzymes and metalloproteins [40, 65]. Another source of metals in animal food products are the machines used during processing, from which minor amounts of iron can get into the products either by abrasion or due to acidic dissolving of metals from the surface. A third source can be migration of metals from the packaging. These metals are present in so low amounts that they do not have a physiological effect; however, they can have pro oxidative effects [66].

4.1.2. Salt

Salt is used for the preservation of meat and fish. Due to its water activity lowering effect and the withdrawl of free water, salt decreases the solubility of oxygen as well as the activity of enzymes and bacteria. In addition chloride ions are also toxic to certain microorganisms. However it is also a pro-oxidant [67].

4.1.3. Oxygen, light and temperature

The more and longer a product is exposed to light and oxygen, the higher is the risk and speed of oxidation. When fish or meat is cut into pieces or minced, the surface is substantially increased and thereby the accessibility for oxygen. As light and increased temperature enhance oxidation [40, 68], during processing temperature and the processing time should be kept as low and short as possible respectively.

4.2. Different preservation and processing techniques:

Various processes including cooling, salting, drying, smoking and heating have been used for a long time to preserve meat and fish and to obtain a variety of products with characteristic organoleptic characteristic [2, 69, 70] Processing is a primarily way to preserve meat, but also adds to its value. However, different processing steps can also negatively affect meat quality, and change for example lipid quality traits. Heating of meat and meat products e.g. hot smoking, can disrupt the cell membranes and promote lipid oxidation [71], which affects the nutritional and sensory properties of the meat product. Use of antioxidants during processing or alternative more gentle processing methods can reduce these negative effects.

4.2.1. Chilling or cooling

Fresh meat is sold chilled at a temperature of about +4 °C. Preservation of meat quality is an important criterion for its shelf life, since raw, chilled meat has traditionally been a perishable product [1, 72]. In order to prolong the chilled storage time advanced packing techniques or various additives are used in addition, which will be described in more detail in the following.

Fresh fish is usually transported and sold on flaked ice, keeping the temperature slightly above 0°C; more recently also ice slurries have been used [2, 73]. To make these ice slurries even more effective different additives to fish as well as to the ice slurry have been used. Examples are natural antioxidants, ozone or organic acid mixtures. [73] evaluated the effect of organic acids mixed into the ice slurry on lipid oxidation and found slightly decreased oxidation. On the other hand [74] showed a significant decrease of lipid oxidation when fish was stored in ice made with water extracts from rosemary or oregano. [2] gives a good review on different additives to slurry ice and summarizes among others that addition of ozone declined microbial spoilage and did not increase oxidation. Addition of antioxidants directly to the fish will be discussed further on in this chapter.

4.2.2. Super chilling or deep chilling

Super chilling or deep chilling means in general to chill the products to a temperature close to or just below the initial freezing point, which is for the most food products between -0.5 and – 2.8 °C (reviewed by [75]). In regard to lipid oxidation it is important that there are no ice crystals formed, as they can destroy organelle membranes and thereby release enzymes and enhance oxidation potential [76]. This technique is used for example for deer meat exported from New Zealand to Europe. The international trading demands new techniques to provide longer storage times. Due to long transport distances and high export quantities, deer meat from New Zealand is stored in vacuum packages and deep chilled to -1.5 °C, and can be considered as fresh meat up to 14 weeks after slaughter [77]. The low temperatures, combined with vacuum, retard bacterial growth, lipid oxidation and color deterioration. There is not done much work on lipid oxidation during deep chilling, however [75] suggested that the improved shelf life and quality reported from deep chilled foods is also indirect resulted by a reduction of lipid deterioration.

Also in fish and seafood deep chilling has been applied successfully and shown to retard microbial growth and extend shelf life of for example prawn (*Penaeus japonicas*) [78] salmon and cod (*Gadus morhua*) [79, 80], however without investigating the effects on lipid oxidation.

4.2.3. Freezing

Frozen storage has since long been a method considered sufficient to preserve meat over longer time periods [81], however freezing can also negatively influence structural and chemical properties of meat, e.g. increase content of FFA and lipid oxidation products [82, 83]. [83] reviews some aspects related to lipid oxidation during and after freezing and describes the amount of unfrozen water as one important factor for primary oxidation. The amount of unfrozen water depends on the freezing temperature and in line with that an optimum freezing temperature for meats of -40°C has been suggested by [84]. At this temperature only a minor fraction of the water is unfrozen. In agreement with that [85] showed a significant increase of lipid oxidation products in pork stored at -18°C. Besides the temperature, the formation of ice crystals during freezing is a critical point [76] and the larger ice crystals are formed the higher is the risk of membrane disruption and increased oxidation.

An important element to avoid increased oxidation after thawing should therefore be the formation of small ice crystals during freezing. The faster and more homogeneous the freezing happens, the smaller and more uniform the formed ice crystals will be [86]. Some recent developed fast freezing techniques suitable for muscle foods are high pressure freezing, pressure shift freezing, cryogenic freezing and the already since longer time used air-blast freezing [86, 87]. [87] showed that substantial smaller ice crystals were formed in Norway Lobster (*Nephrops norvegicus*) when pressure shift freezing was used compared to air blast freezing. However, most of the papers, studying effects of freezing, evaluate only texture, drip loss and sensory, therefore not much is known about the effects of different techniques on lipid oxidation.

Once frozen, it is important for the maintenance of the small ice crystals that a stable temperature is kept, as thawing and refreezing as well as temperature fluctuations lead to formation of bigger ice crystals [86 1895]. Beside different techniques of the freezing itself, the injection or dipping of antifreeze proteins for both meat and fish has shown some success to force the formation of preferably small ice crystals (reviewed by [86]). Addition of antifreeze proteins has also shown to inhibit recrystallization of small ice crystals into bigger ones (reviewed by [86]).

4.2.4. Salting and curing

Meat curing and salting of fish are among the oldest preservation techniques man has used [88]. As described above salt has pro-oxidative effects. A demonstration of the pro-oxidative effect of salt in muscle foods can be found in [89]. During the process of meat curing with salt, nitrite is usually added to keep the nice pink color of the meat. The nitrite exchanges the oxygen ligand in the oxymyoglobin (Fig. 8), which is responsible for the bright red colour of fresh meat, and builds another very stable pink colored complex, the nitrosylmyoglobin [88]. [90] showed in addition an antioxidative effect of nitrite in meat and discussed different possible reaction mechanisms.

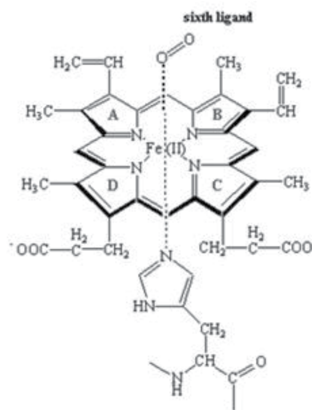

Figure 8. Structure of oxymyoglobine

Salting of fish is very commonly used traditional preservation process [91]. In many cases as for example in the traditional salted herring, salting and ripening takes a quite long ripening time. However [91] showed also that only a modest increase of peroxide values occurred during the ripening time. However as the FFA increased substantially in the brine and as peroxide values show only primary oxidation products substantial oxidation might have happened undetected. An indicator for oxidative stress during the ripening process is the drop of α- tocopherol to approximately 50% after 371 days in that study. However as also pointed out in the section about drying in this chapter, part of the oxidation products might be part of the desired characteristic organoleptic properties.

[66] investigated the effect of trace metals in the used salt during the salting of cod, showing a significant increase of TBARS with increasing copper concentration and during the salting time. Various attempts have been made to study oxidation during various salting processes and to find ways to inhibit or decrease lipid oxidation. [92] showed that salting initially protected chub mackerel (*Scomber japonicus)* from oxidation, however after 12 days of storage TBARS values were significantly higher in salted non-smoked fish compared to unsalted non-smoked fish. However, [93] showed that partial replacement of NaCl with KCl decreased lipid oxidation in salted mackerel as well as addition of ascorbic acid to brine solution. [94] found that EDTA prevented copper induced oxidation in salt brined cod, while added citrate enhanced oxidation and ascorbate had no effect in that study.

Besides the use of salting for preservation, fish is sometimes mildly salted to improve sensory characteristics and water holding capacity, where contents of only 0.1-0.3% can give significantly improved water holding capacity [17] but also resulted in increased oxidation levels in herring (*Clupea harengus*).

4.2.5. Drying

Dry curing and drying of meat also involve pro-oxidative factors, as there are: long exposure to air, dehydration and absence of nitrite [95]. Enzymatic activity can lead to high amounts of FFA, which are more prone to oxidation than TAG [34, 88]. In products such as dry-cured ham or dry-cured salami, a certain amount of volatiles, lipid oxidation compounds and lipolysis products is desired since they are responsible for the particular taste of these products [4, 96]. In line with this [97] showed that the traditional drying process of reindeer meat led to significantly increased oxidation parameters compared to the raw meat or smoked reindeer meat. On the other hand, excessive amounts of oxidation products result in off-flavors and rancid taste [98] and should hence be avoided.

[70] reviewed that in dry cured hams the lipases stay active for several month and hence can produce high amounts of FFA in the tissue. [99] confirmed this in their experiment showing a constant increase of FFA during 24 month of aging of dry cured hams. However TBA values did not increase significantly during that time. Evaluating how to avoid excessive oxidation in dry cured Parma hams, [100] showed that dietary tocopherol could decrease oxidation even in hams with an increased proportion of unsaturated FA.

Also various dried fish products exist, however most work done on dried fish products deals with microbial spoilage or sensory aspects as for example in cod [101, 102] and only few works also evaluate lipid oxidation. But [103] compared different drying methods for dried milkfish (*Chanos chanos*), a traditional Taiwanese product. In their study cold air drying resulted in significant lower TBA values than the traditional sun drying or hot air drying. They conclude that both light and temperature were important factors which induce increased oxidation. These results agreed with [104] who evaluated different drying methods on dried yellow corvenia (*Pseudosciaena manchurica*) and found higer oxidation in products made by the traditional sun drying process. Other works found on dried fish [66, 94] investigated heavily salted cod, and are hence discussed in the previous section about salt.

4.2.6. Smoking

Smoking is another traditional method to preserve meat and fish and create new products. [88] described the antioxidative activity of some of the smokes components. The various techniques and the types of wood used lead to the characteristic taste of the final product [105]. However as hot or warm smoking also includes increased temperature over a longer period and the meat parts are usually salted before smoking, also always some oxidation is initiated. In line with that effects are in general more complex, considering the various pro- and antioxidative aspects of this way of processing. For example [92] showed that smoking initially increased oxidation in chub mackerel (*Scomber japonicus*) but that it had lipid oxidation decreasing effects during storage, leading to lower TBARS values in the smoked fish compared to the unsalted non-smoked fish after 6 days.

In addition the smoke contains also substances that have been associated adverse health effects [106]. Therefore different processing methods as for example the use of liquid smoke have been investigated. [107] showed that a combination of liquid and traditional smoke were more effective inhibiting lipid oxidation in bacon than traditional smoke alone. These results were ascribed to a possible higher content of phenols in the samples processed with the combined smoking procedure. Contradictory [108] showed that traditional smoke resulted in lower TBA values compared to the use of liquid smoke in smoked beef tongue after 5-30 days storage. [109] compared traditional cold smoking and electrostatic smoking of salmon and concluded that electrostatically smoked fillets had a higher loss of lipids, but were less oxidized than traditional smoked fillets.

4.2.7. Packaging

From an oxidation point of view, packing should be tight and compact so that the surface and oxygen access are minimized. However this will not always meet the customers' expectations of product presentation, so naturally compromises have to be made. Packaging systems and technologies have developed rapidly during the last decades [1]. Both in meat and fish the principal function is to limit bacterial spoilage and growth. In red meats also the preservation of a bright red color is important, which is an indicator of freshness for the consumers [7]. This will be reached for example by keeping a high percentage of oxygen in a modified atmosphere package (MAP), while most bacteria are inhibited by an increased

concentration of CO_2 at the same time. For the different types of meat and fish the perfect gas mixture differs. A good overview is given by [110]. In fat fish due to the high oxidation risk a gas mixture without oxygen is generally recommended. [49] tried to use various antioxidants combined with modified atmosphere and showed increased lipid and color stability when a combination of rosemary and ascorbic acid was used in MAP.

Examples for the application of vacuum packing technique are given by [111] for fish burgers and by [80] for salmon fillets. Unfortunately these studies have not investigated oxidation in normal versus vacuum packing. However [112] investigated the effect of different storage conditions on oxidation in burgers made from rabbit meat and found decreased oxidation when vacuum packing was used.

4.2.8. Other preservation methods (irradiation)

Low dose irradiation is a very effective method to kill many bacteria including *Salmonella* and *Escherichia coli*, but it is also known to generate hydroxyl radicals and could hence lead to increased oxidation in meat and fish products [113]. [114] evaluated the effect of low dose irradiation up to 9.43 kGy on different meats (pork, beef, lamb and turkey) and found only low dependency between lipid oxidation values and the irradiation dose. However slightly higher values of malondialdehyd were found in turkey breast with the highest dose compared to the other meats at the same dose. [115] found increased oxidation values in pacu (*Piaractus mesopotamicus*) fish after irradiation. Nevertheless, in the same experiment the researchers showed addition of antioxidants α-tocopherol, BHT or rosemary extract could inhibit the oxidation accelerated by irradiation.

4.2.9. Canned meat and fish products

In canned fish the major part of oxidation seemed to occur due to the heating step before and during sterilization [116, 117]. [117] reviewed that also the storage conditions (time and temperature) before the actually canning do have a significant influence on the final content of oxidation products. The longer the storage time and the higher the storage temperature the more oxidation and lipolysis will take place and the higher the content of easily oxidable FFA will be. Beside these factors also the filling media seemed to have a significant impact [117]. [116] showed a significant increase of TBA values in silver carp canned with brine, sunflower oil and soybean oil while olive oil seemed not to enhance oxidation. On the other hand [117] evaluated the effect of natural antioxidants from the canning oil on canned tuna and found protective effects against lipid oxidation from extra virgin olive oil rich in phenols and also partly from soybean oil rich in tocopherols. Highest oxidation was found in tuna canned in brine in that experiment. [117] ascribed that to a possible accumulation of the PUFA at the oil-water surface. In general the results show that even added antioxidants like spices or other plant antioxidants could have a positive effect against oxidation in canned fish products. However, to our knowledge the effect of the addition of antioxidants or the effect of spices present in the brine has not been investigated yet.

In canned meat products the situation is expected to be similar as in fish, but not as much research as on fish products concerning oxidation has been executed. This might be due to the fact that there are more canned fish products on the market and that fish is known to have higher susceptibility to oxidation due to its higher content of PUFA. However, in one of the few more recent studies [118] investigated the importance of the raw product composition and found lowest oxidation in the product with lowest fat content.

4.3. Ready to eat and fast food products

There is a wide variety of ready to eat products from meat and fish available on the market, such as sausages, meat- or fish balls, paté's and many more. As these products often include minced or grinded meat and several other ingredients beside the raw muscle as well as they require several processing steps, all of these will have an influence on the oxidation behavior. On the other hand this creates a great chance to add antioxidants or to optimize processing techniques and packaging towards the lowest possible oxidation status of the final product. In general it can be said that also in this case the fish products will be the ones which are more prone to oxidation due to their more unsaturated FA composition. However also other aspects play a role as for example [119] found comparable cholesterol oxide values in one of three pork paté's as in a cod paté, while two other pork patés and a tested salmon and anchovy paté had lower values.

Antioxidants additives in fast food products are for example rosemary extract showing an antioxidative effect in mackerel burgers [111] and or as a more novel ingredient yerba mate extracts, which enhanced lipid stability in beef hamburgers [120]. [121] showed protective effects of oregano and thyme oil in ready to eat squid rings and [122] showed antioxidative effects of various herbs in pork patties.

But also processing methods or packaging can be used to increase oxidative stability. For instance [111] used vacuum packing in addition with the applied antioxidants in the mackerel burgers, while [123] evaluated a combination of irradiation and different packing environments to increase shelf life in pork patties.

Sausages are very favorite and omnipresent meat products around the world. A wide variety of categories such as raw, cooked, dry fermented, cooked smoked, raw smoked or precooked sausages exist. Through the addition of especially spices the oxidation in these products can efficiently be decreased [124] for example used Spanish paprika and garlic or a mixture of nitrite, nitrate and ascorbic acid in chorizo type sausages. They concluded that paprika showed a potent antioxidant capacity in this type of product and that a mixture of 3% paprika and 1% garlic had similar antioxidative effects as a traditional used curing mixture of nitrite, nitrate and ascorbic acid. [125] tested rosemary as spice and natural antioxidant in fermented goat sausages and found lower oxidation and increased values of overall sensory. Adding *Palatase M.* (from *Rhizomucor miehei*) to dry fermented sausages in order to improve sensory aspects, resulted in increased FFA content, however no correlation with higher TBARS could be found by [126]. On the other hand, the authors found an increased amount of volatile compounds which could indicate an increased oxidation due to the added enzyme.

5. Antioxidants in meat and fish products

Antioxidants delay or inhibit the process of oxidation, even when present in low concentrations [127]. Some antioxidants function as radical scavengers or peroxide decomposers, while others quench singlet oxygen, remove catalytic metal ions or oxygen, or inhibit enzymes. The cellular antioxidants can be classed as low molecular substances and enzymes that are either water-soluble or fat-soluble.

5.1. Spices and herbs

It is well known that phenolic compounds from spices and herbs have an antioxidative potential due to their possibility to act as a radical scavengers [128, 129]. A short review and a list of some polyphenols with their respective antioxidant activity can be found in [129]. Various spices have hence been tested in a wide range of products from sausages over meatballs to fish fillets and fish oil [124, 125, 130, 131]. [16] showed that 1.5% sage added to meatballs decreased oxidation and limited undesirable changes in the composition.

The advantage in the use of spices and herbs is that they are natural and in case of various products often are anyway included in the spicing or that they blend in to the desired taste of the final product. Consumers appreciate having natural antioxidants in their products over synthetic ones. However a problem might be if the taste of the used spice/herb does not fit with the product or gives a too strong side taste. For example [111] found that addition of 0.4 % rosemary gave improved shelf life of fish burgers with an acceptable taste for the consumers, while 0.8% of rosemary gave a too intense taste.

[132] compared the antioxidative activities of 22 commonly used herbs and spices added in different amounts to pork meat and found highest antioxidant capacity in sansho, ginger and sage. Furthermore also addition of rosemary, thyme, oregano and allspice resulted in up to 64% inhibition of lipid oxidation.

5.2. Fruits and berries

The high antioxidant capacity of berries is particularly due to their content of different phenols, anthocyanins and ascorbic acid [21]. Besides health benefits related to their natural antioxidants, colour attributes of berries are also of interest in food processing, as colour plays a vital role to the acceptability of foods. A wide range of various fruits and berries has shown antioxidant capacity as for example cranberries, elderberries, black currant and many more [133, 134].

For example, grape seed extracts were used to inhibit lipid oxidation in muscle from chicken, beef and pork [14] as well as in turkey meat [135] and polyphenols extracted from grape pomance inhibited lipid oxidation in fish muscle [136].

Even more unusual combinations have been tested successfully, as for example the antioxidative effect of various berry concentrates as marinades for herring fillets [17]. Other applications are cranberry juice powder as antioxidant rich feed for pigs [58], cranberry extract as

additive to separated turkey and ground pork meat [137], grape antioxidant dietary fibre in minced fish [138] or tomatoes in beef patties.

5.3. Antioxidants from other sources

Beside spices, herbs and fruits also teas and other possible sources for natural antioxidants have been evaluated.

Among others, tea catechins have been tested and used as antioxidants in various food products. Extracted tea catechins from green tea showed significant potential to inhibit lipid oxidation in red meat, poultry and in fish muscle [139]. Instant green tea has shown to slow down oxidation in frozen mackerel [140].

Chitosan is the deacetylated form of chitin and has been shown to have antibacterial and antifungal properties and has therefore reached some attention as food additive [141]. In its original form it is ineffective as antioxidant, however [141] have shown that as a glucose complex chitosan exhibited both antimicrobial and antioxidative effects in pork salami.

Besides research is constantly searching for new sources of antioxidants as for example tomato seed oil from tomato pomance (industrial tomato waste) [142] or industrial onion waste [54]. These antioxidant rich waste products could be added to the animals feed as successfully shown by [53] where eggs from chicken fed tomato byproducts contained higher amounts of lycopene compared to normal eggs. Or on the other hand extracts from these byproducts could be used as additives directly to the food products as suggested by [143]. Similarly byproducts from wine and olive oil byproducts inhibited oxidation in minced fish and frozen mackerel fillets respectively [138, 144]. As a more exotic possible additive [145] investigated antioxidant properties of Indian red seaweeds.

5.4. Synergistic effects and interactions

As mentioned before, in addition to the antioxidative effect a substance has alone, there are as well always interactions that can influence the bioavailability, the antioxidative effect and mechanisms between the various nutrients.

For example vitamine C and vitamine E have been found to interact as antioxidants, tocopheroxy radicals are reduced back to tocopherols by ascorbic acid [32]. As in meat and fish products this mechanisms takes place at the border between lipid and water phase, the radical is removed from the lipid phase and the lipid oxidation process due to that radical is terminated.

[146] describes the function of carotenoids in what he calls antioxidant networks, were carotenoids act together with other antioxidants at interfaces as for example xanthophylls and carotenoids in egg yolks and fish. Similar to the synergistic action of tocopherols and ascorbic acid, the more hydrophilic (iso)flavonoids and their glycosides regenerate the lipophilic carotenoids which are active as radical scavengers in the lipid phase. In another mechanism the more hydrophilic xanthophylls act via the membranes between water/lipid interfaces in synergism with more lipophilic carotenoids. [146] defines concluding two types of conditions how carotenoids function: (i) in "equilibrium" with other antioxidants in thermodynamically controlled

networks serving as color indicators of good antioxidant status and (ii) as antioxidants active through radical scavenging in networks with kinetically controlled regeneration. Furthermore carotenoids also showed to enhance antioxidant activity of vitamin E [147]. Moreover [109] reported that astaxanthin and tocopherol act via different mechanisms in salmon and hence improve stability against oxidation at different stages of oxidation.

Squalene has been found to protect α-tocopherol in oxidation processes [148], probably in a similar way of action than those described for the carotenoid networks.

The use of combined added antioxidant and other preserving techniques has also shown effects as earlier presented in the case of [49] where modified atmosphere packaging was used in combination with antioxidants and where also the combination of two different antioxidants, namely rosemary and ascorbic acid gave the best results. Furthermore [93] showed that a combination of various preservation techniques gave the best results against lipid oxidation in salted mackerel. Combined frozen storage at -18°C in a vacuum package and added ascorbic acid at the same time as 50% of the NaCl was replaced by KCl gave the best results in that study.

On the other hand [94] showed that ascorbate might have pro-oxidative effects due to concentration and depending on the presence or absence of other oxidants. For instance did ascorbate concentrations below 50ppm in combination with 5 ppm copper in the brine prevented formation of TBARS while concentrations above 500ppm in absence of copper had pro-oxidative effects.

Effects of other nutrients on antioxidant uptake and accumulation were shown by [109] who showed a positive correlation between fat content and tocopherol accumulation and a negative correlation between fat content and content of ascorbic acid in salmon. [149] showed negative effects of high dietary astaxanthin on α-tocopherol deposition in rainbow trout (*Oncorhynchus mykiss*)

There is still much room for novel combinations that might give improved oxidative stability to varying products and hence more research in that field is strongly needed.

6. Additional value in antioxidant rich foods

Generally, antioxidants maintain product quality by improving shelf-life, nutritional quality and other aspects related to quality. Meat and fish products have been successfully enhanced with different spices and new food ingredients, to prevent oxidation and increase thereby both nutritional value, storage stability and sensory.

The positive effects of tocopherol and ascorbic acid on human health in their property as vitamins are obvious. But beyond that compounds like polyphenols, carotenoids and catechins have shown to influence human health thanks to various other properties beside their antioxidative capacity. For instance [150] gave a valuable review on antioxidant and antimicrobial effects of various berries and their impact on human health and [151] reviewed the

anti-inflammatory properties, effects on cancer, diabetes, the immune system, and ocular health of asthaxanthin. [152] reviews the anti-inflammatory, anti-allergic, antimicrobial and cancer-preventive effects of polyphenols, which are mainly due to their antioxidant activity; and describes that polyphenols furthermore can directly bind with signaling molecules involved in inflammatory mechanisms and carcinogenesis and thereby regulate cell activity. In line with that review, [153] gave an overview about the various positive effects of tea catechins on human health, as for example protection against bacterial induced dental caries and antiviral properties.

Examples for the health effects associated with different berries and fruits are numerous: Cranberries are known for their prevention of urinary tract infections [154]. [155] reviews the antioxidative and cardio-protective actions of Chilean blackberries and [156] reviewed the positive effect of grape juice, berries and walnuts on age related diseases. The authors discuss that beside the antioxidative and anti-inflammatory effects, polyphenols as for example antocyanins and proanthocyanidins enhance neuronal communication and neuronal signaling and decreased oxidative and inflammatory stress occurring due to aging.

As already mentioned before, besides inhibiting oxidation, plant substances can also have protective effect against microbial growth. [141] described the combined antioxidative and antimicrobial effects of chitosan as well as [157, 158] showed that oregano and cranberry inhibit *Heliobacter pylori* and *Listeria* monocytogenes in fish and meat beside their antioxidative capacity. Hence some plants with antioxidant capacity are also protecting from food poisoning.

7. Outlook/novel foods

Considering the various properties of natural compounds as polyphenols, carotenoids terpenes and catechins, waste possibilities for the development of novel foods are still unexplored. For example the importance of a balanced combination of PUFA and antioxidants, both for product stability and human nutrition, was outlined by [12]. When increasing the amount of PUFA and especially the proportion of n-3 also increased proportions of antioxidants are needed to keep a good storage stability of fish and meat products and prevent oxidation [12]. Hence, combining fish or meat and various plant products as berries or spices may be interesting from nutritional, sensory and technological points of view.

Beside this nutritionally packed meals high in PUFA, antioxidants and nutritional beneficial substances may be especially important to people with particularly high nutritional demands, for example elderly people who suffer from malnutrition [29]. The plate of novel dishes that could be developed is broad, an example from recent research are fish dishes rich in polyphenols from berries [17]. Furthermore [159] describes the concept of FOSHU (foods for specified health use) and nine novel meat products that have been approved in Japan claiming to have beneficial effects on various aspects of human health.

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Chapter 7

Value-Added Fruit Processing for Human Health

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Additional information is available at the end of the chapter

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1. Introduction

Fruits are staple food in human diet. There has been a growing interest in the connection of fruit and vegetable consumption and improved health. Research have shown that biologically active components in plant-based foods, particularly phytochemicals such as polyphenolics and carotenoids, have important role in reducing the risks of chronic diseases, including cancer, cardiovascular disease, diabetes and Alzheimer's disease, among others. The first part of the chapter provides a brief update of the links between fruit-based antioxidants and other biologically active compounds and potential health benefits.

Fruit production is increasing globally. Despite the increasing fruit production at the global level, a significant amount of fruit produced is lost or wasted due to poor post-harvest management. The second part of the chapter provides information on current status of post-harvest losses in selected fruits and methods to prevent these losses. Therefore, processing fruits into value-added products is one of the strategies to reduce post-harvest losses and promote consumption of fruits.

Fresh-cut fruits, also called minimally processed fruits, are products that are partially prepared, maintain a fresh-like state and ready for use and eating. Recently, fresh-cut fruits have become popular because they meet the consumer demand for convenient ready-to-eat foods with fresh-like quality. However, fresh-cut fruits are more perishable than whole fruits. The third part of the chapter covers some recently developed approaches for the value addition of fresh-cut fruits with respect to the use of natural antimicrobials, anti-browning agents, edible coating, modified atmosphere packaging (MAP), 1-methylcyclopropene (1-MCP) application and vacuum impregnation (VI).

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2. Fruits and human health

Consumption of fruits and vegetables is increasing because of strong evidence that many beneficial effects for human health are associated with the dietary intake of fruits and vegetables (Kaur & Kapoor 2001, Rupasinghe et al. 2012). As suggested by epidemiological studies, the consumption of fruit and vegetables may lead to prevention of many chronic diseases, including cardiovascular disease (Weichselbaum 2010, Al-Dosari et al. 2011; Thilakarathna and Rupasinghe 2012), type II diabetes (Johnston et al. 2002, Yu et al. 2012b) and some cancers (De Mejía & Prisecaru 2005, Lala et al. 2006, Sun & Liu 2008, Lippi & Targher 2011). These disease prevention effects of fruits could be due to the presence of health promoting phytochemicals such as carotenoids (Chichili et al. 2006), flavonoids (Yu et al. 2012a), other phenolic compounds (Masibo & He 2008) and vitamins (Lippi & Targher 2011, Gutierrez 2008). Furthermore, the health-protective effects may be rather produced by complex mixtures of interacting natural chemicals than a single component in these plant-derived foods (Lila 2007). Table 1 gives a summary of selected fruit-based antioxidants and other health promoting compounds for disease prevention.

3. Fruit production and post-harvest loss

3.1. Fruit production

Fruit production is increasing dramatically worldwide. According to the FAO, the total world fruit production in 2008 was 572.4 million tons, and the number climbed to 609.2 million tons in 2010 (FAO 2010). Among these fruits, thirty percent of which were tropical fruits, with water melon occupied of 59.2%, mongo and guavas of 20.5% and pineapple of 11.4% (Rawson et al. 2011).

Despite the increasing food production at the global level, about one-third of the food produced in the world is lost or wasted (Prusky 2011), among which, post-harvest stage losses and marketing stage losses are major losses.

3.2. Post-harvest loss of fruits

Despite of food production is increasing globally, a significant amount of the food for human consumption is lost or wasted, especially perishable foods such as fruits and vegetables (Prusky 2011). The amount of food lost each year is equivalent to more than half of the world's annual cereals production (2.3 billion tonnes in 2009/2010) (Gustavsson et al. 2011).

It is hard to give precise information on the amount of fruit losses generated globally, because fruit losses vary greatly among varieties, countries, and climatic regions, and there is no universally applied method for measuring losses. As a consequence, the food loss data during post-harvest are mostly estimated and the variations are from 10% to 40% (Prusky 2011). Table 2 lists some examples of post-harvest losses of selected fruits in India, Egypt and United States.

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Source	Active component	Prevention mechanism	Disease	References
Grape	Anthocyanins	Anti-proliferative	Cancer	Lala et al. 2006
	Flavonoids	Inhibition of HNR-adduct formation	Macular degeneration and cataract	Yu et al. 2012a
	Resveratrol	Antioxidant, anti-inflammatory, activation of SIRT1	Alzheimer's	Sun et al. 2010
	Resveratrol	Normalize iron and Ca^{2+}, increase SOD activity	Cardiotoxicity	Mokni et al. 2012
	Resveratrol	Enhance insulin secretion	Diabetes	Yu et al. 2012b
Apple	Polyphenols	Antioxidant, cell cycle modulation	Cancer	Sun & Liu 2008
	Polyphenols	Antioxidant, multiple mechanisms	Cardiovascular	Weichselbaum 2010
	Phloridzin	Anti-inflammatory, bone resorption	Bone protection	Puel et al. 2005
	Polyphenols	Reduce amyloid-β formation	Alzheimer's	Chan & Shea 2009
	Phloretin-2'-*O*-Glucoside	Delay glucose absorption	Diabetes	Johnston et al. 2002
Banana	Lectins (Bioactive protein)	Cell cycle arrest, apoptosis	Cancer	De Mejía & Prisecaru 2005
	Polyphenols	Antioxidant, reduce LDL modification	cardiovascular	Yin et al. 2008
	Polyphenols	Antioxidant	Alzheimer's	Heo et al. 2008
Pineapple	Bromelain	Proteolytic enzyme regulation	Anti-inflammatory	Hale et al. 2010
Mango	Phenolic compounds	Antioxidant, multiple mechanisms	Degenerative diseases	Masibo & He 2008

Table 1. Fruit-based health promoting compounds and postulated disease prevention

Source	Post-harvest loss %				References
	Farm	Wholesale	Retail	Total	
Grape	15.1	6.9	6.0	28.0	Kader 2010
Grape	7.3	4.2	2.9	14.4	Murthy et al. 2009
Grape	N/A	N/A	7.6	N/A	Buzby et al. 2009
Mango	15.6	8.9	5.3	29.7	Murthy et al. 2009
Mango	N/A	N/A	14.5	N/A	Buzby et al. 2009
Banana	5.5	6.7	16.7	28.8	Murthy et al. 2009
Banana	N/A	N/A	8.0	N/A	Buzby et al. 2009

Source	Post-harvest loss %				References
	Farm	Wholesale	Retail	Total	
Papaya	N/A	N/A	54.9	N/A	Buzby et al. 2009
Pineapple	N/A	N/A	14.6	N/A	Buzby et al. 2009
Kiwi	N/A	N/A	12.7	N/A	Buzby et al. 2009
Apple	N/A	N/A	8.6	N/A	Buzby et al. 2009
Avocado	N/A	N/A	9.3	N/A	Buzby et al. 2009
Tomato	9.0	17.9	16.3	43.2	Kader 2010

Table 2. Post-harvest losses in selected fruits

3.3. Prevention and reduction of post-harvest loss

Methods of preventing losses of fruits and vegetables could be found from papers and fact sheet written by Singh and Goswami (2006), Sonkar et al. (2008), Prusky (2011) and DeEII and Murr (2009). These methods include selection of new cultivars with firm fruits and longer postharvest life, minimizing physical damage during harvesting and postharvest handling, control and monitoring of temperature and relative humidity, use of controlled or modified atmosphere storage, use of pre- and post-harvest fungicides (hydrogen peroxide) before and after harvest and use of physical treatment such as ozonation technology. Table 3 gives examples of use of controlled atmosphere storage of selected fruits.

Source	Temp (ºC)	RH (%)	O_2 (%)	CO_2 (%)	Storage life	References
Grape	0-5	90-95	5-10	15-20	> two weeks	Singh and Goswami 2006
Mango	10-15	90	3-7	5-8	> two weeks	Singh & Goswami 2006
Banana	12-16	90	2-5	2-5	> two weeks	Singh & Goswami 2006
Papaya	10-15	90	2-5	5-8	> two weeks	Singh & Goswami 2006
Pineapple	8-13	90	2-5	5-10	> two weeks	Singh & Goswami 2006
Kiwi	0-5	90	1-2	3-5	> two weeks	Singh & Goswami 2006
Avocado	5-13	90	2-5	3-10	> two weeks	Singh & Goswami 2006
Apple (Empire)	1-2	N/A	1.5-2.5	1.5-2.0	5-8 months	DeEII & Murr 2009
Apple (Gala)	0	N/A	1.5-2.5	1.5-2.5	5-8 months	DeEII & Murr 2009
Apple (Golden Delicious)	0	N/A	1.5-2.5	1.5-2.5	5-8 months	DeEII & Murr 2009
Apple (McIntosh)	3	N/A	1.0-2.5	0.5-2.5	5-8 months	DeEII & Murr 2009

* Temp: temperature; RH: Relative humidity

Table 3. Controlled atmosphere storage conditions of selected fruits

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4. Fruit processing and preservation

Processed fruit products generally include minimally processed fruit products such as fresh-cut fruit, fermented fruit products such as cider, wine and vinegar, traditional thermally processed fruit products such as jam, jelly, juice and beverage, novel non-thermal processed fruit products such as juice and beverage, etc. A comprehensive review has been given by the same authors on novel non-thermal processed fruit product preservation including juices and beverages (Rupasinghe & Yu 2012). At the same time, fresh-cut fruit stands out to be a promising food that meets the demand of consumers for convenient and ready-to-eat fruits with a fresh-like quality. In this case, this part of the chapter would give emphasis on fresh-cut fruit processing and preservation.

4.1. Fresh-cut fruit processing

The sales of fresh-cut produce have grown from approximately $5 billion in 1994 to $10–12 billion in 2005, which is about 10% of total produce sales (Rupasinghe et al. 2005). Fresh-cut fruits and vegetables are products that are partially prepared, maintain a fresh-like state and no additional preparation is necessary for use and eating (Watada & Qi 1999). Figure 1 shows the flowchart of fresh-cut fruit processing. It generally includes washing, and/or peeling, cutting, and/or slicing or wedging and packaging. Dipping solutions or edible coating materials could be applied during dipping or coating process.

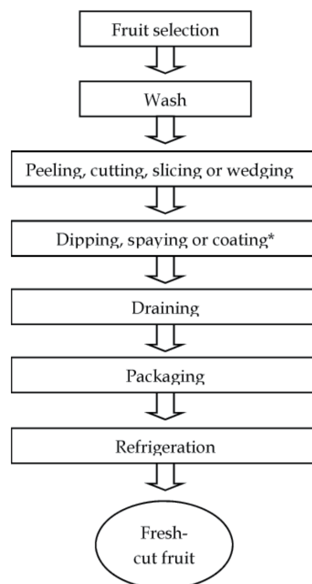

Figure 1. Major steps for fresh-cut fruit processing (revised from Corbo et al. 2010) * During this process, natural preservatives or edible coating materials could be applied

4.2. Fresh-cut fruit preservation

Fresh-cut fruits are more perishable than whole fruits, because the tissue integrity of fruits is more easily altered during processing. Post-cut quality of fresh-cut fruits suffers from wound induced biochemical and physiological changes such as water loss, accelerated respiration and cut-surface browning as well as microbiological spoilage (Kader 2002, Chiabrando & Giacalone 2012). Therefore, preservation of fresh-cut fruits needs combinative efforts of antimicrobial agents, anti-browning substances as well as packaging strategies. A detailed review was given by Oms-Oliu et al. (2010) about recent approaches for preserving quality of fresh-cut fruits.

4.2.1. Antimicrobial agent

During the preparatory steps of fresh-cut fruit processing, the natural protection of fruit is removed and chances of contamination may increase. Damage of tissues allows the growth and fermentation of some species of yeasts such as *Saccharomyces cerevisiae* and the attack by pathogenic microorganisms such as *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus* and *Escherichia coli* O157:H7 (Martin-Belloso et al. 2006). Therefore, the searching for methods to retard microbial growth is of great interest to researchers and fresh-cut industry.

Traditionally, the most commonly used antimicrobials are potassium sorbate and sodium benzoate. However, consumer demand for natural origin, safe and environmental friendly food preservatives is increasing. Natural antimicrobials such as organic acids, herb leaves extracts and oils, chitozan and *bacteriocins* have shown feasibility for use in some food products including fresh-cut fruits (Gould 2001, Corbo et al. 2009). Some of them have been considered as Generally Recognized As Safe (GRAS) additives in foods. Selected natural antimicrobials and their status for GRAS additives are listed in Table 4.

Cinnamon as an antimicrobial agent has been investigated in fresh-cut apple slices (Muthuswamy et al. 2008). Ethanol extract of cinnamon bark (1% to 2% w/v) and cinnamic aldehyde (2 mM) could reduce *E. coli* O157:H7 and *L. innocua in vitro*. Ethanol extract of cinnamon bark (1% w/v) reduced significantly the aerobic growth of bacteria inoculated in fresh-cut apples during storage at 6°C up to 12 days. It was also found that cinnamic aldehyde has greater antimicrobial activity than potassium sorbate (Muthuswamy et al. 2008).

Carvacrol and cinnamic acid could delay microbial spoilage of fresh-cut melon and kiwifruit. Dipping of fresh-cut kiwifruit in carvacrol solutions at up to 15 mM reduced total viable counts from 6.6 to less than 2 *log* CFU/g for 21 days of storage at 4°C. Also, treatment with 1 mM of carvacrol or cinnamic acid reduced viable counts on kiwifruit by 4 and 1.5 *log* CFU/g for 5 days of storage at 4°C and 8°C, respectively (Roller & Seedhar 2002).

Vanillin was also proved to be a practical preservative for processing fresh-cut mango and apples under refrigerated conditions. Fresh-cut mango slices were dipped for 1 min in solutions containing 80 mM vanillin before being packaged. Results indicated that treatment with 80 mM vanillin significantly delayed ($P < 0.05$) the development of total aerobic bacteria and yeast and mold populations of fresh-cut mangoes stored at 5 and 10 ∘C for up to 14 and 7 d, respectively (Ngarmsak et al. 2006). Also, a dip of 12 mM vanillin incorporated with

a commercial anti-browning dipping solution (calcium ascorbate, NatureSeal™) inhibited the total aerobic microbial growth by 37% and 66% in fresh-cut 'Empire' and 'Crispin' apples, respectively, during storage at 4 °C for 19 days. Furthermore, vanillin (12 mM) did not influence the control of enzymatic browning and softening by NatureSeal (Rupasinghe et al. 2006).

Name	Origin	GRAS status
Rosemary	Plant	Yes
Cinnamon	Plant	Yes
Cinnamic acid	Plant	Yes
Clove	Plant	Yes
Lactoperoxidase	Animal	No
Lemon (peel, balm, grass)	Plant	Yes
Lime	Plant	Yes
Nisin	Microorganism	Yes
Chitozan	Animal	No
Carvacrol	Plant	Yes
Citric acid	Plant	Yes
Ascorbic acid	Plant	Yes
Vanillin	Plant	Yes

* Revised from USFDA (2006): Food Additive Status List

Table 4. Selected natural antimicrobial agents and their status for GRAS additives*

4.2.2. Anti-browning agents

Enzymatic browning is also a major concern on the extension of shelf-life of fresh-cut fruit (Oms-Oliu et al. 2010). It is caused by the enzymatic oxidation of phenols to quinones by enzymes, typically polyphenoloxidases, in the presence of oxygen. Quinones are then subjected to further reactions, leading to the formation of browning pigments (Ozoglu & Bayindirli 2002, Jeon & Zhao 2005). Traditionally, sulfites have been used for browning prevention. However, their use on fresh-cut fruit and vegetables was banned in 1986 by the FDA owing to their potential hazards to health (Buta et al. 1999). Therefore, various alternative substances, such as honey, citric acid, ascorbic acid, calcium chloride, calcium lactate and calcium ascorbate, among others, have being used to retard browning in fresh-cut fruit (Jeon & Zhao 2005, Oms-Oliu et al. 2010). These anti-browning products are not often used alone because it is difficult to achieve efficient browning inhibition, and combination of them would give preferable results. Table 5 gives examples of anti-browning treatment on fresh-cut Apples.

Examples of anti-browning treatment on other fresh-cut fruits including banana, kiwifruits, mango, among others could be found in Table 3 in Oms-Oliu et al. (2010)'s paper.

Cultivar of apple	Anti-browning agent	Storage conditions	References
Gala	10% honey solution with vacuum impregnating	3°C for 14 days	Jeon & Zhao (2005)
Granny Smith	1% w/v of citric acid/CaCl2 and 1% of ascorbic acid/CaCl2	4°C for 5 days	Chiabrando & Giacalone (2012)
Granny Smith	0.05% w/v of sodium chlorite and 1% of calcium propionate	10°C for 14 days	Guan & Fan (2010)
Golden Delicious	1% w/v of citric acid/CaCl2 and 1% of ascorbic acid/CaCl2	4°C for 5 days	Chiabrando & Giacalone (2012)
Golden Delicious	80 mg/L acidic electrolyzed water (AEW) followed by 5%calcium ascorbate	4°C for 11 days	Wang et al. (2007)
Granny Smith	1% w/v of citric acid/CaCl2 and 1% of ascorbic acid/CaCl2	4°C for 5 days	Chiabrando & Giacalone (2012)
Red Delicious	300 mg /L sodium chlorite (SC) and 300 mg /L citric acid	5°C for 14 days	Luo et al. (2011)
Scarlet Spur	1% w/v of citric acid/CaCl2 and 1% of ascorbic acid/CaCl2	4°C for 5 days	Chiabrando & Giacalone (2012)

Table 5. Examples of anti-browning treatments assessed on fresh-cut fruits

4.2.3. Edible coating

The incorporation of antimicrobial and anti-browning agents to fresh-cut fruits could be done by dipping, spaying or edible coating treatment. Dipping or spraying aqueous solutions to fruit pieces containing antimicrobial agents, antioxidants, calcium salts or functional ingredients such as minerals and vitamins are widely used to improve quality of fresh-cut fruit. However, the effectiveness of these compounds could be better improved with their incorporation into edible coatings. The application of edible coatings to deliver active ingredients is one of the recent progresses made for shelf-life extension of fresh-cut fruits. Detailed information on edible coating for fresh-cut fruits could be found in review papers from Vargas et al. (2008), Rojas-Graü et al. (2009) and Valencia-Chamorro et al. (2011).

Edible coatings may be defined as a thin layer of material that covers the surface of the food and can be eaten as a part of the whole product. Therefore, the composition of edible coatings has to be food grade or GRAS. Furthermore, the coating materials need to be transparent, odourless, permeable for water vapour and selectively permeable to gases and volatile compounds (Kester & Fennema 1986).

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Ingredients that can be used to form edible coatings include polysaccharides such as cellulose, starch, alginate, chitosan, pectin, carrageenan, gum Arabic, guar gum and xanthan gum, proteins such as zein, gluten, soy, whey protein, lipids such as beeswax, lecithin, cocoa butter and fatty acids (Vargas et al. 2008). Examples of edible coating treatment on fresh-cut apples are listed in Table 6.

Cultivar of apple	Functional ingredients	Concentration (%)	Coating materials	References
Gala	N/A	N/A	Cassava starch, glycerol, carnauba wax, stearic acid	Chiumarelli & Hubinger 2012
Gala	N/A	N/A	Chitosan	Wu et al. 2005
Fuji	Oregano oil	0.1 – 0.5 (v/v)	Apple puree, alginate	Rojas-Graü et al. 2007
Fuji	Lemongrass	1.0 – 1.5 (v/v)	Apple puree, alginate	Rojas-Graü et al. 2007
Fuji	Vanillin	0.3 – 0.6 (v/v)	Apple puree, alginate	Rojas-Graü et al. 2007
Fuji	Cinnamon	0.7 (v/v)	Alginate	Raybaudi-Massilia et al. 2008
Fuji	Clove	0.7 (v/v)	Alginate	Raybaudi-Massilia et al. 2008
Fuji	Lemongrass	0.7 (v/v)	Alginate	Raybaudi-Massilia et al. 2008
Fuji	Cinnamaldehyde	0.5 (v/v)	Alginate	Raybaudi-Massilia et al. 2008
Fuji	Citral	0.5 (v/v)	Alginate	Raybaudi-Massilia et al. 2008
Fuji	Ascorbic acid, $CaCl_2$	1.0 (w/v)	Carrageenan	Lee et al. 2003
Fuji	Ascorbic acid, $CaCl_2$	1.0 (w/v)	Whey protein concentrate	Lee et al. 2003
Fuji	Ascorbic acid, $CaCl_2$	1.0 (w/v)	Whey protein concentrate	Lee et al. 2003
Golden Delicious	Ascorbic acid	0.5-1.0 (w/v)	Whey protein concentrate, beeswax	Perez-Gago et al. 2006
Golden Delicious	Cysteine	0.1-0.5 (w/v)	Whey protein concentrate, beeswax	Perez-Gago et al. 2006
Granny Smith	Ascorbic acid, citric acid	0.5 (w/v)	Pectin, apple purée	McHugh & Senesi 2000

Table 6. Examples of edible coating treatment on fresh-cut apples

4.2.4. Modified atmosphere packaging (MAP) and 1-methylcyclopropene (1-MCP)

The respiration rate of fresh-cut fruits is greater than that of intact fruits (Kader 1986). The increased respiration rate can induce the ethylene synthesis, increase enzymatic activity, promote oxidation of phenolic compounds and microbial growth, and therefore contributes to quality losses such as color and firmness. In this case, the control of respiration is essential for maintaining quality and prolonging the shelf life of fresh-cut fruits (Rocha & Morais 2003).

Modified atmosphere packaging (MAP) is a technology which offers the optimum gas conditions around the product by adjusting the barrier properties of the packaging film (Simpson and Carevi 2004). Various approaches to prolong the shelf life of fresh-cut products, such as edible coatings and refrigeration could be applied in combination with MAP (Rupasinghe 2005).

1-Methylcyclopropene (1-MCP) may retard or inhibit the generation of ethylene, the natural ripening hormone which is undesirable in terms of storage of certain fruits. Therefore, 1-MCP is becoming a commercial tool (SmartFresh, AgroFresh Inc., Philadelphia) for extending the shelf-life and quality of certain fruits and plant products (Rupasinghe et al. 2005). 1-MCP can be applied immediately after harvest (Aguayo et al. 2006; Mao & Fei 2007), just before fresh-cut processing or at both steps (Calderón-López et al. 2005; Vilas-Boas & Kader 2007). However, treatment of intact fruit with 1-MCP before fresh-cut processing is easier and more convenient than after processing. Moreover, the increase in ethylene production promoted by peeling, slicing or wedging could be prevented by the pre-use of 1-MCP (Rupasinghe et al., 2005).

4.2.5. Vacuum impregnation

Osmotic treatments have been traditionally used as a pre-treatment step in freezing, canning and frying to improve the quality of the final produce (Alzamora et al., 2000). Among developments in osmotic treatments of fruit products, vacuum impregnation (VI) may be the latest (Zhao & Xie 2004). The VI technique is performed by applying a vacuum pressure in a tank or oven containing the immersed product for a short time and then restoring the atmospheric pressure with the product remains immersed (Martínez-Monzó et al., 1998). The process of VI is a hydrodynamic mass transfer process based on an exchange between internal gas or liquid and an external liquid phase (Zhao & Xie, 2004). VI technique could be used to develop novel minimally processed fruit products with value-addition since nutritional and bioactive ingredients could be incorporated into the fruit based products during VI process (Xie & Zhao, 2003; Guillemin et al., 2008, Rößle 2011) and which gives a bright future for VI application in fresh-cut fruits. Table 7 gives examples of VI treatment on fresh-cut fruits.

Type of fruit	VI treatment conditions				References
	VI solution	VI pressure (mmHg)	VI time (min)	Restoration Time (min)	
Apple (Gala)	20% (w/w) of HFCS, Ca, Zn	50	15	30	Xie & Zhao 2003
Apple (Gala)	10% (w/w) of honey	75	15	30	Jeon & Zhao 2005
Strawberry	8ºBrix of glucose solution	37.5	5	5	Castelló et al. 2006
Apple (Empire)	15ºBrix of grape juice, 1.6% of $CaCl_2$ (w/v), 0.05% of NaCl (w/v), 0.1% of vitamin E (v/v)	152.4	10	22	Joshi et al. 2010
Apple (Empire)	20-40 % (v/v) of maple syrup, 1.6% of $CaCl_2$ (w/v), 0.05% of NaCl (w/v)	152	10	22	Joshi et al. 2011
Apple (Granny Smith)	50% (v/v) of honey	525	10	10	Rößle et al. 2011

HFCS: High fructose corn syrup

Table 7. Examples of VI treatment conditions on fresh-cut fruits or value-added products

5. Conclusion

Fruits are not only consumed as stable food but also provide desirable health benefits beyond their basic nutrition. However, the quantitative and qualitative losses of fruits are significant during post-harvest, marketing, processing and storage. Prevention of these losses during post-harvest management could be done by multiple steps and methods such as controlled or modified atmosphere packaging and application of ozonation technology.

On the other hand, promotion of minimally processed fruit products such as fresh-cut fruit into the commercial market is a practical, economical, and consumer and environmental friendly approach compared with traditional processing methods. However, fresh-cut fruits are more perishable than whole fruits in terms of biochemical and physiological changes such as water loss, accelerated respiration and cut-surface browning as well as microbiological spoilage. Therefore, preservation of fresh-cut fruits needs combinative efforts of antimicrobial agents, anti-browning substances as well as packaging strategies.

Natural or GRAS additives have been the popular ingredients used as antimicrobial agents and anti-browning agents, or bioactive ingredients. The incorporation of antimicrobial and anti-browning agents to fresh-cut fruits could be done by dipping, spaying or edible coating treatment. The application of edible coatings to deliver active ingredients is one of the recent progresses made for shelf-life extension of fresh-cut fruits. It could be used in combination with modified atmosphere packaging (MAP), 1-methylcyclopropene (1-MCP) and refrigeration for better results.

In addition for edible coating, vacuum impregnation (VI) may be another practical approach for incorporation of health promoting natural ingredients into fresh-cut fruits. VI technique could be used to develop novel minimally processed fruit products with value-addition through incorporation of nutritional and bioactive ingredients.

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Chapter 8

Differentiated Foods for Consumers with New Demands

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Additional information is available at the end of the chapter

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1. Introduction

In recent decades, the food industry has been meeting the growing demand of consumers in search of foods that have benefits that go beyond their nutritional value, and this sector has generated billions of dollars in the global market. Lifestyle, the convenience and speed of the preparation and the modification of eating habits among the population all reflect the increasing incidence of chronic diseases caused by eating high-calorie foods and a lack of exercise.

Advances in food science knowledge have become available to demonstrate the function and mechanism of action of bioactive compounds, and they support the inclusion of ingredients and the design and development of foods that contribute to a healthy diet that is associated with a healthy lifestyle. Although functional foods should be consumed as such and not in the form of supplements or capsules, the introduction of bioactive ingredients or components into the formulation and processes of these supplements can be a tool for industry innovation and contributes to the ability to offer products with additional quality.

Traditionally, dairy products were associated with health benefits, and in part, they still have this status; thus, innovations in this area are generally associated with the use of lactic acid bacteria (LAB) or products containing probiotic microorganisms or the addition of functional ingredients and bioactive metabolites. Various procedures, such as encapsulation, could be used to protect and maintain the viability of microorganisms in foods. There is a

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tendency towards the use of cheap and sustainable new materials with properties consistent with ingredient control release.

The concept of functional starter cultures that *per se* may not be probiotics but may improve product quality or result in physiological effects for the consumer is a possibility that should be explored. In addition to the probiotic properties, other choices include the use of *in situ* cultures that inhibit pathogenic contaminants by antimicrobial action; degrade or remove toxic compounds; produce vitamins or exopolysaccharides (EPSs); contribute to viscosity, body or texture; and facilitate adherence to specific sites in the host.

The action of binding EPS mucoid bacteria to the protein matrix results in increased viscous behaviour, and some EPSs produced by LAB are beneficial to health due to their prebiotic and hypocholesterolemic effects, immunomodulation ability or anticancer activity. Confirming these observations, some authors reported that the production of exopolysaccharides by certain bifidobacteria can increase the viscosity of fermented foods, contributing to the rheological properties, and therefore can be considered to be natural additives preferred by consumers that can replace plant or animal stabilisers.

The use of the special characteristics of LAB to potentiate their effects in foods or food supplies to vegetarians and people with dietary or religious restrictions provides an alternative to differentiated products. This category includes foods that are lactose free, have an increased fibre content, are free of animal products, and have an increased amount of antioxidant bioactive compounds (e.g., isoflavones, aglycones, oligosaccharides). Fruits and vegetables contain high levels of beneficial substances (e.g., antioxidants, vitamins, fibre and minerals), and the addition of LAB and probiotics can add more features. The knowledge of their behaviour in fruit and vegetable matrices as vehicles for the use of probiotics or bioactive ingredients is fundamental and still largely unexplored in the literature or in industrial processes.

There is, however, a need for the emerging pressure or process as a whole to be consistent with sustainable practices throughout the production chain in terms of the economic, environmental or social issues. Each step of the process that adds value to a product or avoids the generation of waste or effluent will be in agreement with the goals of clean production.

This chapter will focus on the recovery of by-products and innovative uses of plant materials and the strengthening of the resources for and beneficial effects of combining foods to obtain value-added functional products and offer alternatives to consumers searching for ways to improve their health through specialty foods.

2. Antioxidants from plant sources

In recent years, natural compounds have generated great interest due to the correlation between carcinogenic effects and the ingestion of synthetic compounds. Natural compounds such as phenolics, carotenoids and organic acids are widely found in plants and vegetables

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and have been the target of numerous studies because they exhibit strong antioxidant activity in addition to the ability to reduce the incidence of cancer in humans [1-4].

There is an equilibrium between the antioxidant defence system and the pro-oxidants in the human body, which are mainly reactive oxygen species (ROS) and reactive nitrogen species (RNS). The majority of reactive species (RS) originate in endogenous metabolic processes, whereas exogenous sources may include excess iron or copper in the diet, smoking, exposure to environmental pollutants, inflammation, bacterial infections, radiation, prolonged emotional stress and unbalanced intestinal microflora. The abnormal formation of RS may occur *in vivo* and cause damage to lipids, proteins, nucleic acids or carbohydrates in cells or tissues, and an imbalance with regard to pro-oxidants gives rise to oxidative stress (OS) [5].

Antioxidants impede or delay the *in vivo* oxidation reactions of lipids and other molecules or foods, inhibiting or retarding the chain propagation of free radicals generated by oxidation, such as hydroxyl radicals (•OH). In general, antioxidants are aromatic compounds that possess at least one free hydroxyl; they may be synthetic, such as BHA (butylhydroxyanisole), or natural, such as terpenes and phenolic compounds [2, 6-9].

Many studies have demonstrated that the consumption of antioxidants in food reduces the effects of the oxidative processes that naturally occur within the organism, aiding the natural endogenous protection mechanisms, such as the activities of superoxide dismutase, catalase and peroxidase, which together with vitamins E, C and A; enzymes; and other antioxidants and reduced glutathione (GSH) constitute the integrated antioxidant defence system (IADS) of the human body [4, 5, 7-8].

Flavonoids belong to the polyphenol group, which can be further divided into 11 smaller classes, including isoflavones, anthocyanins, flavans and flavanones. Their basic structure (Figure 1) comprises a flavone nucleus with 2 benzene rings (A and B) bonded to a heterocyclic pyran ring (C) [10,11].

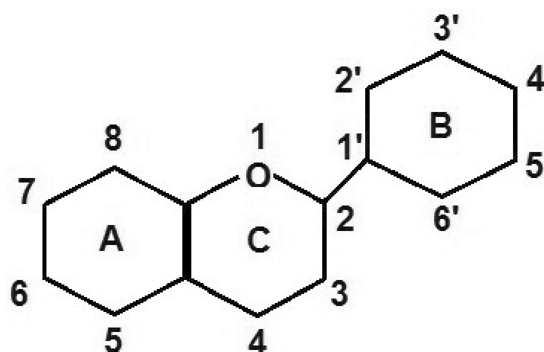
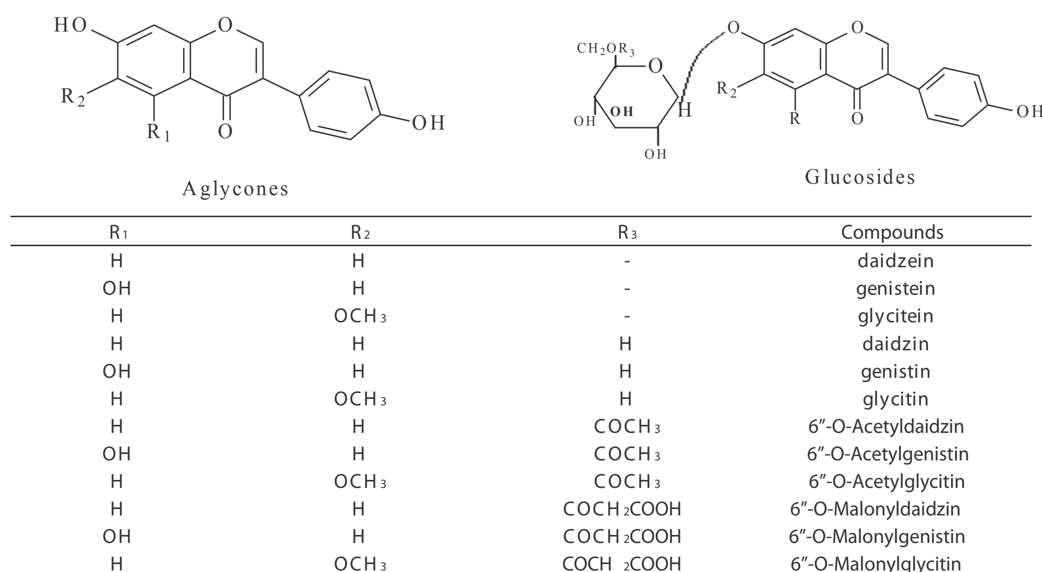


Figure 1. General structure of a flavonoid [10]

Isoflavones are phenolic compounds found mainly in beans and soybean derivatives and vary in concentration from 0.1 to 5 mg/g. They are distinguished by the substituents on the

benzene ring, which are classified into 4 distinct forms: β-glycosides (daidzin, genistin and glycitin), acetyl-glycosides (acetyldaidzin, acetylgenistin and acetylglycitin), malonyl-glycosides (malonyldaidzin, malonylgenistin and malonylglycitin) and aglycones (daidzein, genistein and glycitein). As a result, there are a total of 12 different forms, with the β-glycoside forms bonded at position 7 of the benzene ring to a glucose molecule (Figure 2). The consumption of isoflavones is related to the prevention of several diseases, such as breast cancer, colon cancer and cardiovascular problems. In a study performed by Silva, Carrão-Panizzi and Prudêncio (2009) comparing different varieties of soybean, the authors found a prevalence of glycosidic and malonyl-glycosidic isoflavones in the beans, with higher levels of the aglycone forms in the BRS 267 soybean variety with cooking [10,13-15].

According to Arora, Nair and Strasburg et al. (1998), all isoflavone forms display antioxidant action, which varies widely according to the structure. In addition, the genistein form, with hydroxyl groups at positions 5, 7 and 4, has a greater antioxidant strength, which is evident by its structure, as shown in figure 2.



R_1	R_2	R_3	Compounds
H	H	-	daidzein
OH	H	-	genistein
H	OCH_3	-	glycitein
H	H	H	daidzin
OH	H	H	genistin
H	OCH_3	H	glycitin
H	H	$COCH_3$	6″-O-Acetyldaidzin
OH	H	$COCH_3$	6″-O-Acetylgenistin
H	OCH_3	$COCH_3$	6″-O-Acetylglycitin
H	H	$COCH_2COOH$	6″-O-Malonyldaidzin
OH	H	$COCH_2COOH$	6″-O-Malonylgenistin
H	OCH_3	$COCH_2COOH$	6″-O-Malonylglycitin

Figure 2. Chemical structures of the 12 isoflavones found in soybean [10]

Chaiyasut et al. (2010) evaluated the effect of the time of *Aspergillus oryzae* fermentation in soybean on the isoflavone profile and the antioxidant capacity through ABTS cation (2,2′azinobis-[3-ethylbenzthiazoline-6-sulfonic acid]) and iron reduction (FRAP) assays. According to the authors, there was a significant increase in aglycone isoflavones (daidzein and genistein) and a reduction in glycosilades (daidzin and genistin) with a longer exposure time to the fermentation process. This trend was reflected in the antioxidant activity, with the greater antioxidant capacity displayed by samples with a longer fermentation time due to an increase in the aglycone forms. These results were similar to those found by Barbosa et al. (2006), who evaluated the isoflavone profile and the amount of phenolic compounds in different soybean-based products and the influence of these products on the antioxidant capacity. Their results showed that the antioxidant capacity is related not only to the amount of total phenolic compounds but also to the amount and forms of the aglycones and the types of conjugation.

However, anthocyanins are considered to be natural pigments, as they exhibit colours that are visible to the human eye and may be found in flowers, fruits and vegetables. Anthocyanins belong to the flavonoid group and are not synthesised by the human body; when ingested, they help the immune system by decreasing the action of radicals formed during respiration, and they are naturally found in several plants [19,20].

Anthocyanins are glycosides of anthocyanidins (Figure 3) and may have different sugars bonded to their ringed structure. They are classified as mono, di or triglycosides, and the diglycoside and triglycoside forms are more stable than the monoglycoside forms. They display colour variations according to their structural forms, pH value, number of hydroxyls and methoxyls and temperature [21-23].

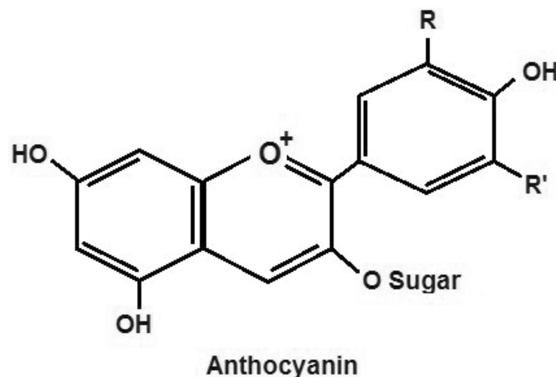


Figure 3. General structure of an anthocyanin [23]

According to Levi et al. (2004), there may be 4 structures in an aqueous medium depending on the pH value: the flavylium cation, the quinoidal base, carbinol and chalcone (Figure 4).

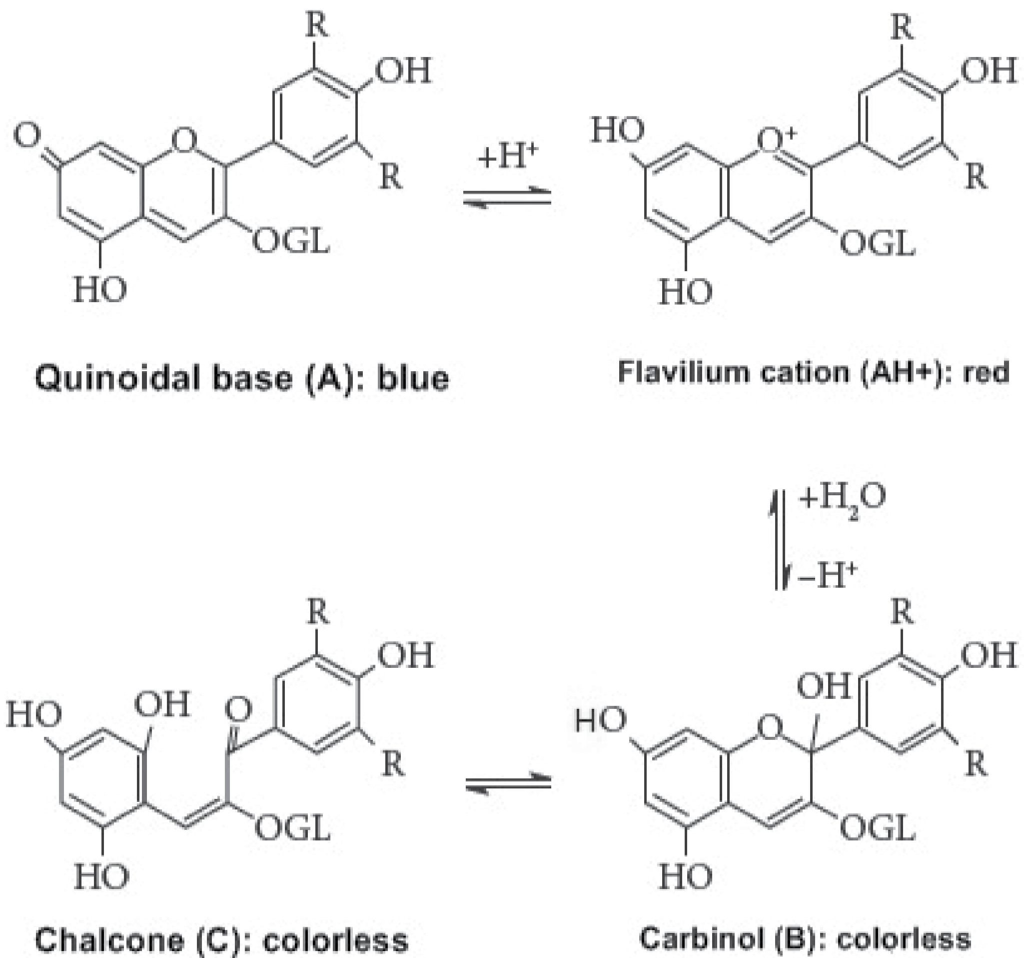


Figure 4. Molecular structures found at different pH values [24]

Kahkonen et al. (2003) isolated and identified the anthocyanins present in bilberry, blackcurrant and cowberry and evaluated their antioxidant activities through *in vitro* DPPH (2,2-diphenyl-1-picrylhydrazyl) assays with emulsified methyl linoleate and LDL (human low density lipoprotein). They found that the amounts of anthocyanins for bilberry, blackcurrant and cowberry were 6000, 2360 and 680 $mgkg^{-1}$ of the fresh weight, respectively; all samples exhibited high activity in the DPPH tests and were effective antioxidants for the emulsion of methyl linoleate and human LDL. Rufino et al. (2010) studied the antioxidant strength of açaí (*Euterpe oleraceae*) with the aim of using it in functional foods and dietary supplements and found an antioxidant capacity for acai oil in the DPPH assay that was higher (EC50=646.3 g/g DPPH) than the value for virgin olive oil (EC50=2057.27 g/g DPPH), indicating its considerable potential for nutritional and health applications.

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3. Non-dairy matrices as vehicles for probiotics and viability

Currently, there is increasing consumer interest in probiotic foods as an alternative to improve health. The majority of probiotic products found on the market are milk based, including milk drinks, yogurts, cheese and ice cream. Despite being an ideal substrate for the growth of these microorganisms, dairy products have several disadvantages, such as the need for refrigerated transportation, their cholesterol content and the restriction of their consumption to individuals who are not intolerant of or allergic to the products [27].

Thus, the development of new alternatives for consumption has increasingly earned the attention of the scientific and industrial communities, and new products, such as those based on soybeans, cereals, fruits, vegetables and meats, are being developed as potential carriers. In addition, these non-dairy matrices contain reasonable amounts of carbohydrates, fibres, proteins and vitamins, which may beneficially favour the growth and maintenance of the probiotics [27].

The viability and stability of probiotics have been a formidable market and technological challenge for food producers, given that probiotic foods should contain specific lineages and maintain an appropriate level of viable cells during the product's shelf life. Before they reach consumers, probiotics need to be produced under industrial conditions and maintain their functionality during storage in the form of a starter culture. Then, they need to be able to survive the processing of the food to which they are added. Finally, when ingested, the probiotics need to survive under the harsh conditions of the gastrointestinal tract and perform their beneficial effects in the host. In addition, they must be incorporated into the foods without producing unpleasant flavours or textures [28].

The application of probiotics in non-dairy matrices must be evaluated, given that several factors may influence the survival of these organisms and their activity when they pass through the gastrointestinal tracts of consumers. The following are among the factors that should be considered: the physiological state of the added probiotic organism as a function of the logarithmic or stationary growth phase; the appropriate concentration of viable cells in the product at the time of consumption; the physical conditions, such as low temperatures, during product storage; and the chemical composition of the product to which the probiotic is added, such as the pH, water content and amounts of carbon, nitrogen, minerals and oxygen [29].

Alternative vehicles for the incorporation of probiotic microorganisms may be fruits and fruit juices, but maintaining their viability is challenging because the pH of fruits and fruit juices is frequently low (< 4.0). They also contain antimicrobial substances. To minimise these factors, fruit juice may be formulated to have a higher pH value and smaller amounts of antimicrobial substances [30]. Sheehan, Ross and Fitzgerald (2007) evaluated the survival of several probiotic lineages in orange, pineapple and cranberry juices and observed that in addition to the juice's pH, the probiotic lineage and type of fruit also influenced the counts of the final product.

Pereira, Maciel and Rodrigues (2011) obtained a survival value of 8 log UFC/mL of *L. case*i in fermented cashew juice when the initial pH was 6.4 and the fermentation temperature was 30ºC. Yoon, Woodams and Hang (2004) also reported viable cell counts of more than 8.0 log CFU/mL in tomato juice. In addition, *L. acidophilus, L. plantarum, L. casei* and *L. delbrueckii* were capable of rapidly using this juice for cellular synthesis without nutrient supplementation. In another study by the same researchers, the fermentation of beets by probiotic bacteria was also investigated, and the authors observed a cellular survival of 10^9 UFC/mL of juice after 48 hours of fermentation in this substrate [34].

Lima, (2010) studied the behaviour of probiotic microorganisms in different tubers. Beetroot (*Beta vulgaris*) displayed the best survival results compared with sweet potato and arracacha. Upon adding pure betaine to the samples before fermentation and dehydration to supplement the amount of betaine already present in beetroot, the counts of *L. plantarum* and *L. rhamnosus* (LPRA Clerici-Sacco culture) were maintained at 8 log CFU/g of dehydrated sample (Figure 5).

To increase the robustness of the probiotic lineage of *Lactobacillus salivarius* UCC118, Sheehan et al. (2007) in a previous study cloned the *betL* gene of *Listeria monocytogenes* enables the system to capture or accumulate compatible solutes, such as betaine. BetL increases the tolerance to salt, low temperature and pressure stress as well as increases the viability of the probiotic in foods.

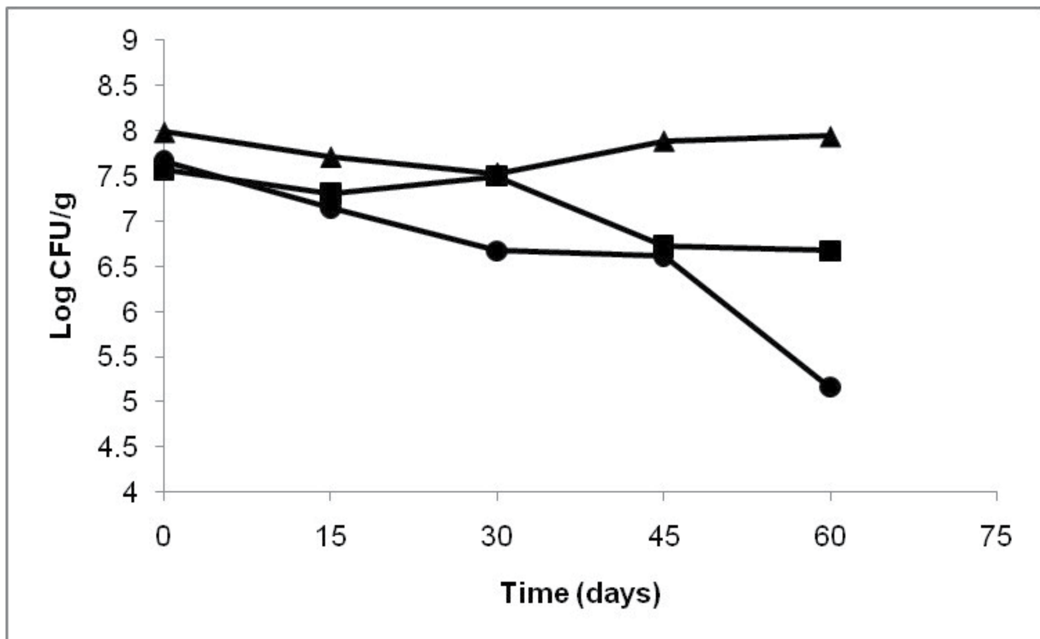

Figure 5. Behaviour of LPRA culture (composed of *Lact. plantarum* and *Lact. rhamnosus*) in assays 1, 2 and 3 during storage for 60 days at 25 °C. Assay 1(●): beet; assay 2 (■): Beet + 0.5 mM betaine; assay 3 (▲): Beet + 2 mM betaine

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In addition to studies focusing only on the survival of probiotics in alternative matrices, for these products to be fit for human consumption and compatible with industrial production, evaluating the sensory quality of the formulated products is important. In this context, Ellendersen et al. (2012) developed and optimised a probiotic drink composed of apple juice and established the sensory profile using quantitative descriptive analysis (QDA). Sensorially, apple juice recently fermented with *L. casei* was characterised as having a thick texture and sweet flavour, but at 28 days of storage, a sour taste was observed by the tasters. When the fermented drink was tested by potential consumers, a rate of 96% acceptance was obtained, indicating that apple juice may be a medium for the inclusion of probiotics. Baptista, (2010) used orange peels with a pectin content of 19.3% (p/p) and a subsequent fermentation by a starter culture (Lyofast M36 LV) of kefir in milk serum and dehydrated the product, which was used to produce a cereal bar. An average acceptance rate of 6.97 (in a structured Hedonic scale of nine points) for samples without the peel and 6.90 for samples containing the dehydrated probiotic was obtained (the samples did not differ between one another at a level of $p<0.05$). The counts of *Lactococcus lactis* found in the product were 5.4×10^7 CFU/g.

Cereals are considered one of the most important sources of proteins, carbohydrates, vitamins, minerals and fibres. The traditionally fermented products of cereals exhibit modified textures, tastes, aromas and nutritional qualities and are widely consumed in Asia, Africa, South America and India. The fermentative process of these foods, in addition to improving the nutritional value, contribute to increasing its preservation via the production of alcohols and acids and reduction in the amount of toxic substances and cooking time for the cereal [29].

According to Charalampopoulos et al. (2002), the possible applications of cereals or cereal constituents in the formulation of functional foods may include the following: (a) as a fermentable substrate for the growth of probiotic microorganisms, especially lactobacilli and bifidobacteria; (b) as a dietary fibre promoting various beneficial physiological effects; (c) as a prebiotic due to its specific non-digestible carbohydrate content; and (d) as encapsulating materials for probiotics to increase their stability.

Thus, several studies have been performed to bind probiotic microorganisms to cereal matrices. Charalampopoulos, Pandiella and Webb (2003) verified the viability of *Lactobacillus plantarum, L. acidophilus* and *L. reuteri* in extracts of malt, barley and wheat for 4 hours in a phosphate buffer with an acidity of pH 2.5. They observed that these cereals displayed a significant protective effect toward the viability of these microorganisms, which may mainly be attributed to the amount of sugar present in these extracts. In 2010, Charalampopoulos and Pandiella evaluated the survival of *Lactobacillus plantarum* in extracts of barley, wheat and malt that were produced in suspensions of flour/water at concentrations of 5%, 20% and 30%, fermented for 24 hours at 37ºC and stored at 4ºC for 70 days. The authors observed that the cells displayed greater survival when they were stored in a medium containing malt extract, and this result was attributed to the higher concentration of sugar and the presence of other unidentified compounds.

Rathore, Salmerón and Pandiella (2012) used malt, barley and a mixture of malt and barley as substrates in the fermentation of *Lactobacillus plantarum* and *Lactobacillus acidophilus* with

the objective of evaluating the influence of the lineages and the matrices used for the production of a probiotic beverage. The authors observed that a higher level of cellular growth was obtained in the medium that contained malt. In addition, these results suggested that the functional and sensory properties of probiotic beverages based on cereals may be considerably modified by changes in the composition of the substrate or the inoculum.

Oats, one of the major sources of beta-glucan, are commonly used in studies with probiotics. Guergoletto et al. (2010a) achieved a high level of survival for *L. casei* attached to oat bran when undergoing the vacuum drying process. Angelov et al. (2006), after optimising several factors such as the concentrations of the starter culture, oat flour and sucrose, developed an oat beverage fermented with *L. plantarum*, obtaining approximately 7.5×10^{10} CFU/mL of probiotics at the end of the process.

Another interesting application of probiotic microorganisms would be the enrichment of chocolate. With the development of technologies modified and adapted to maintain cells, this process may contribute toward increasing the benefit of this product for human health and increasing the consumption of probiotics by children, given that chocolate is one of their favourite products. For this combination to be successful, the sensory attributes of chocolate must remain unaltered, and the probiotic population must remain viable during commercialisation [44].

Finally, the application and development of new probiotic products of a non-dairy origin continue to grow. In light of the studies that have been presented by the scientific community, minimising the difficulties found in the application of these microorganisms in other food segments is possible.

4. Non-traditional fermented soybean-based products

Soybean is a plant that has been consumed since ancient times and is known worldwide for its nutritional benefits; it has a composition of approximately 40% proteins, 35% carbohydrates, 20% lipids and 5% ash [10]. In addition, soybean contains a considerable amount of components that are beneficial to health, such as fibres, isoflavones, essential fatty acids and oligosaccharides.

Traditionally, soybean-based products may be fermented by bacteria and/or fungi, with the most well-known being koji, shoyu, miso, tempeh, natto and sufu. These products are traditionally consumed by the East Asian population and represent an important source of dietary protein.

The search for foods that offer health benefits in addition to basic nutrition has promoted the development of new products that are based mainly on soymilk, which is obtained from the aqueous extraction of the bean's components. Soymilk possesses a chemical composition and appearance similar to those of animal milk and constitutes an appropriate substrate for fermentation; it contains, on average, 3.6% protein, 2.0% lipids, 2.9% carbohydrates and 0.5% ash [10]. Although studies have shown an increase in the consumption of soymilk,

there are still technological limitations with regard to its sensory characteristics due to the perception of undesirable flavours that were inherent in the extract or that were formed during the processing [45,46]. Fermentation, especially by lactic bacteria, has been used to improve the flavour and increase the acceptability of soymilk and sometimes, to decrease the levels of saponin, phytate and oligosaccharides [47, 48].

Soybean-based products that are analogous to the products derived from milk have been developed and are widely consumed. In general, these products are not cheaper than dairy products, yet they meet the growing demand for lactose- and cholesterol-free products. The main products developed in this segment are beverages, yogurt and cheese made from soybean, which are sought by consumers looking for healthier foods. The fermentation of soymilk by lactic bacteria, in addition to increasing shelf-life, is aimed at obtaining products with flavours and textures that are more acceptable to consumers [47]. In general, the microorganisms that are utilised are capable of using soybean sugars, or sucrose may be added as a substrate for fermentation. Table 1 shows several non-traditional fermented soybean products and the respective microorganisms used in their production.

Product	Microorganisms used	Reference
Soy yogurt	*Streptococcus thermophilus, L. delbrueckii subsp. bulgaricus* and *L. johnsonii, L. rhamnosus and bifidobacteria*	Farnworth et al. (2007)
	L. delbrueckii subsp. bulgaricus and *Streptococcus thermophilus*	Rinaldoni et al. (2012)
Fermented soy beverage	Bifidobacteria	Chou and Hou (2000)
	Streptococcus thermophilus and *L. helveticus*	Champagne et al. (2010)
Kefir	*L. delbrueckii subsp. lactis, L. helveticus, L. rhamnosus and Bifidobacterium longum* and *Streptococcus thermophilus*	Champagne et al. (2009)
	Lactococcus lactis ssp. lactis, Lactococcus lactis spp lactis biovar diacetylactis, L. brevis, Leuconostoc and *Saccharomyces cerevisiae*.	Baú (2012)
Custard	Commercial kefir culture - *Streptococcus lactis, Streptococcus cremoris, Streptococcus diacetylactis, L. plantarum, L. casei, Saccharomyces fragilis* and *Leuconostoc cremoris*	McCue and Shetty (2005)
	Kefir grains	Sánchez-Pardo et al. (2010)
Soy cheese	*L. rhamnosus*	Liu et al. (2006)

Table 1. Non-traditional fermented soybean products

Several studies on the survival of probiotic microorganisms indicate that soybean is an appropriate substrate for the growth of several probiotic species, such as bifidobacteria and

several lactobacilli, such as *L. casei, L. helveticus, L. fermenti, L. reuteri* and *L. acidophilus*. Therefore, new probiotic products based on soybean are being continuously developed, exploring the potential that soybean has as a vehicle for functional ingredients.

4.1. Fibre in non-traditional fermented soybean-based products

The development of ingredients and products rich in fibre has significantly increased and involves the incorporation of fibre into a wide variety of products, including those made from soybean, with the aim of improving the dietary habits of the population.

In addition to performing physiological functions that are beneficial to the human body, when added to food products, fibre may change the sensory characteristics and consumer acceptance as well as the product's cost and stability. The addition of fibre may affect the processing and handling of the products, with changes in the viscosity, texture, creaminess, syneresis, acidity, colour and other characteristics [49]. In fermented foods, fibre may change the fermentative ability of the products and, in some cases, may protect probiotic microorganisms under stress conditions. Soluble fibre can also be fermented by bacteria in the colon, giving rise to short-chain fatty acids, mainly acetate, propionate and butyrate. In contrast, insoluble fibres are not very fermentable. Furthermore, some types of fibre may act as prebiotics, selectively stimulating the growth of some probiotic microorganisms.

The main types of soluble fibre added to products include pectin, inulin, oligofructose, gums, β-glucan and some non-digestible oligosaccharides. Insoluble fibre mainly comprises cellulose and hemicellulose, with the most common sources being legumes and cereals, such as soybean, rice, corn, oats and wheat. In general, some sub-products have been used as an alternative for the incorporation of fibre into products as in the case of okara, which is the residue from producing soymilk and has a significant amount of fibre and other important compounds, such as proteins and isoflavones.

In fermented soybean products, the addition of inulin and oligofructose in soybean yogurt has been reported [50], and soybean, oat and wheat fibre have been added to soybean kefir [49]. In the soybean product fermented with kefir, the soybean fibre stimulated the growth of a probiotic microorganism and promoted an increase in firmness and viscosity and a decrease in the synerisis of the product. Yeo and Liong (2010) supplemented WSSE with the prebiotics maltodextrin, pectin, inulin and fructooligosaccharides and observed an alteration in the lactic bacteria count and other characteristics.

Therefore, it is possible different uses of soybean in human food, including being a source of fibre and providing foods with high nutritional value to meet the population's demand for healthy foods.

5. Products developed for individuals with celiac disease

Increasing our knowledge on the relationship between diet and health has caused consumers to look for high nutritional value, additional health benefits, convenience and pleasant sensory characteristics in processed products. In addition to this demand, a portion of the

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population is allergic to gluten. For this group, the treatment is essentially based on diet modification, which consists of eliminating gluten. The appropriate foods for individuals who are allergic to gluten are restricted and normally expensive, given that during processing, naturally gluten-free products may experience contamination that is unacceptable for those with celiac disease.

Celiac disease (CD) is an immune-mediated enteropathy triggered by the ingestion of wheat gluten (*Triticum aestivum* and *T. durum*) and similar proteins from rye (*Secale cereale*) in genetically susceptible individuals. During proteolytic digestion, prolamins (secalins) from rye and those in a subgroup of wheat (a-, b-, g- and w-gliadin) release a family of polypeptides rich in Pro and Gln that is responsible for the auto-immune response in celiac enteropathy [51]. The disease corresponds to hypersensitivity to gliadin (protein portion of gluten), which may be found in wheat, rye, barley and oat, and this hypersensitivity is marked by intense inflammatory processes. The consumption of cereals that contain gluten by individuals with celiac disease harms the small intestine [52], causing atrophy and a flattening of the intestinal villi, thereby leading to a limitation of the area available to absorb nutrients, among other manifestations. Situations such as travelling, eating outside the home and even enjoying relationships with friends and families may represent difficulties for celiac sufferers, thus interfering in their social lives [53]. With this disease, the processes of digestion and absorption may be compromised due to the increase in the immune activation of the intestinal tract. Celiac disease is one of the main causes of malabsorption in developed countries [54].

Therefore, there is a search for healthy foods that contain a variety of sensory attributes to allow for the possibility of providing a diverse selection of these foods. However, even with these possibilities, the celiac population is deprived of the consumption of many foods given that the formulations contain cereal-derived ingredients that contain gluten, such as oat flakes, wheat flour and malt.

Therefore, the development of new products for this population is essential, which may be performed by incorporating ingredients that contribute to an increase in mineral absorption, such as the fructans of inulin and oligofructose and other gluten-free bases. Fructans are soluble dietary fibres that may contribute to an increase in the absorption of minerals through colonic absorption [55, 56]; this effect may be especially important for those with celiac disease, given that the absorption of calcium in the small intestine is impaired in these individuals [57]. Capriles and Gomes Arêas (2010) developed amaranth bars with different flavours through the addition of inulin and oligofructose and observed that the amaranth bars enriched with these fructans may contribute to greater compliance by those with celiac disease to a gluten-free diet and help increase the absorption of calcium. These bars also have a reduced energy content and a high fibre content.

Other alternatives available for the celiac population include the substitution of the wheat flour that is present in several foods, such as breads, cakes, biscuits and pasta, with a mixture of flours that contain rice cream, tapioca flour, potato starch or corn starch, among other products.

Also notable is the use of soluble fibre such as Psyllium – Plantago ovate [59]. The main component of psyllium is mucilage (made up of slightly branched polysaccharides, found in algae and seeds), which represents 10 to 30% of its structure. These types of fibre also contain lipids, proteins, oxalic acid and the enzymes invertase and emulsin. Psyllium is considered to be a prebiotic food and is used either pure or in preparations to improve intestinal constipation [60]. With the double function of substituting for wheat in the development of special foods, psyllium has been added to bread dough, which is traditionally made with wheat flour, to improve the characteristics obtained via water retention and gelatinisation [61].

In a study performed by Zandonadi, 2006, psyllium was added to breads, biscuits, pasta, cake and pizza dough, and these products could be classified as foods for special purposes because they reduce the gluten fraction and exhibit good acceptability both by those with and without celiac disease. In addition, they reduce the lipid fraction and thus the product´s energy values.

Given the importance of seeking alternatives that promote sensory and functional characteristics that are similar to those of products prepared with gluten, Stork et al. (2009) studied two protein and transglutaminase sources in bread from rice flour to produce a better-quality bread. They observed that rice flour may be enriched with albumin and casein modified by transglutaminase to improve the bread's nutritional quality.

Figueira et al. (2011) evaluated the characteristics of gluten-free breads produced with rice flour and enriched with the microalga Spirulina platensis, which is a microalga that has an appropriate composition for use as a food complement, that have a possible use in combating malnutrition [65]. The dry composition of Spirulina platensis contains high amounts of proteins (64-74%), polyunsaturated fatty acids and vitamins [66] and contains antioxidant compounds [67]. This microalga is classified as GRAS ("generally recognised as safe") by the FDA, which ensures it can be used as a food without health risks [68]. The authors recommend the use of S. platensis for the enrichment of gluten-free breads made from rice flour using a suggested microalga concentration of 3%, and these bread are appropriate for celiac patients.

In a study on quinoa flour, Berti et al. (2004) evaluated the triglyceride and free fatty acid levels and glycaemic and insulinaemic responses in individuals with celiac disease and showed that the foods prepared with quinoa flour resulted in improved measures for all of these factors compared with the foods prepared with common flours. They also found that satiety was higher in the ingestion of products prepared from quinoa flour.

The use of kefir, which may act as an anti-inflammatory agent, may provide satisfactory results in patients with celiac disease. For these individuals, kefir may help to combat the nutritional deficiencies resulting from the reduction in intestinal villi because kefir is rich in vitamin B12, thiamine and potassium, which increase the absorption of the vitamin B complex [70,71].

Mixtures of several LAB were capable of hydrolysing 109 out of 129 ethanol-soluble polypeptides of rye, and De Angelis et al. (2006) concluded that long-term fermentation by selected LAB may be a potential tool to decrease the risk of contamination with rye in gluten-free products for patients with celiac disease.

Green banana flour may also be an alternative for the celiac population because the cost is not high, it is easy to prepare, and it exhibits a high amount of resistant starch, approximately 74% of its composition. This high level of starch is related to its glycaemic index and ability to reduce cholesterol levels and promote gastric fullness and intestinal regulation, and its fermentation by intestinal bacteria produces short-chain fatty acids that may prevent the emergence of cancer in intestinal cells [72]. Given these observations, Zandonadi (2012) evaluated the development of a gluten-free pasta alternative for those with celiac disease using green banana flour and demonstrated good acceptance without compromising the product while imparting important nutritional characteristics.

However, in foods that are thermally processed, especially breads, the lack of gluten represents a challenge in maintaining good sensory qualities, especially in the structure or softness during storage. The use of fermented dough (sourdough) by baker's yeast resulted in an improvement of the texture and effectively delayed the hardening of the gluten-free breads [74]. Fermented doughs also provided the breads with characteristics such as starch digestibility and low glycaemic responses, thereby proving to be a promising procedure in the improvement of the texture of gluten-free breads for those with celiac disease [75]. Galle et al. (2012) studied the influence of the in situ formation of EPS from LAB on the rheology of the dough for gluten-free sorghum bread. Among the EPSs, dextran improved the texture quality of the bread in addition to contributing to the nutritional benefits.

Therefore, the search for and development, market availability, diversification, cost compatibility and even improvement of already existing products for the celiac population all need to increase not only to improve the selection or consumption of these foods but also to ensure a better quality of life for the individuals who require a gluten-free diet.

6. Probiotics, metabolic action and vehicles of bioactive compounds

Through fermentation, toxic compounds may be hydrolysed and transformed into derivatives that are more or less absorbable or less toxic. Several studies describe the reduction of toxic or mutagenic compounds following fermentation or in the presence of microorganisms. In most cases, the microbial cells adsorb these compounds, and this process is normally increased with thermal treatment of the cells; the result is the possibility of reducing or degrading the compounds, but this latter mechanism is still not yet completely understood. Franco et al. (2010) observed a gradual increase in the reduction of the percentage of deoxynivalenol in solution depending on whether the LAB cells were viable or thermally inactivated (Table 2).

Other toxic compounds that could be degraded with this approach are toxins produced by algae. Considering the increase in the occurrence of cyanobacterial blooms and the possibility of metabolites being released into water supply sources used for human consumption, Guergoletto et al. (2010b) studied the microcystin (MC) biodegradation activities of microorganisms in water (Figure 6). Their work evaluated the use of the probiotic bacteria *Lactobacillus acidophilus* (La-5) and *Lactobacillus casei* (LC-1) and kefir grains for MC degradation over time. The mixtures were maintained at 27°C and 100 rpm, and samples were collected at 0, 12, 24, 48, 72 and

96 h to determine the level of MCs by immunoassay ELISA. The results indicated that the highest degradation percentage was obtained for kefir grains, reaching 60% and 62% of the total MC degradation for Microcystis sp. and NPLJ4 extracts, respectively, followed by the La-5 strain with levels of 43% and 51%. For LC-1, the degradation activities were 20% and 34% for Microcystis sp. and NPLJ4 extracts, respectively, but significant cellular growth was not verified when compared with the La-5 strain (Figures 7A and 7B).

Microorganism	**Reduction Percentage (%)***		
	Viable cells**	**Unviable cells following pasteurisation*****	**Unviable cells following sterilisation******
Lyofast LPRA	52.07 ± 0.1^{aB}	53.18 ± 2.06^{cB}	70.32 ± 1.65^{aA}
Lyofast BG 112	52.62 ± 4.95^{aB}	67.45 ± 2.95^{aA}	71.19 ± 2.77^{aA}
Lyofast LA3	39.23 ± 2.22^{bC}	60.67 ± 1.34^{bB}	66.71 ± 1.82^{aA}
LC 01	40.61 ± 1.19^{bB}	64.03 ± 0.07^{abA}	66.56 ± 2.43^{aA}
Yo flex YC 180	31.25 ± 0.89^{cC}	57.43 ± 0.95^{bB}	65.64 ± 0.77^{aA}
Florafit LP 115	32.61 ± 1.38^{cC}	40.81 ± 0.95^{deB}	58.51 ± 1.29^{cA}
Yo mix	40.67 ± 0.76^{bB}	41.98 ± 0.45^{deB}	48.75 ± 1.81^{cA}
Choozit Helv A	55.30 ± 1.35^{aB}	59.05 ± 0.45^{bAB}	63.84 ± 0.16^{abA}
L. plantarum TG VIII	29.86 ± 1.18^{cC}	50.38 ± 0.46^{cdB}	56.05 ± 1.86^{bA}
L. plantarum FT VI	34.88 ± 0.94^{bcB}	38.58 ± 1.66^{eB}	55.74 ± 1.25^{bA}
L. plantarum GT III	56.12 ± 1.02^{aB}	62.67 ± 1.09^{abA}	66.79 ± 0.43^{aA}
L. plantarum FTQ VII	39.70 ± 1.93^{bC}	51.37 ± 1.36^{cB}	65.26 ± 1.27^{aA}
L. plantarum FB VII	16.41 ± 5.35^{dC}	48.34 ± 1.46^{cdB}	59.62 ± 1.02^{bA}
L. plantarum FI IX	39.71 ± 0.30^{bB}	44.73 ± 0.29^{bB}	57.68 ± 0.41^{cA}
L. pentosus S I	19.51 ± 4.63^{dC}	35.95 ± 1.57^{eB}	47.48 ± 1.59^{dA}
L. paracasei K VI	29.51 ± 1.16^{cC}	44.98 ± 1.77^{dB}	57.19 ± 1.04^{cA}

The results correspond to the average of duplicates ± standard deviation. Averages ± standard deviation in the same column followed by the same lowercase letter do not differ at p≤0.05. Averages ± standard deviation in the same line accompanied by the same uppercase letter do not differ at p≤0.05.**Viable cells were separated by centrifugation (5 ºC/ 3000 g/ 10 minutes), washed in PBS pH 7.2 and ultrapure water, resuspended in DON solution with ultrapure water at a concentration of 1500 ng ml^{-1} and incubated at 37±1 ºC for 4 hours.***Nonviable cells following pasteurisation (100 ºC/ 30 minutes) were separated by centrifugation, washed in PBS pH 7.2 and ultrapure water, resuspended in DON solution with ultrapure water at a concentration of 1500 ng ml^{-1} and incubated at 37±1 ºC for 4 hours.****Nonviable cells following sterilisation (121 ºC/ 15 minutes) were separated by centrifugation, washed in PBS pH 7.2 and ultrapure water, resuspended in DON solution with ultrapure water at a concentration of 1500 ng ml^{-1} and incubated at 37±1 ºC for 4 hours.

Table 2. Reduction of deoxynivalenol level by LAB viable cells and cells that were heat inactivated (unviable) by pasteurisation or sterilisation

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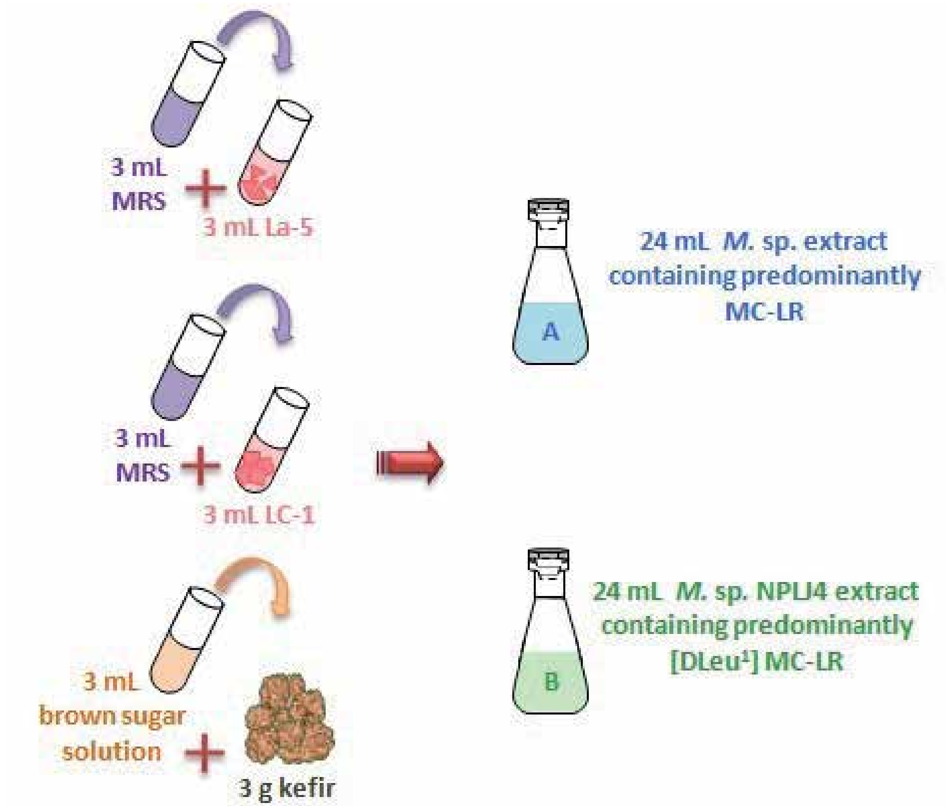


Figure 6. Scheme for the microcystin biodegrading activity experiment *Lactobacillus acidophilus* (La-5), *Lactobacillus casei* (LC-1) and kefir grains

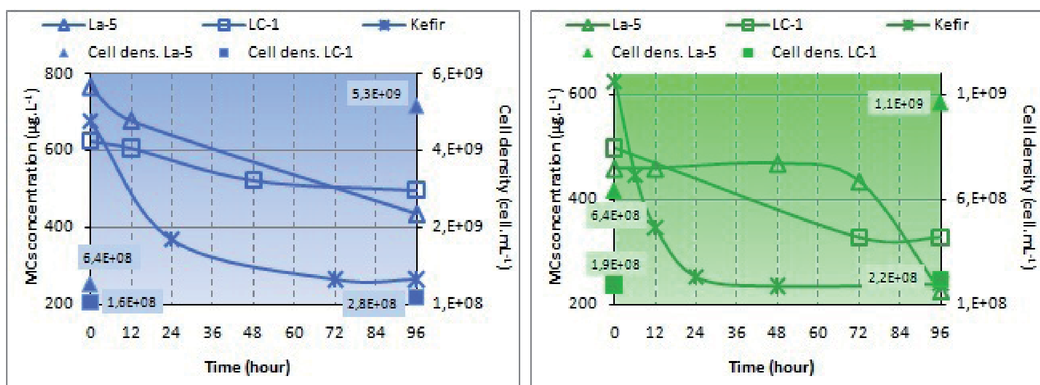


Figure 7. A Degradation kinetics of total MCs by La-5 and LC-1 bacteria and kefir grains during 96 hours of incubation with *Microcystis* sp. (A) extract B Degradation kinetics of total MCs by La-5 and LC-1 bacteria and kefir grains during 96 hours of incubation with NPLJ4 (B) extract

Mutagenic or carcinogenic activity in the caecal or urinary structures may be reduced by the consumption of *L. casei shirota* (LcS). A mechanism to explain the production of mutagenic substances was described by an *in vitro* study [79] in which the LcS was capable of strongly adsorbing and inactivating mutagenic pathogens and carcinogens, such as 3–amino-1,4 dimethyl-5H-pyrido (4,3-b) indole-trp-P-1 and 3-amino-1-methyl 5H pyrido (4,3-b) indole-trp-P-2. LcS also has the ability of binding aflatoxin, a known carcinogen produced by fungi [80].

Multi-functional polysaccharide molecules of plant, algal, bacterial or fungal origins have been extensively studied in recent decades for applications as thickeners, stabilisers, gelling agents, prebiotics and bioremediators or anti-pollutants [81-83]. Until now, plant macromolecules have dominated the market due to their ease and availability and because their purification is cost efficient, as plants are superior primary sources of polysaccharides, including starch, cellulose, pectin and gums. However, because polysaccharides of microbial origin are renewable, have little cost variation and have reproducible physical-chemical properties, they may be of value in certain situations, although they are still not widely marketed and represent an unexplored market [82]. Prasanna et al. (2012) studied the growth, acidification, EPS production and viscosity potential of 22 lineages of *Bifidobacterium spp* of intestinal origin, and EPSs were produced by *Bifidobacterium bifidum* ALM 35, B. breve NCIMB 8807 (UCC 2003), *B. longum subsp. infantis* CCUG 52486 and *Bifidobacterium infantis* NCIMB 702205 in concentrations varying from 25 to 140 mg L-1, producing an increase in the viscosity of dairy products with a low fat content.

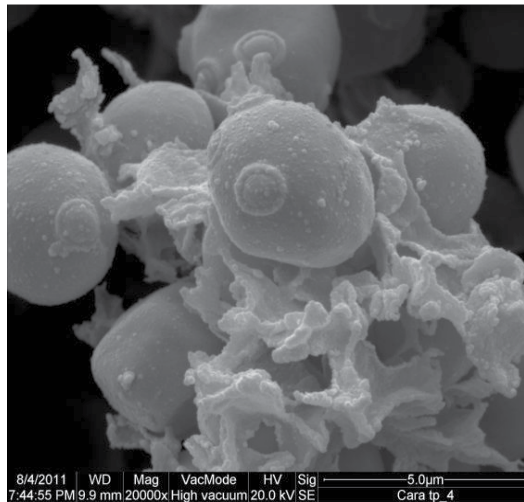
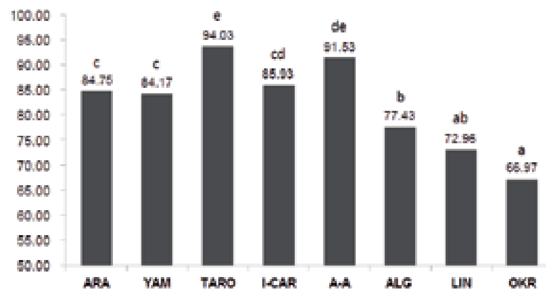


Figure 8. Scanning electron micrographs at magnifications of 20,000x Agar-agar and yam (Dioscorea sp.) microspheres containing *Saccharomyces cerevisiae* Laboratory of Electron Microscopy and Microanalyses – State University of Londrina.

Laurenti, E. (2010) studied the controlled release of probiotic *S. cerevisiae* (Biosaf SC-47) from microspheres of agar-agar added to mucilage (Figure 8) and gums and evaluated the poten-

tial application of these new natural materials for the protection of probiotics using gastrointestinal simulation tests. Okra and flaxseed showed the greatest retention of yeast cells in the microspheres and, consequently, a lower percentage of release at 66.97% and 72.96%, respectively (Figures 9 and 10).



abc Means between columns followed by different lowercase letters are significantly different ($p < 0.05$). ARA acacia gum; YAM *Dioscorea sp*; TARO *Colocasia esculenta*; I-CAR iota carrageenan; A-A agar-agar; ALG alginate; LIN *Linun usitatissimum*; OKR *Hibiscus esculentus.*

Figure 9. Mean release (% log CFU/g) of the probiotic *S. cerevisiae* encapsulated in agar-agar and different gums and mucilages after digestion *in vitro*

Mean release (% log CFU/g) of the probiotic S. cerevisiae encapsulated in agar-agar and different gums and mucilages after digestion *in vitro*

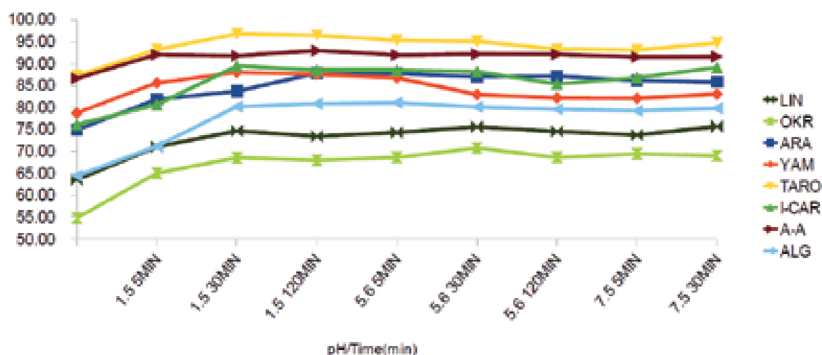


Figure 10. Digestion *in vitro* of the probiotic S. cerevisiae encapsulated in mucilages and gums. ARA acacia gum; YAM Dioscorea sp; TARO Colocasia esculenta; I-CAR iota carrageenan; A-A agar agar; ALG alginate; LIN Linun usitatissimum; OKR Hibiscus esculentus

Free radicals, especially those belonging to the family of ROS, are increasingly implicated or recognised as the cause of aging and in the pathogenesis of different diseases, such as cancer. Oxidative damage to the cellular molecules caused by chain reactions of free radicals may be combatted by antioxidants or by free-radical-sequestering agents. The use of natural antioxidants with less harmful effects and better bio-acceptability is gradually be-

coming important. Many plant or microbial polysaccharides have been demonstrated to exhibit sequestering or antioxidant ability due to the abundance of functional groups in the molecule [86]. Pan and Mei (2010) described the antioxidant action of EPS from *Lactococcus lactis subsp lactis 12*.

New evidence increasingly suggests the correlation of human IADS with the microbial organisms in the gastrointestinal tract. Specific lineages with physiological and antioxidant activities have a major impact on the management of the levels of oxidative stress in the lumen, among the mucosa cells and even in blood to support the functionality of the IADS in the human body. A lineage of *Lactobacillus fermentum* ME-3 (LfME-3) with antioxidant, antimicrobial and antiatherogenic properties was patented by the University of Tartu and proven to be 80 to 100 times more potent *in vitro* in sequestering the superoxide anion radical than Trolox or ascorbic acid. This lineage expresses Mnb superoxide dismutase (Mn SOD) activity, which effectively eliminates hydroxyl and peroxyl radicals and has the complete glutathione system (GSH, GPx, glutathione reductase – Gred) necessary for the recycling, transportation and synthesis of glutathione [5].

According to estimates by the World Health Organization [88], 3.2 million deaths per year are associated with physical inactivity. A sedentary lifestyle, a term derived from the Latin root “sedere”, meaning to be seated, includes physical activities with low energy expenditure that are correlated with obesity, metabolic syndrome, type 2 diabetes and cardiovascular diseases (CVD) [89]. Therefore, new approaches are necessary to reduce the risk of CVD, for which prevention via anti-inflammatory agents and antioxidants is considered to be the “third great wave” [90].

In contrast to the traditional action of probiotics involving a direct interaction with the host, the action of LAB in the cardiovascular system occurs via the release of bioactive peptides from proteins by *L. helveticus* during the fermentation process. A functional dairy product, Cardi-04™, was developed to reduce blood pressure [91]. A functional cheese with *L. plantarum* lineage TENSIA (DSM 21380, property of the Bio-competence Centre of Healthy Dairy Products LLC) may reduce blood pressure, both diastolic and systolic (a dose of 10^{10}UFC of viable probiotic cells per daily portion), in adults with high blood pressure or healthy adults and elderly individuals [5].

Recently, new bioactive compounds have been introduced in different medicinal and therapeutic applications. These molecules have been used due to their antioxidant, anti-tumour, anti-inflammatory and anti-viral activities. The EPSs induce cytosine and interferon activity, inhibit platelet aggregation and modulate the immune system [81]. Polysaccharides of *Lactobacillus sp*. have health benefits. Kefir may be classified as a functional food due to its action at different levels in animals. At doses between 100 and 300 mg/kg in rats, kefiran reduced blood pressure and the levels of blood sugar and cholesterol and displayed a positive effect toward constipation [92]. Other properties were perceived following the oral administration of this polysaccharide, such as anti-inflammatory and anti-tumour effects and the stimulation of immunoglobulin secretion. In addition, diosgenin, a steroid saponin present in yams (*Dioscorea sp.)* and fenugreek, displays properties that

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may be of value in future applications in medicine for the reduction of blood sugar and cholesterol and for the treatment of cholestasis [93].

Hobbs et al. (2012) studied the effect of beet juice and breads with added beets on the change in blood pressure and found strong evidence for a cardioprotective effect and the lowering of blood pressure caused by nitrate-rich plants. Recently, the effect of cardioprotective agents in green-leafed plants and beets has been postulated [95] to be due to the high nitrate content. Given that hypertension is associated with a decrease in the endogenous production of nitric oxide (NO) and that NO can be produced from the nitrate in the diet, new cost-effective strategies for the incorporation of nitrates in the diet are of considerable interest.

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Chapter 9

Quality Management: Important Aspects for the Food Industry

Caroline Liboreiro Paiva

Additional information is available at the end of the chapter

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1. Introduction

Certainly, with the advent of globalization, the market has become more competitive, because it has opened the opportunity for new competitors. This does not necessarily mean risk for the survival of local businesses, but a challenge that they must consider. This challenge relates to the need to create greater consumer loyalty to products and services, greater suitability of the product to the consumer's needs and greater concern about the social impact of the company. Moreover, this global scenario represents some opportunities for the companies to act in the new markets. It is clear that this action will depend mainly on the quality of their own products and services offered.

However, first, the concept of product quality is not so immediate and obvious. Although not universally accepted, the definition for quality with greater consensus is that "suitability for the consumer usage." This definition is comprehensive because it includes two aspects: characteristics that lead to satisfaction with the product and the absence of failures. In fact, the main component consists of the quality characteristics of the product features that meet the consumers' needs and thus it provides satisfaction for the same. These needs are related not only to the intrinsic characteristics of the product, such as the sensory characteristics of a food product, but also to its availability in the market with a compatible price and in a suitable packaging. The other part is the absence of faults, which is related to the characteristics of the product according to their specifications, making the consumer inspired by the reliability of the product, i.e., the consumer is sure that he will acquire a safe product, without health risks, and with the properties claimed on the label.

For these objectives to be achieved it is required an efficient management of quality, which implies continuous improvement activities at each operational level and in every functional area of the organization. The quality management combines commitment, discipline and a

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growing effort by everyone involved in the production process and fundamental techniques of management and administration, with the goal of continuously improving all processes. For that, the industries need to be structured organizationally, establish policies and quality programs, measure customers' satisfaction and even use more quality tools and methodologies. Specifically for the food industry, also involves the knowledge and application of techniques and programs for product safety.

With all that, the purpose of this chapter is to describe the potential use of quality tools in food companies. The study initially intends to contextualize the quality management in the food industry and the activities related to the quality function. In addition, support tools related to quality control in process will be suggested with practical examples of application.

2. Evolution of the quality management: A brief history

It can be said that each company has a particular stage of maturity on the issue of quality management. In general they tend to evolve in four stages, the similarity of ages or how the quality management in the world has evolved over the years. Thus, it is important to highlight these stages of evolution of quality that began with the inspection of products, have passed the statistical quality control, the stage of quality systemic management until the strategic quality management.

Garvin, a scholar of quality management, highlights four ages or stages through which the way to manage the quality has evolving over time in the U.S [1]. The first stage of development was called "era of inspection." In this stage the quality control of products was limited to a focus on corrective inspection, i.e., was a way to check the uniformity of the final product by separating the non-conforming products. According to Garvin in the U.S. only in 1922 the inspection activities were related more formally with quality management, after the publication of the book "The Control of Quality in Manufacturing". For the first time, the quality was seen as managerial responsibility having distinct and independent function in the companies.

Later, the year of 1931 was a milestone in the quality movement and the beginning of the second phase, the Statistical Quality Control. This phase had a preventive approach, centered on the monitoring and control of process variables that could influence in the final product quality through the development of statistical tools for sampling and process control.

The next phase was called Quality Assurance, that was associated with broader control and prevention, which sought through systematic management, ensure quality at all stages of obtaining the product. The quality management became a practice restricted to industrial production management applied to all production support functions. In the U.S., this time started in the late 50's when the quality of the instruments have expanded far beyond the statistics, now covering the quantification of quality costs, total quality control, reliability engineering and zero defect.

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Finally, quality management has been incorporated within the strategic scope of organizations, this phase called Strategic Management of Quality. It represented a vision of market-oriented management, i.e., with a view of opportunities before the competition and customer satisfaction, where market research has become more important for evaluating the market needs and how the competition stands. The strategic approach is an extension of its predecessors, but with a more proactive approach.

Several scholars of quality management are unanimous in emphasizing that the companies in general, and also the food industry, through its organizational structure, the policies adopted, the focus given to the business and the practice of quality control, demonstrate a certain degree of maturity in how to manage quality. Some companies may present practices related to more advanced stages, mature, such as quality assurance and strategic quality management, others may prove more practices related to inspection and process control. Through observation of tools and methods currently adopted in the food industry, it can be inferred that this quality management company is based on the characteristics of a particular stage of the quality evolution.

For example, the control of the raw material and products for inspection, with special attention to satisfy the governmental health rules, is a characteristic of the inspection stage. Likewise, the product control only by laboratory analysis is a feature of this stage. Moreover, quality control practices in process, application of statistical methods for quality control and the adoption of Good Manufacture Practices (GMP) and Hazard Analysis and Critical Control Points (HACCP) denote that the company has a slightly broader approach that inspection, i.e., a more preventive approach control in the production process. But when practice inspection and process control are well established in the company and efforts are directed towards continuous improvement, it can be inferred that the company is evolved into a system of quality assurance. Practices consistent with this era are shown by performing quality audits in different sectors of the company, adoption of quality systems across the supply chain and also implementation of programs for the development of quality suppliers of products and services. Companies that take a strategic quality management are those that use market research and specific indicators to measure customer satisfaction, such as consumer complaints, returns by wholesalers for the time of the product in the inventory and sales below target. Further, evaluate their products compared to competitors' products and apply techniques of sensory analysis to compare products and find sensory qualities required by the market. Concerned to improve their production processes, automate production lines and constantly launch new products into the market.

3. Tasks quality of the sector in the food industry

In general, the operating system of quality control in the food industry must meet some specific tasks. One of the tasks is to ensure compliance with sanitary standards and compliance requirements of the legislation, including with regard to food safety standards, the Good

Manufacturing Practices (GMP) and the system Hazard Analysis and Critical Control Points (HACCP). For this, there is need for procedures to control insects, rodents, birds and other pests, and procedures for cleaning and sanitizing equipment, industrial plant and storage areas. Still, personal hygiene of staff working on process lines and proper habits on food handling should be implemented and monitored to ensure that food safety standards are met. In cooperation with the departments of production, research and development, engineering or operations, the department of quality control analyzes manufacturing processes to "Hazard Analysis and Critical Control Points." The integrity and safety of food products should be ensured through the identification and assessment of all unit operations of the process in order to prevent potential contamination and adulteration that could expose consumers to health risks.

In cooperation with the department of research and development (R&D), production, purchasing and sales, should be prepared written specifications for raw materials, ingredients, packaging materials, other supplies and finished products. Furthermore, should be established in writing form and in cooperation with the departments of production and R&D the procedures for each unit operation of all manufacturing processes of the fashion industry that can be implemented in processing lines. The participation of staff from other departments of the company occurs by the virtue of their expertise in relation to consumer demands or knowledge of product technology and process, and the participation of the operators of the process, because of its experience in the production.

The quality control personnel works in different laboratories performing physical, chemical, microbiological and sensory properties of raw materials, ingredients, packaging materials and finished products. They also work in the factory or processing areas, collecting samples for performance evaluation processes, unit operations, sanitary conditions or levels, verifying compliance with the requirements of food safety and all other operating specifications. It is the responsibility of the department of quality control implementation of Statistical Quality Control (SQC), in which statistical techniques are applied to assessments of control for scientific analysis and interpretation of data. The SQC's functions include the selection of sampling techniques, control charts for attributes and variables, the use of analysis of variance and correlation, among other statistical tools. The methods, procedures and selection of instruments used to measure quality attributes of products and processes are the responsibility of the department of quality control. These techniques can be developed for specific purposes within the production process, to product development or troubleshooting and optimization standards.

The quality control personnel must interact cooperatively with the personnel of the standards and inspection agencies to ensure that the official food law is understood and met. It should also watch the production department in its efforts to increase revenues, reduce losses and improve efficiency of operations. It should also develop, conduct and assist in an organized program, training of supervisors, operators and workers in general, into specific concepts of quality.

The development of an appropriate plan of "recollect" adulterated or defective product in marketing channels and the planning of internal traceability of products is also a function of

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the quality control department. Another assignment of quality control includes reviewing and responding to consumer complaints.

Thus, faced with so many responsibilities, it remains to note that the dynamics of intervention and performance of those who are responsible for the quality department is paramount to the success of the food industry and customer satisfaction.

4. Methodologies in support of the quality management in the food industry

The quality management applies systems and tools that are intended to assist the implementation of quality-oriented way to improve the product and the process, increasing the levels of quality business and ensuring customer's satisfaction.

The purpose of this topic is to describe some tools, techniques and systems that have been more widely used in quality management in the food industry. Besides the methods mentioned, there are others that could be employed by companies. The choice of which implement depends on the company's strategies and know-how of its employees.

4.1. Food security programs

The issue of food safety has been in the public eye as never before. Foodborne disease has an enormous public health impact, as well as significant social and economic consequences. It is estimated that each year foodborne disease causes approximately 76 million illnesses, 325,000 hospitalizations and 5,000 deaths in the U.S., and 2,366,000 cases, 21,138 hospitalizations and 718 deaths in England and Wales [2]. Thus, many food safety programs have been published in order to ensure safe food production and consumer protection.

Safety food programs can be set as the measures to be taken to ensure that food can be eaten without adversely affect to the consumer’s health. These measures aim to prevent food contamination, such contamination are chemical, physical or microbiological. The programs commonly used in this area are Good Manufacturing Practices (GMP), Hazard Analysis and Critical Control Points (HACCP), British Retail Consortium (BRC) and Global Food Safety Initiative (GFSI), frequently found in the food industry, are obligatory by law, and others are implemented voluntarily by the food chain members [3].

4.1.1. Good Manufacturing Practices (GMP)

The Good Manufacturing Practices program is composed of a set of principles and rules to be adopted by the food industry in order to ensure the sanitary quality of their products. The GMP program came at the end of the last century when the U.S. pharmaceutical industry began to define optimal manufacturing practices based on technological knowledge available. In the late 60's, organizations such as the WHO (World Health Organization) and the Food and Drug Administration of the United States, the FDA (Food and Drug Adminis-

tration) adopted the program as a minimum criterion recommended to the manufacture of food products under adequate sanitation conditions and routine inspection. Later in 2002, FDA forms Food GMP Modernization Working Group and announces effort to modernize food GMP´s [4].

The rules establishing the so-called Good Manufacturing Practices involves requirements for industry's installations, through strict rules of personal hygiene and cleanliness of the workplace to the description in writing form of all procedures involved in the product. These standards are characterized by a set of items summarized below.

The projects and industry facilities, in addition to requirements engineering/architecture, must meet requirements to ensure food safety, such as the installation of devices to prevent the entry of pests, contaminated water, dirt in the air, and still be designed to avoid the accumulation of dirt or physical contamination of food that is being manufactured. The equipment and the entire apparatus of materials used in industrial processing should be designed from materials that prevent the accumulation of dirt and must be innocuous to avoid the migration of undesirable particles to foods. On the production line, the procedures and steps for handling the product have to be documented, in order to ensure the standardization of safety practices. Also running records should be implemented as evidence that the job was well done.

Otherwise, the cleaning and sanitizing phases are inherent to the processing and handling of foods, and thus programs for execution on a routine and efficiently must be implemented. Similarly, is required a plan for integrated pest control in order to minimize access vector and reduce the number of possible focus of insects, rodents and birds.

Regarding food handlers, the GMP recommend that training should be given and recycled so the concepts of hygiene and proper handling are assimilated as a working philosophy and fulfilled to the letter.

A control of raw materials should be developed with suppliers, not only in the laboratory, but in a gradual and continuous improvement work, where food security is split with suppliers. Guidelines for the safe packaging of raw materials, inputs and finished products should be followed and extended to the storage and loading area, and to the transportation that reach the consumer.

The Good Manufacturing Practices have wide and effective application when all the elements cited are effectively deployed.

4.1.2. Hazard Analysis and Critical Control Points (HACCP)

HACCP is a system based on prevention of hazards to the industry to produce safe food to consumers. The HACCP involves a complete analysis of the dangers in the systems of production, handling, processing and consumption of a food product. HACCP is widely acknowledged as the best method of assuring product safety and is becoming internationally recognized as a tool for controlling food-borne safety hazards [3].

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In short, this system has a systematic and scientific approach to process control, designed to prevent the occurrence of failures, ensuring that the controls are applied in processing steps where hazards might occur or critical situations. For this, the HACCP system combines technical information updated with detailed procedures to evaluate and monitor the flow of food into an industry.

The new sanitary requirements and quality requirements dictated by the main international markets, led since 1991, to the deployment experimental stage of the HACCP. There are new rules governing the international market, established during the Uruguay Round of Trade Negotiations and applicable to all member countries of the World Trade Organization (WTO). The Codex Alimentarius has become the regulatory body for matters of hygiene and food safety in the WTO. The Codex Alimentarius reflects an international consensus regarding the requirements for protection of human health in relation to the risks of foodborne illness. This measure is accelerating the process of harmonization of food laws of the countries, process that is oriented concerning food security, with the recommendation of the use of the system Hazard Analysis and Critical Control Point, to ensure food safety.

Generally the HACCP system initially involves the creation of a multifunctional team, supported by senior management of the company, and the characterization of all food products that will be included in the system. Also a set of programs, such as Good Manufacturing Practices (GMP) and Sanitation Standard Operating Procedures (SSOP) are universally accepted as prerequisites for the implementation of the HACCP system and therefore should be consolidated. Only then each step of the production process of a product will be analyzed for the possibility of a chemical, physical and microbiological contamination. Thereafter preventive measures are described and identified the Critical Control Points (CCPs). For each critical point is necessary to establish critical control limits, which allow the monitoring of hazards. As there is always a possibility of failure, it is essential to provide corrective measures in order to ensure the process return into a controlled situation. It should also establish procedures for verification of CCP′s and their respective records. After the HACCP plan drawn up, it is validation occur through discussions among team members [5].

Finally, the HACCP plan is disseminated to the production employees and for those responsible for assessing the products quality on the factory floor. Internal and external audits are recommended for periodic maintenance and continuous improvement of the system [5].

4.2. Standardization of processes

Standardization is a management tool involved in the preparation, training and control standards within the company. Such standards are documents containing technical specifications or specific criteria that will be used as a guide in order to ensure that products, processes and services are designed with quality [6]. The main objective of a program of standardization for the food industry is to minimize the variations in quality of production. For this, it is necessary to provide means to standardize both the operational and analytical procedures, as raw materials, machinery and equipment used in the manufacturing process.

The patterns are instruments that indicate the goal and procedures for accomplishment of the work and can be classified as follows:

- Standards of Quality (SQ): refer to the parameters related to quality of products, raw materials and inputs.
- Operation Standards: describe the manufacturing process of a product, the technical parameters of control by the operators and operating procedures. These are divided into Standard Process Technician (SPT) and Operational Procedure (OP). The first document describes the process of manufacture of a product, the quality characteristics and the control parameters. Operating procedures standards are prepared by managers and operators to achieve the objectives proposed in the SPT and SQs.
- Standards Inspection: describe methods and criteria for assessing the degree of success achieved in carrying out an activity, compared to planned levels of quality for the product. The inspection may occur in the process, the finished product and in the raw material.

Through standardization it is achieved greater standardization of products, improved productivity and product quality, cost reduction, simplification and optimization of production processes, increase the technical capacity of operators of process, greater job security, reduction of inventory levels of raw materials and inputs, reducing the preparation time of the machines and self-management by the workers.

Also noteworthy is that the patterns facilitate the transfer of knowledge since all the people and functional units involved in a particular pattern should collaborate, as far as possible, be trained in their preparation and for their use.

4.3. PDCA cycle

The PDCA originated in the 30´s in the laboratories of the United States, becoming known in the fifty decade due to the expert quality, Deming, who was responsible for implementing and disseminating tools of control and quality management in several countries. The PDCA cycle is a method of managerial decision-making to ensure the achievement of goals related to a process, product or service [7].

The letters that form the acronym PDCA mean Plan, Do, Check, Action. The Plan (P) consists in establishing goals, and procedures to achieve them. The stage Do (D) consists in performing the tasks as planned and collect data that will be used in the step control. Thus, in the stage of "implementation" are essential trainings at work. Check (C) consists of comparing the results achieved with the planned goals through quality control tools. Finally, Action (A) is to act correctively in the process in order to correct an unexpected result.

As can be seen in Figure 1, a schematic representation of PDCA cycle translates the dynamism steps purposes. The conclusion of a turn in the cycle continues back to the beginning of the next cycle, and so on. Following in the spirit of continuous quality improvement, the process can always be renewed and a new change process can be started. Continuous improvement occurs the more times the PDCA cycle is run, and optimizes the execution of

processes, enables cost reduction and increases productivity. Moreover, the gradual and continuous improvements add value to the project and ensure customer satisfaction.

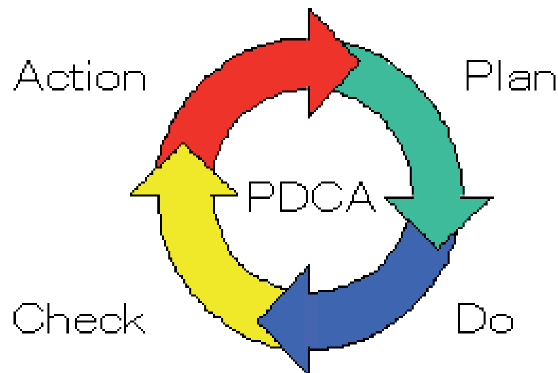

Figure 1. PDCA Cycle

In using the PDCA method may be necessary to use various tools, such as the basic tools for process control as stratification, check sheet, Pareto chart, cause and effect diagram, and scatter plots, histograms, control charts. Other techniques could include analysis of variance, regression analysis, design of experiments, process optimization, multivariate analysis and reliability [8].

Within the food industry, the PDCA cycle can be applied to the standardization or improvement of any product, process or activity the support the production, such as the standardization of procedures for cleaning and sanitizing, pest control, production processes, or improvement in the set-ups of equipment, reduction in losses in production, among others.

4.4. Traceability

The concept of traceability of products originated in the aeronautical and nuclear industries and it is widely practiced in industries. The tool aims to locate the source and the root causes of a particular problem of quality or safety, by the information recorded from a particular product, regardless of the stage of production where it is - whether raw material, in-process product or finished product. Through the traceability of products is possible to develop prevention and improvement actions, so that a specific problem does not occur again.

Traceability can cover only internal actions of the company, or otherwise, may be complete, when it involves the entire chain of production, allowing identifying even basic raw material that led to the final product and locations outside the company where finished products are stored. Consideration as the consumer safety, as the demands of the institutional environment and the costs of implementation of the traceability system will define the scope more suited to be deployed by the company.

4.5. Statistical quality control

The Statistical Quality Control uses statistical tools to control a product or process. To do this, it works with data collection and the interpretation thereof, acting as a fundamental tool to solve problems in critical product and process. Thus, ensures the quality sector the product conformity with the specifications defined as ensures the production sector the information needed for effective control of manufacturing processes providing subsidies to decision making in purchasing processes, receiving raw materials and shipment of products and also in reducing cost and waste. From the identification of the market requirements it is collected sufficient statistical information necessary for the development of new products and assists in monitoring the quality profile of competing products.

Although not a mandatory requirement in the food industry, statistical quality control can prove beneficial to organizations in the sector regardless of their particular specialism and size [9]. According Grigg, the initiatives of training of new graduates entering the industry in the principles of quality assurance and statistical methods and training the existing workforce and management in applying statistical control procedures to processes will make this methods more use of it than they are [9, 10].

The industrial statistic includes descriptive statistics, process capability analysis, measurement system analysis, basic graphics as histogram, scatter, box-plot, Pareto diagram, cause and effect, design of experiments, linear regression and correlation, multiple regression, hypothesis testing, confidence intervals, analysis of variance, analysis of process capability, among other tools [8]. It also covers the sampling techniques and control charts that will be described below, to be very useful to inspection and process control.

4.5.1. Inspection by sampling

The inspection process is to analyze or examine units of a product in order to verify with its quality characteristics are in accordance with technical or contractual specifications. Upon inspection of the product by sampling units are randomly selected to compose the sample batch. Depending on the number of defectives in the sample or the level of quality, that lot is accepted or rejected. Thus, sampling allows, by analysis of a small part of the whole or lot it is possible to draw conclusions about the rest not inspected. Therefore, in the sampling inspection an absolute conclusion about the quality of the lot will never be achieved, there is always a risk rate inherent in the sampling plan and dependent on its discriminatory power.

The current continuous improvement programs that evolve throughout the production chain, call for reducing the use of inspection techniques for the evaluation of the product or process, based on the idea that efforts should focus on "getting it right" in the first time and not in check it, then add value to the product, if it was done properly. However, these inspection techniques for acceptance have restored the importance of quality of audits.

There are two types of sampling plans, sampling plans by attributes and sampling plans by variables. The sampling rate by attributes consists in classifying units of a product just as acceptable or unacceptable based on the presence or absence of a particular feature in each

unit qualitative inspected. The results of the inspection by attributes are expressed in terms of defective/not defective, conforming/nonconforming. In the inspection by variable the characteristics or indicators of quality of the product unit are analyzed and the results are expressed by some continuous numeric scale. While inspection by attributes takes values from the set of integers, inspection by variable takes values in the set of real numbers [11, 12]. Upon inspection by attributes the probability of acceptance of the lot is based on Poisson Probability Distribution. The Poisson Probability Distribution is sometimes used to approximate the binomial distribution when the sample size (n) is too large and the proportion of defectives (p) is small. Otherwise, the use of sampling plans by variable assumes that the Normal Probability Distribution fits well with the distribution of the values of the quality characteristic under study.

Inspections by sampling can be used in finished products, raw materials, manufacturing operations, products in intermediate stages of processing, stored materials, among others. There are situations when only one plan by variable applies, for example, when the buyer will accept the product, but will pay different prices depending on the level of product quality. Also when the analysis result of the product will be expressed as quantitative values. For example, in the determination of chemical composition, weight, volume, and physical and rheological measurements. Therefore, measures such as pH, acidity by titration, soluble solids, fat, objective measurements of color and texture, among others, are typical of the sampling variable. The sampling by attributes can be implemented when it wanted to analyze a quality parameter in qualitative terms. Thus they are quite applied, for example, in visual analysis of packaging, the presence of dirt and physical damage in fruit and vegetables.

The following hypothesis test is linked to inspection for acceptance:

$$\begin{aligned} H_0 &: p = p_0 \\ H_1 &: p > p_0 \end{aligned} \tag{1}$$

Being "p" the proportion of defectives that the process produces. If the process is in control properly, this ratio is around p_0 (hypothesis H_0 true). The risk α, also known as producer's risk is likely rejection of a batch of a process whose average is equal to p_0 defective, that is, the risk that the producer suffers as a result of inspection or analysis of sample can lead to a rejection of a good plot (which meets the specifications). The risk β, also known as consumer's risk is the probability of acceptance of a batch of a process in which the proportion of defectives is greater than p_0, i.e., the result of inspection or analysis of the sample can lead to the acceptance of a batch inadequate; i.e., which does not meet the specifications [13].

A single sampling plan by attributes is defined by two parameters: sample size and acceptance number. The likelihood of acceptance of batches relates to the sample size, the severity in the acceptance criterion and the quality level of the products being analyzed in relation to the predetermined quality parameter [11]. In the sampling plans by variables, the probability of acceptance is related to the quality level of the product under examination and de-

pends on the average of the quality parameter in question and its variability. It also depends on the severity criterion for acceptance of the lot [12].

Finally, it is worth noting that the Codex Alimentarius recommends the use of the ISO 2859 series relating to the procedures for sampling by attributes and the ISO 3951 series for the procedures for sampling by variables [14].

4.5.2. Control chart

The formal start of statistical process control occurred around 1924, when Shewhart developed and applied control charts at *Bell Telephone Laboratories*, a telephone company in the United States [1, 7, 13]. As in the entire production process variability occurs, Chart Control or Control Chart, or Map Control, aims to monitor these changes in processes, as well as to evaluate the stability of this process and eliminate or control the causes of variations. A Control Chart (Figure 2) consists of a Central Line (CL), is a pair of control limits: one above Upper Control Limit (UCL) and one below, Lower Control Limit (LCL), and characteristic values marked on the graph. If these values are within limits, without any particular trend, the process is considered under control. But if the points relate outside the control limits or submit an atypical arrangement, the process is judged out of control.

Variability in process may be classified into two types: the variability caused by random or common cause, which are inherent in the process and will be present even considered that this process is fully standardized. If only this kind of cause is acting in the process, it is said that the manufacturing process remains in statistical control. The other type of variability is caused by remarkable and special causes that arise sporadically due to a particular situation which causes the process to behave in a completely different way than usual, which can result in a displacement of the quality level. Thus, it is said that the process is out of statistical control.

The manufacturing control is exercised by the manufacturer during the industrialization process. The goal is to maintain the quality of the product satisfactorily uniform, preventing the production of items outside specification. The proofing that the process is in control or not is, made by examining unit samples taken periodically out of the production line. If the process is under control, samples that present variability corresponding to samples taken from a normal population, i.e., the variability is attributable only to product that is the sample. The "under control process" supposes, therefore, that the quality characteristic of all units produced has Normal Probability Distribution (Figure 3). Moreover, it also implies that this distribution remains stable, i.e., that its two parameters, medium (μ) and standard deviation (σ), remain constant, which is verified by extracting a sequence of samples. So it is said that in a process under statistical control, the variability is attributed solely to random causes. These causes of variation do not cause appreciable variation in product quality; its elimination is impossible or anti-economical, and therefore, random causes are considered a natural part of the manufacturing process [8].

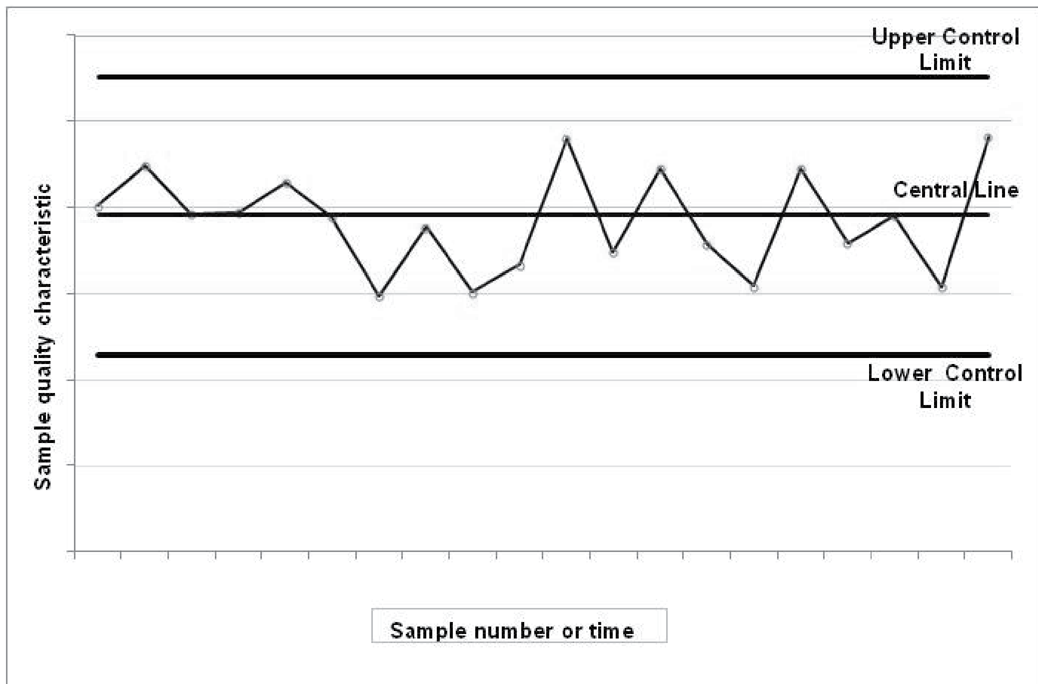

Figure 2. A typical control chart [8]

The Normal Distribution consists of an essential notion in statistical quality control rational. It is known that the items of a Normal Distribution (average μ and standard deviation σ) are distributed around the average, approximately by the following proportions: 68% of the values in the range $\mu \pm \sigma$, 95% in interval $\mu \pm 2\sigma$ and 99.7% in the range $\mu \pm 3\sigma$. Consequently, differences between an observed value X and the average μ, greater than $\pm 3\sigma$ are separated, three times to every 1000 observations, and therefore, the range of variability "normal" in the process under control is μ-3σ and μ+3 σ (Figure 3).

When the variability becomes "abnormal" changes in the quality characteristics of the product are sensitive. The causes of modification can be discovered and are therefore called "identifiable causes". These causes require prompt corrective action, in order to eliminate them. In these situations the samples indicate that the manufacturing process has changed and that the units were produced out of control. Some typical situations in process out of control occur when can be seen points outside the control limits. This is the clearest indication of lack of control of a process, which requires an immediate investigation of the cause of variation. Also can happened of points of the chart represent a trend, which consists of a continuous motion of the points of the control chart in one direction (ascending or descending). Also there is a configuration in sequence in several successive points of the control chart shown in only one side of the center line (eight or more consecutive points on one side of the center line). Another approach is the normality of the control limits, where 2 out of 3 consecutive points are outside the limits of 2σ [8].

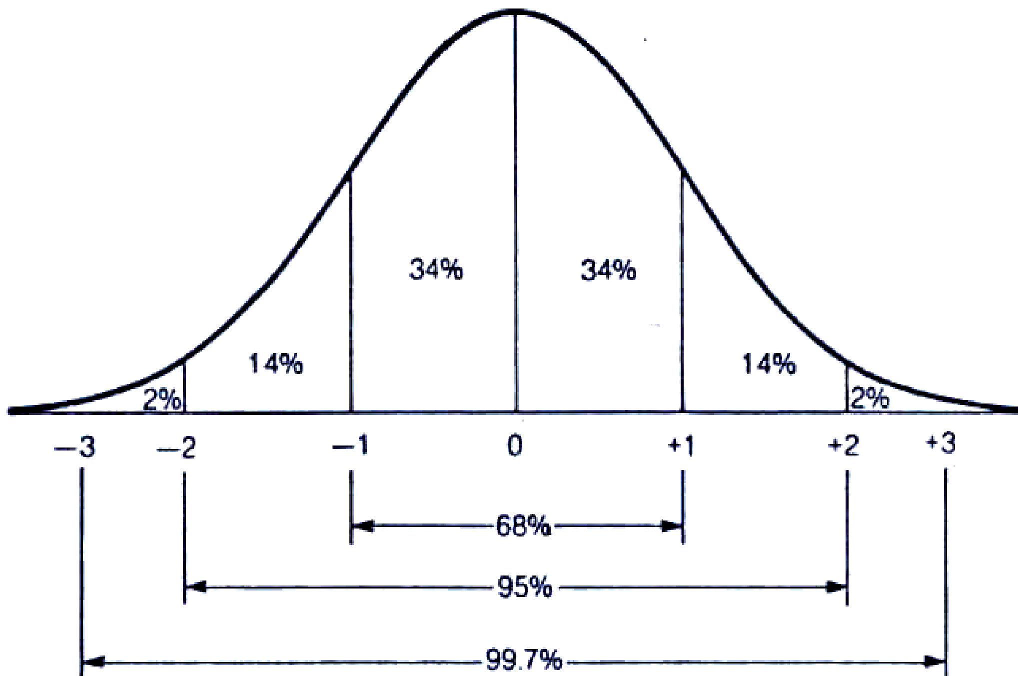

Figure 3. Scheme of Normal Probability Distribution

The food industry use control charts in different ways depending upon their level of maturity in statistical thinking [15]. In a survey conducted in UK food industry, revealed that while there are large differences in process types, quality priorities and key measures among different sub-sectors of the industry, the use of control charts was broadly similar. This generally extended to the use of control charts for recording or monitoring product net weight and volume data [15].

There are two types of quality control charts: control charts for variables and control charts for attributes, which will be described below.

4.5.2.1. Control charts for variables

Control charts for variables are named due to the fact that the quality characteristic being analyzed is expressed by a number on a continuous scale measures. Some examples of control charts are to yield a formulation, to verify the volume of a drink during their bottling, the soluble solids of a sweet after its cooking and the time to deliver a product to the customer.

Some control charts for variables most commonly used are: chart of the average (x), chart of amplitude (R), chart of standard deviation (s). When a quality characteristic of interest is expressed by a number on a continuous scale of measurement, the two control charts most used are the chart of the average (x) and a chart of variability (R or s). The two charts should be employed simultaneously.

Although the benefits of the application of control charts can be obtained in various situations of the food industry, the construction of the charts by variables will be exemplified by a typical situation of the food industry, in a packing operation. Imagine that a poultry slaughterhouse want to control the process of packaging of poultry cuts. In practice, the parameters average μ and standard deviation σ are unknown and must be estimated from sample data. The procedure to estimate μ and σ is to take *m* preliminary samples, each containing *n* observations of quality characteristic considered. These samples, known as rational subgroup should be taken when one believes that the process is under control and the operating conditions kept as uniform as possible. It is usual to consider m = 20 or 25 at least and n = 4, 5 or 6 [7,8].

The procedure for construction of the chart is:

1. Collect the data

Table 1 shows the values x_{ij}, weight of "j" cutting belonging to "i" sample, for 25 rational subgroup size of 4 (m = 25 and n = 4). Therefore, "i" varies from 1 to 25 and "j" from 1 to 4. The sections were collected when the machine was operating within normal procedure, i.e. no stops or apparent defects.

Samples	x_{i1}	x_{i2}	x_{i3}	x_{i4}	R_i
1	250,11	250,30	249,50	248,60	1,70
2	248,00	248,60	249,78	250,15	2,15
3	249,19	250,02	250,84	250,84	1,65
4	251,29	248,86	251,00	249,39	2,43
6	249,33	251,80	249,65	248,31	3,49
7	250,26	248,56	250,43	251,21	2,65
8	250,31	249,11	249,54	249,95	1,20
9	250,72	250,80	249,35	249,35	1,45
10	250,21	248,78	248,99	250,20	1,43
11	251,21	251,45	249,34	250,55	2,11
12	249,22	250,43	250,45	250,78	1,56
13	251,89	250,87	249,65	249,00	2,89
14	250,98	249,01	249,51	249,51	1,97
15	249,00	249,00	251,45	250,00	2,45
16	249,98	249,55	249,67	249,23	0,75
17	248,88	250,43	249,76	249,11	1,55
18	251,65	249,76	249,12	250,32	2,53
19	248,65	248,32	249,00	250,12	1,80

Samples	x_{i1}	x_{i2}	x_{i3}	x_{i4}	R_i
20	248,12	248.15	249,45	249,67	1,55
21	251,13	250,21	249,11	247,88	3,25
22	250,44	251,17	250,01	250,01	1,16
23	250,12	251,98	251,13	251,93	1,86
24	248.56	248.90	248,20	248,98	0,78
25	248,12	248,45	248,90	250,16	2,04

Table 1. Values of x_{ij} and R_i.

2. Calculate the amplitude of each sample R_i

$$R_i = \text{highest sample value - lowest value of the sample} \tag{2}$$

See the values of R_i in Table 1.

3. Calculate the average amplitude of the sample R

$$R = \frac{R_1 + R_2 + \ldots + R_m}{m} \tag{3}$$

Thus the value of R (average amplitude) is R = 1,93.

4. Establish the boundaries of the amplitude chart (Chart of R):

$$\begin{aligned} UCL &= D_4 \times R \\ CL &= R \\ LCL &= D_3 \times R \end{aligned} \tag{4}$$

The values of D_4 and D_3 are tabulated [7, 8]. Thus, D_4 = 2,282 and D_3 = 0.

Therefore:

$$\begin{aligned} UCL &= 2,282 \times 1,93 = 4,41 \\ CL &= 1,93 \\ LCL &= 0 \times 1,93 = 0 \end{aligned} \tag{5}$$

5. Build the chart of amplitude (Figure 4).

6. Analyze the chart.

Analyze the behavior of the points on the chart of amplitude and verify if the process is in statistical control. If necessary, recalculate the chart boundaries after the abandonment of the points there are out of control. Repeat this procedure until the control state is reached.

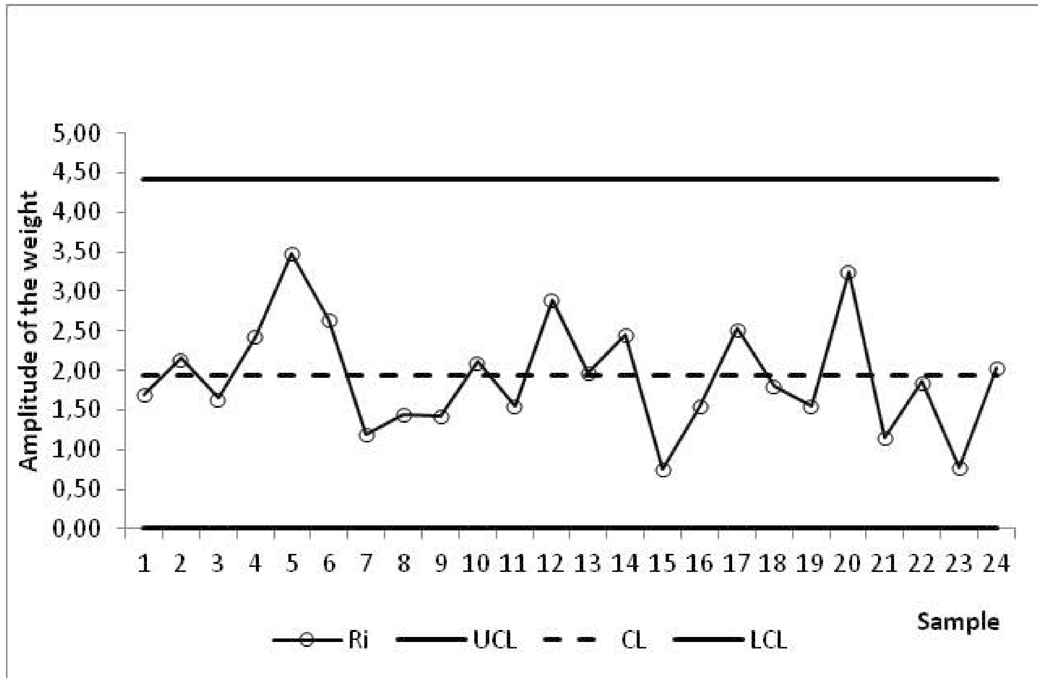

Figure 4. Chart of Amplitude (R) (25 points)

Analyzing the Figure 4, it can be seen that all points present within normal behavior. Now it is necessary to build the chart of average (x). To do this:

7. Calculate the average x_i of each sample (Table 2).

$$x_i = \frac{x_{i1} + x_{i2} + \ldots + x_{in}}{n} \tag{6}$$

8. Calculate the global average $\overline{X}$.

$$\overline{X} = \frac{x_1 + x_2 + \ldots + x_m}{m} = 249{,}83 \tag{7}$$

9. Calculate the control limits of the chart average.

$$ULC = \overline{X} + A_2R$$
$$CL = \overline{X} \tag{8}$$
$$LCL = \overline{X} - A_2R$$

The value of A_2 is a constant tabulated [7, 8]. Thus, $A_2 = 0{,}729$. $\overline{X}$ is the average of averages and R is the average amplitude found in the last chart of amplitude.

Thus:

$$ULC = 249{,}83 + 0{,}729 * 1{,}93 = 251{,}24$$
$$CL = 249{,}83 \tag{9}$$
$$LCL = 249{,}83 - 0{,}729 * 1{,}93 = 248{,}42$$

10. Construct of the average chart (Figure 5).

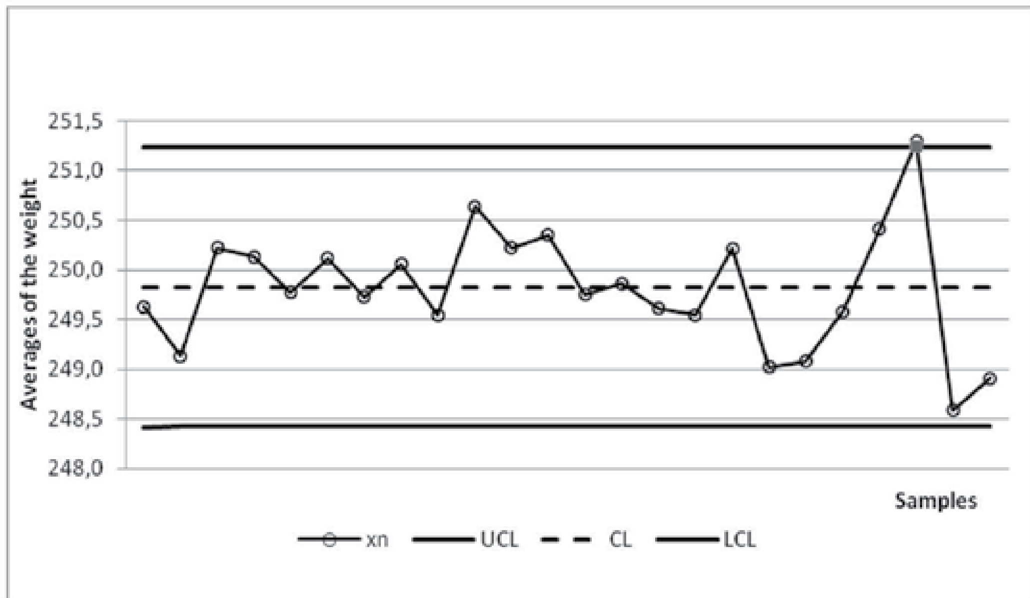

Figure 5. Chart of Average (x)

11. Interpret the chart of average built.

Analyze the behavior of the points on the average chart and whether the process is in statistical control. If necessary, recalculate the chart boundaries after the abandonment of the points there are out of control. Repeat this procedure until the control state is reached.

Analyzing the Figure 5, it can be seen that point 23 is above the UCL and therefore should be eliminated. The boundaries must be recalculated and a new chart of amplitude must be drawn (Figure 6).

New limits of the graph of the average (x) after removal of the subgroup 23.

$$\begin{aligned} ULC &= 249{,}76 + 0{,}729 * 1{,}93 = 251{,}17 \\ CL &= 249{,}76 \\ LCL &= 249{,}76 - 0{,}729 * 1{,}93 = 248{,}36 \end{aligned} \tag{10}$$

Samples	x_{i1}	x_{i2}	x_{i3}	x_{i4}	x_n
1	250,11	250,30	249,50	248,60	249,63
2	248,00	248,60	249,78	250,15	249,13
3	249,19	250,02	250,84	250,84	250,22
4	251,29	248,86	251,00	249,39	250,14
6	249,33	251,80	249,65	248,31	249,77
7	250,26	248,56	250,43	251,21	250,12
8	250,31	249,11	249,54	249,95	249,73
9	250,72	250,80	249,35	249,35	250,06
10	250,21	248,78	248,99	250,20	249,55
11	251,21	251,45	249,34	250,55	250,64
12	249,22	250,43	250,45	250,78	250,22
13	251,89	250,87	249,65	249,00	250,35
14	250,98	249,01	249,51	249,51	249,75
15	249,00	249,00	251,45	250,00	249,86
16	249,98	249,55	249,67	249,23	249,61
17	248,88	250,43	249,76	249,11	249,55
18	251,65	249,76	249,12	250,32	250,21
19	248,65	248,32	249,00	250,12	249,02
20	248,12	248.15	249,45	249,67	249,08
21	251,13	250,21	249,11	247,88	249,58
22	250,44	251,17	250,01	250,01	250,41
23	250,12	251,98	251,13	251,93	251,29
24	248.56	248.90	248,20	248,98	248,59
25	248,12	248,45	248,90	250,16	248,91

Table 2. Values of x_{ij} and $x_{n.}$

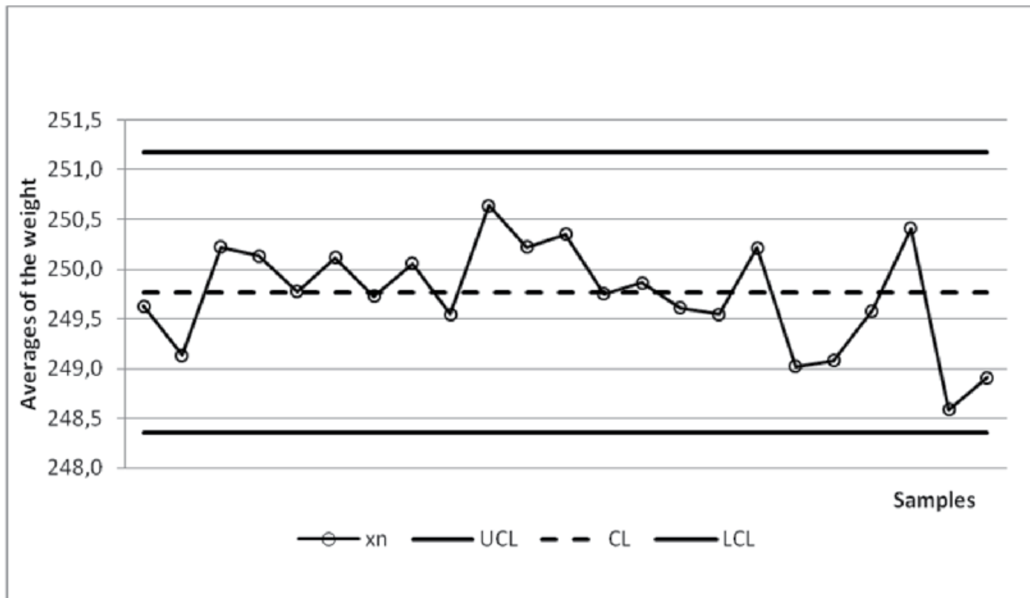

Figure 6. Chart of Average (x) (without the 23th subgroup)

12. Place the final charts of amplitude and average in the production line.

Note that for control of the packaging process of cuts of poultry, it chart has to be placed without padding, only with the UCL, CL and LCL, so that operators or responsible for quality control of packaging can monitor the process.

13. Periodically review the values of the control limits.

4.5.2.2. Control charts for attributes

It is not always by means of measurements that assess the quality of a product. For example, the color of a biscuit or of a sweet can be evaluated sensorially and the result is expressed as conforming or not conforming to a specified standard. Or, a PET bottle can be classified as not defective if it is whole in its structure or defective if it is crushed or broken.

Control charts for attributes can be: chart of the proportion of defective items (Chart p), chart of the total number of defects (Chart np), chart of number of nonconformities in the sample (Chart C) and the chart of number of nonconformities by inspection unit (Chart u) [8, 13].

Also here the construction of a chart for attributes will be exemplified. Suppose a manufacturer industry of biscuits decides to build a control chart *p* to visually check whether the product color after baking, was established as a standard for quality control. The number of defective products is presented in Table 3 and is important to note that the samples were numbered according to the date of production.

http://dx.doi.org/10.5772/53162

Date	Lot	Nº. Biscuit inspectionated	Defective items (x_i)	Proportion of defective items (p)
01/mai	1	200	7	0,035
02/mai	2	200	9	0,045
03/mai	3	200	4	0,02
04/mai	4	200	5	0,025
05/mai	5	200	6	0,03
06/mai	6	200	9	0,045
07/mai	7	200	5	0,025
08/mai	8	200	6	0,03
09/mai	9	200	6	0,03
10/mai	10	200	4	0,02
11/mai	11	200	6	0,03
12/mai	12	200	7	0,035
13/mai	13	200	4	0,02
14/mai	14	200	6	0,03
15/mai	15	200	7	0,035
16/mai	16	200	8	0,04
17/mai	17	200	8	0,04
18/mai	18	200	4	0,02
19/mai	19	200	7	0,035
20/mai	20	200	6	0,03

Table 3. Number of defective biscuits in samples of 100 units

1. Collect the data

Collect m samples of size n. In general m = 20 or 25 at least. Collect the samples at successive intervals and record observations in the order they were obtained (Table 3).

2. Calculate the average proportion of defective items p (average).

$$\overline{p} = \frac{1}{mn}\sum_{i=1}^{n} X_i \tag{11}$$

X_i is the number of defective items in the "i" sample.

3. Calculate the control limits.

$$\begin{aligned} UCL &= \overline{p} + 3\sqrt{\overline{p}(1-\overline{p})/n} \\ CL &= \overline{p} \\ LCL &= \overline{p} - 3\sqrt{\overline{p}(1-\overline{p})/n} \end{aligned} \qquad (12)$$

The LCL is not considered when the value is negative.

4. Draw the control limits. Mark left-hand vertical axis in the scale for horizontal axis p and the number of samples. Draw lines to represent full UCL, CL and LCL (Figure 7).

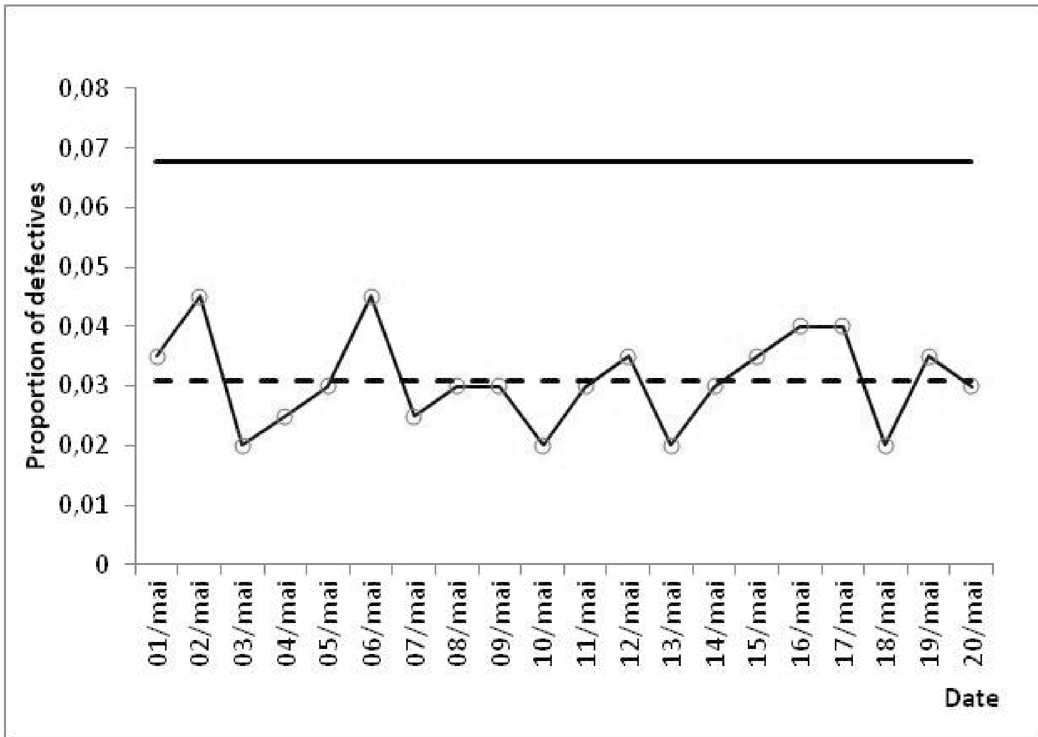

Figure 7. Chart p (proportion of defective products in the sample)

5. Mark the points on the chart.

Represent on the chart the m values of p (Figure 7).

6. Interpret the graph constructed.

To analyze the behavior points on the graph, and verify that the process is in statistical control. If necessary, recalculate the chart boundaries after the abandonment of the points there are out of control. Repeat this procedure until the control state is reached.

7. Check if the control state reached is appropriate to the process. If so, adopt the current control chart. Note that for control of the biscuit color, it chart has to be placed without padding, i.e., only with the UCL, CL and LCL.

8. Periodically review the values of the control limits.

5. Quality management systems

5.1. Total Quality Control (TQC)

In the '50s, the quality control was employed in Japan, by an intensive use of statistical techniques. However, the excessive emphasis on statistical techniques led to some problems, such as low interest shown by senior management of companies, by the quality control, which remained a movement of ground and plant, i.e., to engineers and workers [16].

In 1954 JUSE invited the engineer Juran, one of the masters of quality management, to deliver seminars to senior management. From the visit of Juran, the Quality Control came to be understood and used as an administrative tool, which represented the beginning of the transition of Statistical Quality Control for Total Quality Control as is currently practiced, involving the participation of all sectors and employees[16].

The quality management system proposed by the Japanese model shows how basic features to the participation of all sectors and all company employees in the practice of quality control, constant education and training for all levels of the organization, circles activity of quality control, audits, use of basic and advanced statistical techniques and national campaigns to promote quality control.

The TQC ideas developed by the Japanese were broadcast around the world, being this model capable of being deployed in companies of various sectors, with appropriate adjustments to the corporate culture.

5.2. ISO 9000 series

While the movement occurred in Japan by TQC, in Europe there was a movement around an organizational structure whose purpose was to develop standards for manufacturing, trade and communication in European countries for the increased levels of quality of activities. Thus, in 1947 the International Organization for Standardization was founded, based in Geneva, Switzerland. And in terms of quality control there was difficulty to unify standards that ensure that a product had been manufactured under quality criteria, after several trials, in 1989, was published the standard ISO 9000. The goal was to establish requirements for a quality management system, the implementation of which would extend to all types and business segments. The requirements of the series represented the consensus of different countries of the world.

More specifically, ISO 9001 deals with the requirements of the quality management system for an organization to produce compliant products and get customer satisfaction. Within the

rules of the certification ISO 9001, there are specific requirements regarding the responsibility and involvement of management with the quality system, requirements for preparing and controlling of the documentation, for the critical analysis of contracts and selection of suppliers, to traceability and processes control, for measurement, for inspection and testing, analysis of nonconformities and for continuous improvement, for audits and training.

As ISO 9001 is a rule of general character it contains requirements to serve the most various sectors, it is necessary, once adopted by the food industry, some aspects can be considered in some cases insufficient. There's not in the standard, explicit references to the risks to consumer health, the safe products, the nutritional values, the critical control points, the good manufacturing practices. Food security can be seen as failures risk of deterioration and damage as a result of careless handling and storage inconvenient and not because of contamination and loss of sensory and nutritional values. Thus management systems for food safety have also been employed to address this need [17].

5.3. ISO 22000 series

Aiming to harmonize the international level, the various guidelines related to food safety systems, it was developed the ISO 22000:2005 - Food Safety Management systems - Requirements for any organization in the food chain. This applies the principles of a plan Hazard Analysis and Critical Control Points (HACCP) programs along with prerequisites, such as Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP). The standard has a similar format to the standard of ISO 9001 Quality Management. This similarity allows organizations to implement the specifics of food management system integrated to the quality management system. In this context the ISO 22000 presents as fact the benefits of being recognized internationally, to apply to all elements of the food chain and fill for the food sector, the gap between ISO 9001 and HACCP.

The ISO 22000 standards specifies the requirements to a safety management system that combines elements of food management system to ISO 9001 templates, as already said, and interactive communication, since communication along the supply chain is essential to ensure that all relevant safety hazards of food are identified and controlled. Finally, through concrete measures, tangible and that can be checked in audits, ISO 22000 combines the HACCP plan with prerequisite programs (PRP), since they are keys to an effective management system of food safety.

The ISO 22000 considers that the safety of food is related to the presence of hazards in food at the time of consumption. And because of the dangers that can occur at any stage of the supply chain, the security must be ensured at all levels of the supply chain. So it should be applied to producers of animal feeds and other agricultural products, food manufacturers, packaging, transportation and food warehouses to suppliers of retail and food services. So for its strong integrator character, the success of the implementation depends largely on the acceptance of the various links in the supply chain. Other barriers may arise in terms of local practices and investment cost.

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5.4. Six sigma

The concept of 6-Sigma system was developed by Motorola in the mid 80´s. The 6-Sigma program involves the application of statistical methods to business processes, guided by the goal of eliminating defects. The 6-Sigma focuses on quality improvement (eg, waste reduction) to help organizations produce better, faster and more economical. More generally, the program focuses on defect prevention, reduction of cycle times and cost savings. Unlike careless cost cutting, which reduce the value and quality, Six Sigma identifies and eliminates costly waste, i.e., that do not add value to the customers. With this, the company increases operational efficiency reduces costs, improves quality, increases customer satisfaction and increases profitability [18, 19].

Sigma (σ) is a letter of the Greek alphabet used by statisticians to measure the variance in any process. The performance of a company is measured by the sigma level of their business processes. Organizations that employ the Six Sigma method aim to achieve 3.4 defects per million on manufactured products. This methodology is based on the implementation of a system based on the measurement and monitoring of processes so that deviations from 'normality' are avoided as much as possible.

The Six Sigma methodology is composed by a broad set of tools and techniques for quality improvement, among which there is a strong application of statistical tools and techniques. The cycle of phases, called DMAIC (Define, Measure, Analyze, Improve, Control) is used as a guide for professionals (mainly black belts and green belts) to implement projects that meet the goals most daring and radical pre-set by the company. The DMAIC can be resumed as follows:

- Define: define problems and situations to be improved, including the goals of the activities, as they will be the company's strategic objectives.
- Measure: to establish valid and reliable measurements for information and data.
- Analyze: analyze the information captured in order to identify ways to eliminate the gap between the current performance of the system or process and the desired goal. It should apply statistical tools to aid analysis.
- Increment: deploy processes, it can use management tools of projects or planning and managing to deploy a new approach,
- Control: control the improved processes in order to generate a continuous improvement cycle.

The statistical aspects of six sigma must complement business perspectives and challenges to the organization to implement six sigma projects successfully.In the list of tools and statistical techniques of DMAIC, are included: descriptive statistics, principles of sampling, control charts, process capability analysis, measurement system analysis, basic charts (histogram, scatter, box-plot, Pareto, etc..), cause and effect diagram, statistical process control (SPC), design of experiments, linear regression and correlation, multiple regression, hypothesis testing, confidence intervals, analysis of variance, capability process analysis, among others [18-20].

Factors influencing successful six sigma projects include management involvement and organizational commitment, project management and control skills, cultural change, and continuous training. It is a methodology that crosses the entire company, i.e., it is not the isolated involvement of a team, but the involvement of all in the pursuit of the implementation of continuous improvement and customer satisfaction [19, 20].

The adoption of the Six Sigma methodology as a quality program in all agribusiness chain in general is still new, but it is important to highlight the potential of this method for improving the quality of food products and reduce production costs.

6. Conclusion

The competitiveness of a company can be seen as a reflection of the strategies adopted as a means to adapt to the prevailing standards of competition in the markets in which the organization operates. Certainly, quality is a key factor for the food industry acts in a market increasingly globalized. For that companies must establish competitive strategies and develop an appropriate internal structure.

From these assumptions, this chapter talked about the important aspects and also specific to quality management in the food industry. The reality of each company, in financial terms, cultural, organization and motivation, will determine the degree of maturity and efficiency in quality management. What can be concluded is that the competitive advantage certainly goes through the constant search for new tools and learning management systems that improve the quality of processes and services and consequently the products offered by the food industry.

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Section 3

Food Safety

Chapter 10

Social and Economic Issues – Genetically Modified Food

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Additional information is available at the end of the chapter

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1. Introduction

Food is one of the most important necessities for humans; we eat to live and at least most people are blesses with a meal a day, while some others can afford three or more. Independent of our culture and customs, dinning remains a vital aspect in different festivities across the world between and within families and friends. Furthermore, we want a healthy and nutritious meal but the question is "How safe is the food we are consuming?"

The improvement of plants and livestock for food production and the use of different conservation techniques have been in practice as long as humankind stopped migrating relying on agriculture for survival. With the quest to grow more and better food to meet the demand of our fast growing world population, genetic engineering of crops has become a new platform in addition to plant breeding.

Molecular genetics has been and is a very useful tool used to better understanding of genes underlying quantitative traits associated with increasing crop yields or improving food quality. The eagerness to increase crop products has resulted in the genetic manipulation of plants, which has raised much polemics ranging from political, ethical and social problems. Genetically modified food simply means that the original DNA (deoxyribonucleic acid) structure of plants has been altered or tempered with. Since the DNA is the finger print of every organism consequently, changes made within the genetic code could possible lead to alteration in the quality or characteristic of the plant in question.

Although, there has been steady increase in the total area under genetically modified (GM) crop cultivation, nevertheless, there has been a marked slowdown in the last few years. The most extensively cultivated GM crops include soybean, corn and cotton. Europe is known to grow less than 0.5% of the world's GM crops, primarily because of the

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very rigorous EU regulations imposed on GMO crops in Europe until 2003 and the refusal of European consumers to buy GM products.

Notwithstanding, the essential knowledge and understanding of cell function and heritability combined with genetic engineering offering new possibilities to transfer and or modify DNA between organisms has enabled governments in many countries, for the first time, to be able to provide adequate food supply to their growing population. These advancements have resulted in the development of efficient vaccines and pharmaceuticals, new food technologies and many other products improving the overall standard of life. This is also true of agriculture where genetic engineering of crops can complement traditional plant breeding to suit the needs of today's world. Most of these improvements can be grouped under the term "biotechnology", which aims to use organisms, cells and or part of cells in technical or industrial processes.

2. Regulations and why?

Because genetically modified foods have been one of the most controversial topics that have made news in the last years. Many European environmental organizations, NGOs and public interest groups have been actively protesting against GM foods for months. Beside, recent controversial studies about the effects of genetically-modified food have brought the issue of genetic engineering to the forefront of the public consciousness (Fonseca, Planchon, Renaut, Oliveira, & Batista, 2012; Losey, Rayor, & Carter, 1999; Nykiforuk, Shewmaker, Harry, Yurchenko, Zhang, Reed, et al., 2012). Generally in Europe, the idea of introducing GM food products in the market for human consumption and or as animal feed has not been welcome for health reasons (Maga & Murray, 2010). Although there are no clear research results suggesting the negative effects of GM food to human health, the distancing from GM foods is more or less preventive. Nevertheless, with the growing interest in the use of biofuels as one of the sources of alternative sources energy, genetic engineering then comes in to play for economic reasons.

As a reaction to the growing public concern on GM food and products, many governments across the world have taken different approaches to tackle this hot topic on GM foods. This has resulted in the creation of GMO regulations which are most often country or region specific. The European parliament and council for example have set up regulations regarding GM foods to protect human health and well-being of citizens, and European social and economic interests (McCabe & Butler, 1999). The EU regulations segregates between GM food and feed, it further gives specific instructions on how GM products should be labelled in terms of the amount of modifications involved.

EU GMO regulations suggest for example that it is appropriate to provide the combined level of adventitious or technically unavoidable presence of genetically modified materials in a food or feed or in one of its components is higher than the set threshold, such presence should be indicated in accordance with this regulation and that detailed provisions should be adopted for its implementation (Ramon, MacCabe, & Gil, 2004). The possibility of estab-

lishing lower thresholds, in particular for foods and feed containing or consisting of GMOs or in order to take into account advances in science and technology, should be provided for. In my opinion, the European GM food regulations are the most stringent in the world and it is not quite clear whether or not there is any room for GM products due to the complexity in understanding and implementation of the said regulations. Nonetheless, the EU GMO regulations could be summarized as it is meant to provide the basis for ensuring a high level of protection of human life and health, animal health and welfare, environment and consumer interests in relation to genetically modified food and feed, whilst ensuring the effective functioning of the internal market; lay down community procedures for the authorisation and supervision of genetically modified food and feed; and to lay down provisions for the labelling of genetically modified food and feed.

Similarly, the United States regulation process is confusing because there are three different government agencies that have jurisdiction over GM foods. The Food and Drug Administration (FDA) evaluate whether the plant is safe to eat; the U.S. Environmental Protection Agency (EPA) evaluates GM plants for environmental safety, and the United States Department of Agriculture (USDA) which evaluates whether the plant to be grown is safe (Pelletier, 2005; Strauss, 2006). The USDA has many internal divisions that share responsibility for assessing GM foods. Among these divisions are, the Animal Health and Plant Inspection Service (APHIS), which conducts field tests and issues permits to grow GM crops, the Agricultural Research Service which performs in-house GM food research, and the Cooperative State Research, Education and Extension Service which oversees the USDA risk assessment program (Whitman, 2000). This implies there is a combination of regulations from these three agencies to be followed in order to carry on with GM food. Nevertheless, it is estimated that up to 70% of processed food on US supermarkets shelves ranging from soda to soup, crackers to condiments contain genetically engineered ingredients. Currently, up to 85% of U.S. corn is genetically modified as are 91% of soybeans and 88% of cotton (cottonseed oil is often used in food products) (Whitman, 2000).

In many developing countries whereby due to seasonal changes, there are usually a season of plenty and that of starvation, GM food is less a problem because the goal is to feed the starving population. Although, some of them might have GMO regulations, when food aid is coming into their countries in the moment of disaster, their rules and regulations are not important at that moment. This is understandable because the ultimate goal is saving lives before thinking of any qualms.

Plants have always been able to developed mechanisms over the years to endured environmental stress (drought, predation and pollutions just to name a few) and consequently adapted to the changing environment by developing genes resistant to the different factors. This is supported by the fact that, historically it was assumed that changes in plants as a result of genetic modification in breeding are generally safe and not harmful. Nevertheless, this was eventually challenged with the arrival of rDNA (ribosomal deoxyribonucleic acid) technology in the early 1970s when Cohen and Boyer successfully linked two different pieces of DNA (McHughen & Smyth, 2008).

The scientific world did not acknowledged the positive potentials of genetic engineering to crop breeding but the risks associated with these techniques (Berg & et al., 1974; McHughen & Smyth, 2008).

Over the last century, agriculture in general and plant breeding in particular have enjoyed fast dynamic research, which have been speedy and valuable developments. Traditional forms of crop genetic improvements, such as selection and cross-pollination, remain the standard tools in the breeder's toolbox, but have been supplemented with a range of new and specialized innovations, such as mutation breeding using ionizing radiation or mutagenic chemicals, wide crosses across species requiring human interventions such as embryo rescue and transgenic, commonly called genetic modification.

3. GM food and human health

Food choice is influenced by a large number of factors, including social and cultural factors. One method for trying to understand the impact of these factors is through the study of attitudes. Research is described which utilizes social psychological attitude models of attitude-behaviour relationships, in particular the Theory of Planned Behaviour. This approach has shown good prediction of behaviour, but there are a number of possible extensions to this basic model which might improve its utility. One such extension is the inclusion of measures of moral concern, which have been found to be important both for the choice of genetically-modified foods and also for foods to be eaten by others.

It has been found to be difficult to effect dietary change, and there are a number of insights from social psychology which might address this difficulty. One is the phenomenon of optimistic bias, where individuals believe themselves to be at less risk from various hazards than the average person (Paparini & Romano-Spica, 2004).

This effect has been demonstrated for nutritional risks, and this might lead individuals to take less note of health education messages. Many children in the US and Europe have developed life-threatening allergies to peanuts and other foods. There is a possibility that introducing a gene into a plant may create a new allergen or cause an allergic reaction in susceptible individuals. There is a growing concern that introducing foreign genes into food plants may have an unexpected and negative impact on human health. A recent article published in Lancet examined the effects of GM potatoes on the digestive tract in rats (Brunner & Millstone, 1999).

Another concern is that individuals do not always have clear-cut attitudes, but rather can be ambivalent about food and about healthy eating. It is important, therefore, to have measures for this ambivalence, and an understanding of how it might impact on behaviour (Shepherd, 1999).

One measure of how far we have travelled down that road is that it hardly matters any more whether objections to GMO are based on alleged environmental risks of cultivating GM crops or alleged toxicological hazards of eating them. GMO like 'radioactivity' has become

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an odious, generic shibboleth. Given that millions of people throughout the world are already benefiting from pharmaceuticals made by GM organisms, this is bizarre (Dixon, 2003).

Among the next generation of genetically modified (GM) plants are those that are engineered to produce elevated levels of nutritional molecules such as vitamins, omega-3 fatty acids, and amino acids. Based upon the U.S. current regulatory scheme, the plants and their products may enter our food supply without any required safety testing. The potential risks of this type of GM plants are discussed in the context of human health, and it is argued that there should be very careful safety testing of plants designed to produce biologically active molecules before they are commercially grown and consumed. This will require a mandatory, scientifically rigorous review process (Schubert, 2008).

Nevertheless, advances in our understanding of molecular biology, biochemistry, and nutrition may in future allow further improvement of test methods that will over time render the safety assessment of foods even more effective and informative (Konig, Cockburn, Crevel, Debruyne, Grafstroem, Hammerling, et al., 2004).

4. GM food and environment

Genetic modification and "biosafety" are concepts that have not been well understood by, or accessible to, the non-geneticists working in the fields of conservation science, law, administration and management, and in the scientific, legal, administrative and management aspects of sustainable use.

Genetically modified (GM) plants represent a potential benefit for environmentally friendly agriculture and human health. Although, poor knowledge is available on the potential hazards posed by unintended modifications occurring during genetic manipulation processes, the increasing amount of reports on ecological risks and benefits of GM plants stresses the need for experimental works aimed at evaluating the impact of GM crops on the natural and agro-ecosystems. One of the major environmental risks associated with GM crops include their potential impact on non-target soil microorganisms which plays a fundamental role in crop residues degradation and in biogeochemical cycles (Giovannetti, Sbrana, & Turrini, 2005).

Transformed corn plants with genetic material from the bacterium *Bacillus thuringiensis* (*Bt*) have been reported to represent a risk because most hybrids express the Bt toxin in pollen which could be further deposited on other plants near such corn fields causing non-target organisms that consume these plants (Yu & Shepard, 1998). It is thought that genetically modified plants could be harmful to the environment by depleting soil microorganism which are very important for soil fertility and or influence the micro-environments of other organisms (Giovannetti, Sbrana, & Turrini, 2005). The cultivation of GM seeds and plants could be detrimental to the environment (Losey, Rayor, & Carter, 1999).

The biodiversity debate is at the forefront of the larger question of how humanity can, in an integrated, congruent way, address human livelihoods, while at the same time fulfilling its international mandates to conserve and sustainably use the environment. In a world focused

on issues such as poverty and food security, as well as species loss and ecosystem destruction, these questions are among the most important and the most difficult on the planet.

5. GM food and economic issues

Bringing a GM food to market is a lengthy and costly process, and of course agro-biotechnological companies wish to ensure a profitable return on their investment. Thus many new plant genetic engineering technologies and GM plants have been patented, and patent infringement is a big concern of agribusiness.

Although, genetically modified (GM) plants represent a potential benefit for environmentally friendly agriculture and human health, poor knowledge is available on the potential hazards posed by unintended modifications occurring during genetic manipulation. The major economic fears are the risk of patent enforcement which may oblige farmers to depend on giant engineering companies such as Monsanto for strains when their crops are cross pollinated. Consumer advocates are equally worried that patenting these new plant varieties will raise the price of seeds so high that small farmers and third world countries will not be able to afford seeds for GM crops, thus widening the gap between the wealthy and the poor. It is hoped that in a humanitarian gesture, more companies and non-profits will follow the lead of the Rockefeller Foundation and offer their products at reduced costs to impoverished nations.

These plants would be viable for only one growing season and would produce sterile seeds that do not germinate. Farmers would need to buy a fresh supply of seeds each year, consequently will have to be dependent on the few agric-biotech companies with patent rights. However, this would be financially disastrous for farmers in third world countries who cannot afford to buy seed each year and traditionally set aside a portion of their harvest to plant in the next growing season.

6. Social and cultural aspects on GM foods

With the emergence of transgenic technologies, new ways to improve the agronomic performance of crops for food, feed, and processing applications have been devised. In addition, ability to express foreign genes using transgenic technologies has opened up options for producing large quantities of commercially important industrial or pharmaceutical products in plants. Despite this high adoption rates and future promises, there is a multitude of concerns about the impact of genetically modified (GM) crops on the environment (Paparini & Romano-Spica, 2004). Potential contamination of the environment and food chains has prompted detailed consideration of how such crops and the molecules that they produce can be effectively isolated and contained. One of the reasonable steps after creating a transgenic plant is to evaluate its potential benefits and risks to the environment and these should be compared to those generated by traditional agricultural practices (Poppy, 2004). The precautionary approach in risk management of GM plants may make it necessary to

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monitor significant wild and weed populations that might be affected by transgene escape. Effective risk assessment and monitoring mechanisms are the basic prerequisites of any legal framework to adequately address the risks and watch out for new risks. Several agencies in different countries monitor the release of GM organisms or frame guidelines for the appropriate application of recombinant organisms in agro-industries so as to assure the safe use of recombinant organisms and to achieve sound overall development. We feel that it is important to establish an internationally harmonized framework for the safe handling of recombinant DNA organisms within a few years (Singh, Ghai, Paul, & Jain, 2006).

7. Conclusion

Genetically-modified foods have the potential to solve many of the world's hunger and malnutrition problems, and to help protect and preserve the environment by increasing yield and reducing reliance upon chemical pesticides and herbicides. Yet there are many challenges ahead for governments, especially in the areas of safety testing, regulation, international policy and food labelling. Many people feel that genetic engineering is the inevitable wave of the future and that we cannot afford to ignore a technology that has such enormous potential benefits. However, we must proceed with caution to avoid causing unintended harm to human health and the environment as a result of our enthusiasm for this powerful technology.

In this connection, we find many claims about genetically modified organisms (GMOs) – that they can be a basis for increasing food production, without the need to convert more land to cultivation, for example. These claims, however, are countered by the claims that GMOs may have a variety of impacts on people and animals, and especially on ecosystems and lands not under cultivation, and concerns about whether and how the benefits of GMOs are actually experienced in developing countries.

Furthermore, some of the questions we need to answer to better understand GMOs include;

a. Are the current scope and objectives of the GMO legislation in line with the needs of society, and especially the biotechnology operators and consumers?

b. Are the procedures associated with the legislative framework fit for purpose, in definition and in implementation?

c. Are the procedures for the risk assessment of GMOs and their implementation up to date, are efficient, time limited and transparent known?

d. In design and implementation are provisions governing risk management of GMO marketing up to date, efficient transparent and in line with the general objectives of our legislation?

e. And is the communication of risk concerning the release of GMOs into the environment and the manner in which it has been implemented known?

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Chapter 11

Acidified Foods:
Food Safety Considerations for Food Processors

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Additional information is available at the end of the chapter

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1. Introduction

The food processing industry is one of the United States' largest manufacturing sectors, accounting for more than 10 percent of all manufacturing shipments. Concerns over food safety have increased as the industry has been hit by several high profile and large-scale food recalls. Thus, commercial food processors must be vigilant about ensuring the safety of their products. If inadequate or improper manufacturing, processing or packaging procedures are used in the production of low-acid or acidified canned foods serious health hazards, especially *Clostridium botulinum*, could result. To prevent this, processors must be in compliance with regulations established by the U.S. Food and Drug Administration (F.D.A., U.S. Department of Agriculture) and state agriculture and health departments across the United States (Barron, 2000).

2. Acidified foods

The term "acidified foods" means low-acid foods to which acid(s) or acid food(s) are added. These products include, but are not limited to:

- Pickled beets, cocktail onions, and cherry peppers (normally pickled by the addition of acid);
- Red bell peppers treated in an acid brine;
- Some pears and tropical fruits that have a natural pH greater than 4.6 and are acidified to a pH of 4.6 or below;
- Fermented green olives subjected to processes (such as lye treatment or washing with low-acid foods) that raise the pH above 4.6, with subsequent addition of acid or acid foods to reduce the pH to 4.6 or below;

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- Tomato salsa made from tomatoes with a pH of 4.6 or below and low-acid ingredients, when the amount of low-acid ingredients is not a small amount and/or the resultant finished equilibrium pH differs significantly from that of the predominant acid or acid food; and
- Cold-pack pickles that are subjected to the action of acid-producing microorganisms but require the addition of acid or an acid food to achieve a pH of 4.6 or below.

All acidified foods must have a water activity (a_w) greater than 0.85 and a finished equilibrium pH of 4.6 or below within the time designated in the scheduled process. These parameters must be maintained in all finished foods as outlined in 21 CFR 114.80(a). These foods may be called, or may purport to be, "pickles" or "pickled." However, some barriers exist in the preparation of acidified foods, including inadequate acid in the cover brine to overcome buffering capacity of the food, the presence of alkaline compounds from peeling or other processing aids, and the peels, waxing, piece size or oil in the product which can cause a barrier to penetration of the acid. These barriers may cause the failure to achieve the final equilibrium of a pH value of 4.6 and raise concerns about the growth of pathogens and production of toxins in the finished product.

After proper acidification, all acidified foods must then be heat processed to destroy the vegetative cells of pathogenic microorganisms or other microorganisms that cause spoilage and to inactivate enzymes that might affect color, flavor, or texture of the product. Acidified foods can be heat processed in a boiling water canner or by low-temperature pasteurization. The processing time, temperature, and procedure necessary to safely preserve acidified foods are determined by factors such as level of acidity (pH), size of food pieces (density) and percentage salt. An FDA recognized process authority must review the product and process and make the appropriate recommendations about time and temperature requirements. Processing temperatures higher than 185°F (85°C) could break down pectin and cause unnecessary softening of acidified foods (FDA 2010a).

All commercial establishments engaged in the manufacture of Acidified Foods and Low-Acid Canned Foods (LACF) offered for interstate commerce in the United States are required by 21CFR Parts 108, 113 and 114 to register their facility with form FDA 2541, "Food Canning Establishment Registration," and file scheduled processes for their products with forms FDA 2541a, "Food Process Filing for all Methods Except Low-Acid Aseptic," and FDA 2541c, "Process Filing for Low-Acid Aseptic Systems." The following items are not considered to be acidified foods or low-acid foods.

- Acid foods (naturally acid foods have a pH of 4.6 or less)
- Acid foods (including such foods as standardized and non-standardized food dressings and condiment sauces) that contain small amounts of low-acid food(s) and have a resultant finished equilibrium pH that does not significantly differ from that of the predominant acid or acid food
- Alcoholic beverages
- Carbonated beverages
- Standardized jams, jellies and preserves (21 CFR 150)

- Tomatoes and tomato products having a finished equilibrium pH less than 4.7
- Foods that are NOT packaged in hermetically sealed containers
- Any food prepared under the continuous inspection of the meat and poultry inspection program of the Animal and Plant Health Inspection Service of the Department of Agriculture under the Federal Meat Inspection Act and the Poultry Products Inspection Act
- Foods that are stored distributed and retailed under refrigeration
- Foods with water activity of 0.85 or below
- Food that are not thermally processed

Because these foods are not recognized as acidified foods, commercial processors do NOT have to file and register their processing information for these products with the Food and Drug Administration (FDA 2010b).

3. Pathogens of concern

In 1979, the Code of Federal Regulations (CFR) published the acidified regulations identified today as 21 CFR Part 114. Since then, new food processing technologies and methodologies have been developed and are frequently used in the industry. Furthermore, pathogens, such as *E. coli* 0157:H7 and *Salmonella* spp. have been shown to survive and grow in acidic environments. As a result of changing technologies and emerging pathogens, actions by federal agencies have motivated researchers to investigate new ways to eliminate pathogens, such as *E. coli* and *Salmonella* spp. The following are several research citations that provide a brief history of developments related to pathogens in acidified foods.

In 1996, an outbreak of *E. coli* 0157:H7 was identified when an individual contracted hemolytic uremic syndrome after drinking apple juice packaged in sealed containers. The outbreak affected 45 individuals across the USA and Canada. The product was voluntarily recalled by the manufacturing company (*Centers for Disease Control and Prevention,* 1996)

In 1999, an outbreak of *Salmonella* Muenchen serotype in the United States and Canada caused 298 cases of illness, which were attributed to unpasteurized orange juice. The outbreak affected 17 states, primarily in the Midwest, as well as regions of Canada. The product was voluntarily recalled after unopened product tested positive for the causative serotype (*Centers for Disease Control and Prevention,* 1999).

A study performed on the relative safety of pickled cucumbers from *Clostridium botulinum* infection, as a response to a 1976 study in which the organism was found in sealed containers previously believed to be safe. The study involved introducing *C. botulinum* spores into experimentally packed pickles artificially adjusted to a target pH and checking for growth of the organism. It was reported that any pH less acidic than 4.8 was insufficient to effectively kill *C. botulinum* spores, thus establishing a minimum safe pH for pickled cucumbers. (Ito et al, 1996).

A study investigating the effects of acetic acid on *E. coli* O157:H7 in apple juice and pickle brine found that increasing the pH of the food product yielded an increased inhibitory effect on pathogen growth. The study also demonstrated that acetic acid, a key component in vinegar, had a significant effect on the aforementioned inhibition over other methods of manipulating pH. (Breidt et al, 2004).

A study investigating the thermal resistance of *E. coli* O157:H7 found evidence for the phenomenon known as cross-protection, or the ability of a bacterium to apply resistance to one negative condition against another. These authors reported that microorganisms grown in an acidic environment display increased resistance to killing via thermal methods, indicating an increased threat by these types of organisms against current food safety methods involving both heat and acid (Buchanan and Edelson, 1999).

Recently, a study found that the Breidt model could be used to measure five-log reduction times in a less conservative manner, allowing for a more encompassing approach to determining safe preparation times for various foods. Acidified vegetable products with a pH above 3.3 must be pasteurized to assure the destruction of acid resistant pathogenic bacteria. The times and temperatures needed to assure a five log reduction by pasteurization have previously been determined using a non-linear (Weibull) model. Recently, the Food and Drug Administration has required that linear models be used with online electronic process filing forms for acidified foods. A linear model was developed that is based on the existing safe processing data. The processing times and temperatures meet or exceed the established heat processing conditions needed to assure safety (Breidt et al, 2010).

4. Control measures for ensuring food safety of acidified foods

Control measures for ensuring the safety of acidified foods are well documented in the scientific literature. A simple overview of appropriate measures includes:

- Acidified foods must be properly acidified to a pH below 4.6, but most foods are acidified to a pH of 4.2 or below.
- To assure quick and proper acidification, the food is normally cooked or heated with the acid before being filled into the final container.
- A thermal process or heating step is required to kill all pathogens and any other non-pathogenic microorganisms that could grow during storage of the product. Thermal processing must be completed by hot-filling the product or by the boiling water bath process. The heating temperature and time must be validated by an FDA recognized process control authority and be monitored, controlled and documented.
- The final equilibrium pH must be checked, controlled and documented after the product has completed the thermal processing step. A pH meter with two decimal places accuracy must be used to measure the pH if the final pH is 4.0 or above; other methods can be used such as pH paper or a pH meter with one decimal place, if the final pH is below 4.0.

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- Containers for acidified foods should be such that a hermetic seal is obtained. Vacuum is a good indicator of a hermetic seal and helps to keep the quality of the product.

5. Acified food guidance

Probably the most comprehensive guide to assist food processors in determining what constitutes an acidified food is a document prepared by the FDA in 2010 titled "Guidance for the Food Industry: Acidified Foods." This guidance document provides nonbinding recommendations but nevertheless presents step by step guidelines to determine if a food can be classified as an acidified food. In this document standardized and non-standardized food dressings, such as mayonnaise, and condiment sauces, such as ketchup, are considered acid foods, which have a natural pH of 4.6 or below.

Processors who are not sure if a particular food is classified as an acidified or not, can voluntarily submit the respective FDA forms for a preliminary evaluation. The draft guidance reminds processors that jams, jellies and preserves are excluded from the 21CFR114 as long as these products meet the applicable standard of identity under 21CFR150; otherwise, the non-standardized products are covered by 21CFR114 based on the pH of the fruit, the pH of the final product and the water activity level of the finished product.

Another important aspect to be considered by a food processor is the use of acid foods and small amounts of low amounts of low acid foods as ingredients to produce an acidified food.

There are two basic criteria needed to exclude any food from being subject to 21 CFR Part 114. The first is that acid foods contain small amounts of low acid foods and the second is that acid foods have a resultant finished equilibrium pH that does not significantly differ from that of the predominant acid or acid food.

Fermented foods with a water activity level above 0.85, such as cucumber pickles and green olives, are considered low acid foods subject to the action of acid producing microorganisms to reduce the pH of the food to 4.6 or below. As such, these products are subject to the requirements of 21CFR114. Processors repacking and reprocessing previously acidified foods are also subject to 21CFR114.

Common questions of food processors new to the food processing industry are precisely related to this matter of reprocessing or repacking a previously acidified food and to procedures to determine a finished equilibrium pH. The draft guidance reminds processors about the meaning of equilibrium pH. It is recommended to use a reference temperature of 25°C, commonly used in laboratory measurements. Equilibrium means the acid is fully diffused throughout the food (especially solid particles) and any successive measurements produce the same results. Further recommendations about food preparation for pH measurements and indicated to follow 21CFR114.90 and to ensure the pH of an in process batch to be reduced and reach the 4.6 within 24 consecutive hours. The likelihood that spores of *C. botulinum* will germinate and grow increases with the length of time it takes to reduce the equilibrium pH of a food to 4.6

There are three very important terms embedded in the definition of acidified foods (21 CFR par 114): (1) small amount of low acid food(s), (2) predominant acid or acid food, and (3) pH that does not significantly differ. Regarding the small amount of low acid food(s), it has been recommended to be no more than 10% by weight in the finished product. This recommendation is based on FDA experience when evaluating filed processed. This recommendation has been identified by FDA as the 'small amount provision" which means that acid foods that contain small amounts of low acid food(s) AND have a resultant finished equilibrium pH that does not significantly differ from that of the predominant acid or acid food are excluded from complying with 21CFR114. Some examples under this provision may be products such as tomato puree with added spices, or a salad dressing where the predominant acid is the mixture of all acid ingredients, such as mayonnaise, lemon juice, vinegar and tomato paste, and the small amount of low acid foods are red peppers, onion and garlic.

The acid ingredient, such as vinegar has a pH of 4.6 or below; the acid food such as tomatoes has a natural pH of 4.6 or below. These acid ingredients need to be at least 90% of the total weight of the finished product to be considered predominant.

Regarding the term pH that does not significantly differ from that of the predominantly acid or acid foods, FDA recommends the following criteria:

If the equilibrium pH of the predominant acid or acid food is:	Then one should consider a shift in pH to be significant when:
>4.2	Any shift in pH is present
4.2	The shift in pH is >0.2
≥ 3.8 and < 4.2	The shift in pH is >0.3
<3.8	The shift in pH is >0.4

It is important to consider variability factors, such as the accuracy of the pH meter and variations in the finished equilibrium pH of the food itself. Also, as a reminder to processors, water, being an important ingredient in many acidified foods, it is a low acid food and if it is a predominant ingredient in the finished product, this product is considered a water-based acidified food. Apple juice, bended juices, reconstituted juices and vegetable juices are all considered to be water-based liquids. When the finished equilibrium pH of a water-based liquid that contains acid(s) or acid food(s) is 4.6 or below, the product is subject to 21CFR114, unless the liquid is a carbonated beverage.

The draft guidelines recommend the use of decision tables to determine if a given food, including fermented foods to which low acid foods are added fall under the coverage of 21CFR114. These tables are a step-by-step series of questions that lead to the most probable correct answer about a food being an acidified or not product; however, it is recommended to consider other factors related to the product and the manufacturing process to make the final decision. The guidelines indicate that most acidified foods would require a heat treatment step. This thermal process is to be developed based on the most resistant microorganism that must be controlled under the given pH conditions. For example for a pH range of 4.0 to 4.6 the spores

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of acid tolerant spoilage microorganisms such as *B. licheniformis* need to be destroyed, while at a pH range below 4.0, the vegetative cells of yeasts, molds and non spore forming bacteria such as lactobacillus need to be destroyed.

The thermal destruction of spores and microorganisms can be expressed in terms of heat resistance parameters. The adequate combination of time and temperature (extent of thermal processing) to safely manufacture a commercial food product and is also resistant to spoilage is called thermal process lethality. The draft guidance document provides a table demonstrating relationships between finished equilibrium pH of products and the thermal process lethality of acidified foods. For example, for a pH range between 3.3 and 3.5 the F value of 1 minute is recommended. F being the destruction time desired at reference temperature of 195 F and a Z value of 10 F. This is typically written as F 10/195 =1.0 minutes. The thermal process lethality is part of a scheduled process required by FDA to prevent the growth of microorganisms of public health significance in the thermally processed food. This process need to be established by a competent process authority as defined in 21CFR114.3(e).

The draft guidance also includes final recommendations to address spoilage problems through quality control procedures such as systematically implementing written plans to investigate signs of spoilage and their causes, as well as corrective actions to solve the problem.

6. Recalls

A commercial processor engaged in the processing of acidified foods is also required by 21CFR108.25 to prepare and maintain a written recall plan. Guidelines for product recalls are contained in 21CFR7. This plan will provide a current procedure for implementation, including:

- notifying FDA of any recalls
- a procedure for distributors to follow to recall products which may be injurious to health
- a procedure for identifying, collecting, warehousing and controlling products and a method for determining the effectiveness of any recalls.

Recall is a voluntary action taken by manufacturers and distributors to remove food that is in violation of laws administered by the FDA and USDA. These agencies may request a recall, but cannot order one without a court order. Product recovery is only classified as a recall when the product is violative.

Product Identification. Each batch or production lot must be properly coded. This code will allow the product lot to be identified as to date, batch product personnel production records, and ingredient records.

Records. Records are key to the recall plan and must be maintained for three years. They include:

- Records of examination of raw materials, packaging materials, and finished product along with any supplier guarantees or certifications.
- Processing and production records showing adherence to scheduled processes, including records of pH measurement and other critical factors.
- A log of all departures from scheduled processes, actions taken to rectify them, and disposition records of the portion of product involved.
- Records of initial distribution of the finished product adequate to facilitate separation of food lots which may have become contaminated or otherwise unfit for use.

Notification. Persons to be notified in the event of a recall include FDA and USDA, key company personnel, and distributors. The notification should include the product, container size, and code of affected lots. The extent of the hazard and the level of the recall will be as determined by FDA and USDA. Based on this determination, FDA will approve the recall strategy. The notification will include instructions for consumers and distributors for product recovery and information feedback. The contact person should be listed on all notification forms.

Product Recovery. Plans for recovery include procedures for segregation of affected lots, storage, warehousing, and control. Procedures in place shall allow determination of the effectiveness of the recall. The recall is concluded when FDA and USDA determine that recovery is adequate and there is no longer any threat to the public.

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Chapter 12

Microbiological Contamination of Homemade Food

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1. Introduction

The consumption of healthy food is a consumer's right and the duty of the manufacturing industry. Health authorities are duty bound to prepare and enforce laws to protect the population's health. The supply of food free from health risks to the population is actually a challenge. In fact, contaminated food may cause serious infections and jeopardize the health of the population.

Owing to their frequency, food-caused infections are a very grave issue to public health. They may cause hazards ranging from a simple intestine discomfort to cases that are more serious, such as neurological disorders and death, because of the high number of microorganisms involved in a simple epidemic event.

Fresh or processed animal-derived food may harbor several pathogenic microorganisms that cause physiological disorders in people who consume them. When food eventually contaminated by disease-causing microorganisms is consumed, pathogens or their metabolites invade the host's fluids or tissues and trigger serious types of diseases, such as tuberculosis. They are conveyed by non pasteurized milk or by cheese contaminated by bacterial populations of *Mycobacterium bovis* and *M. tubercolosis* or by *Brucella abortus*, gram negative bacteria, intracellular pathogen that cause undulant fever and arthritis in human beings.

Bacteria, fungi, protozoa and viruses are the main microorganism groups that cause food disorders. Due to their diversity and pathogenesis, bacteria are by far the most important microbial group commonly associated with food-transmitted diseases. High rated agents in food

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infections are *Salmonella* sp., *Campylobacter* sp and *Listeria monocytogenes* due to their importance in eventual sequelae. The microbiological health risks in fowl consumption and its raw products include contamination by the above food pathogens.

Besides being one of the principal causes of food-derived diseases since its attack generally involves a great number of people, the genus *Salmonella* is associated with economic liabilities, commercial damage and decrease in production due to its frequency and extension. These facts occur because of the great number of food products that may be contaminated by this bacterium, namely, food with high humidity, protein and carbohydrate rates, such as beef, pork, chicken, eggs, milk and their derived products, highly liable to deteriorate. The contamination process by pathogenic bacteria in humans may be caused by poor hygiene conditions during processing involving sick people and animals or involving feces from infected agents. Bacteria-contaminated food may also be hazardous to public health due to the excessive growth in bacteria populations at food surface or within the food. These bacteria may come from the environment and cause toxins that develop into serious health problems on intake.

Hand-manipulated meat, sausages, salamis and cheese are among the most consumed products worldwide. They are also liable to high microbiological contamination due to their manufacturing process.

The World Health Organization and the Food and Agriculture Organization of the United Nations have published reports and studies developed in several regions of the planet highlighting the pathogen risks to populations and suggested the protection of food consumers through special industrial, operational, commercial and residence care. The need for great attention in food safety is a self-evident topic. In fact, improvements in food processing methods and conscience-awareness with regard to food safety by all involved in the food production chain will surely reduce the incidence of food-originated diseases.

2. Microbiological contaminants of milk and homemade fresh cheese

Milk is one of the most complete food featuring high levels of protein and mineral salts. However, due to the availability of nutrients and almost neutral pH, milk is highly perishable. It is highly liable to microbial growth and requires thermal treatment for its conservation [1]. Pasteurization prolongs milk conservation time, conserves its natural characteristics and preserves it safe for human consumption. High temperatures are involved so that the product's pathogenic microbiota are eliminated with no changes in its physical and chemical constitution. However, people in rural regions still drink milk in natura and use it thus as prime matter for the manufacture of derived products.

The hygienic obtaining of milk is the first critical factor within the manufacturing process of cheese and other products. In fact, the animal, equipments and environment at milking may be an important contamination source by microorganisms [2]. Faults during milking and processing coupled to inadequate conservation temperatures at the selling outlets are factors that contribute towards the commercialization of milk products with microbiological charac-

teristics that go against health norms and legislation [3]. The quality of milk and that of its products is a highly relevant factor for positive industrialization success since both the dairy and the consumer are interested in the outcome. In some case, however, a significant increase in the price of milk ensues. Milk is a product that should come from healthy herds, with good meals and managements, and from farms with proper technical installations that guarantee conservation during transport up to the dairy factory [4].

Since the number of milk contaminants increases at a slow rate from the moment of their introduction, the importance of adequate conservation of recently obtained milk should be underpinned as a basic practice for the maintenance of its quality. Milk should be submitted at low temperatures immediately after the milking process, with the consequent avoidance of the proliferation of unwanted microorganisms [5].

As a milk-derived product, cheese is frequently a food-originating pathogen vector. This is especially true for handmade fresh cheese manufactured from raw milk, lacking any maturation process. The product's microbial contamination is relevant for the industry because of financial liabilities, and for public health because of the risks in food-transmitted diseases.

Several studies [6] have shown that a product's quality and durability largely depend on the prime matter used in manufacturing. It is practically impossible to improve the qualities of a derived product, such as cheese, with a high number of microorganisms present in raw milk.

3. Fresh Minas cheese

Fresh Minas cheese (traditionally manufactured in the state of Minas Gerais, Brazil, whence its name) is defined by the Brazilian Ministry of Health (Decree 146) as fresh cheese obtained by enzyme coagulation of milk with curds and other appropriate coagulant enzymes, supplemented or not by the activity of specific lactic bacteria. According to the Technical Rules for the Identification and Quality of Milk Products [7], fresh Minas cheese may be classified as cheese with low moisture or semi-hard cheese with moisture ranging between 36 and 45.9%; cheese with high moisture or moderate mass cheese with 46 to 54.9% moisture; and very high moisture cheese or soft mass cheese, with not less than 55% moisture.

The processing of fresh Minas cheese comprises the following stages: milk pasteurization, coagulation, cutting, draining, milling, salting, packing and cooling [8]. Since the manufacturing of this type of cheese is highly simple, many small, medium-sized and large dairies are interested in its fabrication. In fact, it is the most common type of cheese found in fairs, bars and grocers. The cheese is normally placed in a common non-vacuum plastic bag and closed by a metal seal [9].

According to the Brazilian Association of Cheese Industry (ABIQ), Brazil produces 400,000 tons of cheese per year, of which 240,000 tons are produced under federal, state and municipal inspection. Most production (95%) is consumed by common people [10].

The intake of fresh cheese may be risky for the consumer's health. However, Decree 861/1984 basically prohibits the sale of fresh cheese manufactured from the raw milk of cows, goats or sheep, pure or mixed. Milk should undergo pasteurization or other equivalent thermal treatment. Current legislation was published after several registers of human brucellosis caused by fresh cheese. In defiance of the law, the homemade manufacture of cheese in certain regions of Brazil is not done with pasteurized milk. Consequently, the consumption of homemade cheese brings to the fore old dangers such as brucellosis (Maltese fever) and other infectious diseases.

In spite of the legal prohibition against the commercialization of fresh and tender cheese manufactured from raw milk, the sale of homemade fresh Minas cheese occurs openly and everywhere in Brazil [11]. This is partially due to a greater yield, simpler processing and lack of product's maturation in the fabrication of this type of cheese, with low costs for the consumer and a fast return of expenditure to the manufacturer [12].

Food protection authorities classify microbial biological contamination as a main danger to public health. Who has constantly raised its voice on the need to restrict food contamination by health-impairing biological agents. Although microbial quality of food is of paramount importance, registration at the Federal Inspection Service does not guarantee lack of pathogens in food [13].

Food-derived diseases may be caused by several microorganism groups that include bacteria, fungi, yeasts, protozoa and viruses. Due to their diversity and pathogenesis, bacteria are by far the most important microbial group and commonly associated with food-transmitting diseases

Bacteria are microorganisms largely spread throughout the natural world and may be found in every type of environment [14]. They cause diseases in humans, animals and plants and deteriorate food and other materials. On the other hand, they may be useful too when they compose the human being's normal microbiota and are used in the production of food as symbiotic in agriculture and medicine.

In spite of certain unreliable Brazilian statistics, it is believed that food-derived diseases in Brazil are high [15]. In fact, several studies estimate that 12% of hospitalization cases in Brazil occur because of infectious intestinal diseases [16].

Occurrences of food-derived diseases are normally associated with certain risk factors, or rather, procedures that benefit toxin infections. The following may be highlighted: faults in food refrigeration; conservation of warm food at room temperature; food prepared many hours earlier for later consumption with inadequate conditioning during the interval; faults in the cooking process; handling of food by people with inadequate personal hygiene practices, or with lesions or with contaminating diseases; usage of contaminated prime matter; faults in the hygiene of utensils and other equipments in food preparation; favorable environmental conditions for the growth of etiological agents; food obtained from unreliable sources; inadequate storage; use of utensils which release toxic residues; intentional or accidental addition of toxic chemicals to the food; usage of water with uncontrolled drinkability features; water contamination from damages in the supply system [17].

Problems in the manufacture of cheese in Brazil are related to precarious conditions of milk, bad conditions during the manhandling of cheese and the lack or deficiency of refrigeration throughout the production chain. These factors worsen the situation and establish contamination conditions which favor the development of microorganisms at several places [18].

Whereas some microorganisms contribute beneficently towards the processing, safety and quality of certain food products, other organisms are involved in processes with unwanted effects in food and for the consumers' health. There are two categories of food-transmitted microbial diseases: food intoxication and infection by food. In food intoxication, the person ingests toxins that are pre-formed by microorganisms in the food. The toxin causes damage to the organism. Examples comprise botulinum toxin that binds itself to the nerve terminals at the muscle level and impedes the release of acetylcholine neurotransmitter, and staphylococcus toxin that acts on the brain's vomiting-center [19]. Infection by food occurs when the pathogen, such as by *Salmonella typhy* and other serotypes, is ingested and multiplies itself, causing diseases in the intestine tract and often in other organs [20].

The sale of animal-derived food in fair stalls without any refrigeration and without any protection against dust and insects may alter their quality. In the case of cheese, it is sold in portions or slices and thus the external incorporation of biological or non-biological foreign matter is dangerous due to faults in the handling of the product during commercialization, poor hygiene of the stalls and utensils used, and crossed contamination between exposed products [21].

Food microbial contamination is unwanted and dangerous within food microbiology. This aspect should be faced with great strictness. The acknowledgement of possible hygiene deficiency implying in food contamination brings to the fore microorganism groups, comprising indicators, and pathogenic microorganisms that find an excellent environment in food for their development and even for the release of toxic substances [22]. Total and thermotolerant coliforms, such as *Staphylococcus aureus*, fungi, yeasts and even *Salmonella* spp., should be highlighted among the microorganisms whose presence and numbers indicate the quality of the product.

The above mentioned microorganisms, causes of several types of pathogenesis, are transmitted to humans because of lack of hygiene, bad habits of handlers, inefficient production processes, maintenance or re-heating of food at inadequate temperatures and also by non-adequate conditions in industries where the food is produced [23].

Most microorganisms, whose pathogenicity in humans depends on their variegated presence in food, are relatively sensitive to high temperatures. In fact, they are destroyed by the adequate cooking of eventually contaminated food or by pasteurization processes.

The Brazilian Agency for Health Vigilance (ANVISA) established, by Decree RDC 12 of the 2nd January 2001[24], the microbiological Standards for several types of food, described in Table 1.

So that food-caused disease cases and events could be characterized, the populations should be informed on the symptoms of each, such as mild diarrheas and vomiting since these are considered as a "passing illness" and not necessarily associated with food consumption [25].

Microorganism	Quantity
Coliforms at 45ºC	$5x10^2$ MPN/g
Staphylococcus aureus	$5x10^2$ CFU/g
Salmonella sp.	Absence in 25g
* MPN (most probable number), CFU (colony forming unit). Source: ANVISA/2001[24]	

Table 1. Microbiological Standards for Food: cheese with high moisture (55%).

According with registers, more than a billion cases of acute diarrhea are detected in less-than-5-year-old children in developing countries yearly, with 5 million deaths. Between 1999 and 2001, in the state of Paraná, Brazil, 67.1% of food epidemics were caused by bacteria. Moreover, out of 1389 notified epidemics, 38.6 were confirmed in the laboratory; 29/7% were confirmed clinically or epidemiologically suspect and 31.6% were of unknown etiology [25].

World cheese production is slightly above 19 million tons. Cheese production increased more than 76.3% during the last thirty years, or rather, from approximately 10.8 million tons in 1978 to more than 19 millions in 2008. The expansion of milk-producing regions and production increase throughout recent years provided a highly relevant presence of Brazilian production within the world market of milk-derived exports. Concern is therefore high with regard to the quality of commercialized goods for internal and external consumption.

Family-run agriculture in Brazil has an important share in the milk production chain, with approximately 86% of milk producers. However, the production and management of these milk producers are foregrounded on a homemade basis with scanty technical assistance and high influence of cultural factors that may put to risk consumers' health. Technical and educational orientation through the introduction of healthy manufacturing practices are deemed necessary to minimize contamination risks and food intoxication by the product.

Research in all Brazilian regions, where the production and commercialization of cheese is undertaken mainly by small producers, has demonstrated the risk of toxin infections in the consumption of these products by the population.

The curd-cheese is the most produced and consumed milk-derived product in the northeastern region of Brazil. Several investigations [26] have shown that the handling and carelessness in hygiene within the production system have made it foremost as a contamination source. The manufacturers are transmission vectors of the pathogen *Staphylococcus aureus* and others that may cause food intoxication. The presence of positive coagulase staphylococcus witnesses the lack of hygiene and sanitary conditions during the production, processing, distribution, storing and commercialization stages of samples of curd-cheese. Sanitary education of the producers and the spreading of processing techniques based on good manufacturing practices are mandatory.

Researches in the state of Mato Grosso, in the Mid-Western region of Brazil, (Loguercio & Aleixo 2001) [27] have shown the poor hygiene and sanitary conditions that characterize the

production of fresh Minas cheese. *Staphylococcus aureus* bacteria rates higher than those permitted by current legislation are rife. The need for more sanitary surveillance and orientation by government authorities is urgent.

Research work in the southeastern region of Brazil [28] (Salotti et al 2006) evaluated the microbiological quality of fresh Minas cheese samples. Results from the hinterlands of the state of São Paulo, Brazil, showed non-compliance to rules established by the Brazilian Agency for Sanitary Vigilance (ANVISA) for 83.4% of homemade products and 66.7% for industrial samples with regard to thermotolerant coliforms. In the case of positive coagulase *Staphylococcus*, 20% of homemade samples and 10% of industrial products failed to comply with the ANVISA regulations. Microbiological results revealed the potential risk of the product for consumers.

After analyzing samples of fresh Minas cheese in Minas Gerais for coliforms and *E. coli*, a recent study [29] showed the presence of microorganisms, above the rates allowed by current legislation, in 30% of cheese with certificate; 70% of cheese without certificate and 61.4% of mild cheese. Since *E. coli, Proteus*, *Providencia*, *Serratia, Klebsiella* and *Enterobacter* were identified within the Enterobacteriaceae isolated in fresh Minas cheese, the risk to public health when the products are consumed is amply demonstrated.

Was reported [30] on the risk in the consumption of fresh Minas cheese by the population of the state of Paraná, southern Brazil. Samples inspected by the Federal Inspection Service of Santa Helena PR Brazil revealed that only 15% were in accord to ANVISA standards. All homemade cheese samples and 70% of inspected ones were not according to legislation. Studies [31] confirmed the above results and reported that 50% of samples of analyzed cheese had thermotolerant coliforms, 100% had positive coagulase *Staphyloccocus* and 12.5% had *Salmonella* sp. These samples were inadequate for human consumption since they were not consonant to cheese microbiological standards.

4. Microbiological contaminants of jerked beef

One of the most traditional products of the northeastern region of Brazil is jerked beef which may be characterized as a nutrition food with high calorie rates and widely accepted by consumers for its peculiar sensorial features. Jerked beef is produced from cuts derived from all parts of cattle carcass, salted and dried, with longer durability when compared to that of fresh meat [32].

Due to different nomenclature in Brazil, such as 'carne-de-sertão', 'carne serenada', 'carne deviagem', 'carne-mole', 'carne-do-vento', 'cacina' or more simple still, dehydrated meat, jerk beef is often confused with another type of salted beef, albeit industrialized, called 'charque' or dried salted meat [33].

Jerked beef was first used in the northeastern region of Brazil as an alternative to preserve beef surplus which could not be consumed immediately and so that the meat would not deteriorate quickly due to difficulties in its preservation especially among the poor population with no

refrigeration equipments. Favorable climate conditions and availability of seawater salt, fresh meat could be preserved by being dehydrated and salted.

Currently the above-mentioned preservation process is less relevant due to the introduction of refrigeration. However, many people from different regions of Brazil, especially from the northeast, became accustomed to the produce's characteristic taste and continued to produce jerked beef will less amounts of salt and frequently without exposure to the sun.

Each Brazilian state developed its own technology and thus produced jerked beef with different characteristics with regard to aspect, taste, color, amount of salt and shelf life. The states of Rio Grande do Norte and Ceará are the greatest producers of jerked beef mainly due to climatic conditions that favor the food's dehydration. In fact, jerked beef passed from a locally consumed product and used in certain food receipts to wider conditions. In fact, it is appreciated throughout Brazil and in several meal preparations. Jerked beef may be found in big city centers such as São Paulo and Rio de Janeiro, in homes and restaurants, outside the restricted circle of northeastern cuisine [34], and in the menu of the poorest worker [35,36].

Owing to the popularization of homemade salting technique, jerked beef production follows typically regional norms. Consequently, it is produced in a highly rudimentary way under inadequate sanitary conditions [37,38]. Analysis of the hygiene conditions in the production and commercialization of jerked beef in the region of Itapetinga BA Brazil may be brought forward as an example of the popularization of the technique. In fact, 73.3% of the shopkeepers interviewed admitted that they themselves produced the jerked beef on sale in their shops. Whereas 63.6% used non-inspected meat, 27.3% used meat inspected by municipal health officers and only 0.1% was inspected by federal health officers. Jerked beef was stored and commercialized in 71% of the shops at room temperature, which favored the multiplication of contaminant microorganisms and flies. These facts bring health risks to consumers and jeopardize the product's physical aspects [39].

Salting technique consists in the removal of water from the meat tissues; decrease in water activity ensues, inhibits microbial development and the speed of unwanted reactions of the final product. When salted beef is conserved without any type of refrigeration, its shelf life is higher than that of fresh meat [40]. However, jerked beef has low sodium chloride (NaCl) rates, between 5 and 6%, high moisture, between 65 and 70% [35,41,42] and water activity of 0.92. It may be characterized as partially dehydrated meat in which water activity is not sufficient decreased to avoid microbial development (and consequently degradation) or the production of microbial toxins [43,44].

Although the literal translation of the jerked beef in Portuguese is 'meat exposed to the sun', it is actually only rarely exposed to the sunrays during the dehydration process. The end product is a semi-dehydrated homemade product with four-day shelf-life at room temperature and up to eight days under refrigeration [43,45,41].

Data on the physical and chemical qualities of jerked beef sold in butcheries and supermarkets in João Pessoa PB Brazil showed that water activity in all samples was relatively high, between 0.898 and 0.967, and that the rates of sodium chloride (NaCl) ranged

between 3.73% and 9.79%. Consequently, NaCl employed in the process was insufficient to decrease water activity in the product and thus it did not have a significant inhibitory action in the development of most microorganisms in the beef [46]. Lack of standardization in the quality of jerked beef was also assessed in samples collected at inspected shops. Mean rates of water activity were 0.94±0.02. The same average was obtained for samples collected in shops without any health inspection [47]. Variations in sodium chloride rates were also registered in the samples. Techniques for more efficient conservation are required to decrease such risks since it is a type of food with contamination possibilities throughout the manufacturing process.

With regard to the microbiological contamination of jerked beef, the transformation by which meat in natura is processed into jerked beef requires that technological alterations modify the initial microbiota by which the salting and dehydration process selects more tolerant microorganisms for such conditions [48]. Pathogens that may contaminate jerked beef comprise *Clostridium perfringens*, *Staphilococus aureus*, *Salmonella*, verotoxin-producing *Escherichia coli*, *Campylobacter*, *Yersinia enterocolítica*, *Listeria monocytogenes*, *Aeromonas hydrofila*, and other deteriorating bacteria [49]. However, low NaCl rates used in jerked beef is one of the factors that trigger microbiological development since decrease in water activity is insufficient to hinder the development of deterioration-producing bacteria of the genus *Pseudomona*. It also provides proper conditions for the growth of gram-positive bacteria as those of the genus *Staphylococcus* [38].

Samples of jerked beef from the north of the state of Minas Gerais, Brazil, showed that the amount of mesophile aerobic bacteria, an index of food hygiene quality, was between 2.0x104 UFC/g and 8.9x108 UFC/g. Psichrotrophic bacteria were found in 93.33% of samples, between 5.4x103 UFC/g and 2.9x106 UFC/g. Results show poor hygiene in the manufacture of jerked beef [50]. Similar results were reported in samples of jerked beef commercialized in João Pessoa where the number of mesophile bacteria ranged between 1.8x105 and 7.5x107 UFC/g, with a clear correlationship between mesophile contamination and hygiene and sanitary standards [42].

High thermotolerant coliform rates, which also demonstrate unsatisfactory hygiene and sanitary conditions during the processing stages in the manufacture of jerked beef, were also registered in most jerked beef samples sold in butcheries and supermarkets in João Pessoa PB Brazil [46]. However, total coliforms in food did not report recent fecal contamination or the occurrence of enteropathogens [51,52]. However, Brazilian sanitary laws did not regulate the presence of this microorganism group in meat.

The commercialization of jerked beef in health inspected or not in the region of João Pessoa PB Brazil has been evaluated and results showed high rates in both groups. Ninety-six samples were analyzed and high contamination by feces-derived microorganisms was reported. *Staphylococcus ssp*. rates were high in both groups, with a low frequency for *S. aureus* [47]. *Staphylococcus aureus* rates were higher than 5logUFC/cm2 in 50% of jerked beef samples commercialized in butcheries and supermarkets in João Pessoa PB Brazil. The above amounts demonstrate high contamination causing gastrointestinal disorders in consumers [53].

Mesophile microorganisms *Salmonella sp*. and *Staphylococcus aureus* in jerked beef commercialized at room temperature and under refrigeration in Campina Grande PB Brazil showed no significant difference in *S. aureus* counts for samples commercialized at room temperature and under refrigeration. *Salmonella* ssp. was detected in 40% of jerked beef samples commercialized at room temperature and in 30% of samples under refrigeration.

Another source of contamination in the commercialization of jerked beef may be found in supermarkets, open market stalls and butcheries. Data reveal that the utensils used in 75% of these outlets were not exclusively for meat cutting and that the handling of money and food was common practice in 25% of the businesses. Aprons, disposable caps and clean closed shoes were only found in 25% of the shops.

The inadequate washing of hands and other habits such as talking during the handling and commercialization of food were also reported in all commercial enterprises [54]. It has been verified that in João Pessoa, supermarkets had the best hygiene and sanitary profile in jerked beef quality, whereas open markets and stalls in fairs had the worst [42]. In the latter case, meat is exposed without any type of protection and any passerby may handle it at will.

Investigations were carried out with regard to alien matter, such as flies, acarids, larvae, insects, feathers and others, found in jerked beef sold in 20 (90.9%) shops in Diadema SP Brazil, specialized in typical products from the northeastern region of Brazil. Exposure of products without any wrappings is an excellent condition for attacks by insects, especially flies, and rodents, making it improper for human consumption in the wake of health-hazard matter [55].

Almost all jerked beef is manufactured and sold in small shops and specifically prepared for people who appreciate the product. Consequently, lack of sanitary rules for its production, precarious conditions in its commercialization, storage without refrigeration and its exposure without any protection characterize jerked beef in such conditions as haphazard to public health.

5. Microbiological contaminants in meat fillings (sausages made from beef and fowl meat, salami)

Animal-derived food conveys a host of microorganisms dangerous to human health. The incidence of toxin infections in Brazil is high, although statistics are rather lacking on the matter. Bacteria causing toxin infections are widely distributed although their main natural habitat is the human or animal intestine tract [14]. The most common bacteria in food contamination are of the genera *Escherichia, Salmonella, Shigella, Yersinia, Vibrio, Brucella, Clostridium, Listeria, Campylobacter, Bacillus cereus* and *Staphylococcus aureus* [56]. Sausages, widely used in Europe, is a type of food stuffed with meat from swine, fowls, goats, cattle and fish, seasoned with several types of spicy ingredients. Sausages are a highly popular food in Brazil, easily accessible to all classes of people and consumed

throughout the country. Sausages have great acceptance in the southern and southeastern regions due to a more Europeanized culture.

Brazilian swine breeding has a very important role in several sectors of Brazilian economy. It produces jobs and intensifies demand of agricultural products in the industrialization and commercialization of animal-derived products. Besides providing excellent animal protein to the population, the meat industry exports meat and important economical assets are aggregated [57].

Data by the Brazilian Association of Production and Exportation Industry of Pork (ABIPECS) showed that approximately 65% of the Brazilian pork production is directed towards the internal market through industrialized products. Among the processed products, the fresh Tuscan-type sausage, made exclusively from pork, uses the less important animal parts as food, with great acceptance among the population.

Pork and its derived products undergo bacterial alterations owing to several factors such as animal health and fecal contamination by *Escherichia coli* highly relevant worldwide as a microorganism hazardous to animal and population health involving hygiene and sanitary issues [57]. The same author evaluated the occurrence of *E.coli* in swine in the abattoirs of Rio de Janeiro, Brazil, from which the Tuscun-type sausages were made. Different parts of the animal used in the stuffing process were examined and concluded that, depending on the meat and the manufacturing process, sausages were not fit for consumption.

Toxoplasma gondhii in fresh pork sausages commercialized in Botucatu SP Brazil was evaluated by researches [58]. Pork represents one of the main sources of infection by *T. gondii* in humans. Swine were the most important animals in the process of toxoplasmosis transmission [59,60,58,61]. Mendonça's data did not show any evidence of *T. gondii* in the samples, perhaps due to salt, used in the manufacturing process, which eliminated the microorganism.

The occurrence of food infection by pork sausages contaminated with *Salmonella sp*. has been suggested [62]. Brazilian sanitary laws [63] make it mandatory that the microorganism should be lacking in 25% so that human intoxication may occur. However, such possibility may vary since it depends on serotype and the person's health conditions and tolerance. Mürmann's results [62] showed that 24% of pork sausages samples were contaminated by *Salmonella enterica*.

Contamination by *Salmonella sp* in pork may occur in pens through contact with feces, lack of hygiene and sanitation in the installations and by other animals during the transport, waiting or pre-finishing period. A high increase of *S. enteriditis* in food toxin infections in humans and in aviary products has been reported in Brazil since the 1990 [64].

Fecal coliforms, positive coagulase staphylococcus, *Salmonella* spp and *Campylobacter* spp in fresh sausages were evaluated [65]. When the hygiene and sanitary quality among the different types of fresh sausages was compared, pork sausages had the worst scores with regard to risks in public health, as ruled by the RDC n.12 of Anvisa [63].

The authors also registered that most samples were not in accordance to microbiological standards and thus hazardous to consumer's health. Another datum refers to the absence of *Campylobacter* spp in the samples, perhaps due to sodium chloride concentrations over 1.5% that may have inhibited these microorganisms.

Was analyzed [66] the presence of *Listeria* spp, principally *L. monocytogenes*, during the manufacture of fresh mixed-meat sausages in three abattoirs, supervised by state health authorities, in Pelotas RS Brazil. Results showed that all samples from the three abattoirs were contaminated by *Listeria* spp, of which the most frequent species was *L. innocua* (97.6), followed by *L. monocytogenes* (29.3%) and *L. welshimeri* (24.4%).

When the hygiene and sanitary conditions in the manufacture of fresh sausages in the northwestern region of the state of Paraná, Brazil, were analyzed [67] data failed to show any microbiological contamination that would jeopardize the health of the consumer. The manufacture of these samples followed strict handling and processing procedures.

On the other hand, another authors [68] studied the prevalence of antimicrobial resistance by serotypes of *Salmonella* isolated from fresh pork sausages and found significant quantities of the above in samples collected in the southern state of Santa Catarina, Brazil. These serotypes resisted the antimicrobial products sulfonamide and tetracycline (81%); ampicillin (50%) and chloramphenicol (31.25%). Was evaluated the microbiological quality of fresh sausages in two towns of the state of Minas Gerais, Brazil [69]. Results confirmed positive coagulase *Staphylococcus* in 35% of samples which made them improper for human consumption. The same author also demonstrated that 35% of samples were contaminated by thermotolerant fecal coliforms above the maximum limits.

The consumption of chicken meat and its derivates has recently increased considerably in Brazil due to price decrease, good quality and practical cuttings provided [70]. Per capita consumption increased from 10 kg to 35.4 kg, only slightly lower than beef consumption (União Brasileira de Avicultura) [71]. The products' quality is highly important and a great concern to health authorities, food industry and consumers. Chickens bred for human consumption may host several pathogenic microorganisms such as *Campylobacter jejuni, Salmonella* sp and *E. coli* [72,73].

Rall investigated [70] the hygiene and sanitary conditions of chicken meat and several types of sausages commercialized in the interior of the state of São Paulo, Brazil, by determining the Most Probable Number of coliforms at 45ºC. The same authors also analyzed the presence of *Samonella sp* by the traditional method and by PCR. Data showed that 40% of the 75 sausage samples analyzed were improper for human consumption due to excess in coliforms and 7 samples (9.3%) were positive for *Salmonella* sp. (9.3%). Research by PCR increased to 56% *Salmonella*-positive samples. When the frequency rate of *Salmonella* was added to the microbiological limits for coliforms, it might be concluded that 86.7% of sausages were improper for human consumption.

In their research in the northwestern region of the state of São Paulo, Brazil, others authors [74] found contamination by *Salmonella* in 16% of chicken sausages samples. The most relevant item in the above result may be the handling of the product during processing, coupled to the

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exposure of the meat to several contamination sources or to already contaminated chickens that provided the contamination of the final product.

The above authors researched the microbiological quality of industrialized avian products and their derivates in another region of the state of São Paulo. Research determined the presence of *Campylobacter jejuni* and *Salmonella sp.* Sausages samples analyzed were 42.8% positive for *C. jejuni* and 28.5% for *Salmonella sp*.

The presence of microorganisms in the above research works suggests the need for greater care during the handling and preparation of sausages that may be eaten in natura, without any heating treatment that would reduce the number of microorganisms causing toxin infections [75].

Vienna sausage may be defined as an industrialized meat-stuffed product obtained from the emulsion of animal meat to which are added a variety of ingredients and condiments, filling a natural or artificial casing, and submitted to proper thermal process [76]. Vienna sausages are highly popular in Brazil due to their low costs and for the manufacturing of the ubiquitous hot dog.

The physical and chemical characteristics of Vienna sausages should contain a maximum of 65% moisture, 30% fat, 2% starch, 7% total carbohydrates, 12% protein. Fresh sausages should be under permanent refrigeration (0^oC to 5^oC) from manufacture until consumption, with expiry period after 48 hours [77].

Vienna sausages samples of the hot-dog type were analyzed in Niterói and Rio de Janeiro RJ Brazil to detect thermotolerant coliforms, positive coagulase *Staphylococcus*, *Clostridium* spp and *Salmonella* spp by conventional methods with the necessary modifications [78]. When compared to health norms, results showed that 33% of samples were inadequate for consumption due to the presence of their isolated microorganisms.

Salami is another highly appreciated product in southern Brazil. Its homemade manufacture started in the early 20th century with an enormous variety of industrialized types that differed in composition, casing, size of meat and fats, spices, smoking process and maturation period prior to commercialization. Researchers revaluated the various characteristics [79] of salamis produced by small- and medium-sized agro-industries in the southern state of Santa Catarina, Brazil. Bacteria *Staphylococcus aureus*, *Salmonella* spp, *Listeria monocystogenes* and *E.coli* were researched in the products. Although results did not identify contamination by *Salmonella* spp, the *E. coli* and *S. aureus* counts were significant, but within the reliability parameters.

Was analyzed the quality [80] of salami in the interior of the state of São Paulo, Brazil, and verified that, despite samples with *E. coli* and fecal coliforms, all samples were within health standards. Nevertheless, 60% of samples were contaminated by *Staphylococcus aureus* and 22% were unhealthy for consumption.

6. Final considerations

Owing to their importance for public health, the correct handling of meat and milk products required greater attention, care and supervision from the competent health authorities. Since there is great cultural diversity in food manufactured in Brazil, the direct intervention of all the sectors involved within the food production chain is mandatory to warrant healthy and reliable products and thus a decrease in diseases caused by food contamination.

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Chapter 13

Occurrence of Organochlorine Pesticides Residues in Animal Feed and Fatty Bovine Tissue

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Additional information is available at the end of the chapter

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1. Introduction

Nowadays, more than 800 different kinds of pesticides are used for the control of insects, rodents, fungi and unwanted plants in the process of agricultural production. Although most of them leave the products or degrade in soil, water and atmosphere, some trace amounts of pesticide residues can be transferred to humans via the food chain, being potentially harmful to human health. [1] Pest control in intensive agriculture involves treatment of crops (fruits, vegetables, cereals, etc) pre and post harvest stages, rodenticides are employed in the post-harvest storage stage, and fungicides are applied at any stage of the process depending on the crop. These chemicals can be transferred from plant to animal via the food chain. Furthermore, breeding animals and their accommodation can themselves be sprayed with pesticide solution to prevent pest infestations. Consequently, both these contamination routes can lead to bioaccumulation of persistent pesticides in food products of animal origin such as meat, fat, fish, eggs and milk. [2,3] During the last decades much attention has been given to this group of substances and the international level after it became apparent that they are transported through the environment and critical concentrations have been reached in some areas even in places where they have never been produced or used. Several countries banned the use of Organochlorine Pesticides (OCPs) during the 1970s and 1980s, although many of them continue to been used by other countries. OCPs have been identified as one of the major classes of environmental contaminants because of their persistence, long-range transport ability and human and animal toxic effects. OCPs are carcinogenic in animals as well as in human (International Agency for Research on Cancer, 1987). The immunotoxicity of selected OCPs has been also documented in vitro [4], in vivo [5], as well as in animals, in human fetal, neonatal and infant immune systems [6,7,8,9].

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A growing number of epidemiological studies have investigated blood or adipose levels of OCPs and their metabolites in relation with cancer, neurodevelopmental effects, immunotoxicity and reproductive efficiency [10,11,12]. The main sources of OCPs in the human diet are foods of animal origin and environmental exposure. It has been concluded that humans are exposed to toxic compounds via diet in a much higher degree compared to other exposure routes such as inhalation and dermal exposure. Low volatility and high stability, together with lipophilic behaviour, are responsible critical factor for their persistence in the environment (air, water and soil) and subsequent concentration in fatty tissues through the food chain. Therefore, it's important to identify and to monitor levels of OCPs in foodstuff of animal origin (meat and tissues that contain fat, milk and dairy products, eggs, honey and fish). The main pathway for the OCPs contamination of animal food is the ingestion of the contaminated food and/or water by the animals. [13,14,15] Breeding animals can accumulate persistent organic pollutants from contaminated feed and water, and/or from pesticides application in livestock areas (treatment of cowshed, pigsties, sheepfold etc.).[16,17,18] The use of feedstuffs in farms has become indispensable for animal diet in developed countries because of increasingly higher production requirements. Animal feed plays an important part in the food chain and has implication for the composition and quality of the livestock products that people consume. Therefore, the control of OCPs residues in animal feed is mandatory as well as the control in fatty tissues.

1.1. Organochlorine Pesticides (OCPs)

Organochlorine pesticides (OCPs) were intensively used in agriculture to protect cultivated plants in mid-twentieth century. 1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (DDT), one of the common OCPs, was used to prevent spreading of malaria and other vector-borne diseases such as dengue, leishmaniasis and Japanese encephalitis through the prevention of growth of mosquito.[19,20] After OCPs were used widely in soil and plants for some years and due to their relative stability and bioaccumulation property, these persistent chemicals can be transferred and magnified to higher trophic level through the food chain. Consequently, OCP residues are present in fatty foods, both foods of animal origin such as meat, eggs and milk, and of plant origin such as vegetable oil, nuts, oat and olives. Besides, these chemicals are widely distributed in the environment, which provides another route of unwanted intake in human. [21,22,23] Nevertheless, human exposure occurs still primarily via low level food contamination. Since their mode of action is by targeting system or enzymes in the pest which may be identical or very similar to system or enzymes in human beings, these OCPs pose risks to human health and the environment. [24,25] Thus, monitoring of OCPs residues in food becomes a routine analysis of pesticides monitoring laboratories. All US government pesticides datasets showed that persistent OCP residues were surprisingly common in certain foods despite being off the market for over 30 years. Residues of dieldrin, in particular, posed substantial risks in certain root crops. About one quarter of samples of organically labelled fresh produce contained pesticides residues, compared with about three quarters of conventional samples. [26,27] Among the contaminated organic vegetable samples, about 60% of them were contaminated with OCPs. After some OCPs were banned for use since the 80s, common daily food items such as eggs, milk, poultry, meat and fish have

been used for monitoring the residuals levels of OCPs. As regards food of animal origin, one efficient way to avoid large-scale contamination is to control and monitor the levels of OCPs residues present in animal feeds before being fed to the husbandry animals. [28,29,30]

At the same time, public health safety authorities should constantly monitor the OCPs in animal food commodities as the major source of human background exposure to OCPs is through food of animal origin. Most persistent organic pollutant (POPs) are OCPs, namely, aldrin, endrin, chlordane, DDT and hexachlorobenzene (HCB). They have been banned for agricultural or domestic use in Europe, North America and many countries of South America, in accordance with Stockholm Convention in 1980s. However, some OCPs are still used, e.g. DDT is used to control the growth of mosquito that spread malaria or as antifouling agent in some developing countries. [31,32] Residues of OCPs have been detected in breast milk (including DDT, HCB and HCH isomers) in contaminated areas. Recently, the scope of POPs was extended to include nine plus one chemicals. Among these new POPs, chlordecone, lindane, α-HCH, β-HCH, pentachlorobenzene (PeCB) and endosulfan, also belong to OCPs. [33,34] In order to fulfil the requirements of the Stockholm convention, the participating countries have to develop their own implementation plant to monitor the background level and collate the exposure data. To ensure the pesticide residues are not found in food of feed at levels presenting an unacceptable risk for human consumption, maximum residue levels (MRLs) have therefore been set by the European Commission. [35,36,37] MRLs are the upper legal concentration limits for pesticides in or on food or feed. They are set for a wide range of food commodities of plant and animal origin, and they usually apply to the product as placed in the market. MRLs are not simply set as toxicological threshold levels; they are derived after a comprehensive assessment of the properties of the active substance and the residues behaviour on treated crops. Both the periodic estimation of human exposure to persistent organic pollutants and the establishment by the EU authorities of MRLs in foods have required the development of analytical methods suitable for research purposes and inspection programmes. As an example, the European Union has established maximum contents for these compounds in animal feed which can be as low as 5 μg Kg^{-1} for some OCPs in fish feed and β-HCH in cattle feed. In the rest of feed materials these values can be as low as 10 μg Kg-1 relative to feedstuff with moisture content of 12%. [38,39,40]

1.2. Extraction methods and clean-up of OCPs

Animal feed as well as animal fat are considered a very complex matrices with large number of components especially lipids. Consequently, the development od sensitive methods for its analysis with elimination of interferent compounds and enough efficiency in term of analyte recovery represents an interesting task. [41,42] The most intricate step in these procedures is represented by the sample extraction and clean-up that should be efficient enough to allow a reliable screening of contaminated samples. The selection of suitable solvent (s) and extraction method is critical for obtaining satisfactory recovery of OCPs from the food matrix. Of course, if co-extracted materials are minimised in the extract, the clean-up procedure would became simpler. Owing to the lipo-

philicity of OCPs, organic solvent (s) normally can extract OCPs form food efficiently but lipids are also co-extracted. Solid-liquid extraction method was applicable for extracting OCPs from various types of food samples including vegetables, meats and its products, fish, eggs and animal fats. In addition, several standardised methods, including AOAC 970.52, EN 1528 and EN 12393, have employed such solid-liquid or liquid-liquid extraction techniques for the determination of OCPs in both fatty and non-fatty foods. [43,44,45] In some occasions, sonication or Polytron was also applied to improve the extraction efficiency and recoveries.

1.3. Clean-up methods

Matrix constituents can be co-extracted and later co-eluted with analysed components and can consequently interfere with analyte identification and quantification. Moreover, co-extracted compounds, especially lipids, tend to adsorb in GC system such as injection port and column, resulting in poor chromatographic performance. A through clean-up minimised such matrix issues, improves sensitivity, permits more consistent and repeatable results, and extend the capillary column lifetime. Several approaches have been attempted to eliminated co-extracted interferences from extracts, including freezing centrifugation or filtration, liquid-liquid partitioning, gel permeation chromatography (GPC), solid phase extraction (SPE) and solid-phase microextraction (SPME). The simplest approach to remove the fatty co-extracted is by freezing centrifugation. [46,47] The logic behind is that fatty substances (mainly lipids) have lower melting point than the solvent so that frozen lipids can be removed by centrifugation or filtering while OCPs remain dissolved in the solvent. Different freezing temperatures ranged from -24 °C to -70 °C have been used. However, the solubility of lipids in solvent not only depends on the temperature but also the solubility product. Therefore this technique can remove significant amount of lipids for some food matrix but not for every matrix. Certain amount of lipids would remain in the solvent after the freezing centrifugation step and hence further cleanup is required. Using materials with large surfaces area for absorption of lipids have been employed since early 1970s. These materials include, Florisil, Lipid Removal Agent (LRA) media from Supelco, micro Cel E and Calflo E from Johns-Manville. Micro Cel E and Calflo E and LRA are synthetic calcium silicate while Florisil is a magnesium silicate with high specific surface area. [48,49] They can be applied to remove lipids either in sample preparation, solid phase extraction step or during sample clean-up step, with minimal effect on non-lipid chemicals. When food sample is mixed with these lipids absorbing materials, edible fat could be removed. Therefore it is common to conduct a clean-up step by solid phase extraction (SPE) nowadays. Both, conventional glass column packed with sorbent(s) and ready-to-use cartridges have been utilised and the common used phases are silica, Florisil, alumina and C18-bounded silica. Doong and Lee compared the cleaning efficiency of ready-to-use cartridge filled with three different adsorbents for shellfish extract. [50,51,52] Their results demonstrated that out of 14 OCPs tested, two were retained in the C18-cartridge. As for alumina and Florisil SPE, though all 14 pesticides tested could be recovered, Florisil provide better results in term of recoveries, repeatability and removal of interfering substances. Similarly, Hong et al., also

showed that Florisil had better cleaning efficiency of fatty acids in fish extract when compared with C18. Besides, recoveries of some OCPs were poor with hexane as eluent and these more polar OCPs could be eluted out from the column with acetone. Bazlic et al., reported also that the quality of Florisil was important in avoiding possible interference and misinterpretation of results. Even though GC-MS was employed as the detection system, poor quality Florisil could introduce false positive results for lindane and dieldrin. [53,54] To sum up, the combination of sorbent(s) and eluting solvent(s) have to be chosen very carefully. Otherwise, some OCPs or their metabolites/derivatives would be lost during the clean-up step. [55,56,57] These OCPs could either break down or adhere to the sorbent material, leading to low or even no recovery. Finding of the optima clean-up conditions is an art itself. As the targeted OCPs might cover a wide range of polarities, it is quite difficult to find the best combination of SPE column material and eluting solvent, which permits recovering the polar OCPs (but leaving the polar interferents behind on the column), as well as recovering the non-polar OCPs (without eluting any residual oil present in the extract from the column).

1.4. Detection techniques of OCPs

A number of different selective detectors can be coupled with GC for analyzing OCPs, including electron capture detector (ECD), halogen specific detector (XSD), electrolytic conductivity detector (ELCD) and atomic emission detector (AED). GC-ECD is the most commonly used detection method with low detection limits. It is particularly useful for detecting halogen containing molecules. However, other organic molecules, such as aromatic compounds, would also give positive signal. Users have to confirm the presence of OCPs by another confirmative technique. Even though the above-mentioned selective detector can be used for quantification, it is unlike to fulfil the European Commission's stringest requirements as set for pesticides analysis. Confirmation with GC-hyphenated with mass spectrometric (MS) detector is normally required. Single quadrupole MS detector running in electron ionisation (EI) mode with target analytes monitored by selective ion monitoring (SIM) becomes a routine monitoring tool for OCPs nowadays. Since some OCPs are electronegative in nature, GC-MS detector under negative chemical ionisation mode with methane as reagent gas could provide better sensitivity. [58,59,60,61] To further increase confidence in confirmative analysis, GC coupled with tandem Ms is one of suitable techniques. Besides providing a more definitive detection tool, tandem MS also decrease matrix interferences, improves selectivity and achieves higher signal-to-noise ratio and subsequently improves the detection limit. Both tandem-in-time (ion-trap) and tandem-in-space (triple quadrupoles) detector have been applied for OCPs residues analysis in different matrices. The determination of pesticides residues in the environment and in food is necessary for ensuring that human exposure to contaminants, especially by dietary intake, does not exceed acceptable level for health. Consequently, robust analytical methods have to be validated for carrying out both research and monitoring programmes, and thus for defining limitations and supporting enforcement of regulations. In this field, reproducible analytical methods are required to allow the effective separation, selective identification and accurate quantification of pesticides analyses at low levels in food-stuff including food of animal origin.

2. Aim of the research

The aims of the present work were:

- To develop and optimise a simple extraction and clean-up method to quantify non-polar chlorinated compounds in high lipid containing samples (animal feed and subcutaneous fat bovine tissue).
- To validate a multiresidues method for the simultaneous determination of 20 OCPs by using GC-MS/MS in term of repeatability, precision, limit of detection (LOD), limit of quantification (LOQ) etc. The coupling of this detection mode is very useful for the analysis of these complex samples allowing the separation, identification, quantification and confirmation of a large number of pesticides at trace level.
- To monitor the OCPs level in animal feed samples used in bovine farm.
- To monitor the OCPs level in subcutaneous fat bovine tissue to asses and to verify the concentration phenomena of these persistent pollutants.

3. Experimental

3.1. Feed and subcutaneous bovine fat samples

25 feed samples used for bovine with different composition were obtained from intensive livestock farming. An example of feed mixture was shown in figure 1. 35 fat samples were obtained from bovine for slaughter (18-24 month age) and presented in figure 2.

Figure 1. Feed sample mixture

http://dx.doi.org/10.5772/54182

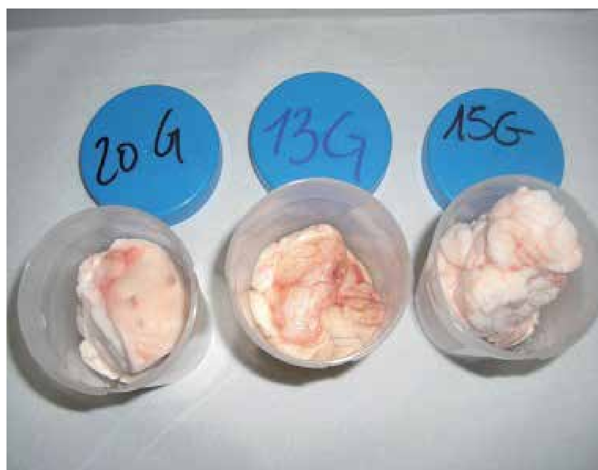

Figure 2. Subcutaneous fatty tissue sample

3.2. Chemicals and reagents

All OCPs were purchased from Supelco Inc.: mix 32094 and 32412 (Bellefonte, PA, USA). The figure 3 displays the structures of these OCPs considered in this study. Purities of pesticides standards were greater than 99%. Working standard solution was prepared at concentration of 0.1-5 μg mL^{-1} by volume, dilution with acetone and hexane. Organic solvents (hexane, acetone and acetonitrile) were of pesticide residue analysis grade (Sigma Aldrich, USA). All glassware was cleaned with laboratory reagent, sequentially rinsed with distilled water, acetone and methanol and finally baked in a oven at 300 °C. Distilled water was obtained with a Milli-Q system (Millipore, Bedford, MA, USA). For SPE, Florisil 5 g was purchased from Supelco.

3.3. Equipments

Ultrasonic bath (Branson) was used for the extraction of chlorinated pesticides form feed and fat samples. The generator of ultrasonic bath has an output of 150 W and a frequency of 35 kHz. Rotary evaporator (Buchi, Swiss) was used for the concentration of organic solvent. High intensity planetary mill Retsch (model MM 400, Retsch, GmbH, Retsch-Allee, Haan) was used to obtain representative aliquots of feed samples powder.

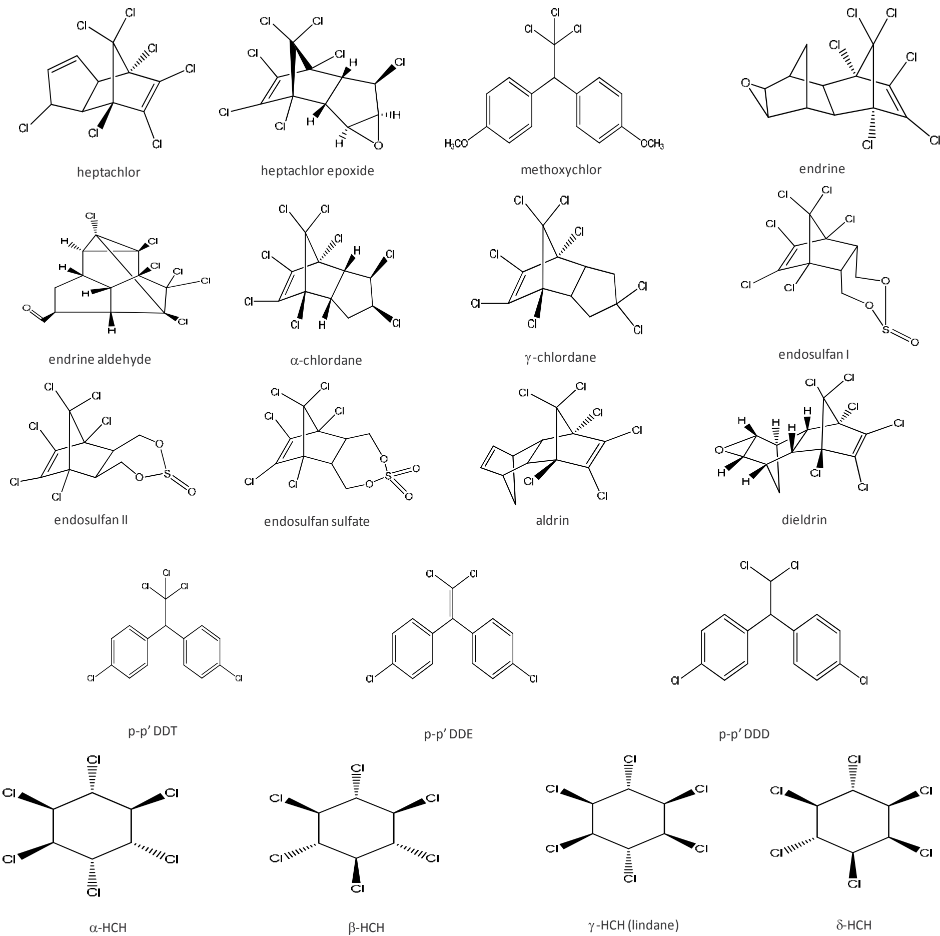

Figure 3. Chemical structures of chlorinated pesticides investigated in this study (19 OCPs)

3.4. Sample extraction, delipidation and clean-up procedure

3.4.1. Superfine Grinding (SFG) of feed sample

In order to obtain a representative feed sample a superfine powder was prepared from feed using mechanical grinding-activation in an energy intensive vibrational mill. 50 g of different feed sample were ground in a high intensity planetary mill. The mill was vibrating at a frequency of 25 Hz for 4 min using two 50 mL jars with 20 mm stainless steel balls. Pre cooling of jars were carried out with liquid nitrogen in order to prevent temperature increasing during the grinding process. The speed differences between balls and jar resulted in the interaction of frictional and impact forces, releasing high dynamic energies. The interplay of all these forces resulted in the very effective energy input of planetary ball mills. The appli-

cation of mechanochemistry deal with the physical changes of substances in all state of aggregation, for instance occurring with the combined action of pressure and shear in energy-intensive grinding mills. Mechanochemical technology has been developed and applied in different fields (synthesis of superfine powder, surface modification and drug modification) and could represent a novel tool of research. [62,63,64] The procedure is presented in Fig.4.

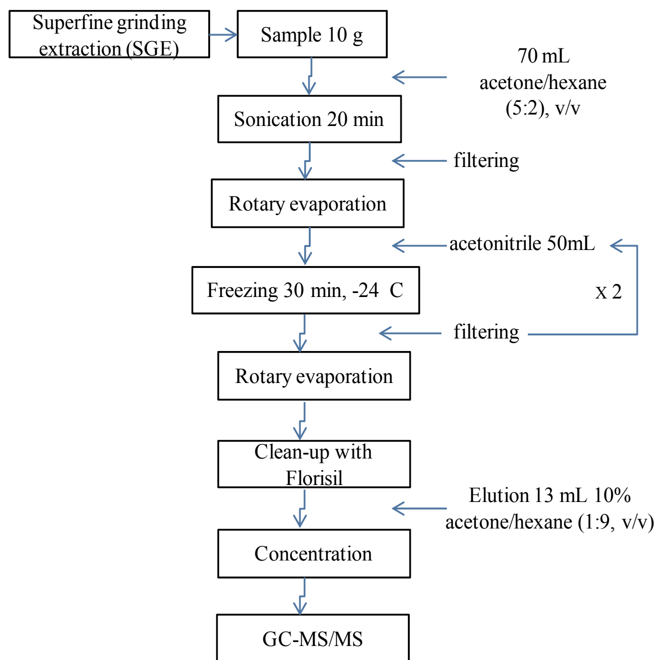

Figure 4. Analytical procedure for the extraction and purification of OCPs from feed and subcutaneous fatty tissue samples

3.4.2. Samples extraction

10 g of subcutaneous fat tissue (homogenised in a cooled mixer) or feed sample finely grounded and prepared with the procedure described above (SFG) were extracted by ultrasonic agitation with a mixed solvent of 70 mL of acetone-*n*-hexane (5:2, v/v) for 20 min. Extract was filtered to remove traces of water with filter paper containing 5 g of sodium sulphate, and then transferred into a 250 mL round flask. The extraction was repeated one more time. Extracted solvent was dried and redissolved in 50 mL of acetonitrile that has low solubility for lipids. Acetonitrile extract was stored in the freezer at -24 °C for 30 min to freeze lipids. Most of the lipids were precipitated as pale yellow, condensed lump on glassware surface. Cold extract at -24 °C was immediately filtered with filter paper to remove frozen lipids. The precipitated lipid on glassware surface was redissolved in 50 mL of acetonitrile to perform filtration again by same procedure. The filtered extracts were combined and concentrated to a final volume of 1 mL by a rotary evaporator to follow Florisil-SPE clean-up.

3.4.3. Sample clean-up

The SPE cartridge was cleaned with 12 mL of n-hexane and air dried by positive pressure prior sample application. 5 mL of hexane were used to condition the cartridge. After sample loading, the cartridge was air dried for 10 min. Desorption of the OCPs, which had been concentrated on the Florisil sorbent, was carried out using 13 mL of acetone-*n*-hexane (1:9, v/v) mixture at a flow of 1 mL min-1 and collected in a 50 mL round flask. The eluate was then concentrated at 45 °C under nitrogen stream until just the disappearance of the last drop of solution. Finally, the residue was redissolved in 1 mL hexane Pestanal prior to its injection in GC-MS/MS system.

3.5. GC-MS/MS analysis and detection

A Varian GC 3800 gas chromatograph coupled to a Varian Saturn 2000 ion trap mass spectrometer was used for the analysis and detection of the OCPs. The gas chromatograph was equipped with a Rtx-5 fused-silica capillary column (30 m x 0.25 mm i.d., 0.25 um film thickness) obtained from Restek. Helium (purity 99,99%) was the carrier gas at constant flow of 1 mL min^{-1}. The GC injector temperature was maintained at 280 °C. The oven program temperature was: initial temperature 120 °C increased by 5 °C min^{-1} to 280 °C and held for 10 min. The ion trap spectrometer was operated in electron ionisation (EI) mode.

The ionization energy was set at 70eV. The detector range was *m*/*z* 40-650. The transfer line and trap temperature were 250 °C and 170 °C respectively.

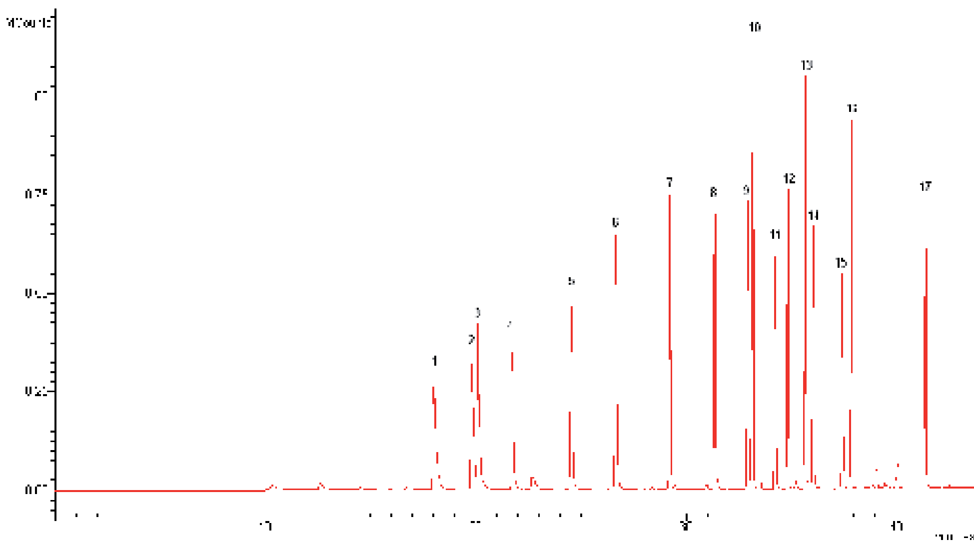

Figure 5. GC-MS chromatogram (TIC mode) of a standard OCPs mixture (MRL 0.5 mg kg^{-1}) used in the present study. 1:α-HCH, 2:β-HCH, 3:γ-HCH, 4:δ-HCH, 5:Heptachlor, 6:Aldrin, 7: Heptachlor epoxide, 8:Endosulfan I, 9:Dieldrin, 10:p-p'DDE, 11:Endrin, 12:Endosulfan II, 13:p-p'DDD, 14:Endrin Aldheyde, 15: Endosulfan Sulphate, 16:p-p'DDT, 17:Methoxychlor

4. Results and disussion

4.1. Key results about extraction and clean-up method

Two extraction and clean-up methods have been developed, tested and optimised for the extraction of 20 OCPs from animal feed sample and subcutaneous fatty tissue samples from bovine.

Large amounts of lipids were extracted when n-hexane or acetone was used as extraction solvents. In general, complex mixtures of of several types of lipids were co-extracted during the extraction of chlorinated pesticides from biological sample. Triglycerides and sterol esters are the major components in meat fats.

The key point of the extraction method take advantage of significant difference of melting points between lipids (below about 40 C) and chlorinated pesticides (above 260 C), so that lipid components can be easily separated from chlorinated compounds. After extraction, lipids in organic extracts were precipitated as frozen at -24 C in the freezer, while chlorinated compounds were still dissolved in cold organic solvents. Thus frozen-lipids can be removed just by filtering extracts. During overall process, approximately 90% of lipids were eliminated without any significant loss of pesticides. After freezing-lipid filtration, the remaining interferences were successfully removed by a solid-phase (SPE) Florisil cartridge.

Sample clean-up was necessary for the removal of polar coextracted substances. Florisil cartridges have been employed for that purpose since that adsorbent has proved to be very efficient for the clean-up of food samples.

4.2. Optimisation of MS/MS transitions

From full scan spectra, the most intense higher mass precursor ions were selected for development of MRM method. For the most of the analytes these were the base peak ions in the mass spectra, but in some cases higher mass ions of lower intensity were selected to minimise the possibility of matrix interferences. Precursor ions were examined using different collision energies (automated method development) and the most intense product ions were selected for each precursor ion. The products ions for all OCPs determined in this study are summarise in table 1.

For quantification of the target analytes linear calibration curves for all pesticides over six calibration levels (0.005 mg kg^{-1}-1.5 mg kg^{-1}) using a feed and fat blank samples were prepared taking also in consideration the MRLs levels for each compounds. In quantitative analysis one of the main problems is the suppression/enhancement of the analyte response caused by sample matrix components. Calibration curves were performed by using matrix-matched (in each matrix) because the feed and fat samples contain many compounds that are co-extracted in the extraction organic solvent. The use of Florisil-SPE tries to avoid matrix effect using a clean-up step, but this not eliminates completely the problem. A matrix effect on the analytical signal due to the matrix was noticed for most pesticides.

OCPs	R.T. (min)	Precursor ion (m/z)	Product ions (m/z)	Excitation voltage (V)	Linearity fat (r^2)	Linearity Feed (r^2)
α-BHC	18.01	181	109, 142	1.0	0,9997	0,9974
Hexachlorobenzene	18.41	286	214, 249	1.0	0,9974	0,9951
β-BHC	19.82	181	109, 145	1.0	0,9980	0,9950
γ-BHC	20.12	181	109, 145	1.0	0,9984	0,9985
δ-BHC	21.73	181	109, 145	1.0	0,9994	-
Heptachlor	24.54	272	100, 237	0.4	0,9987	0,9984
Aldrin	26.63	293	220, 255	0.8	0,9992	0,9951
Heptachlor epoxide	29.21	353	263, 334	0.7	0,9982	0,9977
γ-Clordane	30.66	375	266, 301	0.8	0,9944	0,9945
Endosulfan I	31.37	241	170, 260	0.9	0,9993	0,9978
α-Clordane	31.61	375	266, 301	0.8	0,9935	0,9910
Dieldrin	32.96	263	193, 228	0.7	0,9988	0,9979
p-p' DDE	33.16	318	246, 283	0.7	0,9974	0,9987
Endrin	34.23	263	193, 228	0.7	0,9982	0,9975
Endosulfan II	34.84	241	170, 260	0.9	0,9951	0,9973
p-p' DDD	35.66	235	165, 199	0.6	0,9958	0,9976
Endrin aldheyde	36.03	345	243, 279	0.7	0,9968	-
Endosulfan sulphate	37.43	387	251, 289	0.6	0,9988	0,9925
p-p' DDT	37.84	235	165, 199	0.6	0,9996	0,9971
Methoxychlor	41.04	227	196, 212	0.7	0,9982	0,9991

Table 1. Summary of precursor ions and products ions selected for analysis of OCPs n EI mode and linearity for fat and feed sample calibration curves.

The linearity of the curves was studied for each pesticide considering the area of the peak relative to the internal standard. The calibration data are given in table 2, showing a good linearity of the response for all pesticides at concentration within the interval tested.

LOD and LOQ were evaluated taking into account the baseline noise variations in the chromatogram obtained from the analysis of blank feed and blank fat samples (n=10). The LOD and LOQ were defined as the concentration of the analyte that produced a signal-to-noise ratio of 3 times and 10 times the standard deviation respectively above the blank signal. Table 2 shows the values in mg kg^{-1} of feed and fat sample calculated with blank sample extracts. The values are similar to those obtained by other authors for the LOD and LOQ in feed animal samples. LOD and LOQ values for subcutaneous fat sample are not present in literature. Our results are very similar to that obtained in fish muscle and meat.

	Subcutaneous fat tissue			Animal feed		
OCPs	MRL* (mg kg^{-1})	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	MRL** (mg kg^{-1})	LOD (mg kg^{-1})	LOQ (mg kg^{-1})
α-BHC	0.5	0.007	0.024	0.02	0.002	0.016
Hexachlorobenzene	0.2	0.007	0.026	0.01	0.003	0.011
β-BHC	0.1	0.012	0.041	0.01	0.003	0.010
γ-BHC	0.02	0.001	0.006	0.2	0.012	0.04
δ-BHC	0.5	0.007	0.023	0.02	-	-
Heptachlor	0.2	0.004	0.010	0.01	0.001	0.005
Aldrin	0.5	0.003	0.010	0.01	0.004	0.015
Heptachlor epoxide	0.2	0.002	0.008	0.01	0.002	0.009
γ-Clordane	0.05	0.005	0.019	0.02	0.003	0.013
Endosulfan I	0.05	0.003	0.012	0.1	0.007	0.024
α-Clordane	0.05	0.005	0.019	0.02	0.005	0.017
Dieldrin	0.2	0.002	0.008	0.01	0.002	0.007
p-p' DDE	1	0.001	0.005	0.05	0.003	0.012
Endrin	0.05	0.002	0.007	0.01	0.004	0.013
Endosulfan II	0.05	0.003	0.010	0.1	0.004	0.016
p-p' DDD	1	0.002	0.008	0.05	0.002	0.008
Endrin aldehyde	0.05	0.003	0.011	0.02	-	-
Endosulfan sulphate	0.05	0.002	0.006	0.1	0.007	0.023
p-p' DDT	1	0.004	0.001	0.05	0.006	0.02
Methoxychlor	0.01	0.002	0.008	0.5	0.002	0.007

*= MRLs of EU regulation guidelines (CE 32/2002); ** = MRLs of EU regulation guidelines (CE 396/2005)

Table 2. MRLs, limits of detection (LOD) and limits of quantification (LOQ) for OCPs in fat and feed samples.

4.3. Occurrence of OCPs in animal feed samples and subcutaneous fat samples

The OCPs residues may concentrate in the adipose tissue and in blood serum of animals leading to environmental persistence, bioconcentration and biomagnifications through the food chain. Pesticides contamination of meat as well as chicken resulting from feeding a diet containing a low concentration of pesticides is a well established fact. [63,64] OCPs residues in feed may be ingested bi herbivores and eventually find their way into the animal body which ultimately results in contamination of milk, meat eggs, etc. consumed by human being. [65,66]

The most pesticides detected in animal feed were p-p’ DDT, heptachlor followed by lindane, methoxychlor and aldrin. The frequency of detection is presented in figure. 6.

In subcutaneous fat sample the most detected OCPs were heptachlor, hexachlorobenzene detected in all samples followed by p-p′ DDE, p-p′DDT, methoxychlor, lindane and p-p′ DDD as shown in figure 7. Aldrin was detected both in feed samples and animal fat. The presence of aldrin in meat indicates the need for concern from the public health point of view because of its much higher toxicity than other OCPs. [67,68] These results are in accordance with other author that found HCHS and DDTs the most compounds detected in meat samples. In general, it was observed that the p-p′ isomers of DDE, DDT and DDDwere detected in samples. All detected pesticides in feed samples and fat samples did not exceed the MRLs established by the European Union for each compounds (Fig 8, 9). The concentration of detected pesticides in the samples are summarised in table 3.

	Fat samples		**Feed samples**	
OCPs	**mean**	**sd**	**mean**	**sd**
	(n=35)	(±)	(n=25)	(±)
Σ-Heptachlor	4.11	1.15	2.16	1.02
Σ-DDT	38.68	6.60	4.12	1.79
Σ-Aldrin	8.46	6.01	4.53	1.12
Σ-Endosulfan	9.30	1.36	nd	-
α-HCH	1.32	0.07	nd	-
β-HCH	3.07	0.69	nd	-
δ-HCH	5.67	1.51	nd	-
γ-HCH	11.27	1.21	5.17	1.29
Endrin	16.91	2.82	4.15	0.63
Endrin aldheyde	6.89	1.60	12.99	1.57
Methoxychlor	3.78	1.08	nd	-
Hexachlorobenzene	11.73	1.20	nd	-

nd= not detected; sd=standard deviation

Table 3. Mean organochlorine residues levels (μg kg^{-1}) in subcutaneous fat and feed samples

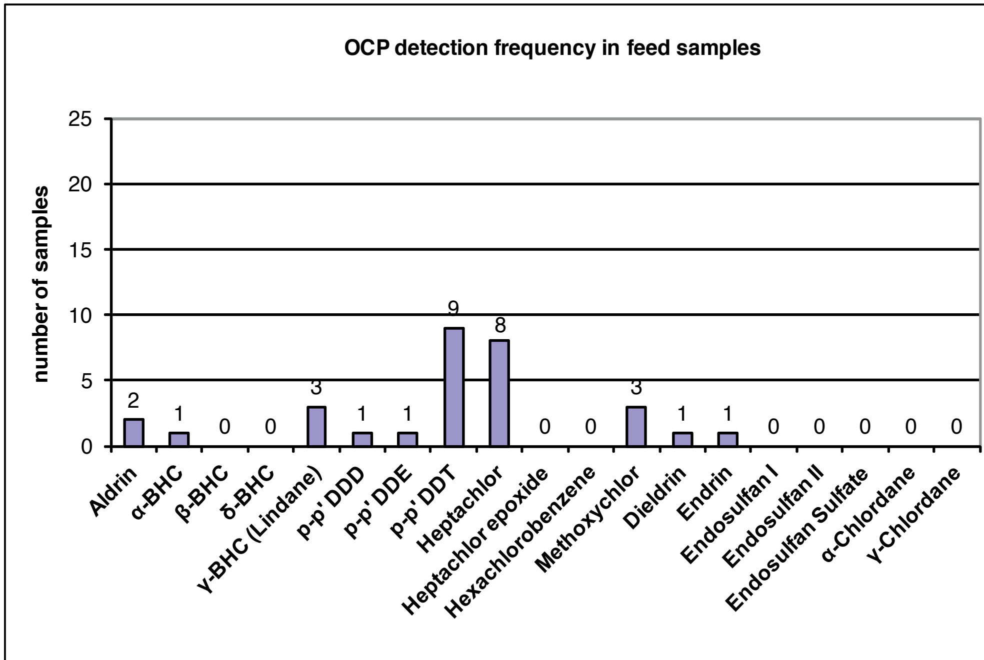

Figure 6. OCPs detection frequency in feed samples

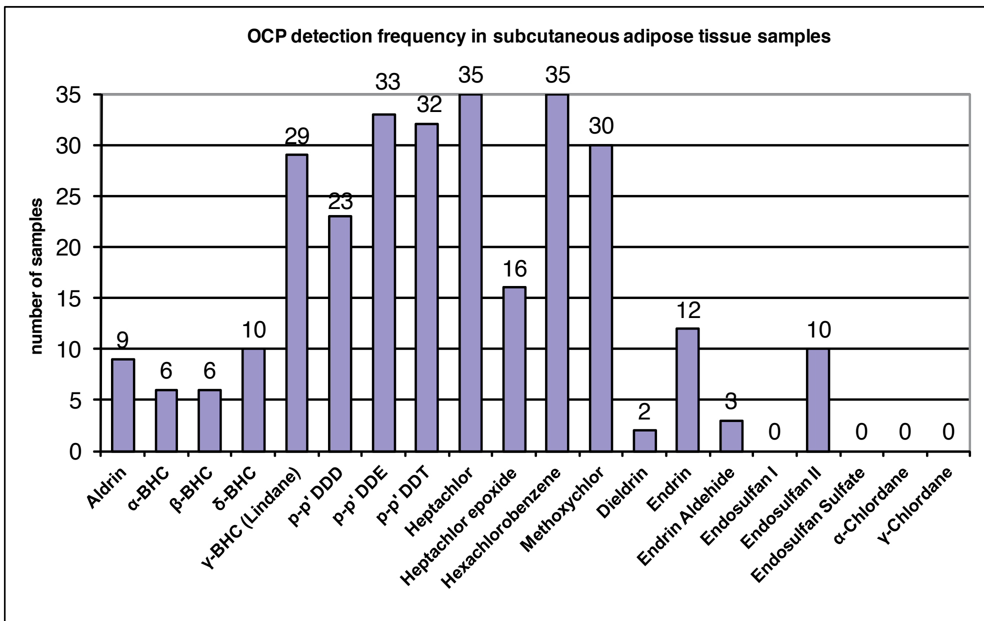

Figure 7. OCPs detection frequency in subcutaneous fat samples

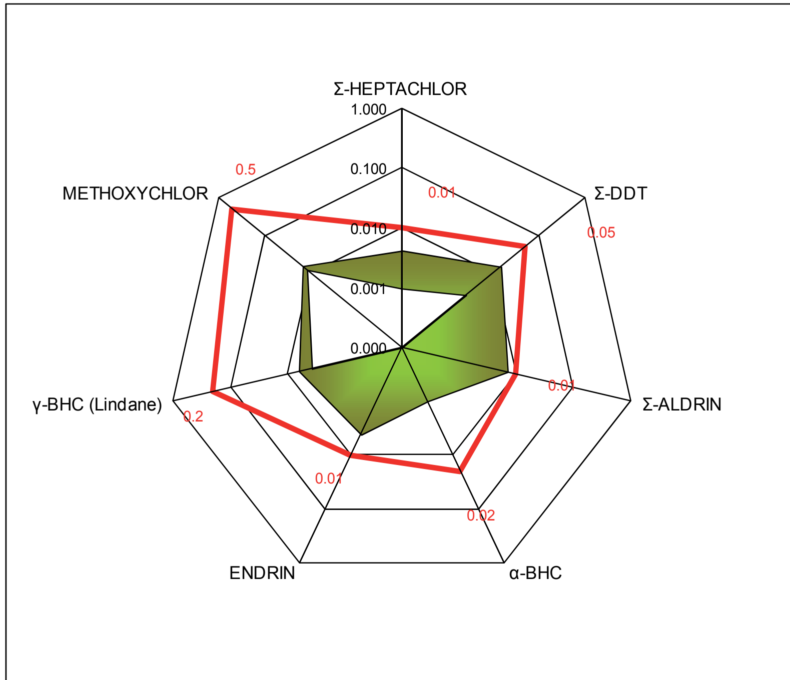

Figure 8. Radar plot of detected OCPs content in (mg kg $^{-1}$) feed samples in relation to MRLs (red line)

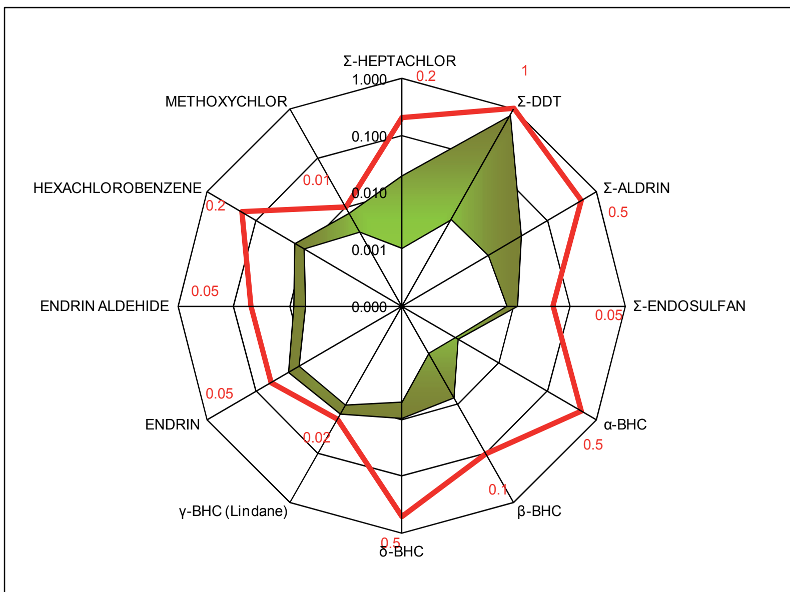

Figure 9. Radar plot of detected OCPs content (mg kg $^{-1}$) in subcutaneous fat tissue samples in relation to MRLs (red line)

In conclusion a rapid extraction, freezing lipid filtration and GC-MS/MS measurement methods were developed and used to measure chlorinated pesticide levels in animal feed sample and subcutaneous fatty tissue in order to assess the possible concentration phenomena of these persistent compounds. The freezing lipid filtration combined with Florisil-SPE cartridge enabled efficient removal of lipids extracted from feed and fat samples without significant loss of pesticides. Hence, the method offers a rapid and valid screening tool with high sensitivity for determination of organochlorine pesticides based on GC-MS/MS detection.

The subcutaneous fatty bovine tissue has been confirmed as target organ able to concentrate pesticides with lipophilic behaviour like organochlorine residues. The feed could also represent a possible source for contamination of OCPs through the food-chain. Therefore, the determination of pesticides residues in feed and food is today necessary for ensuring that human exposure to contaminants, especially by dietary intake, does not exceed acceptable levels for heath. One analytical challenge in the food safety is to present reliable results with respect to official guidelines.

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Section 4

Food Processing

Chapter 14

Enzymes in Bakery: Current and Future Trends

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Additional information is available at the end of the chapter

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1. Introduction

The use of enzymes dates from much longer than their ability to catalyze reactions was recognized and their chemical nature was known. The first completely enzymatic industrial process was developed in the years 1960 [1]. Starch processing, which is undertaken in two steps, involves liquefaction of the polysaccharide using bacterial α-amylase, followed by saccharification catalyzed by fungal glucoamylase.

After the Second World War, enzyme applications rose due to advances in industrial microbiology and biochemical engineering [1]. Nowadays, enzymes are employed in many different areas such as food, feed, detergent, textiles, laundry, tanning, as well as pharmaceuticals, cosmetics, and fine-chemicals industries. Industrial applications account for over 80% of the global market of enzymes [2]. At least 50% of the enzymes marketed today are obtained from genetically modified organisms, employing genetic and protein engineering. Food enzymes are the most widely used and still represent the major share in enzyme market.

Developments in process technology allied to the use of recombinant techniques during the last decades allowed for considerably improved yields by fermentation, increased stability, and altered specificity and selectivity of enzymes [3-5]. Those techniques thrust forward and are continuing to broaden the applications of enzymes in food technology and many different areas.

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There are two scenarios regarding the use of enzymes, either the enzymes are used to convert the raw material into the main product, or the enzymes are used as additives to alter a functional characteristic of the product. In the first case, the enzymatic process is undertaken in optimized and controlled conditions to enhance the catalytic potential of the enzyme, whereas in the second situation it is more difficult to assure optimal conditions and to control the enzymatic reaction [1]. An example of the first case is the use of immobilized glucose isomerase for the production of high-fructose syrups (HFS), and an example of the second scenario is the use of fungal proteases in dough making [1,6,7].

Enzymes are an important ingredient used in most bakery products. More recently enzymes have assumed an even greater importance in baking, due to the restrictions on the use of chemical additives, especially in the manufacture of bread and other fermented products [8].

The aim of this review is to discuss current applications of enzymes in the bakery industry and to explore future trends in this sector of food industry.

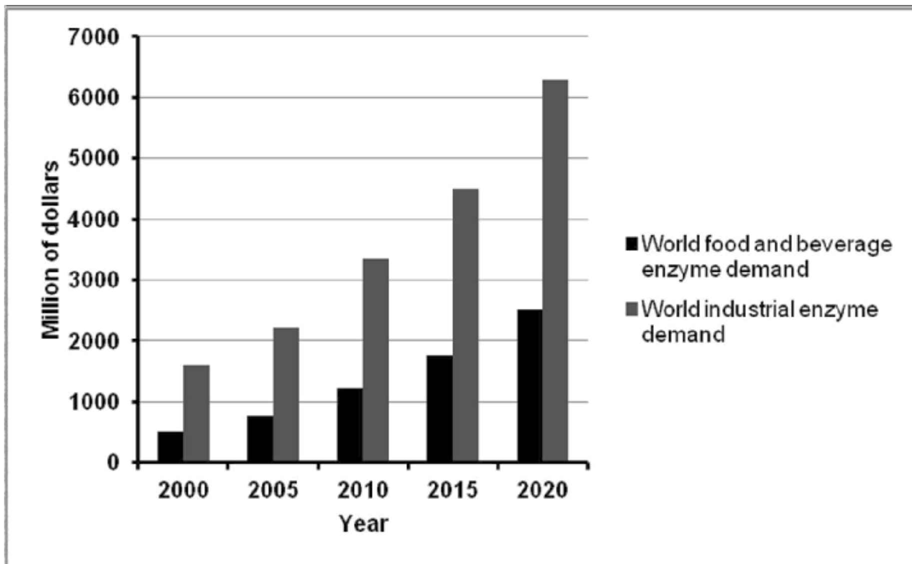
2. Bakery enzymes market

The development of bread process was an important event in mankind. After the 19th century, with the agricultural mechanization, bread's quality was increased while its price was reduced; thereby white bread became a commodity within almost everyone´s reach [9]. An important aspect that contributed to evolution of the baking market was the introduction of industrial enzymes in the baking process, where bakery enzymes represent a relevant segment of the industry.

Among the main industrial enzyme producers, according to Novozymes S/A report 2011 [10], Novozymes S/A occupies 47% of the market, DuPont 21%, DSM 6% and the rest is occupied by other players. Furthermore, in that year, food and beverage enzymes represented 29% of enzyme business and biobusiness sales by the industry [10].

The world enzyme market is in evolution and a growth of 6.8% per year is expected [11]. The world food and beverage enzymes demand requires attention, because it represented $1,220 million dollars in 2010, around 36.5% of the total world industrial enzyme demand, estimated in $3,345 million dollars. Moreover, the world food and beverages enzymes demand is expected to be responsible for 40.1% of the world industrial enzyme demand in 2020, accounting for $2,520 of $6,280 million dollars of the world industrial enzyme market (Figure 1) [11].

Table 1 summarizes the world bakery and enzyme demand between 2000 and 2020, segmented according to products. It is possible to observe that the enzymes market for baked goods is expected to increase from 420 million dollars in 2010 to 900 million dollars in 2020, although maintaining its representativeness in this segment, varying from 34.4 in 2010 to 35.7% in 2020 [11].



Source: Adapted from The Freedonia Group Inc., World Enzymes to 2015.

Figure 1. Estimated world food and beverage enzyme demand participation on the world industrial enzymes in million dollars from 2000 to 2020.

Item	Years				
	2000	2005	2010	2015	2020
World food and beverage enzyme demand	520	760	1220	1770	2520
Baked goods	140	250	420	625	900
Dairy	180	260	360	465	610
Other foods and beverage	200	250	440	680	1010

Source: Adapted from The Freedonia Group Inc., World Enzymes to 2015.

Table 1. Estimated demand of baked goods, dairy and other food & beverage enzymes in million dollars from 2000 to 2020.

3. Main constituents of baked products

Baking is a common name for the production of baked goods, such as bread, cake, pastries, biscuits, crackers, cookies, pies and tortillas, where wheat flour is both the most essential ingredient and key source of enzyme substrates for the product [12]. Even though based on cereals other than wheat, baked goods such as gluten-free products or rye bread are also considered to be baked products [8]. Baked goods formulations vary significantly depending on the desired

final product, and typical ingredients, apart from starch, can include wheat flour (8-16% protein, 71-79% carbohydrate), fats, sugars, eggs, emulsifiers, milk and/or water [13].

Bread is usually made from wheat flour as raw material, which is a mixture of starch, gluten, lipids, non-starch polysaccharides and enzymes. After flour, yeast and water are mixed, complex biochemical and biophysical processes begin, catalyzed by the wheat enzymes and by the yeast, characterizing the dough phase. These processes go on in the baking phase, giving rise to bread. Extra enzymes added to the dough improve control of the baking process, allowing the use of different baking processes, reducing process time, slowing-down staling, compensating for flour variability and substituting chemical additives [14]. Starch is the main component of products such as bread and other bakery goods and is added to different foods, acting as a thickener, water binder, emulsion stabilizer, gelling agent and fat substitute [15]. It is the most abundant constituent and most important reserve polysaccharide of many plants, including cereals, occurring as intracellular, semi-crystalline granules. On a molecular level, its major components are the glucose polymers amylose and amylopectin [16]. Amylose is an essentially linear molecule, consisting of up to 6000 glucose units with α-(1,4)-glycosidic bonds (Figure 2). On the other hand, amylopectin is a highly branched polysaccharide constituted of short α-1,4 linked linear chains of 10–60 glucose units and α-1,6 linked side chains with 15–45 glucose units (Figure 3), containing on average 2 million glucose units [17].

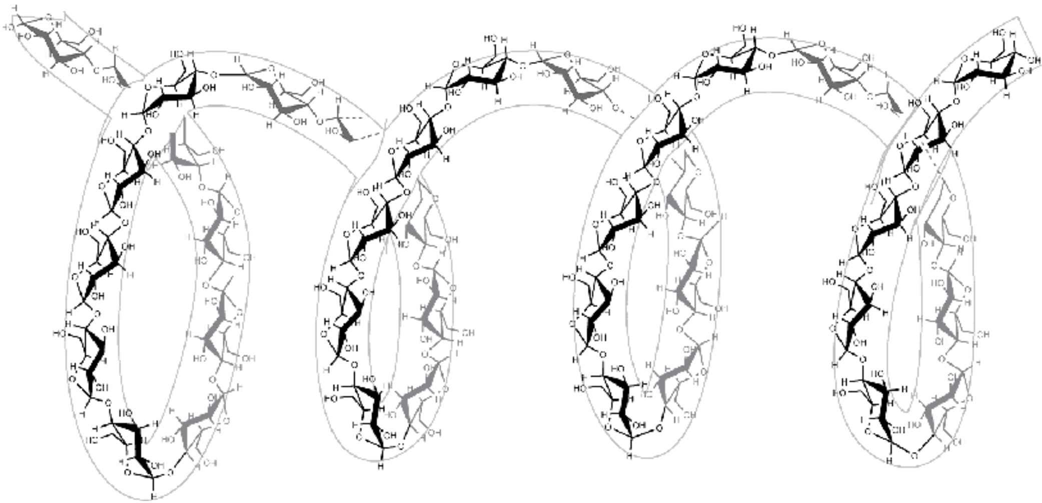


Figure 2. Structure of amylose chain, assumed as a left-handed spiral due to α(1→4) glycosidic bonds (n = 500 - 6000 α-D-glucopyranosyl units).

Even though many flour components such as starch, arabinoxylans and lipids affect dough rheological properties [16,18-20], gluten provides dough with extensibility, viscosity, elasticity, cohesiveness and contributes to its water absorption capacity [21]. The unique ability of wheat flour to form visco-elastic dough with gas-holding properties is mostly due to the gluten proteins, the major storage proteins of wheat, which have an essential role in breadmaking [22].

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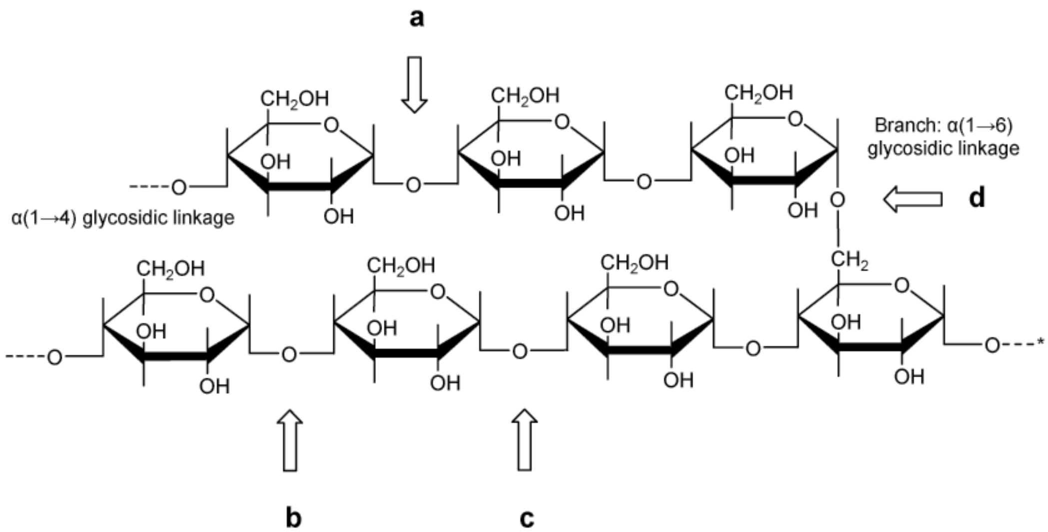

Figure 3. Partial structure of amylopectin with amylolytic enzymes action sites represented by arrows: (a) α-amylases; (b) amyloglucosidases; (c) β-amylases; (d) isoamylases and pullulanases. Both α(1→4) glycosidic linkages between the glucose units in the linear chain and one α(1→6) glycosidic linkage to a side chain of the polysaccharide are represented.

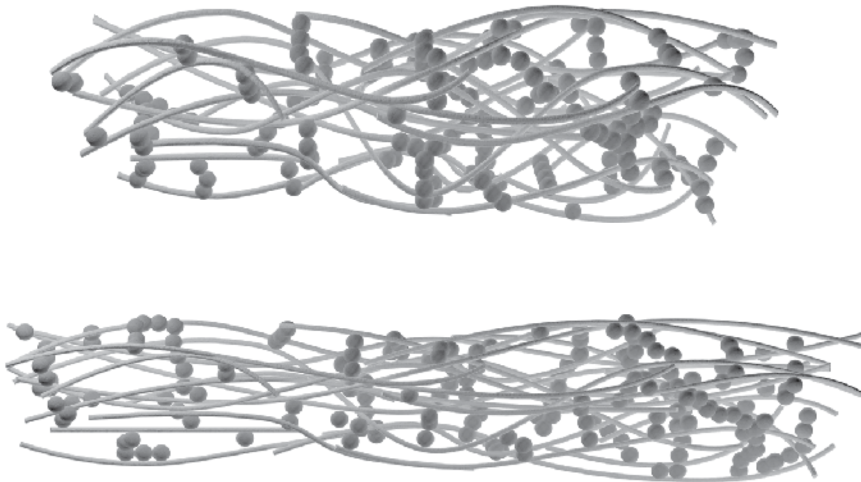

Figure 4. Schematic drawing of gluten proteins structure, where gliadins are represented by spheres and glutenins by filaments. The bulkier structure in the upper part shows the gas retained in the gluten network and consequent dough volume expansion observed in the baking process. The slimmer structure in the lower part represents plasticity, extensibility and viscous properties of the gluten matrix.

Gluten proteins can be divided into monomeric gliadins and polymeric glutenins, based on solubility in 70% aqueous ethanol solutions [23]. Gliadins are globular proteins with molec-

ular weights ranging from 30,000 to 80,000, and are further classified into three groups: α-, γ- and ω-gliadins [24,25]. Except for the ω-gliadins which lack cysteine residues, gliadins contain intramolecular disulfide bonds [21]. Glutenins consist of a heterogeneous mixture of linear polymers with a broad molecular weight range from ca. 80,000 up into the millions [22], made up of disulfide cross-linked glutenin subunits which are biochemically related to the gliadins. The intermolecular disulfide bonds stabilize the glutenin polymers [21]. Gliadins mainly impart the plasticity, extensibility and viscous properties to wheat flour dough whereas glutenins are mostly responsible for the elasticity and cohesive strength of dough (Figure 4) [21,22]. Aspects such as the glutenin polymer structure, size distribution and subunit composition, as well as the gliadin/glutenin ratio are important to determine gluten quality and, consequently, the breadmaking potential of wheat flour [25-29].

Cereal non-starch polysaccharides are dietary fibre constituents, mostly composed of arabinoxylans, β-glucan and arabinogalactan-peptides. Arabinoxylans make up the largest non-starch polysaccharide fraction of cell walls of many cereals, such as wheat and rye [22,30]. They are polydisperse polyssacharides with similar structural properties, which are present in water-extractable (WE-AX) and water-unextractable (WN-AX) forms [16]. Arabinoxylans consist of a β-1,4 linked D-xylopyranosyl backbone substituted with α-L-arabinofuranose residues at the C(O)3 and/or C(O)2 positions [31-33]. Arabinose residues can be further coupled at the C(O)5 to ferulic acid through an ester linkage [34] (Figure 5). Even though minor flour constituents, arabinoxylans have the capacity to significantly affect the properties of dough and the final baked product [18]. Arabinoxylans and arabinogalactans possess important functional properties for the cereal industry. They can improve dough development and dough stability, by enhancing the water absorption capacity of the dough. These polysaccharides also confer viscosity and may increase gas permeability by contributing to the elasticity of the protein film around them. Additionally, during breadmaking they improve loaf volume, crumb firmness, reduce retrogradation and therefore, enhance the shelf life and storage stability of bread [35].

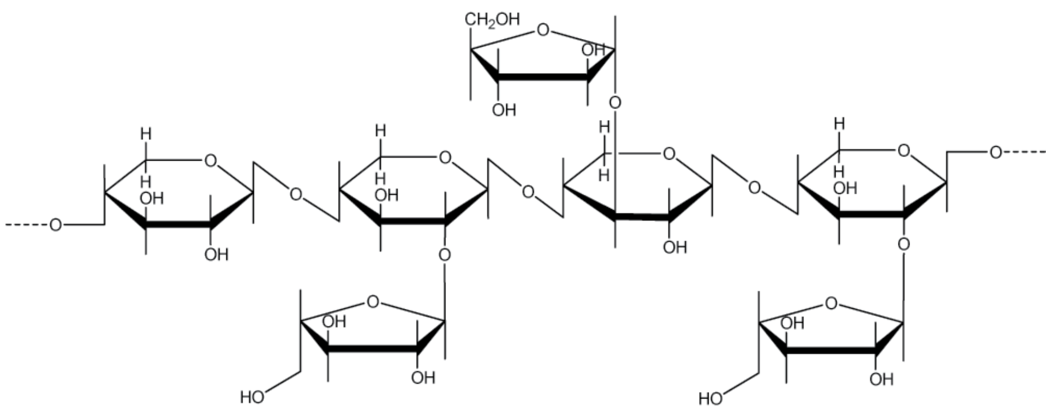

Figure 5. Partial structure of an arabinoxylan: a linear main chain formed by xylan (a pentosan consisting of D-xylose units connected by β(1→4) linkages), randomly attached to L-arabinofuranose residues by α(1→3) or α(1→2) linkages.

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In addition to starch, gluten proteins and wheat flour non-starch polysaccharides such as arabinoxylans, lipids and enzymes can considerably improve the breadmaking performance [16,18,22,36,37]. Lipids are important components in breadmaking because they provide a variety of beneficial properties during processing and storage. In bread, lipids come from multiple ingredients, largely wheat flour, shortening and surfactants in a typical bread formula [38]. Wheat flour contains about 2% lipids [23], which occur free and bound to other wheat constituents. They are classified as starch lipids and free and bound non-starch lipids, based on their solubility in solvents of different polarities [39]. The bound non-starch lipids are mainly associated with flour protein and consist predominantly of non-polar lipids, while free non-starch lipids comprise mostly polar glyco- and phospholipids [40].

4. Baking process

Bread processing can be divided into three basic operations mixing, fermentation (resting and proofing) and baking. Through baking the mainly fluid dough or batter is transformed into a predominantly solid baked product. Indirectly, baking alters the sensory properties, improving palatability, and extending the range of tastes, aromas and textures of foods produced from raw materials [41].

Although baking has been practiced for a very long time, the whole process is not completely understood, possibly due to the occurrence of several coupled complex physical [42] and molecular processes [43]. The baking process therefore results in a series of physical, chemical and biochemical changes in the product. These changes include volume expansion, evaporation of water, formation of a porous structure, denaturation of protein, gelatinization of starch, crust formation and browning reactions [44].

Bread consists of an unstable, elastic, solid foam structure, containing a continuous phase made up of an elastic network of cross-linked gluten protein molecules and of leached starch polymer molecules, mainly amylose, uncomplexed and complexed with polar lipid molecules, and also a discontinuous phase of entrapped, gelatinised, swollen, deformed starch granules [45]. The nature and properties of the final product are influenced by physical and mechanical mixing, chemical reactions (including enzyme-catalyzed reactions), and thermal effects (baking time and temperature).

The simplest breadmaking procedure is a straight-dough system where all bread formula ingredients are mixed into developed dough [46]. A second process is the sponge and dough method where mixing of ingredients is performed in two steps. Leavening agent is prepared in the first step, by mixing together the yeast and certain quantity of water and flour. The mixture is left to develop for a few hours and then it is mixed with the other ingredients [42]. A third procedure is the Chorleywood method in which all the ingredients are mixed for a few minutes in an ultrahigh mixer [47].

In conventional breadmaking, the most commonly used leavening agent is the yeast *Saccharomyces cerevisiae*, although other *Saccharomyces* species such as *S. cariocanus*, *S. mikatae, S. para-*

doxus and *S. kudriavzevii* can be also employed [48]. Furthermore, lactic acid bacteria, mainly *Lactobacillus* species are used as leavening agents for sourdough bread production [49].

The breadmaking process begins with the formation of dough through mixing of flour, water, yeast, sugar, salt, shortening and other ingredients. Flour particles are hydrated and sheared during mixing, and dough develops when gluten proteins form a continuous cohesive network in which the starch granules are dispersed [40]. Depolymerisation and polymerisation reactions possibly give rise to the gluten network, mostly made up of glutenin [50]. Incorporation of air during dough mixing is extremely important, affecting the final crumb structure because the carbon dioxide produced by yeast during fermentation diffuses to pre-existing air bubbles [40,51]. An optimal gluten network confers dough machinability, good gas retention, high bread volume and fine crumb structure [29]. After resting, the dough is divided into loaf-sized pieces, rounded, moulded, placed on a baking tray, proofed and baked.

The combined effects of heat, moisture and time induce starch gelatinisation and pasting which together with heatsetting of gluten proteins occur during baking, giving rise to the typical solid foam structure of baked bread [22]. The partially crystalline starch is converted into amorphous, transient, gelatinised starch networks. The swollen gelatinised starch granules are deformed, part of the starch polymers leach out of the granules and form a continuous network in the bread crumb [40,52]. Besides accumulation of amylose outside the granules, the presence of an amylose-rich region in the centre of gelatinised starch granules was found after baking [22,52].

During baking the transient gluten network formed in dough is transformed into a continuous, permanent network probably due to modifications in protein surface hydrophobicity, sulfhydryl/disulfide interchanges and formation of new disulfide cross-links [22,38,50,53]. Moreover, heat-induced sulfhydryl-disulfide exchange reactions can lead to incorporation of α- and γ-gliadins into the glutenin network [54]. Gas cell opening occurs, and besides becoming gluten continuous the bread is also gas-continuous [38,40].

Macroscopic changes during baking include further expansion of the dough and crust formation and browning [40]. The oven spring is due to continued production of carbon dioxide by yeast, its expansion by heating and vaporisation of ethanol and water. The bread bakes from the outside to the inside, resulting in a baked crumb [38].

The crust browning is directly related to the reducing sugars (glucose, fructose, maltose, etc.) formed by hydrolysis of starch and complex sugars of the flour, during dough making and leavening. Under heating, the sugars can undergo caramelisation, and/or the reducing sugars can react with the free amino acid groups of proteins in the Maillard reaction [54,55]. Besides, different flavour compounds are produced, giving bread its appealing smell and taste [55].

Additional interactions between biopolymers in the bread crumb occur during cooling. Amylose chains form helices, self-associate and crystallise [22,52,57]. Moreover, amylose may form more inclusion complexes with polar lipids. As a consequence, a permanent and in part crystalline amylose network is formed, providing a soft crumb in fresh bread. The gluten network organized during baking and the amylose network developed while cooling thus account for the plasticity of freshly baked bread [22].

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Fresh bread consists mainly of a continuous gluten network, which forms a compressed matrix between the swollen, gelatinised starch granules, and the starch network, consisting of entangled, gelatinised starch polymers [22]. It usually presents an appealing brownish and crunchy crust, a pleasant aroma, fine slicing characteristics, a soft and elastic crumb texture, and a moist mouthfeel [47]. However, when a loaf of bread is removed from the oven after baking, a series of undesirable changes called staling starts, eventually leading to deterioration of quality [46].

Staling implies a relatively short shelf life for fresh bakery products. The loss of freshness is paralleled by an increase in crumb firmness and a decrease in flavour and aroma, leading to loss of consumer acceptance. Loss of moisture and starch retrogradation are accepted as two of the basic mechanisms in the firming of the crumb [58]. This subject has been extensively reviewed and discussed in [16,22,45]. In this context, mechanization, large scale production and increase in consumer demand for consistent product quality and longer shelf life of baked goods have led to the use of a wide range of additives (bread improvers) in the baking industry, which include emulsifiers, soy flour, chemical redox agents and enzymes [29,42,59].

5. Enzymes used in baked products

Baking comprises the use of enzymes from three sources: the endogenous enzymes in flour, enzymes associated with the metabolic activity of the dominant microorganisms and exogenous enzymes which are added in the dough [60].

The supplementation of flour and dough with enzyme improvers is a usual practice for flour standardization and also as baking aids. Enzymes are usually added to modify dough rheology, gas retention and crumb softness in bread manufacture, to modify dough rheology in the manufacture of pastry and biscuits, to change product softness in cake making and to reduce acrylamide formation in bakery products [8]. The enzymes can be added individually or in complex mixtures, which may act in a synergistic way in the production of baked goods [60-62], and their levels are usually very low.

5.1. Hydrolases

Enzymes as technological aids are usually added to flour, during the mixing step of the breadmaking process. The enzymes most frequently used in breadmaking are the α-amylases from different origins [63].

5.1.1. Amylases and other starch-converting enzymes

The industrial processing of starch is usually started by α-amylases (α-1,4-glucanohydrolase). Most of the starch-converting enzymes belong to the α-amylase family or family 13 glycosyl hydrolases (GH), based on amino acid sequence and structural similarities [64,65,66,67].

α-Amylases (EC 3.2.1.1) are endoenzymes that catalyze the cleavage of α-1,4-glycosidic bonds in the inner part of the amylose or amylopectin chain. The end products of α-amylase action are oligosaccharides, with an α-configuration and varying lengths, and α-limit dextrins, which are branched oligosaccharides [17]. These enzymes can be obtained from cereal, fungal, bacterial and biotechnologically altered bacterial sources. Differences in the number of binding sites and location of catalytic regions determine substrate specificity of α-amylases, the length of the oligosaccharide fragments released after hydrolysis and, consequently, the carbohydrate profile of the final product. The different forms of α-amylases also have diverse thermal stability profiles [15].

Also part of the GH13 family are the exoenzymes maltogenic α-amylase (glucan 1,4-α-glucanhydrolase, EC 3.2.1.133) and other maltooligosaccharide forming amylases (EC 3.2.1.60, for instance). While maltogenic α-amylase mainly releases maltose from starch, maltooligosaccharide producing amylases give rise to maltotetraose or maltohexaose, among others. On the other hand, debranching enzymes, such as pullulanase (EC 3.2.1.41) and isoamylase (EC 3.2.1.68), grouped as well in the GH13 family, hydrolyse α-(1,6)-bonds removing the side-chains from amylopectin [16,17].

β-Amylases (EC 3.2.1.2) and glucoamylases (EC 3.2.1.3) are encompassed in the GH14 and GH15 families, respectively. Both are exoamylases that employ the inverting mechanism to cleave α-glycosidic bonds at the non-reducing ends of amylose and amylopectin, producing low molecular weight carbohydrates in the β-anomeric form [15,68]. β-Amylases are unable to cleave α-1,6-linkages and the final products consist of maltose and β-limit dextrin. Therefore hydrolysis of amylopectin is incomplete, resulting in only 50-60% conversion to maltose. In the case of amylose, the maximum degree of hydrolysis is 75-90% due to the slightly branched structure of this polysaccharide [15]. On the other hand, glucoamylase has a limited activity on α-1,6-linkages and would possibly be able to catalyse total conversion of starch into β-glucose [16].

Malt and microbial α-amylases have been widely used in the baking industry. The malt preparation led the way for the commercial use of many other enzymes in baking [69]. Fungal α-amylases or malt are usually added to optimize amylase activity of the flour, initially aiming to increase the levels of fermentable and reducing sugars. In view of their lower thermostability, fungal α-amylases are more appropriate than malt amylases for flour standardization. The α- and β-amylases have different but complementary functions during the breadmaking process [70]. The supplemented α-amylases break down damaged starch particles into low molecular weight dextrins during the dough stage, while endogenous β-amylase converts these oligosaccharides into maltose which is used as fermentable sugar by the yeast or sourdough microorganisms [15,16]. The increased levels of reducing sugars lead to the formation of Maillard reaction products, intensifying bread flavour and crust colour. In addition, these enzymes can improve the gas-retention properties of fermented dough and reduce dough viscosity during starch gelatinization, with consequent improvements in product volume and softness [8,22,71].

Certain amylases are able to decrease the firming rate of bread crumb, acting as anti-staling agents. Amylase-containing anti-staling products typically consist of bacterial or fungal α-

amylases with intermediate thermostability [16,22]. In this context, one of the most effective anti-staling amylases is the *Bacillus stearothermophilus* maltogenic α-amylase [22]. The anti-staling action of amylases has been attributed to the modified retrogradation behaviour of the hydrolysed starch [72-74]. Yet, other researchers ascribe the effect to the interference of the low molecular weight dextrins with starch-starch and/or gluten-starch interactions [74-76].

5.1.2. Proteases

Proteases can be subdivided into two major groups according to their site of action: exopeptidases and endopeptidases. Exopeptidases cleave the peptide bond proximal to the amino or carboxy termini of the substrate, whereas endopeptidases cleave peptide bonds distant from the termini of the substrate [77]. Most of the proteolytic activity of wheat and rye flours corresponds to aspartic proteases and carboxypeptidases, which are both active in acid pH. Additionally, aspartic proteases of wheat are partly associated with gluten [78]. Nevertheless, the proteolytic activity of sound, ungerminated grain is normally low [79].

Proteases are used on a large commercial scale in the production of bread, baked goods, crackers and waffles [80]. These enzymes can be added to reduce mixing time, to decrease dough consistency, to assure dough uniformity, to regulate gluten strength in bread, to control bread texture and to improve flavour [16,60]. In addition, proteases have largely replaced bisulfite, which was previously used to control consistency through reduction of gluten protein disulfide bonds, while proteolysis breaks down peptide bonds. In both cases, the final effect is a similar weakening of the gluten network [79].

In bread production, a fungal acid protease is used to modify mixtures containing high gluten content. When proteases are mixed in the blend, it undergoes partial hydrolysis becoming soft and easy to pull and knead [7,60]. Proteases are also frequently added to dough preparations. These enzymes have great impact on dough rheology and the quality of bread possibly due to effects on the gluten network or on gliadin [7].

Proteases are also applied in the manufacture of pastries, biscuits and cookies. They act on the proteins of wheat flour, reducing gluten elasticity and therefore reducing shrinkage of dough or paste after moulding and sheeting [8,81]; for instance, hydrolysis of glutenin proteins, which are responsible for the elasticity of dough, has considerable improving effects on the spread ratio of cookies [81].

5.1.3. Hemicellulases

Hemicellulases are a diverse class of enzymes that hydrolyse hemicelluloses, a group of polysaccharides comprising xylan, xylobiose, arabinoxylan and arabinogalactan [82]. This group includes xylanase or endo-1,4-β-xylanase (4-β-D-xylan xylanohydrolase, EC 3.2.1.8), a glycosidase that catalyses the endohydrolysis of 1,4-β-D-xylosidic linkages in xylan and arabinoxylan.

Xylanase, also designated endoxylanase, was originally termed pentosanase [83]. A wide variety of xylanases have been reported from a plethora of microorganisms including bacteria,

archaea and fungi [84]. These enzymes are mainly classified in the glycosyl hydrolase (GH) families 10 and 11 [16,64,65], although putative xylanase activities have been reported in GH families 5, 7, 8 and 43 [84,85]. GH10 xylanases are regarded to have broader substrate specificity and release shorter fragments compared to GH11 xylanases, while the latter enzymes are more susceptible to steric hindrance by arabinose substituents [86,87]. In addition, different endogenous xylanase inhibitors occur in cereals: *Triticum aestivum* L. xylanase inhibitor (TAXI) [88,89], xylanase inhibitor proteins (XIP-type inhibitors) [90] and TLXI-type (thaumatin-like endoxylanase inhibitors) [91].

The complete hydrolysis of arabinoxylans requires the concerted action of different enzymes. The xylan backbone will be cleaved randomly by endo-1,4-β-xylanases, the main arabinoxylan hydrolysing enzymes, yielding arabinoxylo-oligosaccharides. β-D-xylosidases (EC 3.2.1.37) cleave xylose monomers from the non-reducing end of arabinoxylo-oligosaccharides. The arabinose residues are removed by α-L-arabinofuranosidases (EC 3.2.1.55), while ferulic acid esterases (EC 3.1.1.73) cleave ester linkages between arabinose residues and ferulic acid [30,83].

Xylanases were introduced to the baking segment in the years 1970 and are most often used combined with amylases, lipases and many oxidoreductases to attain specific effects on the rheological properties of dough and organoleptic properties of bread [85]. These enzymes have also been used to improve the quality of biscuits, cakes and other baked products [71].

The most favourable xylanases for breadmaking are those that preferentially act on WU-AX and are poorly active on WE-AX, because they remove the insoluble arabinoxylans which interfere with the formation of the gluten network, giving rise to high molecular weight solubilised arabinoxylans, resulting in increased viscosity and thus enhancing dough stability [92-94]. As a consequence, a more stable, flexible and easy to handle dough is obtained, resulting in improved oven spring, larger loaf volume, as well as a softer crumb with improved structure [43]. Moreover, the addition of xylanases during dough processing is expected to increase the concentration of arabinoxylo-oligosaccharides in bread, which have beneficial effects on human health [95].

The potential of GH family 8 xylanases as technological aids in baking was shown for a psychrophilic enzyme from *Pseudoalteromonas haloplanktis* and a mesophilic enzyme from *Bacillus halodurans*. Although both enzymes had a positive effect on loaf volume, psychrophilic GH8 xylanase was apparently much more efficient than the mesophilic enzyme from the same family, because much lower concentrations of the former enzyme were required to produce a similar increase in bread volume. Additionally, a psychrophilic GH10 xylanase from *Cryptococcus adeliae* was found to be ineffective [85].

Recently, a purified GH11 xylanase from *Penicillium occitanis* was evaluated as an additive during mixing of wheat flours. Significant improvements of bread characteristics, including higher final moisture content, volume and specific volume, were observed. Enhancements in sensory and textural properties were also obtained [96].

5.1.4. Lipases

Lipases (EC 3.1.13) or triacylglycerol acylhydrolases hydrolyse triacylglycerols (TAG) producing monoacylglycerols (MAG), diacylglycerols (DAG), glycerol and free fatty acids. These enzymes are widely found in nature [97]. Besides TAG lipases there are phospholipases A1 (EC 3.1.1.32), A2 (EC 3.1.1.4), C (EC 3.1.4.3), D (EC 3.1.4.4) and galactolipases (EC 3.1.1.26). Even though they are present in all cereal grains; lipase activity of white flour is usually low enough to avoid rancidity due to hydrolysis of native lipids and of baking fat [71,79,98].

The use of lipases in the baking segment is much more recent in comparison to α-amylases and proteases. The first generation of commercial lipase preparations was introduced to the market in the years 1990 and recently a third generation became available [59]. The latter are protein engineered enzymes, claimed to give a better effect in high speed mixing and no-time dough processes. Moreover, third generation lipases have lower affinity for short chain fatty acids, which reduces the risk for off-flavour formation on account of prolonged storage of the baked goods and the use of butter or milk fat in baked products [12].

Lipases (TAG lipases) of the first generation are 1,3-specific, removing preferentially fatty acids from positions 1 and 3 in TAG. These enzymes can improve dough rheology, increase dough strength and stability, thus improving dough machinability [62,99,100]. In addition, lipases lead to an increase in volume which results in an improved, more uniform crumb structure; hence a softer crumb is obtained [99].

The second generation lipases act simultaneously on TAG, diacylgalactolipids and phospholipids, producing more polar lipids, providing a greater increase in volume, better stability to mechanical stress on the dough, and a fine, uniform bread crumb structure compared to the first generation lipases [43,59,101]. Moreover, a third generation lipase was found to increase expansion of the gluten network, increase the wall thickness and reduce cell density, enhancing volume and crumb structure of high fibre white bread [102].

The surface active properties of the hydrolysis reaction products (MAG, DAG, monoacylgalactolipids and lysophospholipids), along with modifications on the interactions between lipids and gluten proteins caused by the lipases, as well as the effect of these enzymes on the incorporation of air during mixing are possible mechanisms by which they affect bread volume [101]. In this context, the roles of lipids and surfactants in breadmaking have been extensively reviewed elsewhere [38,45].

The addition of lipases has been claimed to retard the rate of staling in baked products [8,103,104]. The effect of these enzymes has been attributed to *in situ* production of MAG following TAG hydrolysis, although this mechanism is not completely accepted because the amount of MAG would be insufficient to account for the antistaling effect [45,99]. Lipases may also be used for the development of particular flavors in bakery products [100].

The effect of a third generation lipase on the quality of high-fibre enriched brewer's spent grain breads has been evaluated. The enzyme produced beneficial effects during bread making, positively affecting loaf volume, staling rate and crumb structure [102].

A recent study compared three generations of lipase enzymes with the emulsifier, diacetyl tartaric esters of monoglycerides (DATEM), on white wheat flour bread. Lipases and DATEM improved most aspects of bread quality. In shorter fermentation times, DATEM, a second generation (Lipopan F-BG) and a third generation (Lipopan Xtra-BG) lipase were more effective. In longer fermentations, unlike the third generation lipase (Lipopan Xtra-BG), moderate amounts of the second generation lipase (Lipopan F-BG) significantly increased the bread volume [59].

The application of lipase and MAG to produce fiber enriched pan bread using the straight dough method was assessed. The use of lipase dosages up to 50 ppm and MAG up to 2% indicated the possibility of replacement of MAG by lipases in fiber enriched pan bread [105].

Recently, the effects of two lipases and DATEM on the rheological and thermal properties of white and whole wheat flour doughs were compared. Lipases were able to cause modifications in the dough components (gluten proteins and starch). The enzymes improved dough handling properties to a similar or greater extent than DATEM, increasing dough stability, maximum resistance to extension and hardness, and decreasing softening degree and stickiness. The possible role of lipases in delaying starch retrogradation was indicated by the greater extent of formation of amylose-lipid complexes promoted by lipases in comparison to DATEM [106].

5.2. Oxidoreductases

5.2.1. Lipoxygenases

Lipoxygenase (linoleate oxygen oxidoreductase, EC 1.13.11.12) is a non-heme iron-containing dioxygenase, found in a wide variety of plant and animal tissues, which with molecular oxygen catalyses the oxidation of polyunsaturated fatty acids (PUFA) containing a *cis,cis*-1,4-pentadiene system, such as linoleic or linolenic acid, to form fatty acid hydroperoxides [107,108]. These enzymes are abundant in grain legume seeds (beans and peas) and potato tubers, being minor constituents of wheat flour [107]. Multiple isoforms of lipoxygenases are found in plants; for example a multigene family encodes soybean lipoxygenases, three members of which encode the three major seed isoforms L1, L2 and L3 [109].

The main commercial sources of lipoxygenases are enzyme-active soybean flour and, to lower extent, flour from other beans, such as fava beans [12]. Wheat lipoxygenase catalyses the oxidation of PUFA in the free or MAG forms [110] while soybean or horse bean lipoxygenases also catalyse the oxidation of PUFA present in TAG [111]. The transient alkyl, peroxyl and hydroxyl radicals formed during lipoxygenase catalysed reactions are able to oxidise carotenoid pigments and sulfhydryl groups in peptides and proteins present in the dough, mainly giving rise to hydroxyacids [112].

In fact, the initial application of lipoxygenases in doughs was based on their ability to bleach fat-soluble carotenoid flour pigments, through co-oxidation of carotenoids with PUFA [113,114]. However, since the endogenous lipoxygenase content of wheat flour is insufficient to give enough bleaching effect, enzyme-active soybean or fava bean flour is added [114].

Lipoxygenases are also employed to improve mixing tolerance and dough handling properties [115]. In this case, the effect of these enzymes may be explained by oxidation of thiol groups of gluten proteins which can lead to rearrangement of intra- or inter-chain disulfide bonds [21] and also to formation of tyrosine cross-links [116], with consequent strengthening of the gluten network. As a result, improvement in dough rheology occurs, with increase in dough strength through proofing and baking, finally leading to improved loaf volume.

On the other hand, the action of lipoxygenase can lead to undesirable flavors in bread [79,114]. These flavors are possibly due to some of the breakdown products (ketodienes) formed during the anaerobic reaction [117,112].

5.2.2. Glucose oxidase

Glucose oxidase (β-D-glucose:oxygen: 1-oxidoreductase; EC 1.1.3.4) catalyzes the oxidation of β-D-glucose to D-glucono-δ-lactone and hydrogen peroxide [118,119]. This enzyme has been obtained from different fungal sources, mainly from genus *Aspergillus* and *Penicillium*, being *Aspergillus niger* the most commonly used [120-123].

Glucose oxidase has been used successfully to remove residual glucose and oxygen in foods and beverages aiming to increase their shelf life. The hydrogen peroxide generated by this enzyme presents antimicrobial properties, and is easily removed by catalase utilization, which is an enzyme that catalyzes the conversion of hydrogen peroxide to oxygen and water [12,124-127]. Glucose oxidase can be used as alternative oxidizing agent instead of potassium bromate in breadmaking. Potassium bromate is an oxidizing agent that was traditionally used in baking, and its use was prohibited in many countries after it was recognized as carcinogenic [128,129].

Although the mechanism of action of glucose oxidase is still not completely understood, a possible explanation is that the hydrogen peroxide formed during catalysis promotes, indirectly, the formation of either disulfide bonds or dityrosine crosslinks, or both, in the gluten network [116,130,131]. Therefore, the increase in disulfide crosslinking and/or promotion of gelative oxidation on the gluten matrix confers dough machinability, good gas retention, high bread volume and fine crumb structure [54,132-134]. Addition of increasing glucose oxidase concentrations to wheat flour dough produced significant changes on dough rheology and bread quality; and the extent of the effect was highly dependent on the amount of enzyme and the original wheat flour quality [130]. Furthermore, glucose oxidase was able to recover the breadmaking ability of damaged gluten [135]. Another possibility to explain the improvements on crumb properties, in both bread and croissants, as a result of glucose oxidase catalysed reactions would be the crosslinking of the albumin/globulin fraction with both disulfide and non-disulfide bonds, and the slight occurrence of non-disulfide crosslinking in the gluten proteins [131].

5.3. Other enzymes

Among the enzymes which have attracted attention for use in bakery is asparaginase. Differently from other enzymes, its use is not associated with improved bread volume, crumb softening

or reduced staling. Instead, asparaginase is claimed to have a high potential of reducing formation of acrylamide during baking [136-138]. Asparaginase (L-asparagine amidohydrolases, EC 3.5.1.1) catalyses the hydrolysis of asparagine to aspartic acid and ammonium, removing the precursor of acrylamide formation [139]. Acrylamide, classified as a probable human carcinogen, is formed in heated foods via Maillard reaction between asparagine and a carbonyl source [137,138,140,141]. Although asparaginase can be found among living organisms, including animals, plants and microorganisms, filamentous fungi as *Aspergillus oryzae* and *A. niger* have been explored for enzyme preparation aiming commercial purposes [142-144].

Enzyme (classification)	**Substrate in foods**	**Reaction**	**Applications in baked products**	**References**
Amylolytic enzymes	*Starch*	*Hydrolysis of linkages*		
α-Amylases (EC 3.2.1.1) or α-(1,4)-glucanhydrolases	Amylose and amylopectin	α(1→4)-D-glycosidic [endo], *liberating α-dextrins*	Generation of fermentable compounds; Increase in bread volume; Reduction in fermentation time; Improvement in dough viscosity, rheology and bread softness; Improvement in bread texture; Formation of reducing sugars and subsequent Maillard reaction products, intensifying bread flavor and color; Decrease of bread crumb firming rate; Anti-staling effects.	[16,17,22,48,74,174]
β-Amylases (EC 3.2.1.2)	Amylose and amylopectin	α(1→4)-D-glycosidic [exo], *liberating β-dextrins and β-maltose*		[16,17,22,175]
Glucoamylase (EC 3.2.1.3) or amyloglucosidase	Amylose and amylopectin	α(1→4)- and α(1→6)-D-glucosidic, *liberating β-glucose*		[16,17,22]
Pullulanase (EC 3.2.1.41)	Amylopectin	α(1→6)-D-glycosidic		[16,17,22]
Isoamylase (EC 3.2.1.68)	Amylopectin	α(1→6)-D-glycosidic		[16,17,22]
Maltogenic α-amylase (EC 3.2.1.133)	Amylose and amylopectin	α(1→4)-D-glycosidic, liberating maltose		[16,17,22,74,175]
Maltooligosaccharides forming amylases (glucan 1,4-α-maltotetraohydrolase) (ex., EC.3.2.1.60)	Amylose and amylopectin	Liberation of maltotetraose or maltohexaose		[16,17,22,175]
Transferases Amylomaltases (EC 2.4.1.25) Amylosucrases (EC 2.4.1.4) Ciclodextrin glycosyltransferases (EC 2.4.1.19)	Amylose, amylopectin and dextrins	Hydrolysis of α(1→4) glycosidic bonds and transference of a reducing group to a non-reducing acceptor (monosaccharide unit)		[17]

Table 2. Applications of starch modifying enzymes in baking.

Transglutaminases (EC 2.3.2.13) from microbial sources also have potential for application in bakery products. Food proteins can be modified through cross-linking by transglutaminases, resulting in textured products, protecting lysine in food proteins from undesired chemical reactions, encapsulating lipids and lipid-soluble materials, forming heat and water resistant films, improving elasticity and water-holding capacity, modifying solubility and functional properties, and producing food proteins of higher nutritive value [29,145-153].

Laccase (EC 1.10.3.2) is a copper containing enzyme that catalyses the oxidation of a wide variety of phenolic compounds via one-electron removal, generating reactive phenolic radicals [29,154]. This enzyme is very interesting for baking due its ablity to cross-link the esterified ferulic acid on the arabinoxylan fraction of dough, resulting in a strong arabinoxylan network [155]. It was also reported that laccase may improve crumb structure and softness of baked products. Futhermore, increases in strength and stability, as well as reduced stickness of dough, which confers improvement of machinability, have been described [149,155-157].

A summary of the main applications of different classes of enzymes in the baking industry is presented in tables 2, 3 and 4.

<table>
<tr><th>Enzyme (classification)</th><th>Substrate in foods</th><th>Reaction</th><th>Applications in baked products</th><th>References</th></tr>
<tr><td>Cellulases and Hemicellulases</td><td>Non-starch components of cereals</td><td>Hydrolysis of linkages</td><td></td><td></td></tr>
<tr><td>Cellulase (EC 3.2.1.4)</td><td>Cellulose and β-glucan</td><td>β(1→4)-D-glycosidic [endo]</td><td rowspan="6">Removal of insoluble arabinoxylans, contributing to gluten network formation; Increase in dough viscosity, stability, with better moldable form; Improvements on rheological properties of dough; Reduction in fermentation time; Increase of bread volume; Synergistic action of glucanases on xylanolytic attack of cereals structure, providing more soluble dietary fiber in bread products; Production of prebiotic oligosaccharides in bread.</td><td>[30,71,83,95]</td></tr>
<tr><td>Lamarinase (EC 3.2.1.6)</td><td>β-glucans</td><td>β(1→3)- and β(1→4)-D-glycosidic</td><td>[71]</td></tr>
<tr><td>Lichenase (EC 3.2.1.73)</td><td>β-glucans</td><td>β(1→3)- and β(1→4)-D-glycosidic</td><td>[30,71,83]</td></tr>
<tr><td>Endo β(1,4)-D-xylanase (EC 3.2.1.8)
or endoxylanase</td><td>Arabinoxylan</td><td>β(1→4)-D-xylosidic bonds</td><td>[71,83,85,95,165,176,177]</td></tr>
<tr><td>α-L-Arabinosidase (EC 3.2.1.55)</td><td>Arabinoxylan</td><td>Terminal α-L-Arabinofuranoside residues</td><td>[71,83,95,102]</td></tr>
<tr><td>β-D-Xylosidase (EC 3.2.1.37)</td><td>Arabinoxylan</td><td>β(1→4)-D-xylosidic bonds (non-
reducing end)</td><td>[83,95]</td></tr>
</table>

Table 3. Applications of cellulases and hemicellulases in baking.

Enzyme (classification)	Substrate in foods	Reaction	Applications in baked products	References
Proteases (EC 3.4.)	Gluten proteins Gliadin and glutenin	*Hydrolysis of peptide bonds*	Reduction of dough mixing time; Control of dough rheology or viscoelastic properties of gluten strength in bread; Enhance dough extensibility; Increase loaf or bread volumes; Formation of aminoacids and flavors; Crispness feature on bread crust; Production of gluten-free products.	[78,79,101,178, 179,180]
Transglutaminases Protein-glutamine γ-glutamyl-transferase (EC 2.3.2.13)	*Gluten Proteins*	Acyl-transfer reaction between γ-carboxyamide and primary amines	Cross-link between gluten and other peptides, forming a new protein network; Increase volume and improve structure of breads, better retention of gas; Improve bread crumb strength, height increase in puff pastry and croissants volume; Improve dough stability; Improve properties of gluten-free breads; Protect frozen doughs from damage.	[29,145-150]
Lipases and esterases	*Lipids*	*Hydrolysis of ester bonds*		
Lipase (EC 3.1.1.3)	Triacylglycerols	Liberation of free fatty acids	Improvement in bread volume and dough stability; Formation of emulsifiers; Retard staling; Development of flavors.	[59,106,158]
Oxidoreductases	*Various*	*Oxi-reductions*		
Glicose oxidase (EC 1.1.3.4) β-D-glucose:oxygen 1-oxidoreductase	β-D-glucose	Oxidation of β-D-glucose to gluconic acid	Control on browning for Maillard reaction; Improvements in crumb properties.	[130,131,181]
Lipoxygenase (EC 1.13.11.12) linoleate:oxygen 13-oxidoreductase	Polyunsaturated fatty acids	Oxidation of fatty acids	Bleaching of fat-soluble flour pigments; Hydroperoxides formed can oxidize sulfhydryl groups in proteins.	[79,182]
Laccase (EC 1.10.3.2)	Feruloyl esters of arabinoxylans;	Oxidation of phenol groups	Dough strength, stability and reduced stickiness; Increase in volume; Improved crumb structure and softness.	[155,163]

Enzyme (classification)	Substrate in foods	Reaction	Applications in baked products	References
or benzene-diol:oxygen oxidoreductase	sulfhydryl groups in gluten proteins			
Sulfhydryl oxidase	Sulfhydryl groups in proteins	Oxidation of sulfhydryl groups	Help gluten network formation and increase dough stability.	[79,162]

Table 4. Applications of proteases, transglutaminases, lipases and esterases, and oxidoreductases in baking.

5.4. Use of enzyme combinations

It is common practice to use mixtures of enzymes, some of which are commercially available. The enzymes may act individually or present a synergistic effect. The trend is to choose and control the use of complex mixtures of enzymes which may act in a synergistic way and can exert a better effect (than the individually used) on the different flour components [60]. Recent advances in understanding of the dough forming and overall baking processes at the molecular level have focused attention on improvements that can be achieved by application of more specially tailored enzymes alone or in combinations. Usually, integrated experimental design and optimization followed by chemical analyses, rheological experiments and baking trials are necessary in order to provide answers to the more complicated questions [158].

The use of a combination of enzymatic preparations of amylases, xylanases and lipases has been reported by different authors [60,102,159]. This specific mixture is claimed to increase bread volume and shelf-life. The use of α-amylase and glucose oxidase to replace bromate led to a significant improvement in dough extensibility and bread volume [160]. Addition of commercial enzyme mixtures, containing α-amylase and lipase activities to produce bread samples, using the straight dough method, had a beneficial effect on bread keeping properties and resulted in the formation of a more thermostable amylose-lipid complex compared to the control bread [161]. Amylopectin retrogradation was inhibited by the use of the enzyme combinations and this effect was strongly related to a decrease in crumb-firming rates.

The combined use of different enzymes, classified as gluten degrading (like proteases) or adjuvants, such as amylases and xylanases, with a group of crosslink promoting enzymes, such as transglutaminases and glucose oxidase, was also studied [149]. Better shaped bread could be obtained after the use of gluten degrading or adjuvant enzymes, and association with transglutaminase resulted in improvements on texture and rheological properties. The crumb firmness which can further lead to staling, can result from transglutaminase action, but it may be reversed with opposite amylase, xylanase and protease effects.

In a similar way, combinations of enzymes classified as carbohydrate degrading, including amylases and xylanases (pentosanases), and crosslink promoting enzymes, like transglutaminases and oxidases, including glucose oxidase, laccase [149], lipoxygenase and sulfhydryl oxidase [79,162] were evaluated. The most frequent associations contained xylanases and glucose oxidase, but addition of laccase and transglutaminase was also employed. The hy-

drogen peroxide formed by glucose oxidase catalysis may interfere in gluten network, via oxidized glutathione reaction, leading to gluten disulfide bonds formation [43], and it also interferes in the formation of a soluble pentosan gel (from xylans) that increased dough consistency [146]. Because both oxidases and xylanases influence the xylan properties, xylanases and oxidases could be used advantageously in combination, resulting in a mesh of gluten and gelified xylans matrix, which increases gas retention, dough stability and bread volume. Laccase is reported to catalyze dimerization of feruloylated esters in feruloylated arabinoxylans in doughs [163,164], forming a xylan network, contributing to increase strength of dough and volume.

Lipoxygenases oxidize polyunsaturated fatty acids during dough mixing. The hydroperoxides formed can oxidize the sulfhydryl groups of gluten proteins and thus be advantageous in the formation of the gluten network of dough. Sulfhydryl oxidase combined to glucose oxidase and xylanases has been used to strengthen weak doughs [79,162].

The use of a combination of commercial preparations of glucolipase, hemicellulase and hexose oxidase in formulations of frozen pre-baked French bread, substituted with whole wheat flour, improved parameters such as proofing time, oven spring and cut opening and cut height [158]. An interaction among the three enzymes was observed for most of the parameters, because the responses of each enzyme to variations in dosing were influenced by the doses of the other two.

6. Future trends

Besides the demand for replacement of chemical additives by others from natural sources, there is an increasing concern among the consumers and consequently an increased demand for preservation and/or enrichment of foods with products that have beneficial effects on human health. Regarding baked goods, the use of enzymes to obtain dietary fiber enriched bread [102,165], for the development of gluten free products [145], to obtain products with increased contents of arabinoxylan oligosaccharides with prebiotic potential [165], has been reported.

Several aspects can be pointed out for the development of enzyme preparations able to provide the desired effects or with adequate characteristics for use under process conditions. Some of the possible strategies include selection of novel enzymes from different sources [166], especially from microorganisms obtained from the vast biodiversity of the planet, production of recombinant proteins from genetically modified organisms [167], as well as protein engineering.

Psychrophilic enzymes usually have higher optimal activity and stability at lower temperatures than their mesophilic counterparts [168]. Due to the fact that the temperatures most frequently used in dough mixing and proofing are around or below 35 °C, it has been suggested that psychrophilic enzymes would be advantageous candidates for use as additives in the baking industry [83,85]. In this context, researchers have shown that much lower dos-

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ages of psychrophylic xylanases than of the mesophilic enzymes could be used to attain maximal bread volumes [85,169,170].

Directed evolution is a powerful tool of protein engineering to design and modify the properties of enzymes [171]. This technology can be employed for a wide range of proteins, most of which are of interest for biocatalytic processes. Within a decade, directed evolution has become a standard methodology in protein engineering and can be used in combination with rational protein design and other standard techniques to meet the demands for industrially applicable biocatalysts capable of withstanding process conditions such as high substrate concentrations, high temperatures and long-term stability, as well as presenting desired specificity and/or selectivity [172]. For instance, a recent study reported the combined use of directed evolution and high-throughput screening to improve the perfomance of a maltogenic α-amylase from *Bacillus* sp. for low pH bread applications. One of the resulting variants showed an important increase in thermal stability at pH 4.5 and a considerable antistaling effect in low pH breads [173].

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Grinding Characteristics of Wheat in Industrial Mills

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Additional information is available at the end of the chapter

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1. Introduction

Grinding of cereal seeds is due to the mechanical action of several forces: compression, shearing, crushing, cutting, friction and collision, to which seeds are subjected, depending on the design if the mill used for grinding (roller mill, hammer mill, stones mill or ball mill). By applying these forces, when the mechanical resistance of the particles is exceeded, their division happens in a number of smaller particles of different sizes, geometric shapes, masses and volumes.

An industrial wheat mill has several technological phases, starting with coarse grinding of seeds to fine grinding of the resulted milling products, after their sorting in fractions of different sizes. The first technological phase of grinding process, in wheat mills, is gristing or coarse grinding phase, which also consists of several technological passages.

A technological passage consists of a grinding machine (roller mill), a machine for sifting and sorting of the resulted milling fractions (plansifter compartment) and, eventually, a machine for the conditioning of semi-final product (semolina machine or bran finisher). In a technological passage, intermediate fractions are obtained, which, by a new grinding, lead to the obtaining of high-quality flour at milling passages (fine grinding).

Wheat processing requires a long and gradual transformation into flour. This process takes place after a gradual crushing schedule, from fine to finer, from machine to machine, of wheat seed, respectively of the crushed particles resulting from it. Each grinding operation is immediately followed by a sorting operation by sifting (fig.1) because during grinding, a wide variety of grinded seed particles is obtained.

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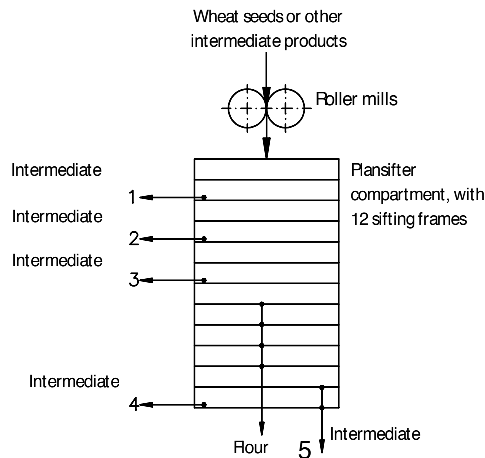

Figure 1. Schematic diagram of a grinding passage

Before the grinding process is started, grains must undergo the cleansing process. This is followed by a conditioning process that ensures a uniform moisture content for the entire lot of grains, helping endosperm softening and cover harshening, which improves the separation process.

One of the fractions resulting from a plansifter compartment is composed of flour particles (with sizes under 160 μm), in a higher or lower percentage of the total flour that can be withdrawn in the industrial mill. To extract the full amount of flour from the wheat berries, multiple passes (passages) are required. Some passages are part of coarse grinding phase (gristing), where the milling rollers have fluted surface, while other passages are part of milling phase (fine grinding), where the milling rollers have smooth surface.

Intermediate milling products are, mainly, grists (seed particles with various sizes), semolina (large, average and small) and dunsts (harsh and smooth). They all return in the grinding process for flour extraction, but the grists are grinded by mills with fluted rollers (gristing passages), while semolina and dunsts are grinded by mills with smooth rollers (milling passages). Semolina and dunsts, as intermediate milling products, are particles of clean endosperm or with a small percentage of cohesive coat.

Particles obtained by grinding have sizes in a fairly wide range (1200-160 μm, within the mentioned fractions), average size of the particles of resulted fraction being determined by granulometric analysis using sieve classifier.

In roller mills, wheat seeds are grinded in the gristing phase by pairs of fluted rollers, thus being obtained a wide range of particles with sizes from < 200 μm to > 2000 μm, [1], consisting in coat particles (of larger sizes) and endosperm particles (of smaller sizes), to be further separated with plansifters. The milling process aims to grind the endosperm into finer particles of flour and semolina, while the coating and the seed particles must remain in large sizes to be separated by sifting, [2]. In gristing passages, milling rollers with fluted surface are used, and in milling passages, rollers with smooth surface are used. The quality of wheat milling process

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is influenced by the physical and mechanical properties of seeds and of the intermediate products (size distribution, seeds hardness, moisture content) and by the design and functional parameters of the roller mill (mutual arrangement of the rollers, differential speed, distance between the rollers, flutes profile, mutual position of the flutes), [3,4]. Effects of these factors are manifested in the size distribution of material particles, compositional distribution of the material, wear degree of the rollers, energy consumed for grinding, [4].

Fang, Campbell et al. (2002) showed that if the distance between rollers increases from 0.3 mm to 0.7 mm, wheat seeds breakage in the gristing phase has a lower intensity, resulting in more particles of large sizes and less particles of smaller sizes. Distance between rollers indirectly influences the specific surface and energy consumption per mass unit and directly influences the specific energy, [5]. Different flutes arrangements on the rollers lead to the obtaining of different size distributions. If the roller flutes are arranged in blade/blade position results in a relatively uniform size distribution, and back to back arrangement lead to a deep parabolic distribution, [1].

Differential speed of milling rollers has a significant effect on the grinding of semolina, flour and wheat bran. With the increase of differential speed of rollers, it also increases the amount of semolina and decreases the amount of flour and wheat bran, [6]. This is due to the difference between shearing and compression forces which are applied on the particles.

It is very important to know the size distribution of the material subjected to grinding, as well of the grist, so that appropriate adjustments can be made to roller mills, and also to choose the fabrics for the sieving frames of plansift compartments. Particles size distribution of the granular material can be determined using superposed sieve classifiers (sieve shakers), with different sizes of sieve holes. This can be assessed by various mathematical functions, from which, most used is the Rosin-Rammler function.

Experiments were performed on the material subjected to grinding (before and after grinding) and cumulative distribution curves were drawn for the sieved material, by computer aided regression analysis of the experimental data with Rosin-Rammler function. Based on the data obtained from particle size distribution were also determined other physical characteristics of the analyzed material: average particle size, grist modulus, specific surface of the granular material, surface increasement resulted from grinding within a passage (break), bulk density and specific mass.

Within this chapter are presented the flow diagrams for two wheat mills of different capacities, one of 100 tons / 24 hours and one of 220 tons / 24 hours, from which it can be estimated the movement of products within the mill.

There are also presented the experimental results obtained from the particles size distribution of the material subjected to grinding and of the resulted grist, in both technological phases, for the two mills, as well as particles size distribution of the material for various grinding machines of the analyzed mills.

Knowing of the mechanical characteristics of wheat seeds and of the grist particles, and also their size characteristics, volume and mass of the wheat seeds, is useful for estimating the energy required for crushing.

For this purpose, in this paper are presented the results of some experimental research on the behaviour of wheat seeds in uniaxial compression tests between parallel plates. There are also presented the curves of variation for the crushing force and energy absorbed until the crushing point of seeds.

The results presented and the obtained data are of real interest for the designers of roller mills, as well as for the manufacturers and users of such machines.

2. Technological diagrams for wheat grinding

The technological passage consists of one or two pairs of milling rollers, both processing the same product, combined with one or more plansifter compartments for sieving.

Gristing is the technological phase aiming to fragment the wheat seed in particles of different sizes and to remove the endosperm from the coating. Particles resulted from first, second and third grinding phase vary in size, from breakages like half seeds to flour particles with very fine granulometry. As gristing is repeated, particles will get increasingly finer, the amount of white flour decreases, and seeds coating reaches the penultimate and last phase as fine dust, [7]. Thus, grist is the intermediate product obtained in the milling industry, by grinding grains by mean of roller mills with fluted surface.

Fig. 2 presents the technological diagram of gristing phase of the wheat in an industrial mill with the capacity of 220 t/24 h.

Milling unit consists of 9 double roller mills, of which the first processes, in both sections, the same material (whole seeds), two plansifters, together amounting 14 compartments, three double semolina machines and five brushes and bran finishers. The three phases of the process (gristing, milling, sorting) can be observed in fig.3 – fig.5.

Gristing phase consists of six simple mills with fluted rollers, four full and two half's of plansifter compartments and four bran finishers which process the coatings resulted from multiple grinding operations. The seeds are processed in a mill with double rollers placed in horizontal plane, noted by B1–B2.

The first grist is processed in passage B3, and the fractions obtained here will follow different routes, to the milling passages, or to the semolina machines or bran finishers, passages B4gr and B5f being responsible for the processing of material particles with high coating content, and passage B4f processes the second refuse from gristing passage B3, with fractions having the same characteristics processed in plansifter compartments. The development of gristing phase in directly connected to the type of meal and the degree of flour extraction. Products resulted from gristing are named intermediate products and they consist of: big grist, fine grist, big semolina, middle semolina, fine semolina, big dunst, soft dunst, flour and bran, [7].

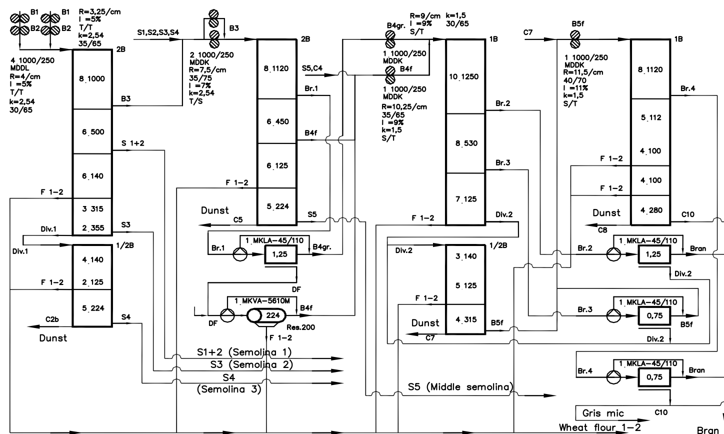

Figure 2. Technological diagram of gristing phase for a Bühler mill with capacity of 220 t / 24 h, [8]

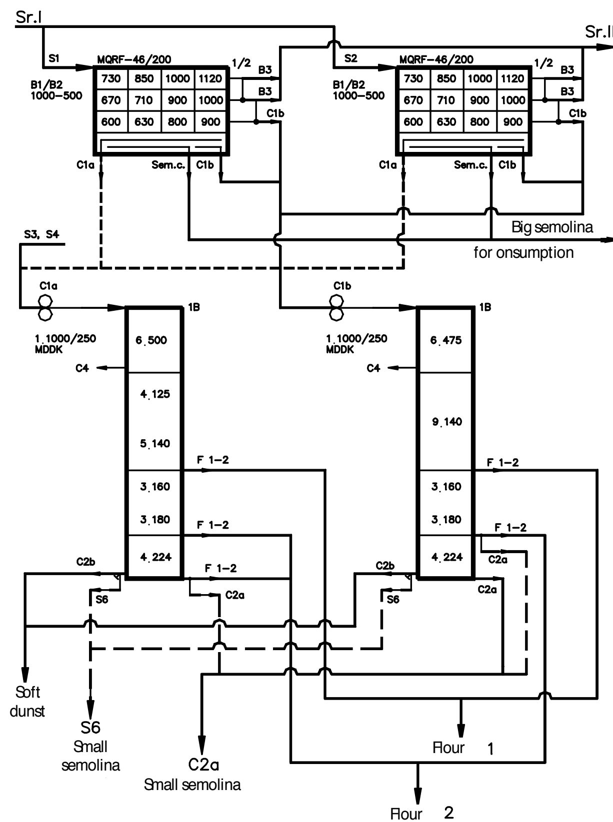

Figure 3. Technological diagram for sorting of big semolina in Bühler mill, [7]

Particles size of these components, resulted from sieving process, is determined by the size of the sieve holes used in sieving compartments. Depending on the particles size, semolina and dunsts can be classified as: big semolina with average size of 1200-630 μm; middle semolina 630–400 μm; fine semolina 400–310 μm; big dunsts 310–245 μm; soft dunsts 245–160 μm. Semolina sorting is dome in sorting phase (fig.2.) A clear delineation between soft dunsts and flour can not be practically achieved, and therefore, are cases when soft dunsts (d_m = 220 μm) are considered to be flour (flours granulosity is given by the sieves, with mean equivalent size of the particles below 160 μm).

Particles of intermediate products can be highlited not only by their size, but also by shape, volume, specific mass, aerodynamic properties. Particles with rich coating have irregular shape in the form of foils with rolled or folded edges. Particles of clean endosperm have polyhedral shape with sharp edges and convex lateral surfaces.

Semolina is an intermediate product obtained in percentage of 25…30% in industrial wheat milling, is found as small granules and after cleaning is further milled to obtain flour or a food product known as "kitchen semolina". This is obtained in percentage of 2...3 % at wheat milling and it is cleaned in special semolina machines in order to remove coating particles by the combined action of sieving and airflows. Dunst is a fine semolina obtained as intermediate product from the grinding of wheat or semolina.

After gristing phase it is important to sort the milling products using a wide range of sizes for sieve holes (1000...224 μm), followed by the cleaning of semolina and dunsts, the phase of semolina opening being no longer necessary, since most coating was already removed in the gristing phase (fig.3).

The unit is fully automated, all mill equipments starting and stopping from the computer, starting with the equipments from the final technological phases (bagging, flour homogenization, sieving with plansifters, semolina cleaning, bran finishers, etc.) from the circuit of flour or intermediate products, while stopping begins with the first pair of rollers, i.e. reverse of start up.

In fig.4 and fig.5 is presented the technological flow for a wheat mill with capacity of 100 t/24 h, in grinding phases (fig.4) and in the milling (breakage) phase of semolina (fig.5), [10].

The technological flow of wheat mill is ensured by 12 processing passages, with 12 pairs of milling rollers (6 double rollers of Buhler type) from which 5 gristing passages and 7 milling passages. In addition, the technological flow is fitted with a sorting passage (separate compartment of plansifter).

Apart from the 12 technological passages, each consisting in a pair of roller mills and one plansifter compartment, the mill also has a double machine for semolina, three bran finishers and other auxiliary equipments (detachers, wheat brushes, filters and cleaning cyclones, etc.), as well as the proper elements for the pneumatic transport system from one equipment to another, according to the technological flow.

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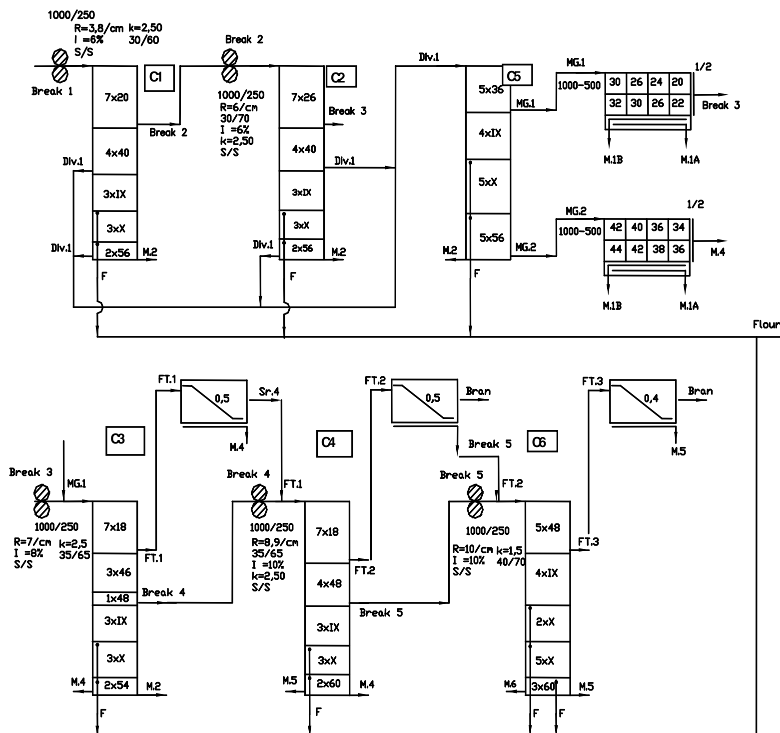

Figure 4. Technological flow of semolina grinding phase in wheat mill, capacity of 100 t/24 h, [11]

In breakage phase the technological diagram of mill contains five pairs of rolls, filled with one compartment of plane sieve, two semolina machines and three wheat bran finishers. The technological breakage phase is completed with one compartment of plane sieve without grinding machine, in which the material is sorted by fractions of different sizes as well as the other compartments of plane sieve.

The first grist, obtained from seeds processing with the pair of fluted rollers Sr.1, is processed in passage Sr.2, and from here the fractions follow various routes, to grinding passages, to semolina machines to wheat bran finishers. The sifting material from the second and the last set of gristing passage Sr.1, is send to a plansifter compartment for division in fractions (Div.1), which next reach the MG1 and MG2 semolina machines. The refuse from the last set of frames in the first passage is then sent to the M2 grinding passage.

The circulation of grist intermediate products in the technological diagram is shown in fig.4 and fig.5.

In the grinding phase (fig.5), the technological diagram of milling unit consists of seven simple roller mills, each fitted with one plansifter compartment for sorting in fractions of the grinded products and the extraction of flour from these products.

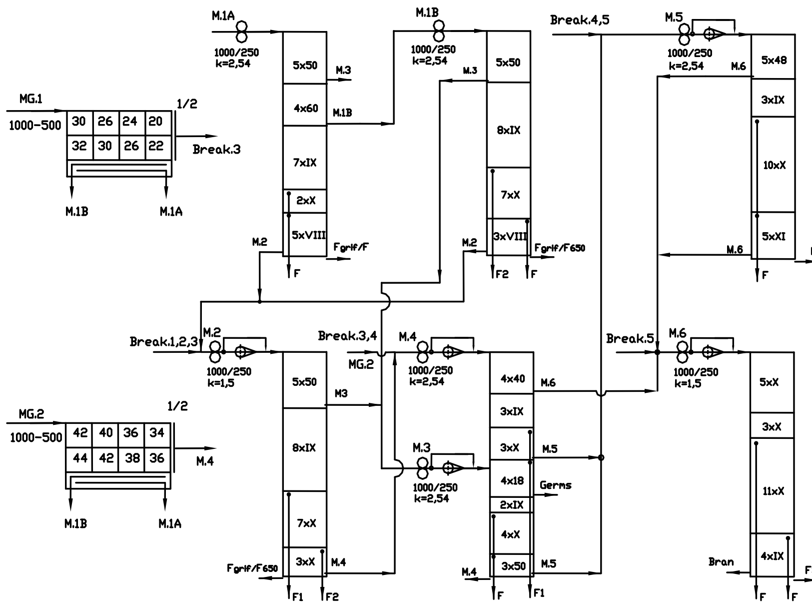

Figure 5. Technological flow of the semolina grinding phase in a wheat mill with 100 t/24 h, [9]

All roller mills of both technological phases have the length of 1000 mm and diameter of 250 mm, with fluted surface, in the gristing phase, respectively smooth surface without flutes in the grinding phase. In the gristing phase, the ratio of the tangential speeds of fluted rollers is k=2.54, and in the grinding phase, for five pairs of rollers, k=2.54, and for two pairs of rollers k=1.5.

As shown in fig.5, the products to be grinded into the grinding phase are products arriving from gristing phase (or breakage phase), inclusive from grists (Sr.1-6) or from semolina machines and bran finishers. The siftings from MG1 and MG2 semolina machines, which are semolinas with sizes below 0.8-1.0 mm, are grinded in the first technological passages M1A and M1B, while the siftings from FT1 and FT3 bran finishers go to the last two grinders M4 and M5, which processed products with higher content of bran. In diagram, the first refusal from M1A and M1B grinders is led to M3 grinder, working with half compartment of plane sieve. It is noted that to grinders which grinded smaller particles of endosperm (about 0.40 mm), after the mill rollers in technological flow are placed detached of material, due to agglomerations arising from the compression of smaller particles of endosperm in the action zone of grinding rolls.

In fig.6 is shown the arrangement of rollers to a mill with 100 t /24 h capacity, where the samples for our determinations were collected.

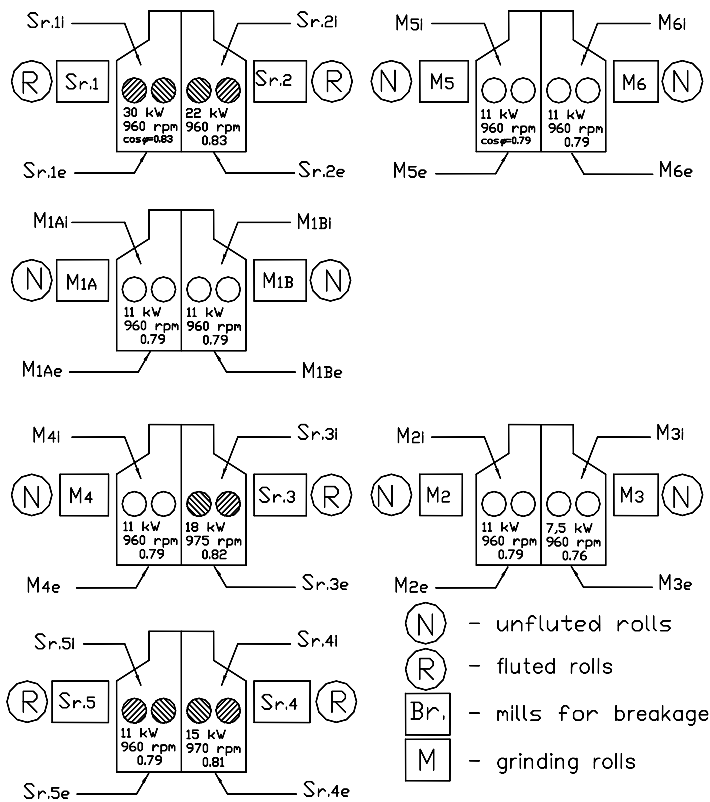

Figure 6. Arrangement of roller mills for the mill with capacity of 100 t /24 h

Plansifters are driven by electric motors of 4 kW, cos φ = 0,81 and speed of 960 rot/min. Double machine for semolina is driven by two moto-vibrators of 400 W and speed of 960 rpm. Characteristics of driving motors for mill rollers are given in table 1.

Passage	I (A)		P, kW	n, rpm	cos φ	Passage	I (A)		P, kW	n, rpm	cos φ
	No-load	Load					No-load	Load			
Sr. 1	19	45	30	960	0.83	M1 B	10	15	11	960	0.79
Sr. 2	21	37	22	960	0.83	M2	11,9	16	11	960	0.79
Sr. 3	23	32	18	975	0.82	M3	10,9	18	7,5	960	0.76
Sr. 4	13	30	15	970	0.81	M4	10,9	15	11	960	0.79
Sr. 5	13	17	11	960	0.79	M5	12	16	11	960	0.79
M1 A	10	15	11	960	0.79	M6	12	17	11	960	0.79

Table 1. Characteristics of electric motors for the drive of mill rollers, for wheat mill with capacity of 100 t/24 h [9]

According to relevant regulations, on the technological diagram (fig.4 or fig.5) should be written the characteristics of grinding rollers: length, diameter (ex.1000x250, in mm), number of flutes and their inclination (ex.7/cm, I=8%), flute angles (ex.35/65), mutual arrangement of the flutes (ex.S/S), speed ratio (ex.k=2.5), and the characteristics of fabrics used in plansifter frames (ex.3x46 – 3 frames with 46 wires per inch or 3xX for flour frames), at semolina machines (ex.42, which represents the number of wires per inch or 1000-500, which is frame size) or at bran finishers (ex.0.5 – size of fabric hole).

Sieve frames from top of compartments are fitted with metal mesh as they separate seed brokens of relatively large sizes (which would wear quite quickly the textile fabrics), while flour frames from the lower set are fitted with frames with plastic or textile fabrics.

Lately, textile fabrics have been replaced with sieve frames with meshes of plastic fabric. According to literature, fot the technological diagram of the analyzed mill, the equivalence between the sieve number and the size of its holes, as they are specified in the diagram, is shown in table 2.

Sieve no.	18	20	26	36	40	46	48	50	54	56	60	VIII	IX	X	XI
Hole size (μm)	1170	1050	780	520	470	390	370	350	320	310	280	180	170	150	130

Table 2. Equivalence between sieve number and hole sizes

3. Physical and granulometric characteristics of seeds and grinding products

In the grinding process is necessary to know the physico-mechanical characteristics of the material at the entry and exit from a processing machine, in this case, roller mills.

Main factors influencing the process of grain grinding are the physico-mechanical properties of seeds and of the grinding products, the constructive and functional characteristics of the grinding machines as well as the technological regime, most of those factors having a random character.

As a result of grinding it is obtained a mass of particles with various smaller sizes and different geometrical shapes (grist).

Granulometric distribution of the grinded material and of the material leaving the grinding process can be assessed by the cumulative weight (%) of material passing through the sieve holes of classifier T(x) or which are refused by its sieves R(x), calculated on base of mass weight (%) of the fractions from the sieve. (R(x)+T(x)=100). The mathematical expression of granulometric distribution in case of grinded biological materials, is based on laws of mathematical statistical method of small particles, [11-14].

There will be defined three usual types of laws of cumulative granulometric distribution.

- The Rosin-Rammler distribution, for material particles with larger sizes than sieve holes, is expressed by the relation:

$$R(x) = 100 \cdot e^{-b x^n} \tag{1}$$

where: R(x) is the mass percentage weight of fraction with larger particles than x (which remained on the sieve with meshes with size x); x – is the sieves meshes size by which the particles rest; b and n are the own coefficients of grinding material.

- The Schuhman distribution is defined by the relation:

$$R(x) = 100 \cdot \left\{1 - \left(x/k\right)^a\right\} \tag{2}$$

where: R(x) and x have the significance from to relationship (1), k - the module product particles size (the size of sieve mesh through which, theoretical, pass all the sample particles (100%)), a - the distribution module.

- The logistics type distribution with two parameters is defined by the relation:

$$R(x) = 100 \cdot \frac{e^{\alpha + \beta x}}{1 + e^{\alpha + \beta x}} \tag{3}$$

where: R(x) and x have the significance from relationships (1-3) α and β are logistical constants.

Of these characteristics are important: the bulk density, ϱ_v (kg/m^3), of the material to be processed, the density of the material, ϱ (kg/m^3); the equivalent sizes of material particle at entry and exit of the grinding machine, dm (mm); angle of internal friction of particles appreciated by natural slope angle, ψ (°); angle of material friction with the surfaces working components, φ (°); material porosity, ε (%) and others.

Of particular importance is the equivalent size of seeds subjected to grinding in the first technological passage.

The density is the ratio between the sample mass and the volume of the particle in it. To determine the densities of wheat seeds, respectively the grinding products, the pycnometrical method was used (xylene 0.8254 kg/cm^3).

The porosity is the property of granular materials, respectively of the grains, to not occupy the entire volume of storage, with an intergranular space. Knowing the values of bulk density and material density, the porosity was evaluated using the following relation, [15]:

$$\varepsilon(\%) = \left(1 - \rho_v / \rho\right) \cdot 100 \; (\%) \tag{4}$$

The static friction coefficient. The most common method for determining the coefficient of static friction is inclined plane method which was used in this paper. It was used a device with adjustable incline plane, [15]. Two sets of determinations were realized on three types of surfaces: glossy fiberglass, steel sheet and cotton canvas.

Assessing parameters of the grinding process are: grinding degree, grinding finesse and specific energy consumption at grinding.

Grinding degree and grinding finesse are determined by granulometric analysis, using a sieve overlay classifier with oscillatory movement.

Grinding degree is defined by the λ index and represents the ratio between equivalent sizes of particles before and after grinding, D_e, respectively d_m, or the ratio between the outer surface of the particles resulted in the grinding process and the initial surface of the particle subjected to grinding, S_f, respectively S_i:

$$\lambda = D_e / d_m = S_f / S_i \tag{5}$$

Absolute value of the increase of particles outer surface in the grinding process ΔS, is given by:

$$\Delta S = S_f - S_i = S_i(\lambda - 1) \tag{6}$$

The grinding finesse has been appreciated by the geometric mean diameter d_m of the grinding particles which was determined by the size distribution analysis, using the relation of weighted average:

$$d_m = (1/100) \cdot \sum_{i=0}^{n} p_i d_i, \tag{7}$$

where: p_i is mass weight of fraction remaining on the sieve *i* of the classifier, d_i, is diameter (average value) of fractions particle on the sieve *i*, considered the arithmetic average of the sieves holes size that contain fraction *i*.

The surface areaand the surface increase. Knowing the mean diameter of particles of a granular mixture, their specific surface $S_{e.m}$ is determined with the relation, [10,15]:

$$S_{e.m.} = 6 / \rho \cdot d_m \ (m^2 / kg), \tag{8}$$

where: ρ is the density of the particles.

There are presented the results of some experimental research on the physical characteristics of grinding products on the technological flow of gristing phase of wheat from a mill with capacity of 100 t / 24 h (SC Spicul Rosiori de Vede, Teleorman, Romania).

The material tested in the experiments was taken from the entry, respectively from the exit of each pair of milling rollers (from the five pairs of the phase).

The experimental data characterizing the physical properties of the grist obtained are shown in table 3. Also, in table 4 and table 5 are presented the results of size distribution analysis on mixtures of material entering and leaving the rolls placed in the technological grinding phase.

Break		**Static friction coefficient μ**			**Natural slope angle ψ**
		Cotton canvas	**Glossy fiberglass**	**Steel sheet**	
M1A	E	1.74 - >1.76	68,7	0.61 – 0.85	39,43
M1B	I	1.24 – 1.82	56,4	0.45 – 0.67	30,19
M2	I	>1.76	61	0.6 – 0.86	43,97
M3	E	>1.76	72,4	0.58 – 0.82	38,35
M4	E	>1.76	67,5	0.58 – 0.73	41,96
M5	I	>1.76	64,3	0.60 – 0.88	46,62
M6	E	>1.76	69,5	0.54 – 0.71	40,17

Table 3. The values of static friction coefficient and natural slope angles [15]

From table 3 it is noted that the static coefficient values, on the glossy fiberglass and metal are within the limits set in various specialized papers, while the values obtained in the experiments on cotton canvas fall in broad limits, probably due to material fractions moisture, but also because of its granularity, this phenomenon is observed, especially, to flours and relatively small particle fractions of endosperm.

l_i (mm)	**M1A - I**		**M1A – E**		**l_i (mm)**	**M1B – I**		**M1B - E**	
	p_i (%)	**T_i (%)**	**p_i (%)**	**T_i (%)**		**p_i (%)**	**T_i (%)**	**p_i (%)**	**T_i (%)**
0,00	0,90	0,00	34,40	0,00	0,00	0,40	0,00	7,70	0,00
0,18	1,00	0,90	16,30	34,40	0,13	0,60	0,40	8,00	7,70
0,25	3,70	1,90	10,30	50,70	0,18	3,90	1,00	23,80	15,70
0,32	33,30	5,60	15,80	61,00	0,25	22,10	4,90	35,60	39,50
0,50	49,10	38,90	12,50	76,80	0,32	48,90	27,00	21,90	75,10
0,71	12,00	88,00	10,70	89,30	0,40	24,10	75,90	3,00	97,00
	d_{M1AI} = 0,55		d_{M1AE} = 0,33			d_{M1BI} = 0,36		d_{M1BE} = 0,26	

Table 4. The ponder values (p_i) of the fractions from the sieving machine classifier sieves and of the cumulative weights T_i(%) for the collected gritting, at entrance "I" and exit "E" from the mentioned rolls (only M1A, and M1B), [9]

Break	Equivalent size(I/E)	Grinding degree	Bulk density	True density	Specific surface	Surface increase	Porosity
	mm	λ	g/dm^3	g/dm^3	x10^3 m^2/kg	x10^3 m^2/kg	%
M1A	0,55-0,33	1,68	560,0-389,5	1344,9-1247,1	8,13-14,72	6,595	58,3-68,7
M1B	0,36-0,26	1,385	583,5-499,0	1338,7-1372,0	12,34-16,72	4,38	56,4-63,6
M2	0,19-0,17	1,113	480,5-437,5	1233,3-1313,4	26,04-27,55	1,513	61-66,7
M3	0,35-0,45	0,788	363,5-308,5	1252,9-1119,8	13,55-11,95	-1,605*	71-72,4
M4	0,22-0,24	0,940	452,5-419,5	1290,6-1290,6	21,02-19,76	-1,252*	64,9-67,5
M5	0,22-0,24	0,924	430,5-419,5	1205,4-1210,2	22,74-20,89	-1,854*	64,3-65,3
M6	0,24-0,27	0,903	416,0-373,0	1274,4-1224,5	19,40-18,23	-1,164*	67,4-69,5

The sign * in table 3, for negative values of specific surface increases, means that at the passage through milling rollers with smooth surface, agglomeration of gritting particles occurs.

Table 5. The values of grinding degree, specific surface, surface increase and porosity

Based on the data obtained from the experiments and presented in table 6, were mapped graphics, using MS Excel version 7.0 program (fig.6), the variations of mean diameter and bulk density to technological breakage passage of milling unit.

Physical characteristic	Sr.1-I	Sr.1-E	Sr.2-I	Sr.2-E	Sr.3-I	Sr.3-E	Sr.4-I	Sr.4-E	Sr.5-I	Sr.5-E
Bulk density, ρ_v (kg/m^3)	713.0	381.5	482.0	346.5	267.8	292.0	255.0	257.0	269.0	266.0
Density, ρ (kg/m^3)	1239	1250	1219	1200	1100	1063	1016	1130	1100	1191
Equivalent size, (mm)	3.76	2.13	2.23	1.22	1.51	0.90	1.06	0.84	0.65	0.63
Grinding degree, λ	1.76		1.83		1.67		1.26		1.03	
Specific surface, (m^2/kg)	1.29	2.25	2.21	4.10	3.61	6.27	5.57	6.32	8.39	8.00*
Surface increase, ΔS (m^2/kg)	0.96		1.89		2.66		0.75		–0.39*	
Natural slope angle, ψ (gr.)	21.8	37.8	37.1	37.5	44.6	39.0	41.1	39.2	42.6	44.4
Porosity, ε (%)	42.5	69.5	60.5	71.1	75.7	72.5	74.9	77.3	75.5	77.7

Table 6. Physico-mechanical characteristics of grinding products at gristing passages of wheat, from the mill with capacity of 100 t / 24 h, [10]

Correlation between individual volume of the seeds, calculated with the relation: $V = (1/6)\,\pi\, l\, w\, t$ (where: l, w, t represent the measured length, width, and thickness of each seed, and the seeds are assimilated with ellipsoid geometrical bodies) and their weight is presented in fig.7.

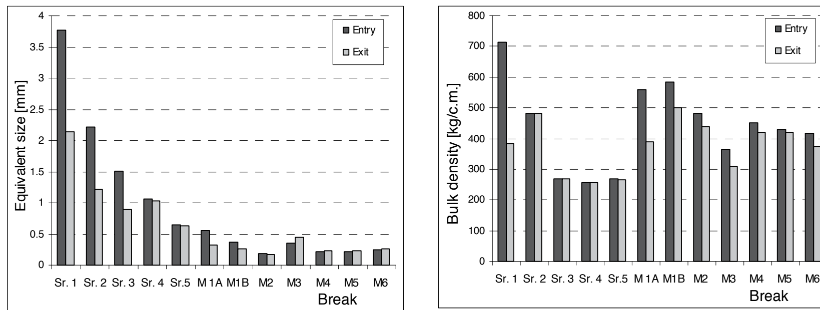

Figure 7. Variation of mean diameter and bulk density of grinding intermediate products on the grinding technological flow with grinding rollers [15]

Sieves used in granulometric analysis with sieve classifier and the results obtained by analysis are given in table 7, for each of the five technological passages, at the entry end exit from the respective mill rollers.

l_i (mm)	Sr.1 - E		l_i (mm)	Sr.2 - I		Sr.2 - E		l_i (mm)	Sr.3 - I		Sr.3 - E	
	p_i (%)	Ri (%)		p_i (%)	Ri (%)	p_i (%)	Ri (%)		p_i (%)	Ri (%)	p_i (%)	Ri (%)
0.00	24.20	0.00	0.00	2.00	0.00	34.70	0.00	0.00	13.40	0.00	43.10	0.00
1.00	8.40	24.20	0.71	6.00	2.00	11.50	34.70	0.71	22.50	13.40	20.60	43.10
1.40	15.10	32.60	1.00	19.20	8.00	22.20	46.20	1.00	22.80	35.90	23.00	63.70
2.00	20.10	47.70	1.40	13.90	27.20	11.50	68.40	1.40	12.00	58.70	5.10	86.70
2.80	27.00	67.80	2.00	29.50	41.10	14.90	79.90	2.00	20.10	70.70	7.20	91.80
4.00	5.20	94.80	2.80	29.40	70.60	5.20	94.80	2.80	9.20	90.80	1.00	99.00
d_{1E} = 2.13 mm			d_{2I} = 2.23 mm			d_{2E} = 1.22 mm		d_{3I} = 1.51 mm			d_{2E} = 0.90 mm	
			l_i (mm)	Sr.4 - I		Sr.4 - E		l_i (mm)	Sr.5 - I		Sr.5 - E	
				p_i (%)	Ri (%)	p_i (%)	Ri (%)		p_i (%)	Ri (%)	p_i (%)	Ri (%)
			0.00	26.00	0.00	42.60	0.00	0.00	5.20	0.00	5.80	0.00
			0.71	25.20	26.00	27.50	42.60	0.25	3.90	5.20	3.90	5.80
			1.00	28.30	51.20	20.70	70.10	0.32	21.10	9.10	22.60	9.70
			1.40	11.90	79.50	3.60	90.80	0.50	28.20	30.20	29.70	32.30
			2.00	7.80	91.40	5.00	94.40	0.71	35.30	58.40	32.70	62.00
			2.80	0.80	99.20	0.60	99.40	1.00	6.30	93.70	5.30	94.70
			d_{4I} = 1.06 mm			d_{4E} = 0.84 mm		d_{5I} = 0.65 mm			d_{5E} = 0.63 mm	

Table 7. Values of weights (%) p_i for the fractions on the shaker sieves of the sifter machine and of the cumulative percentages R_i (%) for the collected grinded products, at entry "I" and exit "E" from pairs of mentioned rollers (Sr.1… Sr.5), [10]

Based on the results obtained by granulometric analysis with the sieve classifier were tested by nonlinear regression analysis, the three laws of cumulative distribution for the refuse of the sieves R(x) (Rosin-Rammler function, Schuhman function and two parameters logistical function), for products entering the process, and for the products leaving the pairs of rollers, in the gristing phase of the grinding process. Experimental points and the curves of cumulative distribution for the refuse of the sieves (R(x)), using the three functions (eq.1, eq.2, eq.3), for some grinding products are presented in fig.8.

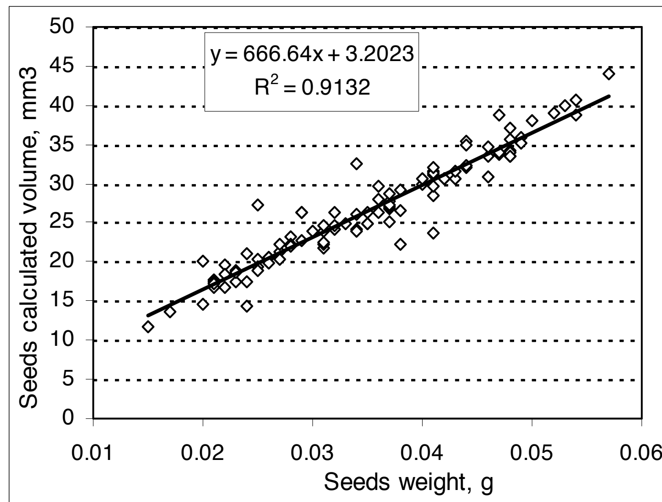

Figure 8. Correlation between volume and the mass of wheat seeds in an technological mixture (before grinding) [16]

The coefficient values k, a, b, n, α and β, from the cumulative distribution relations Rosin–Rammler, Schuhman and the two parameters logistical function, as well as the R^2 correlation coefficient values (which verifies the distribution adequacy degree expressed through the (1), (2), (3) relations), correspondent for the nine analyzed probes (from the five roll pairs) are presented in table 8.

From the analysis and interpretation of the obtained data for the 9 probes, which come from the mill rolls with rifles (for the coarse gritting in the breaking passages) (fig.9), following conclusions were found:

- For the vast analyzed material probes, from the mills flux, the best law of cumulative distribution is the Rosin-Rammler (1) with a correlation coefficient $R^2 \geq 0.982$, time in which the Schuhman type distribution law with a correlation coefficient $R^2 \geq 0.933$ (usually $R^2 \geq 0.956$) can be used with satisfactory results, in these cases;

- For the two parameter distribution law, the R^2 correlation coefficient presents close values from the ones obtained through the Rosin-Rammler function, $R^2 \geq 0{,}963$, at half the probes being very close;

- The total grinding degree of the wheat breakage phase at the analyzed mill is approximately $\lambda = 7$, correspondent to a coarse gritting (crushing);

- It is appreciated that, in all cases, at seeds wheat grinding in the complex roller mills, we can consider that the best law of distribution is the Rosin-Rammler (1), ($R^2 \geq 0{,}982$), but the other methods, Schuhman and two parameter logistic, also can be used with satisfactory results.

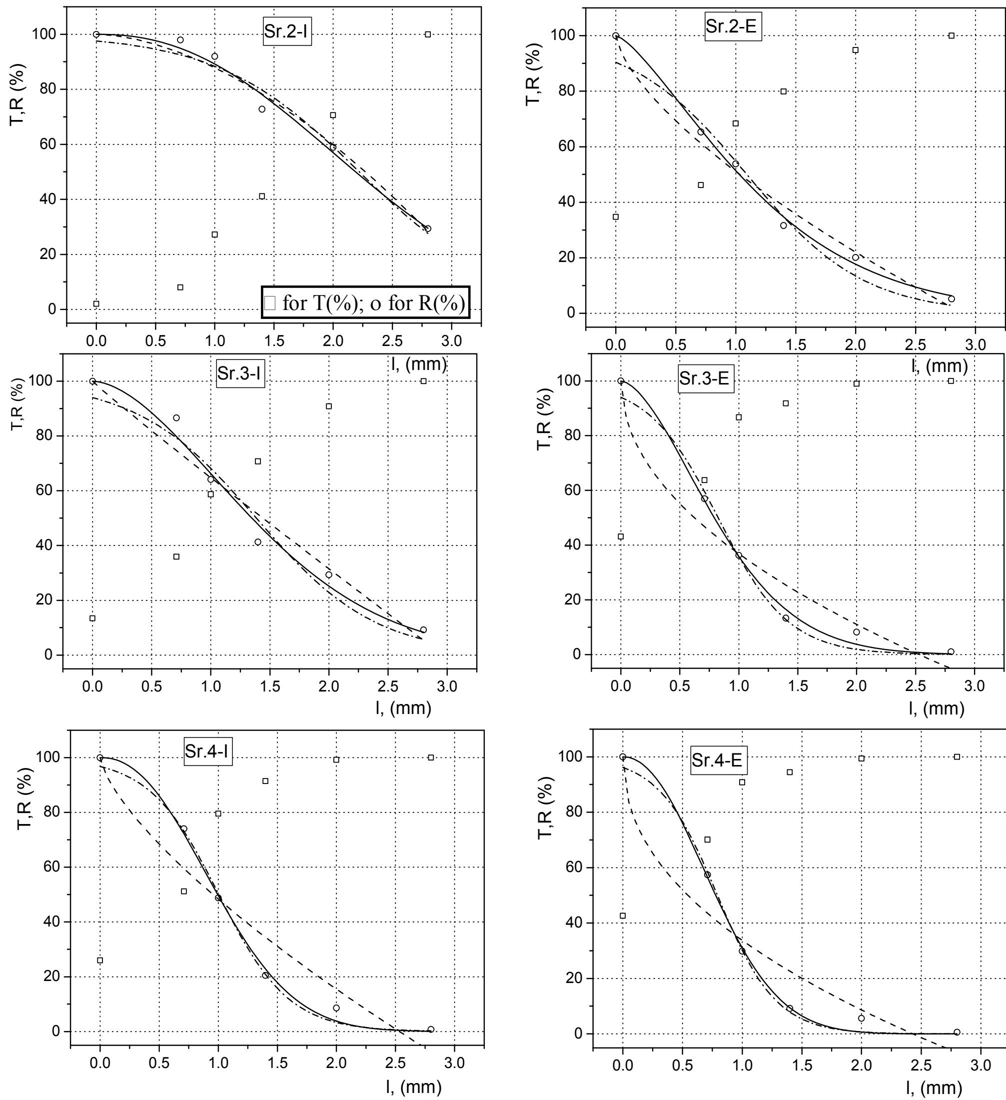

Figure 9. The curves described by the cumulative distribution laws (1), (2), (3) towards the experimental points R(%) for the gritting product from the five roll pairs (Sr.2…Sr.5) [10]; (I-entrance; E-exit); ———Rosin-Rammler; - - - - Schuman; — · — · logistical function)

Law type	Coeff.	Sr.1-E	Sr.2-I	Sr.2-E	Sr.3-I	Sr.3-E	Sr.4-I	Sr.4-E	Sr.5-I	Sr.5-E
Rosin-Rammler (eq.1)	b	0.224	0.114	0.665	0.411	1.025	0.701	1.169	2.472	2.652
	n	1.659	2.302	1.382	1.747	1.682	2.220	2.093	2.852	2.817
	R^2	0,988	0,987	0,996	0,982	0,996	0,996	0,996	0,998	0,999
Schuhman (eq.2)	k	4.201	3.398	2.893	2.966	2.531	2.532	2.431	1.025	1.016
	a	0.996	1723	0.674	0.960	0.495	0.711	0.464	1.710	1.639
	R^2	0,999	0,981	0,985	0,956	0,958	0,933	0.940	0,991	0.987
Logistic with two parameters (eq.3)	α	2.573	3.701	2.243	2.744	2.760	3.397	3.216	4.347	4.303
	β	-1.245	-1.666	-2.053	-1.981	-3.345	-3.380	-4.056	-6.739	-6.878
	R^2	0.984	0,974	0.972	0.963	0.988	0.995	0.994	0.997	0997

Table 8. The coefficient values a, k, b, n, α and β and of the R^2 correlation coefficients, for the three size distribution laws tested, for the gritted products from the „I" entry to the „E" exit between the mentioned roll pairs (Sr.1...Sr.5), [10]

In plansifter compartments, material fractions are separated and sorted, as any granular material is made of particles with sizes between a minimum and a maximum value, in the interior of the mixture the size distribution being characterised by various distribution laws.

It must be mentioned that material particles, being extracted from various areas of the seed (from exterior to interior) have different mechanical characteristics and composition. This, and the different sizes of particles gives a different behaviour of the particles during grinding.

Thus is important to study and to know the size distribution of the particles of each fraction obtained in each frame set of the six plansifter compartments.

Size of sieve holes used for the experiments and the amount of material fractions on each sieve (individual and cumulative) for the separated material are presented in table 9.

In every fraction there is a percentage of material with sizes smaller than the size of the sieve hole, which means that sieving is incomplete, even if the number of frames is quite high. However, the average particle size of fraction C1–Break 2 is 2.27 mm, much larger than the opening of sieve holes of the package (1.05 mm). This shows that here are obtained the parts of seed with quite large sizes, which must be reintroduced in the grinding process at the passage Break 2.

At the second set of sieving frames of plansifter compartment C1, the opening of fabric holes is 470 μm (no. 40), but mean size of particles of fraction C1-DIV1′ is 0.58 mm, slightly larger than the opening of the holes. It is noticed (Table 9) that there are particles with sizes smaller than the size of holes which remain unseparated (at least 8.4%). This phenomenon is valid for all sets of sieves in the plansifter with six compartments, as can be seen from the analysis of the results presented in table 9.

Composition of fraction C1-DIV1″ of plansifter compartment C1 consists of the refuse of sieve frames no. 56 (with holes opening 0.31 mm), after the sieved of the second set, consist-

ing of particles that passes through sieve no. 40 (with holes opening 0.47 mm) was extracted flour F (mean size of particles 0.08 mm). This fraction with fraction C1-DIV1′ and with the two fractions C2-DIV1 of the second plansifter compartment are directed to the sorting-dividing compartment DIV1 (compartment C5). Mean particle sizes of fraction DIV1″, from compartment C1, are 0.31 mm (equal to the opening of sieve holes which refused them, proving that here also the sieving is incomplete).

l_i (mm)	C1 Entrance		l_i (mm)	C1 Break 2		l_i (mm)	C1 DIV1′		l_i (mm)	C1 F		l_i (mm)	C1 DIV1″		l_i (mm)	C1 M2	
	p_i (%)	T_i (%)		p_i (%)	Ti (%)		p_i (%)	Ti (%)		p_i (%)	Ti (%)		p_i (%)	Ti (%)		p_i (%)	Ti (%)
0.000	24.20	0.00	0.000	10.20	0.00	0.000	1.10	0.00	0.000	4.20	0.00	0.000	6.00	0.00	0.000	0.60	0.00
1.000	8.20	24.20	1.000	21.30	10.20	0.180	2.30	1.10	0.045	45.10	4.20	0.125	8.00	6.00	0.090	1.90	0.60
1.400	15.10	32.40	1.400	14.60	31.50	0.250	5.00	3.40	0.063	24.30	49.30	0.180	12.80	14.00	0.125	41.50	2.50
2.000	20.20	47.50	2.000	21.60	46.10	0.400	51.70	8.40	0.090	18.80	73.60	0.250	24.50	26.80	0.180	15.00	44.00
2.800	27.10	67.70	2.500	20.70	67.70	0.630	28.60	60.10	0.125	6.30	92.40	0.315	32.40	51.30	0.200	30.10	59.00
4.000	5.20	94.80	4.000	11.60	88.40	0.710	11.30	88.70	0.160	1.30	98.70	0.400	16.30	83.70	0.250	10.90	89.10
d_{1E} = 2.13 mm			$d_{1Break2}$ = 2.27 mm			$d_{1DIV1'}$ = 0.58 mm			d_{1F} = 0.08 mm			$d_{1DIV1''}$ = 0.31 mm			d_{1M2} = 0.19 mm		
l_i (mm)	C2 Entrance		l_i (mm)	C2 Break 3		l_i (mm)	C2 DIV1′		l_i (mm)	C2 F		l_i (mm)	C2 M2		l_i (mm)	C2 DIV1″	
	p_i (%)	Ti (%)		p_i (%)	Ti (%)		p_i (%)	Ti (%)		p_i (%)	Ti (%)		p_i (%)	Ti (%)		p_i (%)	Ti (%)
0.000	34.40	0.00	0.000	23.50	0.00	0.000	5.60	0.00	0.000	19.10	0.00	0,000	17.70	0.00	0.000	0.30	0.00
0.710	11.50	34.40	1.000	30.10	23.50	0.180	4.00	5.60	0.045	34.90	19.10	0,090	9.40	17.70	0.125	1.60	0.30
1.000	22.20	45.90	1.400	13.50	53.60	0.250	7.70	9.60	0.063	21.60	54.00	0,125	22.30	27.10	0.180	5.60	1.90
1.400	11.60	68.10	2.000	17.60	67.10	0.400	26.10	17.30	0.090	16.10	75.60	0,180	9.60	49.40	0.250	19.20	7.50
2.000	15.00	79.70	2.500	12.40	84.70	0.500	49.20	43.70	0.125	6.70	91.70	0,200	25.10	59.00	0.315	44.50	26.70
2.800	5.30	94.70	3.150	2.90	97.10	0.710	7.40	92.60	0.160	1.60	98.40	0,250	15.90	84.10	0.400	28.80	71.20
d_{2E} = 1.22 mm			$d_{2Break3}$ = 1.56 mm			$d_{2DIV1'}$ = 0.52 mm			d_{2F} = 0.07 mm			d_{2M2} = 0.17 mm			$d_{2DIV1''}$ = 0.37 mm		

Table 9. Values of weights p_i(%) of sieved fractions and of the cumulative weights T_i (%) for products collected at the entrance, respectively exit of plansifter compartments, C1 and C2

The last components of plansifter compartments in gristing passage shave higher content of coating particles which are found in the upper layers of material on the frames, thus being recommended that they do not separate through the holes, even if their sizes are about the size of endosperm particles, to be further removed in semolina machines (sieving motion leads to the layering of mixture components by density). Flour particles have mean sizes under 0.18 mm in all plansifter compartments, while particles of last refuse from the five passages fitted with pairs of rollers have mean sizes over 0.37 mm (see Table 9). Values of

coefficients b and n in the equation of relationship Rosin–Rammler cumulative distribution law (eq.1), for the material which passed through sieve holes in granulometric analysis, and the correlation coefficients R^2 and χ^2 have high values which show the adequacy degree of the given function with the experimental data. In all cases, for all fractions obtained during gristing phase of wheat in the studied mill, the correlation is very good, appreciated by values of coefficient $R^2 \geq 0.926$.

As it can be noticed from fig.10, there are fractions having most particles of sizes close to the minim value of sieve classifier holes, but there are also components with particles with sizes from the mean size to the maximum size of the sieve holes used for granulometric analysis.

However, most components show mean profile (with central inflection point) of the separation curves which demonstrates the correct choosing of sieve classifier sizes (made from a set of 30 sieves by trying to take into consideration the arrangement in geometric distribution with holes ratio of $\sqrt{2}$).

From the analysis of coefficients b and n from Rosin–Rammler law (eq.1) it is noticed that values of coefficient b are 0.2–1.5 10^3 for most analyzed fractions, generally with high values, for the small size components of the particles (flour or dunsts), 1 10^6 – 5 10^7, giving the size characteristics of such particles (Table 10).

Plansifter compartment		b	n	R^2	χ^2	Plansifter compartment		b	n	R^2	χ^2
C1	C1 Entrance	0.222	1.663	0.988	17.039	C4	C4 Entrance	0.621	1.958	0.988	21.914
	C1 Break 2	0.169	1.964	0.987	18.890		C4 F	$2.06 \cdot 10^3$	5.665	0.997	1.144
	C1 DIV1″	37.812	3.412	0.993	9.147		C4 M4	$1.15 \cdot 10^3$	3.967	0.976	36.414
	C1 DIV1′	15.782	6.033	0.997	6.245		C4 FT2	0.027	4.557	0.996	9.071
	C1 F	$2.2 \cdot 10^3$	3.051	0.938	142.827		C4 Break 5	2.590	2.983	0.999	1.091
	C1 M2	$2.86 \cdot 10^3$	5.028	0.988	20.440		C4 M5	$1.6 \cdot 10^3$	6.054	0.999	3.370

Table 10. Values of coefficients b and n and correlation coefficient R^2 for Rosin – Rammler granulometric distribution, for the granulometric distribution law for fractions of the two plansifter compartments

Values of exponent n indicate the uniformity or the irregularity degree of particles from the analyzed fractions.

The analysis of this exponent values for the fractions of each plansifter compartment (Table 9) shows that they have a wide range of values, even for the same type of grinding product (for example flour – F), which shows the irregularity of particles, both for a given fraction and between fractions.

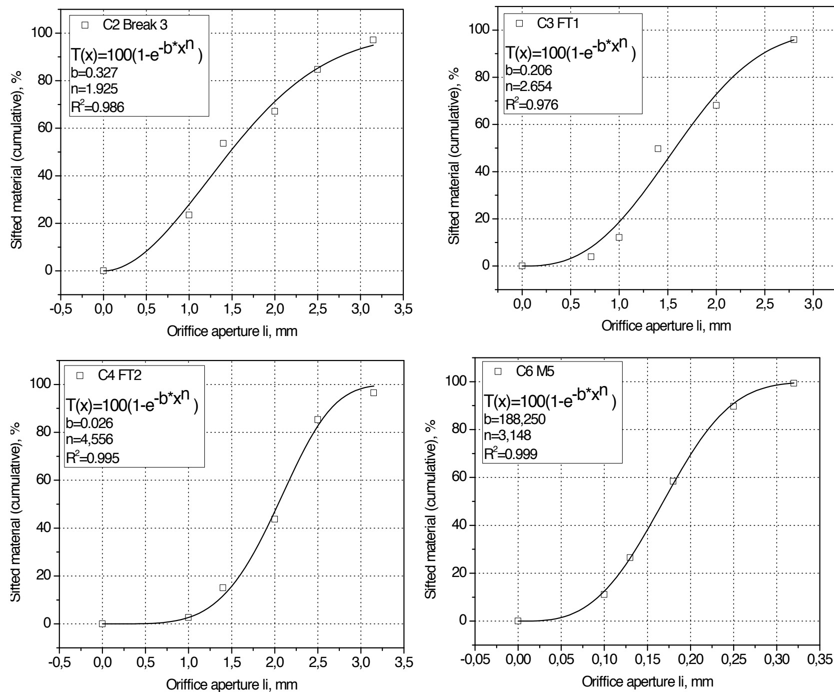

Figure 10. Curves of granulometric distribution given by eq. (1) in correlation with experimental data for grinding fractions in plansifter compartments during gristing phase of wheat in a mill with capacity of 100 t /24 h

4. Some mechanical characteristics of wheat seeds in uniaxial compression tests

Main stress to which seeds are subjected, while passing through mill rollers, is given by the type of rollers surface, namely smooth or fluted. Regardless the surface type, one of the main stress during grinding is compression (or crushing), especially if the mill rollers have smooth surface. To estimate the behaviour of seeds while passing through the rollers, experimental research is required on the compression stress of seeds from various wheat varieties, knowing that not all varieties have similar mechanical characteristics. Even seeds from the same variety have different behaviour, due to the irregular development stage in the ear, and also from one ear to another.

The compression of wheat seeds is performed in three different stages: the first stage is elastically deformation, characterized by the proportionality between the compression force and the deformation; the second stage is plastic deformation, characterized by large increases of seed deformation at small increases of compression force; the last stage consists in cracking or rupture, being characterized by seed crushing when reaching a certain value of compression force, [17-20].

Compression test is an objective method for determining the mechanical properties of cereal seeds and also one of the best techniques for determining the modulus of elasticity by the study of their behaviour at compression stress, using force-deformation curve, [21,22].

By performing uniaxial compression tests on wheat seeds, force-deformation curve is obtained, giving the possibility to determine hardness, apparent modulus of elasticity, crushing resistance, force and deformation and energy consumption in various specific points of the curve (i.e. rupture point) and maximum stress in the material, [21,23].

Cereal seeds have a different behaviour under the action of compression forces, depending on their moisture content, [17,20], variety, development stage, geometric sizes, individual mass, glassiness, (soft cereals and hard cereals) etc.

In fig.1 is presented a typical force-deformation curve for compressed Flamura wheat seed.

The bioyield point is the point on the force – deformation curve at which the force decreases or remains constant with increasing deformation. Force in the rupture point (rupture force) is the minimum required force for the wheat seed to break (rupture). Deformation at bioyield and rupture points is the deformation at loading direction, [24,25]. Values of force and deformation to bioyield and rupture points are directly read from force-deformation curve and recorded by machine used for compression test, [21].

Energy absorbed in bioyield and rupture points could be determined from the area under the force-deformation curve between the initial point and the bioyield and rupture point, respectively, using equation [24,25]:

$$W = \frac{F\,D}{2}(\mathrm{mJ}) \tag{9}$$

where: W is energy absorbed (mJ), F is force in bioyield or rupture point (N), D is deformation in bioyield or rupture point (mm), (see fig.11).

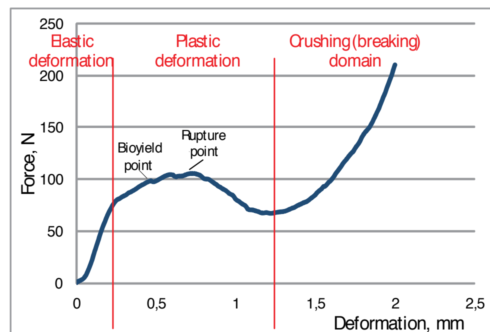

Figure 11. A typical force –deformation curve of wheat grain (type Flamura), [20]

Based on a standard method (ASAE 2008, [21]), for a seed placed between two parallel plates, the modulus of elasticity could be calculated with following equation, [20,21,21]:

$$E = \frac{0.338 k_u^{3/2} F\left(1-\mu^2\right)}{D^{3/2}} \left[\frac{1}{R'} + \frac{1}{R_1'}\right]^{1/2} \tag{10}$$

where: E – modulus of elasticity for cereal seeds, (MPa); k_u – coefficient which depends on the geometrical properties of wheat seeds (k_u = 1,303 - adapted from calculus tables of Kozma and Cunningham, 1962); *F* – compression force, (N); *D* – seed deformation (m); μ - Poisson ratio, (μ = 0,3 for wheat seeds); *R*' and R'_1 – small and large radius of the curvature of convex surface seed in contact with the flat surface, (m), (see fig.12, left).

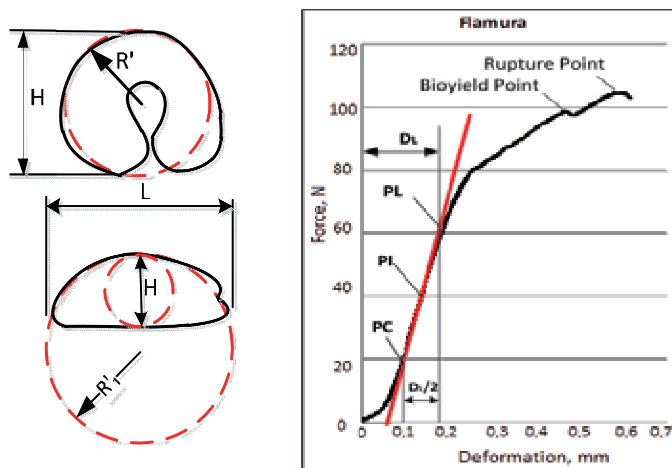

Figure 12. Estimation of curvature radius and force-deformation curve of wheat seed, (adapted from [25,26]) PL – proportional limit; PI – point of inflection; P_c – point of calculation

According to the standard method (ASAE 2008, [21]), also presented by Mohsenin in [25,26], curvature radius of convex surface, R' and R'_1 (fig.12) can be calculated using relations (11) and (12):

$$R' \cong \frac{H}{2} \tag{11}$$

$$R_1' \cong \frac{H + L^2/4}{2H} \tag{12}$$

where: *H* is seed thickness, (m), and *L* is seed length, (m), in undistorted state.

This method was used by many researchers to determine the modulus of elasticity for different agricultural products, [27-30].

According to the standard method (ASAE 2008), values of force *F* and deformation *D*, from equation (2) are calculated for the proportionality area of force-deformation curve in the point of calculation P_c (fig.12). The position of this point is estimated visually, as the point is located halfway between curve origin and proportionality limit P_L (fig.12, right). It was found that the point of calculation P_c is located lower than the point of inflection, also established visually, [21].

To determine the variation of mechanical resistance characteristics of wheat seeds from the same variety, compression tests were performed for sets of 100 seeds of three varieties of Romanian wheat (Flamura, Glosa and Trivale – soft wheat), using Hounsfield mechanical testing machine, at a constant speed of the crushing device of 5 mm min^{-1}, using a force cell of 1000 N. Were graphically plotted the force-deformation curves for each seed, and from each diagram were collected data about: force, deformation and energy absorbed in the bioyield point (F_1, ε_1, W_1), respectively in the final point (rupture), (F_2, ε_2, W_2).

The analysis of measured data showed that the seeds of Flamura variety were larger than Trivale variety, for all three main sizes, and for their volume. The same goes for seeds mass. Flamura variety seeds were more uniform as size and mass. Regarding the mechanical characteristics of wheat seeds, it was found that compression forces, for bioyield point and for final seeds crushing, were smaller for Trivale variety than Flamura. The same goes for energy absorbed to the bioyield point, respectively to crushing. Since the sizes of Trivale seeds were smaller than Flamura seeds, the deformations carried to the bioyield point, respectively to crushing, were smaller for Trivale than Flamura, but the standard deviation of the values was smaller for Flamura for deformations, showing that Flamura seeds were more regular in terms of deformations (until crushing).

In fig.13 are presented two examples of force-deformation curves for two varieties of wheat, and in fig.14 are presented the histograms of bioyield force and energy absorbed for seed crushing.

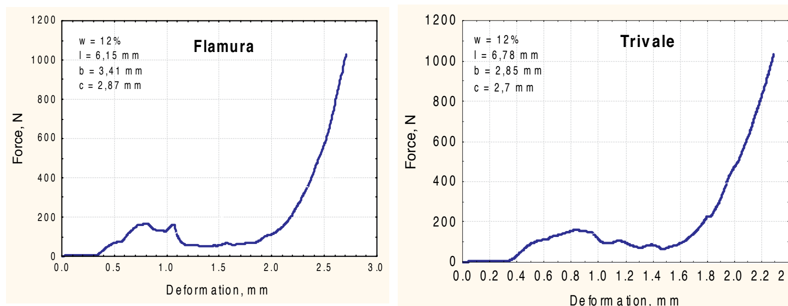

Figure 13. Examples of force-deformation curves for the two wheat varieties, [23]

On the histograms were traced the variation curves for the analyzed parameters by regression analysis of the values given by the histogram, using the normal function presented in equation (13), [23]:

$$p_x = 100a \frac{1}{\sqrt{2\pi}\, b} e^{-\frac{1}{2}\left(\frac{x-c}{b}\right)^2} \tag{13}$$

where: p_x(%) is the percentage weight of each class interval (number of seeds with values in the considered class interval); a – class interval for each analyzed parameter; b and c are regression coefficients of the analyzed function (*b* is the standard deviation, *c* is the values mean).

Values of coefficients for the regression function (eq.13) used in statistical analysis and values of correlation coefficient R^2 for data given by histograms are presented in Table 11.

Measured parameters of wheat seeds	Flamura wheat variety				Trivale wheat variety			
	a	b	c	R^2	a	b	c	R^2
Length l, (mm)	0.20	0.404	6.443	0.989	0.20	0.501	6.186	0.971
Width w, (mm)	0.10	0.202	3.429	0.974	0.20	0.284	2.994	0.981
Thickness t, (mm)	0.20	0.248	3.058	0.975	0.20	0.315	2.664	0.983
Mass m, (g)	0.01	0.008	0.051	0.988	0.01	0.009	0.037	0.981
Volume V, (mm^3)	5.00	5.870	35.57	0.968	5.00	6.207	26.23	0.985
Bioyield force F_1, (N)	20.0	41.36	122.64	0.921	20.0	39.48	104.70	0.888
Bioyield energy W_1, (J)	0.01	0.024	0.036	0.923	0.01	0.017	0.026	0.884

Table 11. Values of coefficients for regression equation (eq.13) and its correlation with experimental data [20]

Analysis of histograms and variation curves, as well as of data in table 11, shows that all analyzed parameters have almost normal distribution, assessed by values of correlation coefficient R^2.

Using standard method (ASAE 2008, [21]) and equations (10), (11) and (12) were determined the values of modululs of elasticity for wheat seeds of Flamura, Trivale and Glosa varieties, in this paper being presented their mean values, (table 12).

Fig.15 shows the machine used for uniaxial compression tests between parallel plates of weat seeds and their position.

From the sample of 100 determinations for each variety of wheat, were selected the 50 most representative determinations, being kept the values found for force and absolute deformation of the seed.

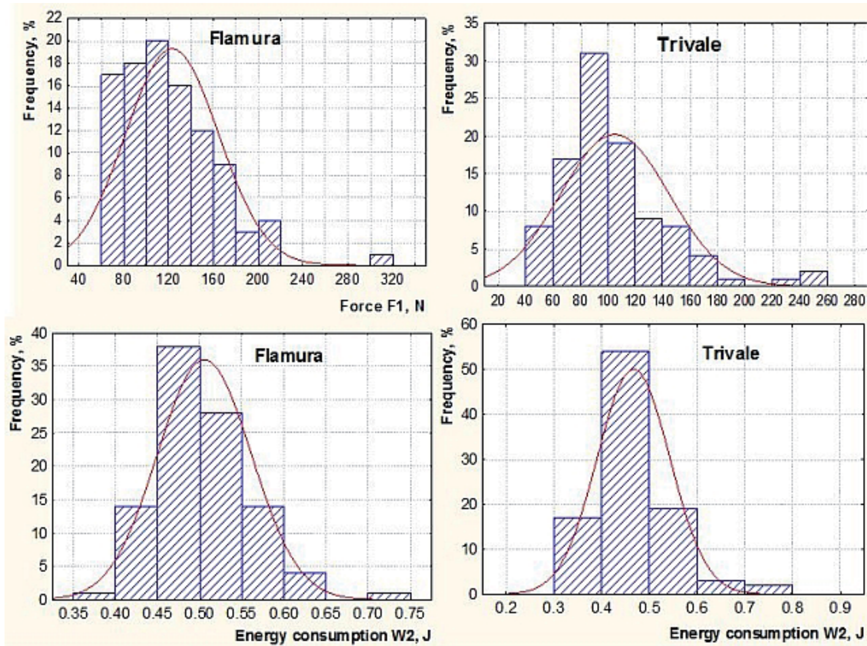

Figure 14. Histograms and variation curves for the force in bioyeld point and the energy consumption in rupture point for wheat seeds [23]

Force-deformation curves, for each of the 50 determinations (of a variety) were processed so that each has the same origin (same starting point), and the intervals of reading (recorded) to be the same. Values for the parameter on the ordinate (forces in the mentioned points) were averaged (arithmetic average for the 50 determinations was calculated) for the same value of deformation (parameter on the abscissa), and these values were used to retrace the force-deformation curve, which respresents the curve of mean values of compression force (fig.16). Using the approximately normal distribution, were statistically estimated the limits within which the mean force-deformation curve is found, for a confidence interval of 95%. For normal distribution, the confidence interval corresponding to 95% confidence level ranges between +/- 1,96, considered standard deviations. Thus, the confidence interval of mean curve was calculated using the following equation:

$$\mu = m \pm 1.96 \frac{\sigma}{\sqrt{n}} \tag{14}$$

where: μ is the confidence interval, and m is the mean value of the analyzed parameter (in this case, the compression force) and $\sigma / \sqrt{n} = S_m$ is the standard error of the mean, σ – standard deviation, and n –number of seeds from each variety of wheat (in this paper, n = 50).

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On the curve of mean values (fig.16), were determined the values of mechanical characteristics mentioned before (forces and deformations in the characteristic points) and it was calculated the value of modulus of elasticity using the standard method (ASAE 2008, [21]), for mean curve (for the three varieties of wheat).

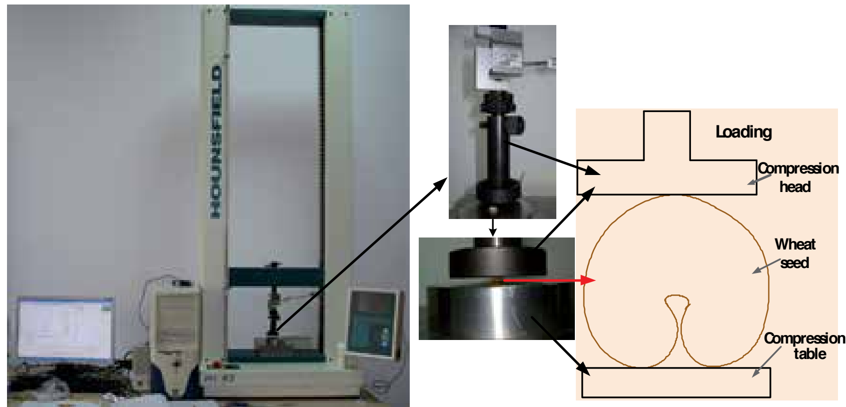

Figure 15. Hounsfield - Mechanical testing machine used in compression test [20]

Knowing the forces and deformations in the points of bioyield and rupture, from the area under the force-deformation curve between the initial point and the bioyield and rupture point, respectively, using equation (1), energy absorbed in bioyield and rupture point was determined.

Measured parameters of wheat seeds		Mean of parameters values			Values of parameters read from the mean curve		
		Flamura	Trivale	Glosa	Flamura	Trivale	Glosa
Bioyield force F_b, (N)		93.2	83.1	98.0	98.4	81.1	94.0
Bioyield energy W_b, (J)		-	-	-	0.028	0.018	0.016
Rupture force F_r, (N)		107.8	90.5	103.6	104.2	83.2	94.7
Rupture energy W_r, (J)		-	-	-	0.038	0.018	0.016
Bioyield deformation	Relative deformation, δ_b	0.138	0.092	0.077	-	-	-
	Absolute deformation, D_b (mm)	0.304	0.267	0.260	0.464	0.348	0.292
Rupture deformation	Relative deformation, δ_r	0.099	0.109	0.086	-	-	-
	Absolute deformation, D_r (mm)	0.419	0.320	0.290	0.576	0.400	0.360
Modulus of elasticity, (MPa)		313	364	486	298	369	468

Table 12. Values of measured and determined parameters in uniaxial compression test [20]

Analysis of data presented in table 12 showed that the values of bioyield force, respectively values of the force in the point of rupture of wheat seeds, determined from the mean curve are very close to the values of these forces obtained from the force-deformation curves for each particular seed.

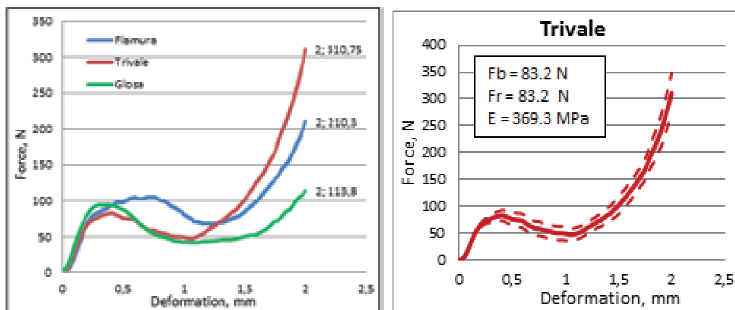

Figure 16. Mean curves force-deformation for three wheat varieties and 95 % confidence interval, [20]

Analysis of curves presented in figure 15 shows that they have similar shapes for the three varieties of wheat, and also within each of them and the force-deformation curves for each individual seed analyzed from each variety of wheat.

As absolute values of the force in the bioyield point, respectively in the rupture point, they are found in between 83.1 N for Trivale variety and 98.0 N for Glosa variety regarding the bioyield force, respectively 90.5 N for Trivale and 107.8 N for Flamura (values calculated with arithmetic average of the 50 determinations). These values are very close to the values presented in literature [31], where is stated that crushing force (rupture) of wheat seeds is of approximately 100 N.

On the relative deformation of seeds, during the compression tests, for the force in the bio-yield point (bioyield force), respectively rupture, data in table 12 also show relatively close values for the wheat seeds of the three varieties.

5. Conclusions

Development of technological gristing process of the wheat in a mill is very important for the entire technological flow of the mill, having a great influence on the degree of flour extraction, without excessive grinding of seed coating.

Based on material samples taken from the entrance and exit of each pair of milling rollers it can be determined, by laboratory analysis, the equivalent average sizes of the material, grinding degree in the passage, and the specific surface of material particles.

Granulometric analysis of the material to be grinded or of the grinded material at mill rollers, and of the sorted fractions in plansifter compartments show a distribution after multiple

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known laws, from which most used is Rosin-Rammler distribution function, with high correlation coefficient R^2.

However, it is shown that there can also be used with good results the Schuhman and logistical two parameters distribution laws, the finding suggest that the type of granulometric distribution law which best describes the size of grinded biological materials depends on material nature and the place and role of roller mill used for grinding in the general technological flow. Knowledge of adequate mathematical models describing the size distribution of grinded materials is useful in all engineering activities related to the processes on the flow of complex roller mills of last generation.

Values of mechanical characteristics of wheat seeds (regardless the variety) are necessary to estimate the energy consumed for their grinding in grain mills. A great influence on the grinding energy is given by the crushing force and their relative and absolute deformation, determined by experimental research of uniaxial compression.

For some wheat varieties presented in this chapter, compression force in the rupture point, determined from force-deformation curves has values of 100-110 N, for seed moisture content of about 12%.

Crushing energy has values of 0.02-0.04 J, for each wheat seed, but it is influenced by the moisture of seeds and by seed arrangement during compression: on width ″sideways″ or on thickness ″laying flat″.

Regarding the modulus of elasticity, its values are between 313-487 MPa, being greater as moisture is lower. It was found that lower moisture content resulted in higher values of modulus of elasticity and to lower values of rupture energy, which confirm that wetter seeds have greater plasticity than dry seeds, so they have higher energy consumption.

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Chapter 16

Technological Options of Packaging to Control Food Quality

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Additional information is available at the end of the chapter

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1. Introduction

The shelf life of perishable foods as meat, poultry, fish, fruit, vegetables and fresh cereal-based products is limited by various factors that generally bring to changes in odor, flavor, color and texture until to their complete unacceptability. Packaging is the main tool to prevent product deterioration and prolong its shelf life. The package protects the food against physical, chemical and biological damage. It also acts as a physical barrier to oxygen, moisture, volatile chemical compounds and microorganisms that are detrimental to food. The package has to be considered as an integral part of the preservation system because it provides a barrier between the food and the external environment. It is usually a composite item meeting several different needs [1]. What we call the preservation role is a fundamental requirement of food packaging, since it is directly related to the safety of the consumer. Package performance depends on numerous variables, such as the initial food quality, the processing operations, the size, the shape and appearance of package, the distribution method and the disposal of packages. Generally specking, the properties which determine their adequacy to meet performance requirements can be grouped into the following categories: mechanical, thermal, optical and mass transport properties. Mass transport phenomena are of great importance to food packaging with plastics, since a polymeric matrix is permeable to moisture, oxygen, carbon dioxide, nitrogen and other low molecular weight compounds. Glass and metal packaging materials are not permeable to low molecular weight compounds, whereas paper-based materials are too permeable. Hence, these last types of materials do not provide an opportunity for the designer to optimize the barrier properties for various applications. The polymers can provide a wide range (by three or four orders of

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magnitude) of permeability for different applications, thus justifying studies aimed to ensure adequate barrier protection. Therefore, in situations where food deteriorations are driven by either gas or moisture permeation to the ambient environment, an accurate choice of packaging mass transport properties may bring about an increase to product shelf life. Each category food has its specificity in quality attributes, storage conditions, expected shelf life and packaging tools applied. Together with transport properties of film, another valid packaging option to maintain product quality is represented by the proper selection of headspace conditions. Vacuum packaging and modified atmosphere packaging (MAP) are two widely used strategies for food preservation [2]. The first strategy means a complete lack of gas in the package whereas, under MAP, headspace environment may change during storage but there is no additional manipulation of the internal environment. Packaging under these conditions can protect products against deteriorative effects, which may include discoloration, off-flavor and off-odor development, nutrient loss, texture changes, pathogenicity, and other measurable factors. With the increasing demand for fresh and natural products without addition of dangerous chemicals, MAP or vacuum seem to be ideal methods of preservation for many foods, being simple and cheap to be applied. The few disadvantages are related to need of equipments and proper packaging materials and, in the specific case of MAP, to the limitation on retail for the increased pack volume of bags.

Today the efforts to improve the performances of packaging with clear effects on food quality can be directed towards two many working areas, green polymers and active packaging. The performance expected from bio-plastic materials used in food packaging application is containing the food and protecting it from the environment while maintaining food quality. It is obvious that to perform these functions is important to control and modify their mechanical and barrier properties, that consequently depend on the structure of the polymeric packaging material. In addition, it is important to study the change that can occur on the characteristics of the bioplastics during the time of interaction with the food. Studies of the literature show up that only a limited amount of biopolymers are used for food packaging application [3, 4]. Unlike the usual wrap, films, labels and laminates came from fossil fuel resources, the use of biodegradable polymers represents a real step in the right direction to preserve us from environmental pollution. This kind of packaging materials needs more research, more added value like the introduction of smart and intelligent molecules able to give information about the properties of the food inside the package and nutritional values. It is necessary to make researches on this kind of material to enhance barrier properties, to ensure food properties integrity, to incorporate intelligent labeling, to give to the consumer the possibility to have more detailed product information than the current system [5, 6].

Active packaging is the most relevant innovative idea applied for consumer satisfaction. It has been defined as a system in which the product, the package and the environment interact in a positive way to extend shelf life of product or to achieve some characteristics that cannot be obtained otherwise [7]. In many present-day active packaging technologies the active agent is placed in the package with the food, in a small sachet, pad or device manufactured from a permeable material which allows the active compound to achieve its purpose but prevents direct contact with the food product, protecting the food from con-

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tamination or degradation. Active packaging developments are now focusing on incorporating the agents into the polymeric matrices which constitute the package walls; the resulting materials act by releasing substances which have a positive effect on the food or by retaining undesired substances from the food or the internal atmosphere of the package. The migration of a substance may be achieved by direct contact between food and packaging material or through gas phase diffusion from packaging layer to food surface. Although the former is the packaging situation usually meets, the latter solution has exerted interesting effects due to simple and wide applications. Among the migratory agent categories, a further division would be made between controlled and uncontrolled release systems. Even though uncontrolled delivery packages intended for food applications are more abundant, controlled release systems are of industrial relevance due to their aptitude to prevent sensorial or toxicological problems or inefficiency of the system, caused by a too high or a too low concentration of delivered substance [8]. The active packaging technology provides several advantages compared to direct addition of active compounds, such as lower amounts of active substances required, localisation of the activity to the surface, migration from film to food matrix and elimination of additional steps within a standard process intended to introduce the active compounds at the industrial processing level such as mixing, immersion, or spraying. New regulations, the Commission Regulation (EC) No 450/2009 (EC, May 2009), and the Question Number EFSA-Q-2005-041 (EFSA, July 2009), together with Regulation 1935/2004 (EC, October 2004) make active packaging possible within the European Union [9].

The current work aims to overview the main technological options of packaging to control food quality. In particular, the attention will be focused on the use of proper headspace conditions and applied active packaging systems. Case studies are given for main food categories, such as dairy products, meat, fish, fruit and vegetables.

2. Headspace conditions

Vacuum, gas flushing or controlled permeability of the pack are valid techniques to control biochemical, enzymatic and microbial degradations so as to avoid or decrease the main degradations that might occur in food. This allows the preservation of fresh state of the food product without temperature or chemical treatments used by competitive preservation techniques, such as canning, freezing, dehydration and other processes. MAP is the replacement of air in a pack with a single gas or mixtures of gases; the proportion of each component is fixed when the mixture is introduced. No control is exerted over the initial composition, and the gas composition is likely to change with time owing to the diffusion of gases into and out of the product, the permeation of gases into and out of the pack, and the effects of the product and microbial metabolism [10]. MAP was first recorded in 1927 as an extension of shelf life of apples by storing them in atmosphere with reduced oxygen and increased carbon dioxide concentrations. In 1930s it was used to transport fruit in the holds of ships. Increasing the carbon dioxide concentration surrounding beef carcasses transported long distances an increase in shelf life by up to 100% was shown [11]. Marks and Spenser intro-

duced MAP for meat in 1979; the success of this product led, two years later, to the introduction of MAP for bacon, fish, sliced cooked meats and cooked shellfish. MAP techniques are now used on a wide range of fresh or chilled foods, including raw and cooked meats and poultry, fish, fresh pasta, fruit and vegetables and more recently coffee, tea and bakery products. The advantage of MAP for the consumer are:

- increased shelf life allowing less frequent loading of retail display shelves;
- reduction in retail waste;
- improved presentation-clear view of product and all round visibility;
- hygienic stackable pack, sealed and free from product drip and odor;
- easy separation of sliced products;
- little or no need of chemical preservatives;
- increased distribution area and reduced transport costs due to less frequent deliveries;
- centralized packaging and portion control;
- reduction in production and storage costs due to better utilization of labor, space and equipment.

The disadvantages of MAP are:

- capital cost of gas packaging machinery;
- cost of gases and packaging materials;
- cost of analytical equipment to ensure that correct gas mixtures are used;
- cost of quality assurance systems to prevent distribution of leakers;
- increase of pack volume which will adversely affect transport costs and retail display space;
- benefits of MAP are lost once the pack is opened or leaks.

MAP does not increase significantly the shelf life of every type of food since some products that undergo processes such as smoking, curing, etc., already have extended shelf lives because of pre-packaging treatments. In these cases MAP may improve other quality aspects such as color stability or slice separation. The safety and the stability of foods depend on microorganisms initially present, being unable to overcome various adverse factors, both extrinsic and intrinsic to the food. Modification of the atmosphere surrounding the food may provide one condition to inhibit microbial growth. In table 1 there is a description of the principal degradations that take part in a common food product and the role of MAP in contrasting these factors. The combination of chilled temperatures and MAP generally results in a more effective and safer storage regime and longer shelf life [12]. Atmospheres within the product are influenced by the type of material used in the package and the initial gas mixture used. Some materials allow diffusion of gases in and/or out of the package during stor-

age while if the film is fully permeable, the atmosphere inside the pack becomes the same as the air outside. In semi-permeable films, the atmosphere in the pack rise to a gas equilibrium. Packs for MAP are made from one or more polymers: polyvinylchloride (PVC), polyethylene terephthalate (PET), polyethylene (PE), and polypropylene (PP), depending on the characteristics desired for the final use. There are many factors which must be taken into account:

- barrier properties: permeability to various gases and water vapor transmission rate;
- machine capability: capacity of trouble-free operation (resistance to tearing, possibility to be heat-formed)
- sealing reliability: ability to seal to itself and to container;
- anti-fog properties: good product visibility;
- special characteristics: possibility of heating without removing product from the packaging and easy-peel seals for convenient opening.

	Spoilage	Rancidity	Enzymatic browning
Effects on products	change in smell, taste, texture, appearance, toxicity	less nutritional value, rancid taste	browning of vegetables
Type of products	vegetables, bakery cooked products	products containing vitamins and fats	vegetables
Role of MAP	gas mixture mainly constituted by CO_2	N_2 or other neutral gas replacement of air	N_2 or other neutral gas replacement of air

Table 1. Main degradation factors and roles of MAP

The main gases used in MAP are: oxygen, nitrogen and carbon dioxide. These three gases are used in different combination according to the product and the needs of manufacturer and consumer. The choice for a particular combination is influenced by the microbiological flora and the sensitivity of the product to gases and color stability requirements. The basic concept of MAP of fresh foods is the replacement of the air surrounding the food in the package with a mixture of atmospheric gases different in proportion from that of air. Oxygen is the most important gas being used by both aerobic spoilage microorganisms and plant tissues and taking part in some enzymatic reactions responsible for food deterioration. For these reasons, under MAP, oxygen is either excluded or set as low as possible. This gas is generally set at low levels to reduce oxidative deterioration of foods, particularly in high fat product. Oxygen generally stimulates growth of aerobic bacteria, inhibiting growth of anaerobic bacteria, although there is a wide variation in the sensitivity of anaerobes to this gas. The exceptions occur when oxygen is needed for fruit and

vegetable respiration, color retention as in the case of red meat or to avoid anaerobic conditions in white fish [13]. One of the main function of oxygen is the maintenance of myoglobin in its oxygenated form, oxymyoglobin because this is the form responsible for the bright red color, which most consumers associate to fresh meat. Carbon dioxide is both water and lipid soluble and although is not bactericide or fungicide, it has a bacteriostatic and fungistatic properties. The effect on microorganisms consists in the extension of the lag phase and a decrease of growth rate. The effectiveness of this gas is influenced by its original and final concentrations, the storage temperature, the partial pressure of carbon dioxide, the initial bacterial population, the microbial growth phase, the growth medium used, the acidity, the water activity and the type of product being packaged [10, 14-16]. Yeasts which produce carbon dioxide during growth are stimulated by high levels of carbon dioxide and thus for some products where they are potentially a major cause of spoilage, MAP may not be an advisable option. Also *Clostridium perfrigens* and *botulinum* are not affected by the presence of carbon dioxide and their growth is encouraged by anaerobic conditions. In general, carbon dioxide is most effective in foods where the normal spoilage microorganisms consist of aerobic and gram negative psychrotropic bacteria [17]. For maximum antimicrobial effect, the storage temperature of the product should be kept as low as possible, because the solubility of carbon dioxide decreases dramatically with increasing temperature, thus improper temperature could eliminate the beneficial effects of carbon dioxide. The absorption of carbon dioxide is dependent on the moisture and fat content of the product. If product absorbs excess carbon dioxide the total volume inside the package will be reduced, giving a vacuum package look known as pack collapse. Excess carbon dioxide absorption in combination with package collapse can also reduce water holding capacity of meats, resulting in unsightly drip. Genigeorgis [18] suggested that the antimicrobial activity of carbon dioxide was a result of the gas being absorbed onto the surface of the product forming carbonic acid, subsequent ionization of the carbonic acid and a reduction in pH. Other theories have been summarized:

- alteration of cell membrane function including effects on nutrient uptake and absorption;
- direct inhibition of enzyme systems or decreases in rate of enzyme reactions;
- penetration of membranes resulting in changes of intracellular pH;
- direct changes to physic-chemical properties of proteins.

Nitrogen is an inert gas which has been used as a packaging filler for many years to prevent pack collapse because of its low solubility in water and lipid. In MAP products, especially fresh meat packed in high concentrations of carbon dioxide, pack collapse occurs because of the solubility of carbon dioxide in meat tissue. Nitrogen is also used to replace oxygen in MAP products, to prevent rancidity and inhibit growth of aerobic organisms. The gas combination depends on product characteristics. Table 2 reports a comparison between storage in MAP and in air for different products. Some relevant case-studies that highlight the benefits and limits of MAP for main food categories are reported hereinafter.

PRODUCT	Temperature	Shelf life under MAP	Shelf life in AIR
Toast bread	Room	2-3 months	10 days
Cake	Room	40-60 days	-[a]
Croissant, milk bread	Room	6 weeks	Several days
Pizza	4-5°C	30 days	Several days
Hamburger, hot dog rolls	4-5°C	30 days	1 week
Cakes with cream	Room	25-30 days	-
Emmenthal	2-4°C	4-5 weeks	A few days
Bovine mozzarella	2-4°C	6-8 days	3 days
Robiola, Crescenza	2-4°C	3-4 weeks	1 week
Cheese slices	2-4°C	2-3 months	2-3 months
Gorgonzola	2-4°C	30 days	10 days
Parmesan in pieces	2-4°C	40-60 days	-

[a] - means that air was never used to store product.

Table 2. Examples of food shelf life under MAP and air

The properties of meat that are important in determining shelf life include water binding capacity, color, microbial quality, lipid stability and palatability. The variables that influence the shelf life of packaged fresh meat are: product, package and headspace, packaging equipment, storage temperature, and additives. Plastic film properties, shrinkage, strength, oxygen transmission, moisture transmission, and anti-fog agents are important for meat package materials [19]. Fresh meat packaging is only minimally permeable to moisture to prevent desiccation, while gas permeability varies with the applications. MAP (commonly 70-80% O_2 and 20-30% CO_2) and vacuum packaging are widely used methods for packaging meat. Packaging under high oxygen concentration, however, may cause an increase in the lipid and protein oxidation. These reactions affect the functional, sensory and nutritional quality of meat products. Lipid oxidation leads to discoloration, increase drip-loss, off-odors and production of toxic compounds. In addition, these modifications can negatively affect the sensory quality of meat products in terms of texture, tenderness and color [20]. Protein oxidation can also result in the loss of enzyme activity and protein solubility, as well as in the formation of protein complexes and non enzymatic browning products. In a recent study [21], the effects of MAP (70% O_2 and 30% CO_2) and vacuum skin packaging on protein oxidation and texture of pork were investigated. Packaging under MAP containing high level of O_2 resulted in protein cross-linking, which reduced tenderness and juiciness of pork. Rowe et al. [20] proposed that the oxidation of muscle proteins may have a negative effect on beef tenderness that was attributed to an inactivation of μ-calpain with a subsequent decrease in proteolysis. Zakrys et al. [22]compared the effects of high levels of oxygen (80% O_2 20% CO_2) with vacuum packaging and showed that high O_2 levels lead to high myosin inter-

molecular cross-links, low free thiol groups and high carbonyl content, demonstrating that a significant level of protein oxidation occurred. This protein oxidation was found to have a negative effect on meat tenderness. Results from this study suggested that high oxygen induced changes in myosin and intermolecular cross-linking, increased disulphide bond formation, protein oxidation and drip loss compared to vacuum packaged. Color of meat is a very important quality attribute that influences consumer acceptance of meat. The surface color of meat depends on the quantity of myoglobin present, on its chemical state and also on the chemical and physical conditions of other components. Meat showing a bright red color is assumed to be fresh, while oxidation of heme iron to form methamyoglobin produces the brown color which consumers find undesirable. An interesting study conducted by Mastromatteo et al. [23] evaluated the combination of different MAPs (from 20% to 40% of CO_2 ; from 5% to 20% of O_2 and from 75% to 40% of N_2) with natural essential oils on shelf life of reduced pork back fat content sausages. They found that lemon and thymol recorded the highest sensory score while all the investigated MAPs showed an antimicrobial effect; moreover, low carbon dioxide concentrations caused low color variations during storage. The combination of MAP and thymol was able to further improve the shelf life of meat, in fact the microbial threshold was never reached. A shelf life of more than 5 days for thymol-MAP samples was obtained, respect to the other investigated samples (2 days). To sum up, integration of meat characteristics with available packaging materials, equipment into current cold chain logistical and information systems have resulted in a sufficiently high state of complexity that has caused uncertainty and confusion among industry, regulatory agency, and consumer segments [13]. Meat and packaging industry must continue to work on systems that will ensure safe and palatable products. The review of Belcher [24] well summarized packaging developments that are resulting from numerous trends taking place in the meat industry and in the retail sector. Moreover, alternative non-thermal preservation technologies such as high hydrostatic pressure, super chilling, natural biopreservatives and active packaging have been proposed because they are also effective against spores. To increase their efficacy, a combination of several preservation technologies under the so-called hurdle concept has to be investigated [25].

As regard fresh-cut fruits and vegetables, process increases respiration rate and causes major tissue disruption as enzymes and substrates normally sequestered within the vacuole. Processing also increases wound-induced ethylene, water activity and surface area per unit volume, which, may accelerate water loss and enhance microbial growth since sugars also become readily available. These physiological changes may be accompanied by flavor loss, cut surface discoloration, color loss, increased rate of vitamin loss, shrinkage and shorter shelf life. MAP is largely used for minimally processed fruit and vegetables. It relies on the modification of the atmosphere inside the package in order to extend the food shelf life by reducing the respiration of the product and consequently its degradation rate. The effect of MAP on quality of many fresh-cut products has been studied and successful applications include mushroom [26], apples [27], tomato [28], butterhead lettuce [29], potato [30], or kiwifruit [31]. These products are metabolically active for long periods after harvesting due to both endogenous activity, such as respiration, and external factors such as physical injury, microbial flora, water loss and storage temperature. Respiration may result in anaerobiosis,

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being quickly established if the produce is sealed in an impermeable film with low initial O_2 concentration. Subsequently anaerobic respiration of the produce will be initiated at very low O_2 concentrations, resulting in the accumulation of ethanol, acetaldehyde and organic acids and deterioration of organoleptic properties. Rate of respiration is influenced by the initial gas concentration so that, for example, reducing the oxygen to 2% and increasing the carbon dioxide concentration to 5%, results in more than 10-fold reduction of respiration rate in vegetables such as broccoli [32]. The maintenance of color is important and in red peppers, MAP has been shown to increase carotenoid retention and reduce browning [33]. The combination of storage time and temperature has been shown to be important in extending the shelf life of fruit in terms of texture, weight loss, pH and other nutritional changes. Temperature is also a factor in determining respiration rates of fruits. In freshly harvested beansprouts for example, which not only have a high respiration rate but also are characterized by high initial microbial populations, Varoquaux et al. [34], observed a 10-fold increase in respiration rate at 16.5 °C. In the case of fruit and vegetables with high respiration rates, there is an optimum initial atmosphere concentration to ensure minimal growth of aerobic spoilage bacteria together with an optimal film permeability to delay the development of anaerobic respiration and necrosis of vegetable. One of the obvious ways in which produce may be assessed for freshness is in terms of wilting and shriveling which are due to loss of moisture. Fruits and vegetables lose moisture when the relative humidity in the packaging is less than 80-95% of saturation and reduction in quality occurs if 3-6% of the produce moisture is lost [35]. Most films used for MAP of fruit and vegetables are relatively good water vapor barriers and are able to maintain a high relative humidity inside the pack. The relative humidity within a pack is influenced by the rate at which the product loses water vapor and by vapor transmission rate of the packaging film. Successful applications include broccoli florets, cauliflower florets, carrots, peeled garlic [36]. LDPE was found a good alternative to PVC for wrapping these vegetables. Comparative evaluation of the effect of storage temperature fluctuation on MAP of selected fruit and vegetables like mushrooms and mature green tomatoes was also studied by Tano et al. [37]. The quality of the products stored under temperature fluctuating regime was severely affected as indicated by extensive browning, loss of firmness, weight loss increase, ethanol level in plant tissue and infection, due to physiological damage and excessive condensation, compared to products stored at constant temperature. It was clear that temperature fluctuation can seriously compromise the benefits of MAP and safety of the product. Temperature is the most effective environmental factor in the prevention of fruit ripening. Both ripening and ethylene production rates increase with increase in temperature. To delay fruit ripening, temperature should be held as close to 0 °C as possible. The use of MAP as a supplement to proper temperature maintenance in the effort to delay ripening is effective for all fruits. Reducing oxygen concentration below 8% and/or elevating Carbon dioxide concentration above 1% retards fruit ripening. Successful applications include broccoli slaw, coleslaw, dry slaw, casserole mix, and mixed salads. Degradation of cut vegetables in terms of appearance was delayed by N_2 gas packaging and vegetables remained acceptable at temperature below 5 °C after 5 days. MAP may have the effect of increasing shelf life of some vegetables in terms of sensory properties but does not reduce growth of some microorganisms such as *L. monocytogenes*

and *Salmonella* Enterica. Therefore, the use of appropriate pre-harvest and postharvest sanitation practices to prevent contamination remains the most important measure for ensuring the microbiological safety of ready-to-eat fresh-cut products.

Shelf life of milk and milk-based products is limited because of their high water content and favorable pH for microbial growth [38-40]. The rapid spoilage adversely affects flavor and texture along with visual color changes of refrigerated raw and pasteurized milk, cottage cheese and other similar products. The responsible microorganisms include psychrotropic Gram negative bacteria, yeasts and moulds. These organisms produce extracellular protease and lipase activity, which reduce the functionality of milk proteins and fat and often produce undesirable aromas. Gram positive bacteria particularly those producing lactic and acetic acids, can spoil dairy foods, but the number of organisms required are generally higher than for Gram-negative bacteria, and the changes can be less noticeable. It has been reported that the product shelf life increases by low oxygen atmospheres because of the reduction in aerobic microorganisms. The antimicrobial effect of Carbon dioxide occurs near 10% level, and further increase in carbon dioxide affects growth of *Pseudomonas* and *Moraxella*. The largest inhibition by carbon dioxide occurs with Gram negative psychrotrophs bacteria [41]. The protective role of carbon dioxide is also important for mould proliferation; its function in creating an anaerobic environment with the displacement of existing molecular oxygen, its extra and intracellular pH decreasing effect and its destroying effect on the cell membrane make carbon dioxide an inhibitory substance towards microorganisms. The antimicrobial effect of carbon dioxide is dependent on many factors, including the partial pressure, application time, concentration of gas, temperature of the medium [42], volume of headspace, acidity, water activity of the medium and type of organism present [43]. The applied composition for packaging of dairy products can vary from 10% to 100% carbon dioxide, balanced with N_2 as inert gas filler, to prevent package collapse as a result of carbon dioxide solubilization in the cheese. MAP has been applied to the packaging of cheese. The packaging of each type of cheese needs to be consider separately. Another fact to be considered is that some cheeses are carbon dioxide producers, while other not. It is important that the levels of carbon dioxide are controlled because for certain cheese high levels of carbon dioxide have been found to impart off-flavor [44-46]. Cheese stored under carbon dioxide contained high concentrations of aldehydes and fatty acids and lower concentrations of alcohols and esters than cheeses stored under nitrogen. Hard and semi-soft cheeses, such as cheddar, are commonly packed in 100% carbon dioxide or mixtures of carbon dioxide and nitrogen. Soft cheese have also a limited shelf life. An alternative to conventional packaging is to use MAP. Carbon dioxide acts both directly on moulds and indirectly by displacing oxygen. Vacuum packaging does not remove all of the oxygen and thus, moulds and yeasts can still occur [47]. The gas mixture typically used is 70% N_2 and 30% CO_2 to inhibit mould growth, to keep the package from collapsing and to prevent shred matting. Alves et al. [48] reported that atmospheres ≥ 50% carbon dioxide were more effective than air or 100% nitrogen in improving shelf life of sliced mozzarella cheese. High carbon dioxide atmospheres have been shown to inhibit growth of lactic and mesophilic bacteria [49]. Piergiovanni et al. [50] compared Taleggio cheese packaged under four modified atmospheres and stored at 6 °C to conventional paper wrapping and found that samples packaged in MAP had satisfac-

tory quality. Gammariello et al. [51] evaluated the shelf life of Stracciatella cheese packaged in four different gas mixtures at 8 °C and showed that MAP 50:50 and 95:5 (O_2:CO_2) prolonged the sensorial acceptability limit by delayed growth of spoilage bacteria, without affecting the dairy microflora. Del Nobile et al. [52] suggest that MAP of Ricotta with 95% carbon dioxide inhibits microbial growth without effects on lactic acid bacteria, probably due to their facultative anaerobic nature, and also maintains the natural color of Ricotta.

MAP found wide application also for fresh fish. Fish such as herring and haddock benefits from being packaged under MAP since this reduces the production of peroxides which affect fish sensory characteristics and hence shelf-life [53]. However, high levels of carbon dioxide may result in carbon dioxide dissolution into the fish flesh, causing deformation or collapse of the packaging and also affecting the product color. The resulting drop in pH of the tissue may cause a decrease in the flesh's water holding capacity and drip may occur, reducing shelf-life. Fresh hake stored in up to 60% carbon dioxide exerted a shelf life significantly longer than those stored in air. MAP inhibits bacterial growth, reduces the formation of total volatile bases and trimethylamine and delays alterations in protein functionality [54]. In cooked fish such as smoked blue cod and smoked atlantic and silver salmon, a high concentration of carbon dioxide increases shelf life without showing drip or muscle exudate observed in fresh fish. carbon dioxide extents fish shelf life due to the inhibition of Gram negative and lactic acid bacteria. carbon dioxide concentrations in all MAP fishery products should be carefully monitored, especially when stored for long periods of time, because carbon dioxide does not inhibit *C. botulinum* and the effect of temperature abuse may increase the risk of botulism in those products which contain spores of non-proteolytic *C. botulinum* [55, 56].

3. Active packaging

Active packaging has been classified as a subset of smart packaging and referred to as the incorporation of certain additives into packaging film or within packaging containers with the aim of maintaining and extending product shelf life [57-59]. Another definition states that packaging may be termed active when it performs some desired role in food preservation other than providing an inert barrier to external conditions [60]. Hence, active packaging includes components of packaging systems that are capable of scavenging oxygen; absorbing carbon dioxide, moisture, ethylene and/or flavor/odor taints; releasing carbon dioxide, ethanol, antioxidants and/or other preservatives; and/or maintaining temperature control and/or compensating for temperature changes (Table 3). In the food and beverage market, growth of active packaging concepts is being driven by the growing use of packaged food, increasing demand for ready-prepared foods such as microwave meals, and increasing use of smaller package sizes. Although many active packaging technologies are still developmental, there are commercial successes, particularly in oxygen scavengers. Oxygen scavengers are easily oxidizable substances included in the packaging system to remove oxygen by means of a chemical reaction. The substance is

usually contained in sachets made of a material highly permeable to air but it can also be included in bottle closures or in the plastic film matrix. Different studies show that the use of scavengers led to faster reduction and to lower levels of residual oxygen, as compared to nitrogen flushing. The most common substances used are iron powder and ascorbic acid. Scavengers also differ in the reaction speed, from immediate action (0.5 to 1 day) to slow action (4 to 6 days), on the application, particularly the moisture content of the food, and on the function, i.e., oxygen scavenging only or dual function, such as absorbing or generating carbon dioxide, besides removing the oxygen. The scientific literature contains a number of references which examine the influence of oxygen scavenger sachets on fresh beef discoloration. Gill and McGinnis [61] performed an oxygen absorption kinetics study with a commercial oxygen scavenger (FreshPaxTM 200R) and reported that discoloration could be prevented in ground beef if large numbers of scavengers were used in each pack to bring residual oxygen to <10 ppm within 2 h at a storage temperature of -1.5 °C. The inclusion of oxygen scavengers (Ageless® SS200) in master packs flushed with 50% carbon dioxide and 50% nitrogen significantly improved color stability of M. longissimus dorsi and M. psoas major, relative to controls [62]. In addition to fresh beef oxygen scavenging technology has also been applied to pork [63] and pork products, where, Martìnez, Djenane, Cilla, Beltràn, and Roncalès [64] reported that fresh pork sausages stored in 20% CO_2 and 80% N_2 plus an oxygen scavenger (Ageless® FX-40) for up to 20 days at 2 ± 1 °C reduced psychrotrophic aerobe counts and extended shelf life in terms of color and lipid stability. An alternative to sachets involves the incorporation of the oxygen scavenger into the packaging structure itself. This UV light-activated oxygen scavenging film, which structurally is composed of an oxygen scavenger layer extruded into a multilayer film, can reduce headspace oxygen levels from 1% to ppm levels in 4–10 days, compared to oxygen scavenging sachets. The OS2000TM scavenging films found applications in a wide variety of food products including dried or smoked meat products and processed meats [65]. Berenzon and Saguy [66] evaluated the applicability of oxygen absorbers for extending shelf life of military ration crackers packaged in hermetically sealed tin cans and stored at 15, 25 and 35 °C for up to 52 weeks. Sensory evaluations suggested that crackers stored without oxygen absorbers developed oxidative rancid odors after 24 weeks at 25 and 35 °C. Independently of storage temperatures, no oxidative rancid odors were observed after 44 weeks with oxygen absorbers. Opposed to the currently available chemical oxygen scavengers, systems based upon natural and biological components could have advantages towards consumer perception and sustainability [67]. A model system for a new oxygen scavenging poly(ethylene terephthalate) (PET) bottle is proposed using an endospore-forming bacteria genus [68]. Incorporated spores could actively consume oxygen for minimum 15 days, after an activation period of 1–2 days at 30 °C under high humidity conditions. Although the system shows some clear opportunities, such as being a biological based system and its capability of solving polymer compatibility and recyclability issues, towards the current chemical systems, further investigations are necessary to determine a possible interaction between spores and food product.

Active packaging	Action mechanisms	Food applications
Oxygen scavengers	Slowed food metabolism, reduced oxidative rancidity, inhibited undesirable oxidation of labile pigments and vitamins, controlled enzymatic discoloration, inhibited growth of aerobic microorganisms.	Bread, cakes, cooked rice, biscuits, pizza, pasta, cheese, cured meats and fish, coffee, snack foods, dried foods and beverages.
Carbon dioxide scavengers/emitters	Slowed respiration rate, inhibited microbial growth.	Coffee, fresh meats and fish, nuts and other snack food products and sponge cakes.
Ethylene scavengers	Slowed respiration rate, thus slowed softening and ripening	Fruit, vegetables and other horticultural products.
Preservative releasers	Antimicrobial and antioxidant effect.	Cereals, meats, fish, bread, cheese, snack foods, fruit and vegetables.
Ethanol emitters	Effective against mould, can also inhibit the growth of yeasts and bacteria.	Pizza crusts, cakes, bread, biscuits, fish and bakery products.
Moisture absorbers	Inhibited microbial growth and moisture related degradation of texture and flavor.	Fish, meats, snack, cereals, dried foods, sandwiches, fruit and vegetables.
Flavor/odor absorbers	Malodorous constituents causing off-flavors are absorbed.	Fruit juices, fried snack foods, fish, cereals, poultry, dairy products and fruit.
Temperature control	Able to maintain chilled temperature.	Ready meals, meats, fish, poultry and beverages.
Temperature compensating	Gas permeability responding to temperature changes to avoid anoxic conditions.	Fruit, vegetables and other horticultural products.

Table 3. Examples of active packaging systems

Another important example of scavenger is the packaging with ethylene scavenger property. Ethylene has long been recognized as a problem in post-harvest handling of horticultural products because it is responsible for a wide variety of undesirable effects: it accelerates the respiration of fruits and vegetables, as well as softening and ripening, and it is responsible for a number of specific post-harvest disorders. The removal of this gas from storage chambers and packages of fruits and vegetables is, therefore, of the utmost importance, and it is done as a regular practice in the case of chambers, although is only more recently done in the case of removal from a single package. Ethylene is a very reactive compound that can be altered in many ways, such as chemical cleavage and modification, absorption, adsorption, etc. This creates a diversity of opportunities for commercial applications for the removal of ethylene [69]. Most substances designed to remove ethylene from package are delivered either as sachets that go inside the package or are integrated into the packaging material, usually a plastic polymer film. The most commonly used are based in potassium permanganate, activated carbon and activated earth. Meyer & Terry [70] studied the effect of 1-methylcyclo-

propene (1-MCP) and a newly developed palladium (Pd)-promoted ethylene scavenger (e + ®Ethylene Remover) on changes in firmness, color, fatty acids and sugar content of early and late season avocado (*Persea americana Mill.*), cv. Hass, during storage at 5 °C and subsequent ripening at 20 °C. Results have shown that the ®Ethylene Remover is effective at delaying ripening of avocado at low temperature, similarly to 1-MCP; however, subsequent ripening was not impaired. Similarly, but to a lesser extent and concomitant with trends in firmness retention and color changes, ®Ethylene Remover led to greater maintenance of mannoheptulose and perseitol than that of controls. Initial findings have demonstrated for the first time that the presence of a palladium-based scavenger was effective at removing ethylene to below physiologically active levels for preclimacteric green bananas and green avocado fruits. Reduced carbon dioxide production and control of color change from green to yellow was observed for the preclimacteric bananas. Results suggested that the normal and expected climacteric respiratory rise has been disrupted. Therefore, for the first time an ethylene scavenger has been shown to be capable of extending shelf life even when the climacteric respiratory rise has already been initiated [71].

In order to suppress spoilage and remove offensive odors in fresh products carbon dioxide absorbers are used. On the other hand, carbon dioxide emitters are useful in modified atmosphere packaging, because carbon dioxide suppresses the bacteria that cause spoilage. Both carbon dioxide generators and absorbers are available in sachet format [72].

Ethanol emitters are particularly effective in extending the shelf life of high water activity baked products. The use of ethanol generating sachets or strips avoids the ethanol spraying directly onto the product surface prior to packaging [60]. The ethanol is absorbed or encapsulated in a carrier material enclosed in sachets of selective permeability to ethanol to allow for ethanol accumulation in the headspace. The level of ethanol in the packaging headspace depends obviously on the sachet size and on product water activity.

Fragrances incorporated in packaging also found commercial use in food, personal care, pharmaceutical, and nutraceutical packaging. In food packaging, fragrances are being used as a marketing tool to create consumer awareness and to enhance brand image. Because cyclodextrins are able to form inclusion complexes with various compounds, they present a potential interest as agents to retain or scavenge substances such as odors, bitter compounds, lactose, cholesterol, etc., or to add aromas, colors, or functional ingredients whose release could enhance the quality of the packaged product and extend its shelf life [73-75].

Control of moisture is also important for food preservation. In most cases, the packaging material itself is responsible for the control of moisture transfer between the internal and external environment, providing an adequate barrier. There are situations however, where a greater control is needed to avoid the build-up of liquid water inside the package, therefore requiring liquid water control or humidity buffering as in the case of transpiration of fresh produce, melting of ice in fish transportation, temperature fluctuation in high water activity food packages and drip of tissue fluid from cut meats and produce [60]. To this aim, absorbent pads or sheets, anti-fog additives in the polymer film, humectant between two layers of a highly water vapor permeable film or sachets of inorganic desiccant salts, are generally used to accomplish liquid removal or humidity buffering.

Antioxidant food packaging films were produced by incorporation of ascorbic acid, ferulic acid, quercetin, and green tea extract into an ethylene vinyl alcohol copolymer matrix. The efficiency of the films developed was determined by real packaging applications of brined sardines. The evolution of the peroxide index and the malondialdehyde content showed that, in general, the films improved sardine stability. Films with green tea extract offered the best protection against lipid oxidation [76]. A natural citrus extract was also sprayed onto the surface of polyethylene terephthalate trays to delay lipid oxidation of cooked turkey meat slices, stored at 4 °C over 4 days [9]. The high surface roughness, demonstrated by optical profilometry, and the high level of solubility of the antioxidant in water allowed a good effectiveness of the citrus extract coating. Patties made of minced chicken breast and thigh packed in standard vacuum-packaging or in antioxidant active packaging containing rosemary extract were subjected to high pressure treatment (800 MPa, 10 min, 5 °C) and subsequently stored at 5 °C. The active packaging was able to delay surface lipid oxidation up to 25 days. The migration of α-tocopherol from a multilayer active packaging made up of high density polyethylene, ethylene vinyl alcohol and a layer of low density polyethylene containing the antioxidant, was studied. The antioxidant delivering system delayed the lipid oxidation of whole milk powder and it was more effective at temperatures higher than 20 °C [77].

The most investigated active systems are the packaging with antimicrobial properties, even if there has been little commercial activity in North America or Europe. Japan has historically been a leader in antimicrobial use. To date the literature counts various research works and review papers dealing with advances in antimicrobial packaging. Generally specking most of them are focused on *in vitro* test and can be subdivided in various categories: (i) there are studies aimed to develop new active systems with recently exposed natural compounds; (ii) studies finalized to underline the release kinetic of the active agents from the matrix to the food, with the intent to realize controlled release systems; (iii) studies aimed to develop bio-based active systems and (iv) works that use the nanotechnology approach. Among the abundant list of research articles and reviews available in the scientific literature on this topic, some recent works have been selected. Kanatt et al. [78] studied active films of chitosan and polyvinyl alcohol containing aqueous mint extract/pomegranate peel extract. Ramos et al. [79] studied antimicrobial active films based on polypropylene (PP) and containing thymol and carvacrol at three different concentrations. Trans-2-hexenal was encapsulated into β-cyclodextrins and incorporated into a poly(L-lactic acid) matrix by extrusion and casting [75]. A newly sinthetized polyester (poly-butylene adipate) containing covalently bound quaternary phosphonium groups was developed by Anthierens et al. [80]. The resulting polyester showed great antimicrobial activity through direct contact without any migration of active groups. Among the bio-based active systems, various bio-active packaging were developed to control insect pest in granary weevils [81, 82]. The efficacy of edible films produced from whey protein isolate and glycerol, including incorporation of lactic acid, propionic acid, chitooligosaccharides and natamycin was assessed by Ramos et al. [83]. Suppakul et al. [84] studied the diffusion of linalool and methylchavicol from thin antimicrobial low-density polyethylene-based films. Cellulose acetate-based mono and multilayer films including potassium sorbate were prepared using dry phase inversion technique [85].

Monolayer films, prepared using powdered cellulose and poly(vinyl) alcohol were coated with cellulose membrane to obtain multilayer films and sorbic acid was incorporated as antimicrobial agent [86]. Cerisuelo et al. [87] studied by a mathematical model the release of carvacrol from an ethylene-vinyl alcohol coating on a polypropylene film while Del Nobile et al. [88] studied the release of thymol from zein-based films. Bierhalz et al. [89] studied release behavior in water and diffusion coefficients of single and composite films based on alginate and pectin containing natamycin. Organic/inorganic compounds, essential oils, bacteria originated antibacterial proteins, enzymes and fruit extracts have shown great potential in inhibiting microbial growth in food stuff. However, the development of new resistant strains of bacteria to current antibiotics has led to the search for new bactericides that can effectively reduce the harmful effects of microorganisms. With the emergence of nanotechnology, the search for effective biocidal agents has focused on the development of nanostructures of coinage metals like silver, copper, zinc and gold [90]. ZnO nanoparticles loaded starch-coated polyethylene film were developed by Tankhiwale and Bajpai [91]. Montmorillonite nanoclay and rosemary essential oil were incorporated into chitosan film to improve its physical and mechanical properties as well as antimicrobial and antioxidant behavior [92]. Silver nanoparticles (AgNPs) have been abundantly exploited for technological applications as bactericidal agents. Recently, AgNPs were incorporated with success into bio-based materials [93] and into a hydroxy-propyl methylcellulose matrix [94]. Although numerous antimicrobial systems continue to be investigated in food simulating models, real applications are limited by technical, aesthetic and regulatory barriers. To this regards, a few recent examples can be cited. Microencapsulated beta-cyclodextrin and trans-cinnamaldehyde complex was incorporated into a multilayered edible coating made of chitosan and pectin to coat fresh-cut papaya that was then packaged in Ziploc trays with Ziploc lids for 15 days. The layer-by-layer assembly with incorporation of microencapsulated antimicrobial was effective in extending shelf life and quality of fruit [95]. The antimicrobial proteins lysozyme and lactoferrin were incorporated into paper containing carboxymethyl cellulose [96]. The antimicrobial activity on common food contaminants was also retained in the released protein, and a synergism between the two proteins was evident in tests carried out with paper containing both proteins. Lysozyme was most effective in preventing microbial growth when the system was applied to thin meat slices laid on paper sheets containing either or both antimicrobial proteins. Cellulose/silver nanocomposites were investigated to decrease the microbial loads in minimally processed foods and meat [97]. The active systems were synthesized by means of reduction by UV/heat of silver nitrate adsorbed on fluff pulp cellulose fibres. Minimally processed fruits and meat products were packaged in trays containing commercial absorbent pads or silver loaded absorbers and in contact with silver loaded absorbers, spoilage counts were significantly reduced. Active packaging based on silver nanoparticles, obtained by allowing silver ions from nitrate solutions to replace the Na+ of natural montmorillonite and then reduced by a thermal treatment, were applied to fruit salad [98] and fresh dairy products [99, 100]. The striking feature of these works is the interesting antimicrobial effects, without compromising sensory properties. The antimicrobial effectiveness is usually complicated by several factors, including temperature, moisture levels, chemistry of the antimicrobial agent and release mechanism. Moreover, it is necessary to

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consider odor or color change that an antimicrobial could provoke in the packaged product. The cost-benefit ratio of antimicrobials is also a limiting factor for commercial growth and rates of return in the food industry are small. All these considerations explain the limited diffusion of active systems, although several antimicrobials have been successful in the laboratory. Applications with good potential are value added products such as pre-sliced and prepared foods.

4. Final considerations

Packaging design is clearly a fundamental part of a new launch product. Considering the importance of packaging in determining product shelf life, the correct approach allows considering on the same level of importance the product development and its packaging system. The key to successful packaging is selection of materials and designs that best balance the competing needs of product characteristics, marketing considerations including distribution and consumer needs, environmental and waste management issues, and cost. Food packaging technologies also require integration with other processing and preservation activities such as freezing, irradiation, pulsed electric fields, high pressure processing, and pulsed light. Globalization, packaging life cycles, and requirement for strict safety measures are increasing the pressure to produce new packaging systems able to transport food items and that also allow the traceability along the food distribution chain. Due to the diversity of product characteristics and basic food packaging demands and applications, any packaging technologies offering to deliver more product and quality control in an economic and diverse manner would be favorably welcomed. To meet tomorrow's concerns, there continues to be a large amount of research to evaluate areas such as active packaging, traceability, sustainable resources and antimicrobial packaging. Advances in these areas will continue to give us a safe and sustainable food supply. The use of modified atmosphere technique can extend shelf life. Its use does not eliminate the need for proper control of storage conditions, especially temperature, nor for the adequate training handlers at sensory characteristics and shelf life of many food products, inhibiting the growth of pathogenic bacteria. MAP will continue to be used in the future, most probably with several different MAP formats in use around the world. Mechanistic, logistical, and perception obstacles will require effort and ingenuity to overcome existing package and system difficulties and promote implementation of new processing and packaging technologies. Moreover, the concept of combining antimicrobial/antioxidant agents within the package to control the deterioration and growth of microorganisms in food, will have a strong impact on both shelf life prolongation and food safety. Although the evidence suggests that active packaging is a promising technology, its potential cannot be fully realized unless major technical problems are overcome. More research related to the control of the migration of the active agents at rates suitable for different real food systems is still needed. Recognition of the benefits of active packaging technologies by the food industry, development of economically viable packaging systems and increased consumer acceptance opens new frontiers for active packaging technology.

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Chapter 17

Moisture-Dependent Engineering Properties of Chia (*Salvia hispanica* L.) Seeds

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Additional information is available at the end of the chapter

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1. Introduction

Salvia hispanica L., whose common name is chia, is an annual herbaceous plant belonging to the *Lamiaceae* or *Labiatae* family. This botanical species, native to southern Mexico and northern Guatemala, was an important crop in pre-Columbian Mesoamerica in conjunction with corn, beans and amaranth. Chia seeds were valuated not only for food, but also for medicines and paints [1]. Its cultivation was banned by Spanish conquerors and replaced by exotic crops (wheat and barley) [2]. Nowadays, chia seeds are being reintroduced to western diets in order to improve human health.

These seeds have been investigated and recommended due to their oil content with the highest proportion of α-linolenic acid (omega-3) compared to other natural source known to date [3, 4], and also because of their high levels of protein, antioxidant, dietary fiber, vitamins and minerals [5, 6]. Chia seeds from Argentina exhibited 30.0 - 38.6 g oil/100 g, with 60.7 - 67.8 g/100 g of α-linolenic acid [7, 8]. Figure 1 shows the chemical composition of chia seed [9].

Chia seed is traditionally consumed in Mexico, the southwestern U.S., and South America, but it is not widely known in Europe. However, in 2009, the European Union approved chia seeds as a novel food, allowing them to comprise up to 5% of a bread product's total matter [10]. Today, chia is mostly grown in Mexico, Bolivia, Argentina, Ecuador, Australia, and Guatemala, and it has been demonstrated that the species has great potential as a future crop plant [7, 11].

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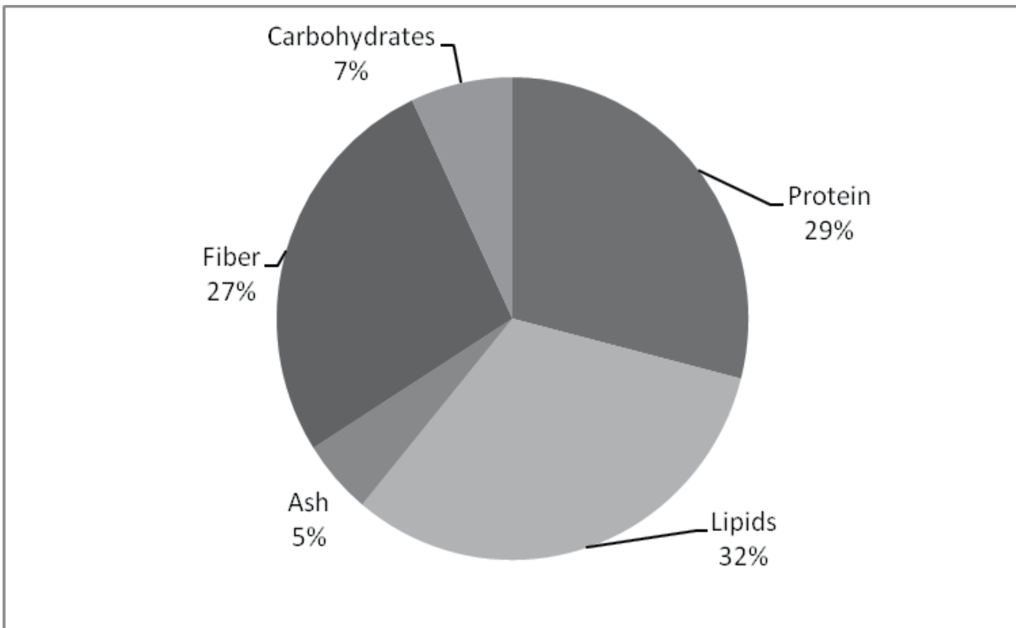

Figure 1. Chemical composition of chia seed (% d.b.)

Salvia hispanica fruit consist in four nutlets, similar to an indehiscent achene, which contains a single seed. These nutlets, are commercially named as seeds, and in the text, we will use this last term. The plant produces small white and dark seeds. Most of chia population that is commercially grown today contains a low percentage of white seeds. Their shapes are oval and in general, the white seeds are somewhat larger than the black ones. Ixtaina *et al.* [12], reported length, width and thickness value of 2.11, 1.32 and 0.81 mm for dark seeds and 2.15, 1.40 and 0.83 mm for white seeds, respectively. Chia seeds are shown in Figure 2.

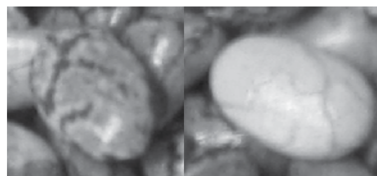

Figure 2. Photographs of dark and white chia seeds (13x)

The knowledge of engineering properties constitutes important and essential data for the design of machines, storage structures, and processes. The value of this basic information is not only important to engineers but also to food scientists, processors, and other scientists who may exploit these properties and find new uses.

Engineering seed properties and their dependence on moisture content are necessary in the design of equipment for transporting, storage and/or processing. The knowledge of the morphology and size distribution of chia seeds is essential for the adequate design of the equipment for cleaning, grading and separation. Gravimetric properties are useful for the design of equipment related to aeration, drying, storage and transport. Bulk density determines the capacity of storage and transport systems while true density is useful for separation equipment; porosity of the mass of seeds determines the resistance to airflow during the aeration and drying of seeds. The frictional properties, such as the angle of repose and the static coefficient of friction, are important for the design of grain bins and other storage structures whose operation is influenced by the compressibility and flow behaviour of materials. Several researchers investigated the moisture dependence of engineering properties of seed or grain and reported different behaviour of these properties as a function moisture content.

The aim of this work was to evaluate the engineering properties of dark chia seed as a function of the moisture content and to compare their behavior with that of other grains.

2. Materials and methods

2.1. Seeds

Chia seeds (20 kg) were obtained from commercial sources in Salta, Argentina (25° S, 65.5° W). Seeds were packed in hermetic plastic vessels and stored at 5 ± 1°C until use. The seeds were manually cleaned to remove all foreign matter, such as stones, dirt, and broken seeds. In this way, a randomized sample of chia seeds (about 2 kg) was picked by a sample splitter (CPASA, Centro Proveedor Agropecuario, Buenos Aires, Argentina). The seeds were manually separated according to their white or dark pericarp surface.

2.2. Sample preparation

The dark chia seeds were further divided into seven lots and they were conditioned to obtain a moisture content range of 4.6 - 17.7% (d.b.).To obtained a less moisture content, a predetermined quantity of seeds was dried down to the desired moisture content (convection air oven, 40 - 45°C). Higher moisture contents were reached indirectly through the saturation of the atmosphere in contact with the seeds. For this purpose, small, clean and dry vessels containing the seeds were placed into a container with 100 cm^3 of water, which was then hermetically sealed for 36 - 240 h. These moisture levels were selected according to the conditions usually applied in harvesting and most processing operations of grains [13].

2.3. Moisture content

The moisture content of the samples was determined by the ASAE standard method used for rapeseed [14], using a convection air oven at 130 ±1°C for 4 h.

2.4. Engineering properties

The engineering properties of seeds were assessed at all moisture levels, as described below.

In order to determine the average size of the seeds, a sample of 100 seeds was randomly selected. For each individual seed, the three principal dimensions, namely length (L), width (W) and thickness (T), were measured using a digital micrometer (least count 0.01 mm). The three principal dimensions were used to calculate the geometric mean diameter (D_g) and surface area (S) of individual grains by assuming that the seeds were ellipsoid.

The geometric mean diameter (D_g) of chia seeds was calculated using the following relationship [15]:

$$D_g = (LWT)^{1/3} \quad (1)$$

The specific surface area (S) of chia seeds was calculated it according to equation 2 [16]:

$$S = \pi D_g^2 \quad (2)$$

To determine the mass of a thousand seeds (W_{1000}), a set of 100 randomly selected seeds was weighed with an analytical balance (0.0001 g accuracy) and then extrapolated to 1000 seeds. This process was replicated 30 times [13].

The bulk density (ρ_b), considered as the ratio of the mass sample of the seeds to its total volume, was determinate using a weight per hectoliter tester [16] (equipment of 90 cm^3 of total volume). The true density (ρ_t) defined as the ratio of the mass of the sample to its true volume, was determined using an electronic balance reading 0.001 g and a pycnometer (50±0.1 cm^3 capacity, liquid displacement method)[17]. The toluene (C_7H_8; ρ = 0.867 g cm^{-3}) was used as a solvent to prevent water absorption in the seeds during the experiment [16]. Both measurements were performed in triplicate.

The porosity value (ε) defined as the fraction of space in the bulk seed which is not occupied by the seed [18], was determined from bulk and true densities using the relationship given by [19] and [20] as follows:

$$\varepsilon = \left(\frac{(\rho_t - \rho_b)}{\rho_t} \right) \times 100 \quad (3)$$

The seed volume (V) was determined from the following relationship [21]

$$V = \left(\frac{m}{\rho_t}\right)10^3 \tag{4}$$

where m is the unit mass of the seed (g) determined in samples used to calculate the true density.

The equivalent diameter (D_e) and sphericity (ϕ), defined as the ratio between the surface area of the sphere having the same volume as that of the seed and the surface area of the seed, were calculated using equation 5 and equation 6 respectively [18]:

$$D_e = \left(\frac{6V}{\pi}\right)^{1/3} \tag{5}$$

$$\phi = \left(\frac{D_e}{L}\right) \times 100 \tag{6}$$

Seed	Moisture Content (%d.b.)*	Reference
Cumin	7.0 - 22.0	Singh and Goswami, 1996. [13]
Sunflower	4.0 - 20.0	Gupta and Das, 1997. [15]
Fenugreek	8.9 - 20.1	Altuntaş et *al*., 2005. [16]
Safflower	3.7 - 15.6	Baümler et *al*., 2006. [17]
Sunflower	2.0 – 18.8	de Figueiredo et al., 2006 [18]
Sunflower	2.9 – 20.1	de Figueiredo et al., 2006 [18]
Quinoa	4.6 - 25.8	Vilche et *al*., 2003. [22]
Soybean	8.7 - 25.0	Deshpande et *al*., 1993. [23]
Amaranth	7.7 - 43.9	Abalone et *al*., 2004. [24]
Chia	4.6 - 17.7	Guiotto et *al*., 2011 [25]
Cotton	8.3 - 13.8	Özarslan, 2002. [26]
Flaxseed	6.1 - 16.2	Coşkuner and Karababa, 2007 [27]
Rapeseed	4.7 - 24.0	Çalişir et *al*., 2005. [28]

* Moisture content range considered in each study

Table 1. References studying the engineering properties of several seeds

The static coefficient of friction was measured using two structural materials, namely galvanized iron and aluminum. These materials are commonly used for transport, storage,

and handling operations of grains, pulses, and seed and for building storage and drying bins. A PVC cylinder (50 mm diameter, 50 mm high, open at both ends) was placed on an adjustable tilting table, faced with the test surface, and filled with the sample. The structural surface with the cylinder resting on it was tilted gradually with a screw device until the cylinder just started to slide down [22]. The angle (α) was read on a graduated scale and the friction coefficient was calculated using the following relationship:

$$\mu = \tan\alpha \tag{7}$$

where μ is the static coefficient of friction, and α is the angle of tilt (degrees). The static coefficient of friction was replicated reading ten times for each moisture content.

Different authors evaluated the engineering properties of several seeds as a function of seed moisture content (Table 1). These data were considered to compare the behavior of different grain properties with regard to moisture content.

3. Results and discussion

Dark seeds represented 89% by mass of the samples studied. The initial moisture content was 10.0% and 10.9% d.b., for dark and white seeds, respectively. Averages of the three principal dimensions were L = 1.97 mm, W = 1.29 mm, and T = 0.84 mm for dark chia seeds. The statistical analysis (ANOVA) detected significant differences ($p \leq 0.05$) between different moisture levels for each principal dimension. Figure 3, shows a linearly increasing tendency between the dimensions (L, W, and T) and moisture content ($R^2 > 0.95$, $p \leq 0.005$). Similar trends of increase have been reported in fenugreek [16], quinoa [22], soybean [23] and amaranth [24] seeds. Knowledge of the seed axial dimensions is important, for instance, in determining aperture sizes in the design of grain handling machinery.

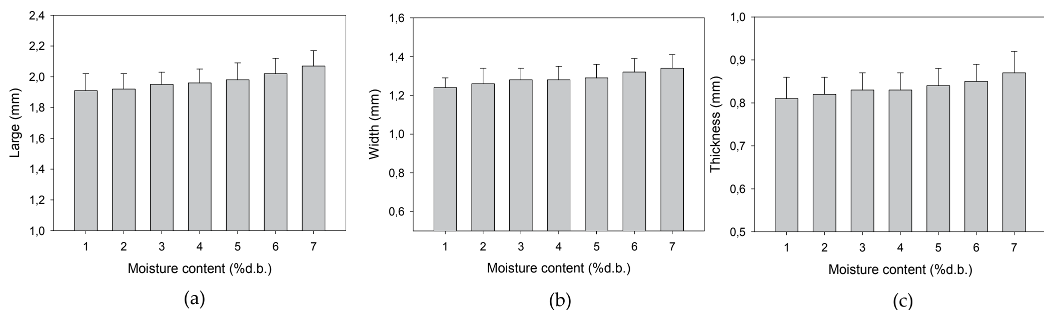

Figure 3. Principal dimensions (a) length (L); (b) width (W); (c) thickness (T) of dark chia seeds with different moisture content (1) 4.6%, (2) 6.5%, (3) 8.7%, (4) 10.0%, (5) 12.5%, (6) 15.3%, (7) 17.7%).

The geometric mean of the axial dimensions is useful in estimating the projected area of a particle moving in the turbulent or near-turbulent region of an air stream. It is therefore gen-

erally indicative of its pattern of behavior in air streams, especially with respect to the ease of separating extraneous materials from the particle during cleaning by pneumatic means [26]. Significant differences (p < 0.05) were found between different moisture contents for geometric diameter ($D_{g,}$ mean 1.28 mm) and for specific area (S, mean 5.20 mm^2). Both properties, D_g and S, increased linearly 8.14% and 16.9%, respectively, in function of increasing moisture content (R^2 > 0.97, p < 0.005) (Table 2 and Figure 4). This D_g trend was similar to that observed for amaranth, flaxseed, quinoa and soybean. Similar behavior of S was reported for fenugreek and soybean seeds. The increase of S may be due to seed dilatation during moisture sorption, resulting in contact area enhancement.

Seed [Reference]	Regression equation	R^2
Amaranth [24]	1.071 + 0.00385 x	0.99
Chia [25]	1.2075 + 0.0057	0.97
Flaxseed [27]	1.941 + 0.011 x	0.97
Quinoa [22]	1.359 + 0.009819 x	0.99
Soybean [23]	4.882 + 0.0253 x	0.99

Table 2. Regression equation as a function of moisture content (x, %d.b.) with their respective coefficient of determination (R^2) for geometric diameter ($D_{g,}$ *mm*).

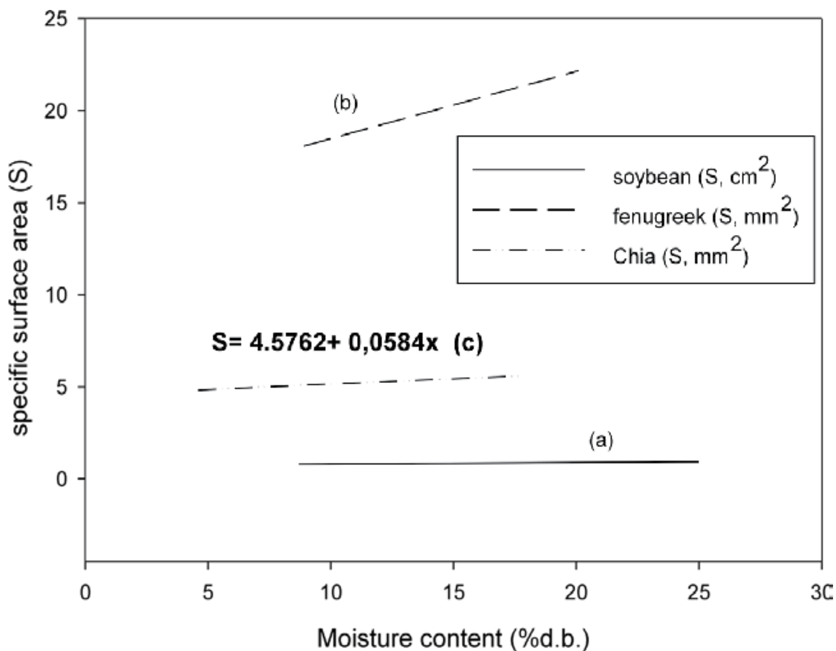

Figure 4. Effect of moisture content (x, % d.b.) on specific area (S). Date correspond to the adjusted function reported by (a) [23]; (b) [16] and (c) [25]

Bulk density, true density, and porosity (the ratio of inter granular space to the total space occupied by the grain) can be useful in sizing grain hoppers and storage facilities; they can affect the rate of heat and mass transfer of moisture during aeration and drying processes. The theories used to predict the structural loads for storage structures have bulk density as basic parameter. Bulk density and porosity are major considerations in designing near-ambient drying and aeration systems, as these properties affect the resistance to airflow of the stored mass. Grain bed with low porosity will have greater resistance to water vapor scape during the drying process, which may lead to higher power to drive the aeration fans.

True density average of dark chia seeds with different moisture content was 1.069 g cm^{-3}. Statistical analysis showed significant differences ($p \leq 0.05$) between different moisture levels. The true density of seed was found to increase linearly at a decreasing in moisture content from 4.6 to 17.7% d.b. The relationship between true density (ρ_t, g cm^{-3}) and the moisture content (x, % d.b.) of the seed can be represented for $\rho_t = 1.1457 - 0.008x$ ($R^2 = 0.9912$, $p < 0.0001$) [25]. This trend can be attributed to minor volumetric product contraction during drying with respect to the decrease of seed mass due to water loss. The negative linear relationship of true density with moisture content was also observed by other authors for fenugreek and soybean, but it was different for cumin, flaxseed, quinoa and sunflower seeds, which increased linearly. However, a non-linear decreasing relationship of true density with moisture content was reported for amaranth and safflower seed (Table 3).

Seed [Reference]	Regression equation	R^2
Cumin [13]	1010 + 6.05 x	0.98
Flaxseed [27]	826.92 + 28.91 x	0.99
Fenugreek [16]	1275.8 – 37.555 x	0.99
Quinoa [22]	853.19 + 13.16 x	0.98
Sunflower [15]	694.6 + 3.72 x	0.92
Sunflower* [18]	706.12 + 4.06 x	0.94
Soybean [23]	1254.8 – 5.258 x	0.99
Amaranth [24]	(1411(100 + x))/ (100 + 1,25 x)	0.99
Safflower [17]	0,7887+(0,000298 x)-(0,0004551 x^2)	0.99

* black-hull oilseed

Table 3. Regression equations as a function of moisture content (x, %d.b.) with their respective coefficient of determination (R^2) for true density (ρ_t, kg m^{-3}).

Chia seed bulk density decreased from 0.713 to 0.644 g cm^{-3} as a function of the increase of moisture content. Table 4 shows the relationship of bulk density with moisture content reported by different authors for several seeds. Regarding bulk density, chia seeds presented a behavior similar (non-linear) to those of cumin and safflower seeds. The negative linear relationship of bulk density with moisture content was observed by other authors for amaranth, flaxseed, fenugreek, quinoa, rapeseed, sunflower and soybean (Table 4). The decrease in bulk density of seed with increase in moisture content indicates that the increase in volumetric expansion is greater than weight.

Seed [Reference]	Regression equation	R^2
Amaranth [24]	869 – 3.50 x	0.87
Chia [25]	697.7+ 5.0 x – 0.4 x^2	0.99
Cumin [13]	407 + 15.67 x – 0.70 x^2	0.97
Flaxseed [27]	822.43 – 15.308 x	0.99
Fenugreek [16]	726.76 – 27.675 x	0.98
Quinoa [22]	771.5 – 3.94 x	0.98
Rapeseed [28]	616.74 – 1.4518 x	0.93
Safflower [17]	452.2 – 0.12 x^2	0.96
Sunflower [15]	472.4 – 1.69 x	0.88
Sunflower* [18]	446.35 – 201 x	0.87
Sunflower** [18]	528,75 – 2.24 x	0.80
Soybean [23]	748.9 – 1.6626 x	0.99

* black-hull oilseed, **striped-hull oilseed

Table 4. Regression equations as a function of moisture content (x, %d.b.) with their respective coefficient of determination (R^2) for bulk density (ρ_b, kg m^{-3}).

These discrepancies observed for as much true density as for bulk density could be due to cell structure and the volume and mass increase characteristics of the seeds as moisture content increases [29].

The average porosity observed for all dark samples was 35.3%, with significant differences ($p \leq 0.05$) among moisture contents. The polynomial relationship between the porosity (ε, %) and moisture content (x, % d.b.) for chia seeds can be represented by $\varepsilon = 40.0745 - 1.0991\ x + 0.0049\ x^2$) ($R^2 = 0.9701$, $p < 0.0010$) [25]. Nevertheless, different authors reported linear increase in porosity with increase in moisture content for amaranth, cumin, flaxseed, fenugreek, quinoa, rapeseed, safflower, sunflower seeds, and trend linear decrease in porosity for soybean (Table 5).

Seed [Reference]	Regression equation	R^2
Amaranth [24]	28 + 0.16 x	0.75
Cumin [13]	48 + 0.643 x	0.93
Flaxseed [27]	11.453 + 2.7621 x	0.99
Fenugreek [16]	42.987 + 0.555 x	0.95
Quinoa [22]	13.1 + 1.22 x	0.98
Rapeseed [28]	44.659 + 0.6656 x	0.99
Safflower [17]	39.53 + 0.342 x	0.93
Sunflower [15]	32.27 + 0.54	0.95
Sunflower* [18]	36.99 + 0.58 x	0.92
Sunflower** [18]	30.10 + 0.37 x	0.91
Soybean [23]	40.5 – 0.1365 x	0.98

* black-hull oilseed, **striped-hull oilseed

Table 5. Regression equations as a function of moisture content (x, %d.b.) with their respective coefficient of determination (R^2) for porosity (ε, %).

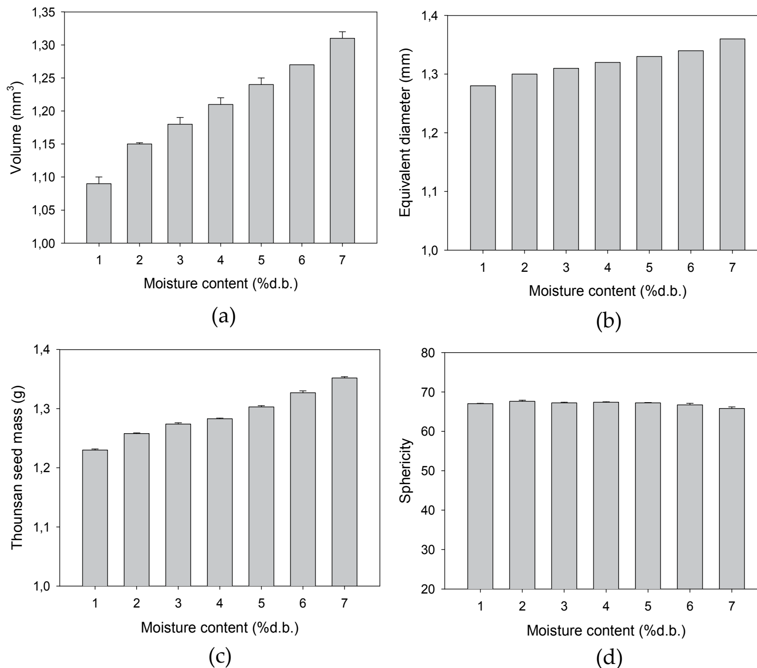

Figure 5. (a) volume (*V*), (b) equivalent diameter (*De*), (c) thousand seed mass (W_{1000}), and (d) sphericity ( ) of dark chia seeds with different moisture content (1) 4.6%, (2) 6.5%, (3) 8.7%, (4) 10.0%, (5) 12.5%, (6) 15.3%, (7) 17.7%).

Since porosity depends on bulk and true densities, the magnitude of its variation depends mainly on these properties. Therefore, the porosity of each type of seed or grain could respond differently with increasing moisture content. This fact could be attributed to the seeds' morphological characteristics; the relative changes in their length, width, and thickness; and the associated bulk and true densities. Taking into account the high level of polyunsaturated fatty acids, chia seeds can be easily affected by temperature. For this reason, aeration is an important process to maintain a low uniform temperature and prevent the moisture migration. The resistance to airflow or pressure drop is affected by different factors, such as the bulk density, porosity, and moisture content. Due to the low bulk density and size of chia seeds, the grain bed will have an important pressure drop, requiring a high level of power for driving the aeration fans [25].

The variation of volume (V), equivalent diameter (D_e), thousand seed mass (W_{1000}), and sphericity (ϕ) of chia seeds with moisture content (4.6 - 17.7 % d.b.) is shown in Figure 5.; the average values were 1.21 mm^3, 1.32 mm, 0.129 g and 66.7 % respectively. Statistical analysis revealed significant differences ($p < 0.05$) between seeds with different moisture content for V, D_e and W_{1000}. Nevertheless, sphericity did not present significant differences ($p > 0.05$).

The sphericity varied between 65.8% and 67.6%, values higher than the data reported for sunflower and safflower seed, but lower than those of amaranth, quinoa, rapeseed and soybean seed (Table 6).

Seed [Reference]	Sphericity (%)
Amaranth [24]	82 #
Fenugreek[16]	60.79 – 64.06
Quinoa [22]	77 – 80
Rapeseed [28]	93 – 92
Safflower [17]	58 – 62
Sunflower* [18]	49 – 52
Sunflower** [18]	47 – 50
Soybean [23]	80.6 – 81.6

mean value, * black-hull oilseed, **striped-hull oilseed

Table 6. Sphericity (ϕ) of different seeds

The high ϕ value thus suggests that the seeds tend towards a spherical shape. Thus, the value of the ϕ generally indicates a likely difficulty in getting the seed to roll. This tendency to either roll or slide should be necessary in the design of hoppers and dehulling equipment for the seed.

As can be seen in Figure 6, the equivalent diameter (D_e, mm) increased linearly from 1.28 to 1.36 mm when the moisture content was increased from 4.6% d.b. to 17.7% d.b., representing a variation of 6.3%.

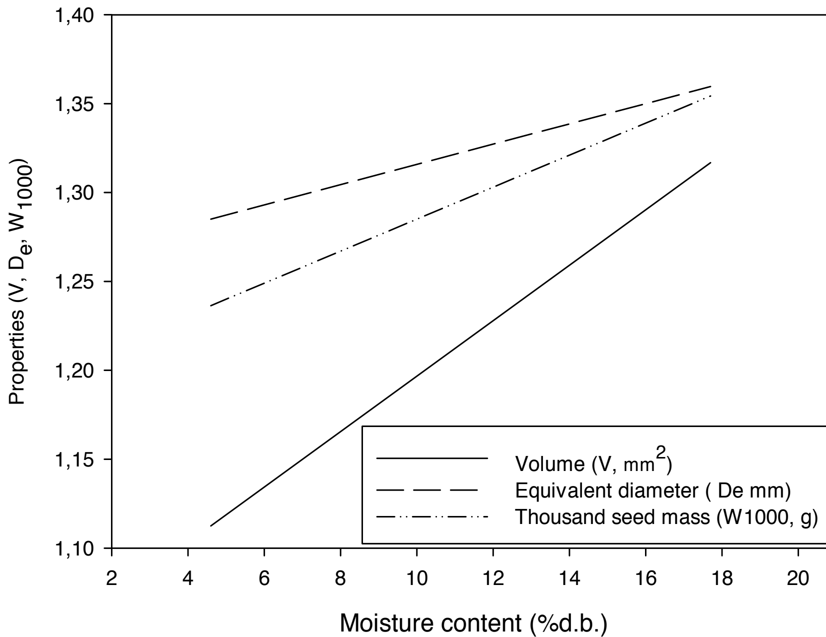

Figure 6. Effect of moisture content (x, % d.b.) on volume seed (V), equivalent diameter (D_e) and thousand seed mass (W_{1000}). Date corresponds to the adjusted function reported by [25].

The V and W_{1000} of dark chia seeds linearly increased with moisture content (Figure 6). The similar trend was reported for fenugreek [16], safflower [17] sunflower [18] soybean [23] and rapeseed [28] seeds.

The frictional characteristics are important for the proper design of agricultural product handling equipment. Friction between a seed and a surface has an influence on the movement of particles on oscillating conveyors, separation on oscillating sieves and unloading and loading operations. The static coefficient of friction of dark chia seeds was determined on two structural surfaces: galvanized iron and aluminum. The values obtained were higher for aluminum (mean: 0.30, minimum: 0.26, maximum: 0.37) than for galvanized iron (mean: 0.28, minimum: 0.25, maximum: 0.34). Increments of 28.4% and 29.5% were recorded for the galvanized iron and aluminum surfaces, respectively, as the moisture content increased from 4.6% to 17.7% d.b. The reason for the increased friction coefficient at higher moisture content may be that the water present in the seed offered a cohesive force on the contact surface and the seed became rougher and sliding characteristics are diminished [22, 26]. For both structural surfaces, the co-

efficient of static friction increased linearly with an increase in moisture content. Similar trends were reported for cumin, flaxseed, quinoa and sunflower (Figure 7).

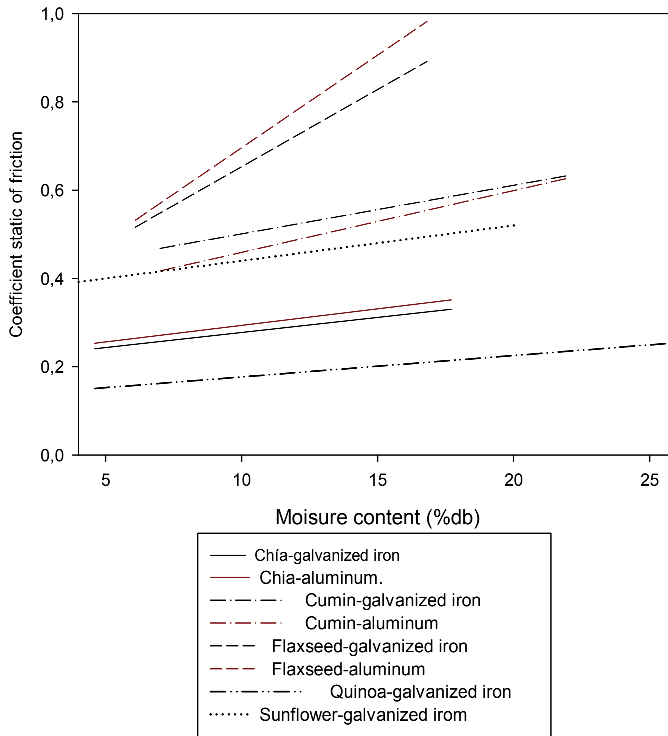

Figure 7. Effect of moisture content (x, % d.b.) on coefficient of static friction. Dates correspond to the adjusted function reported by () [25], () [13], () [27], () [22] and () [15].

4. Conclusions

The engineering properties of dark chia seeds were evaluated as a function of the moisture content, in the range of 4.6% to 17.7% d.b. and their behavior was compared with amaranth, cumin, flaxseed, fenugreek, quinoa, rapeseed, safflower, soybean and sunflower. The principal dimensions of dark chia seed (length, width and thickness), geometric diameter, specific surface area, volume, equivalent diameter, and thousand seeds mass and static coefficient of friction on galvanized sheet and aluminium increased linearly as increasing the seed moisture content. Chia seed is one of the smallest (similar to amaranth and quinoa), and very light.

The sphericity did not present significant differences in the range of moisture content studied for dark chia seed. The most spherical seeds which were compared with chia seed ones were rapeseed, amaranth, soybean and quinoa. An increase in moisture content yields a de-

crease in bulk and true density. The bulk density and porosity varied nonlinearly for chia seeds, showing a quadratic concave behavior as a function of moisture content.

The friction caused by the aluminum surface was slightly higher than that presented by the galvanized iron surface.

In general, the variation of the engineering properties of chia seed with the moisture content showed a similar trend to that reported for other seeds, with some exceptions. Nevertheless, they presented different variation ranges. It could be attributed to the seeds morphological and physiological characteristics.

The comparison of the data of the different seeds can be important for the design and adaptation of equipment for transporting, storage and processing.

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Nomenclature

D_e Equivalent Diameter (mm)

D_g Geometric Diameter (mm)

d.b. dry basis

L Length of seed (mm)

m Unit mass of the seed (g)

S Specific Surface area (mm^2)

T Thickness (mm)

V Seed Volume (mm^3)

W Width of seed (mm)

W_{1000} Thousand seed weight (g)

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x Moisture content (% d.b.)

α Angle of tilt, degree

ε Porosity of seed (%)

ϕ Sphericity of seed

μ Static coefficient of friction, dimensionless

ρ_b Bulk Density (g cm^{-3})

ρ_r True Density (g cm^{-3})

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Chapter 18

Scale Up of Polygalacturonase Production by Solid State Fermentation Process

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Additional information is available at the end of the chapter

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1. Introduction

Polygalacturonases are pectinolytic enzymes that catalyze the cleavage of polygalacturonic acid chain with the introduction of water through hydrogen bonds. These enzymes have technological, functional and organic applications on food processing and plant-fungus interactions.

They are produced by plants, fungi, bacteria and yeasts. However, the fungi are preferred in industrial scale, since about 90% of enzymes produced may be secreted into the medium and produce large amounts of enzymes [1,2].

Polygalacturonases involved in hydrolysis of pectic substances are endo-polygalacturonase and exo-polygalacturonase. Exo-polygalacturonase act on polygalacturonic acid monomers terminals, producing monogalacturonic acids. Endo-polygalacturonase act randomly on polygalacturonic acid, producing oligogalacturonic acid [6].

Pectic enzymes alone are responsible for one quarter of food production enzymes in the world [3]. These are widely used in fruit juices industry to reduce viscosity, improve and increase the efficiency of filtration and clarification [4], preliminary treatment of grape wine industry, extraction of tomato pulp and among others applications [5].

For fruit juices production with pomo (e.g. citrus), and red fruit (e.g. grape) extracts require addition of enzymes to convert viscous macerated or triturated fruit semi gelled (caused by the partial solubility of pectins and the ability high water retention of solids) to maximize the extraction of juice during pressing, subsequent step of process. Pectinases capable to degrade pectins depolymerize with high methylation are more suitable and include endopolygalacturonase and pectin metilesterease [7].

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Principal application of enzymes that pectins hydrolyze is clarification or extraction of juices. The turbidity may be desirable in some juices (e.g. orange), but not for apple and grape juice, in which are translucent over sold. Turbidity is conferred by colloidal particles consisting of proteins coated with pectin. Pectinases depolymerize pectins, promoting flocculation and facilitating clarification [7].

Polygalacturonases produced by fungi are more active in pH range of 3.5 to 6.0 and temperature of 40-55°C. The pratical result of activity of these enzymes is that middle lamella is disrupted and the viscosity of pectin solutions is decreased as the action of enzyme is maintained [7]. In fact, the characteristics of enzyme related with temperature and pH effects will depend on factors of production process, e.g. microorganism used, available nutrients, fermentation temperature, among others.

Industrially, pectinases are produced either by submerged fermentation and solid state fermentation with *Aspergillus niger* strains, however, the solid state fermentation technique is generally considered more susceptible to higher yields of pectin esterase and polygalacturonase. Some authors state that this preference occurs because the solid state fermentation allows the production of crude enzymes more concentrated and therefore a lower cost of extraction and purification [5,8].

Solid state fermentation process is defined as a process that occurs over a non-soluble material, acting as support and nutrients source, with small quantity of water, under the action of fermenting agent [9].

One of the important factors in pectinases production by solid state fermentation is the medium composition. Appropriate balance between sources of nitrogen and carbon is so important to the nutritional requirement of microorganism that the effects of environmental conditions which affect mycelial growth [10].

Water in the system is a limiting factor. This amount of water is related to the medium through of moisture, as regards the percentage of water in total mass. Determination of its value in process is closely related to the substrate nature, requirements of microorganism used and type of end product desired. If moisture level is high, it will result in a decrease of substrate porosity and it will result in lower oxygen diffusion within the medium, consequently, decrease in gas exchange and increases the risk of contamination, especially bacterial. For lower levels of moisture needed by the microorganism, there will be greater difficulty in diffusion of nutrients, resulting in a lower growth and, consequently, lower production of desired product [11].

Temperature is also considered a critical factor, as well as moisture, due to the accumulation of metabolic heat generated during fermentation, which directly affects the microorganism germination and product formation. In composting process, this effect is desirable, however, for biotechnology processes, such as enzyme production, the heat must be dissipated immediately, so that temperature increase does not adversely affect the desired fermentation [11].

There are several types of reactors used in solid state fermentation process. Although there are many projects to industrial bioreactors, these have a limited extent for this type of process [12].

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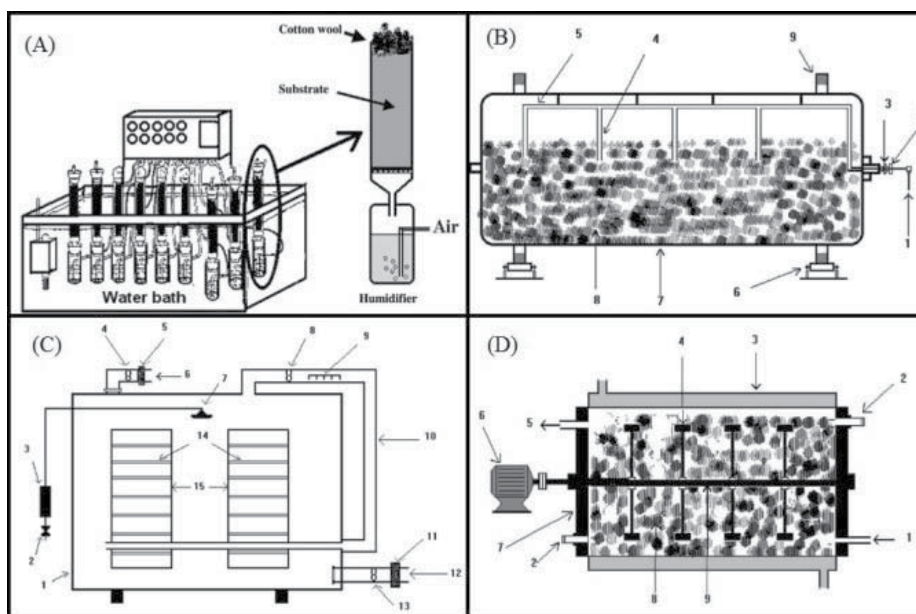

Figure 1. Type of bioreactors: (A) Column; (B) Rotating drum; (C) Koji-type; (D) Stirred horizontal [12].

Tray reactor consists of a chamber in which temperature and humidity air controlled circulates around a series of trays. Each tray contains a thin layer of depth. It is noteworthy that intermittent mixing of medium can be performed, but this generally occurs only once daily [13].

Scale-up using tray reactor cannot be simply by increasing the medium thickness, as this leads to problems of overheating. For this reactor type, scaling-up must be done by increasing the area of trays, which can be achieved using larger trays [14].

As described above, tray reactors are limited by heat transfer, but also by mass transfers, and may develop high temperatures and internal gas concentration gradients in height above 40 mm of substrate [15]. Still about the medium temperature, it is almost impossible to maintain this variable in the optimum value for production. However, fermentative process will vary in temperature that can be of 10°C above the optimum value [16].

Although there are numerous projects for industrial bioreactors, it is observed that these have limited development to processes using solid state fermentation. This occurs because there are some limitations of this process, such as the difficulty to remove heat generated by microbial metabolism, heterogeneity of mixture during fermentation, which makes the control of cell growth and various parameters such as temperature, pH, agitation, aeration, concentration of nutrients and products, making difficult the automation [17].

Despite these difficulties, the use of semi-solid medium may be advantageous, it allows the use of industrial residues (flours, bark and cake) as substrate, which is abundant raw material and low cost at Brazil.

Figure 2. Cashew fruit [22].

Industrial wastes have been used in bioproducts production through fermentation processes. Among these residues may be mentioned the cashew apple (*Anacardium occidentale* L.), which is rich in sugars, organic acids and fiber, that is why it has been used in the production of phenols [18], bioethanol, cashew wine [19], protein enrichment [20], pectinases [21], cellulases, among others.

Therefore, this study aims to characterize the scaling-up of solid state fermentation process for polygalacturonases production, using cashew apple dry bagasse as substrate, *Aspergillus niger* CCT 0916 as microorganism and tray reactor as operating system. For this, it will be performed to characterize of substrate used concerning the physical-chemical properties. It will be constructed and adjusted adsorption isotherms of substrate, showing the relationship with fermentation process. Initially, there will be characterization of factors that most influence the fermentation process in a laboratory scale. By setting these factors, it will be made to scaling-up of solid state fermentation process using tray reactor. Finally, it will be characterized the crude enzyme extract and its stability over temperature and pH.

2. Material and methods

2.1. Substrate

Cashew apple bagasse was obtained from fresh cashew fruit acquired at Empresa de Abastecimento e Serviços Agrícolas (EMPASA) at Campina Grande City, Brazil. First, cashew nut was removed. Next, apple was triturated and pressed to separate the juice. Humid bagasse was dried with air renewal and circulation at 55°C. After drying process, bagasse was ground in TECNAL knife mill.

2.2. Substrate characterization

Measurements of pH, moisture content and mineral waste (MW) followed the standards Brazil [23]. The pectin amount (PC) was determined by gravimetric precipitation method using calcium pectate [24]. Reducing sugars (RS) and saccharose were determined by HPLC (High performance liquid chromatography). Concentration of soluble solids (SS) was obtained by direct reading in refractometer after adding 9 mL of distilled water to 1 g of dry bagasse. It was used 100 g of material to determine the density. This mass was placed in a graduate to determine volume occupied without compression. Size distribution was performed using 100 g of residue in a Cotengo-Pavitest sieve shaker for 1o minutes in 14, 20, 24, 35, 48 and 60 mesh trays. Result was expressed as weight percentage. Protein was determined by semi-micro Kjeldhal method for nitrogen adjusted by spectrophotometry [25]. All characterizations were performed in triplicate. Standard deviation (SD) was based on means values.

2.3. Substrate adsorption isotherms

Triplicate samples were weighed approximately 1 g of product in aluminium crucibles and stored in airtight containers containing saturated salt solutions until reached the equilibrium moisture content for a certain relative humidity range (Table 1). Temperatures of 25, 30, 35 e 40°C were supplied by environmental chamber. Samples were weighed every 24 hours, reached constant weight. After that, samples were transferred to stove at 100°C for determination of dry weight. Equilibrium moisture content (xeq) was calculated by Equation 1.

$$xeq = \left(\frac{mi\ -\ ms}{ms}\right) \times 100 \tag{1}$$

In which: xeq = equilibrium moisture, % dry basis; mi = initial weight of sample, g; ms = dry weight of sample, g.

Saturated salt solutions	Temperature (°C)			
	Relative humidity (%)			
25	30	35	40	
$K(C_2H_3O_2)$	23	23	23	23
$MgCl_2$	33	32	32	31
K_2CO_3	43	42	41	40
NaBr	57	57	57	57
NaCl	75	75	75	75
KCl	86	84	84	83

Table 1. Equilibrium relative humidity of saturated salt solutions [26]

It was used the BET model [27] to fit experimental data (Equation 2):

$$\frac{xeq}{xm} = \frac{C.aw}{1 - aw}\left[\frac{1 - (n+1)(aw)^{n} + n(aw)^{n+1}}{1 - (1 - C)aw - C(aw)^{n+1}}\right] \tag{2}$$

In which: xeq = equilibrium moisture, % dry basis; aw = water activity, adimensional; C = BET constant; xm = moisture in the molecular monolayer; n = number of molecular layers.

Criteria used to observe the fit were the coefficient of determination (R^2) between observed responses and predicted by the fitted model and average percentage deviation (P) (Equation 3). The best fits were those with the highest R^2 and lowest value of P [28].

$$P = \frac{100}{n}\sum_{i=1}^{n}\frac{|Xexp - Xteo|}{Xexp} \tag{3}$$

In which: n = observation numbers; Xexp = humidity of experimental material; Xteo = humidity calculated by adjusted models.

2.4. Fermentation process in laboratory scale

Microorganism used was *Aspergillus niger* CCT0916, donated by Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA, Fortaleza State – Brazil). Spore concentration was adjusted according to experimental design.

Substrate was hydrated with distilled water to obtain moisture content and ammonium sulfate was added to this volume. In a 250 mL Erlenmeyer flask, it was weighed 10 g of sterilized humidified medium. After spore inoculation, this medium was incubated at fermentation temperature by experimental design for 78 hours.

Enzyme extraction for fermented complex was performed by adding 2.5 mL/g of fermented medium using 200 mM acetate buffer pH 4.5. Samples were then left in water bath for 1 hour at 30°C and filtered through Wattman 1 filter paper.

A 2^4 factorial experimental design was conducted with 7 experiments at central point to determine the influence of spore concentration (E), initial moisture (U), ammonium sulfate concentration (N) and fermentation temperature (T_f) on polygalacturonase activity response (Table 2).

One unit of polygalacturonase activity was defined as the amount of enzyme that releases 1 µmol of galacturonic acid per minute of reaction at 35°C for 30 minutes.

2.5. Fermentation process using tray reactor

Microorganism used in this stage was also used for optimization in the laboratory scale. Spores concentration, moisture content and ammonium sulphate concentration were determined based on experiments performed on laboratory scale.

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Substrate was hydrated with distilled water to obtain the initial moisture content and it was diluted ammonium sulphate in this water. On polypropylene trays (Figure 3), it was weighed 500 g of sterilized medium. Substrate thickness was equal to 40 mm. After spore inoculation, the medium was incubated at 23°C for 77 hours. Substrate thickness and fermentation temperature had been selected taking into account studies reported in literature [15,16].

Test	Variables			
	U %(w.b.)	E (mL/g)	N %(w/w)	T_f(°C)
1	30 (-1)	10^6 (-1)	0.5 (-1)	25 (-1)
2	50 (+1)	10^6 (-1)	0.5 (-1)	25 (-1)
3	30 (-1)	10^8 (+1)	0.5 (-1)	25 (-1)
4	50 (+1)	10^8 (+1)	0.5 (-1)	25 (-1)
5	30 (-1)	10^6 (-1)	1.5 (+1)	25 (-1)
6	50 (+1)	10^6 (-1)	1.5 (+1)	25 (-1)
7	30 (-1)	10^8 (+1)	1.5 (+1)	25 (-1)
8	50 (+1)	10^8 (+1)	1.5 (+1)	25 (-1)
9	30 (-1)	10^6 (-1)	0.5 (-1)	35 (+1)
10	50 (+1)	10^6 (-1)	0.5 (-1)	35 (+1)
11	30 (-1)	10^8 (+1)	0.5 (-1)	35 (+1)
12	50 (+1)	10^8 (+1)	0.5 (-1)	35 (+1)
13	30 (-1)	10^6 (-1)	1.5 (+1)	35 (+1)
14	50 (+1)	10^6 (-1)	1.5 (+1)	35 (+1)
15	30 (-1)	10^8 (+1)	1.5 (+1)	35 (+1)
16	50 (+1)	10^8 (+1)	1.5 (+1)	35 (+1)
17	40 (0)	10^7 (0)	1.0 (0)	30 (0)
18	40 (0)	10^7 (0)	1.0 (0)	30 (0)
19	40 (0)	10^7 (0)	1.0 (0)	30 (0)
20	40 (0)	10^7 (0)	1.0 (0)	30 (0)
21	40 (0)	10^7 (0)	1.0 (0)	30 (0)
22	40 (0)	10^7 (0)	1.0 (0)	30 (0)
23	40 (0)	10^7 (0)	1.0 (0)	30 (0)

Table 2. Concentrations and tests from factorial design

Figure 3. Tray reactor

Enzyme extraction for fermented complex was performed by adding 5.0 mL/g of fermented medium, using 200 mM acetate buffer pH 4.5. Samples were left in water bath for 1 hour at 30°C and filtered on Wattman 1 paper filter.

2.6. Enzymatic stability

To check the stability concerning temperature, crude extract samples were taken to a water bath for 20 min at temperatures between 10 and 90 ° C. For pH, crude extract was diluted in the buffers listed below, thus verifying the pH influence on polygalacturonase activity. After reaching the corresponding pH, samples were incubated for 24 hours at 2°C [46]: 0.1 M glycine-HCl pH 2.5; acetate buffer 200 mM pH 3.5-6.5; 0.1 M tris-HCl pH 7.5-8.5; 0.1 M glycine-NaOH pH 9.5. Results of thermostability and stability as to pH were expressed as relative activity (%).

3. Results and discussion

3.1. Physicochemical characterization of cashew apple dry bagasse

In a solid state fermentation process for enzymes production, it is important to know about the substrate composition, because the microorganism uses such as nutrient, for growth and reproduction so as to produce.

In case of pectinases, the inducing substance is pectin. Microorganism will adapt to the environment and for its maintenance, it will produce the enzyme needed to break these substan-

ces. Other substances also welcome in process are reducing sugars. These sugars (glucose, fructose, etc.) are sources of quick energy, and are also consumed by microorganism during fermentation process [21].

However, there must be a balance between nutrient sources. The literature indicates that high concentrations of sugars in fermentation medium, supplying the microorganism needs for its growth, and pectin little used, hence there is little enzymes production [29].

Cashew has on average 30% of reducing sugars (glucose and fructose) and about 10% of pectin in your composition. This implies the need for addition of inducer in a bagasse for use in a pectinases production.

Some authors observed that for pectinases production, using *Aspergillus niger* T005007-2 and wheat bran as substrate through a solid state fermentation process, the citrus pectin addition, up to 16% (w/w) led to increased enzyme production [30]. Based on these data, and in the proximity of same amount with sugar in cashew bagasse, it was decided not to add inductors in fermentation process.

In general, pH is an important variable in any biological process, with optimum values for microorganism growth. Generally, fungi prefer low pH between 4.5 and 5.0 [31,32]. Average value observed for cashew dry bagasse is 4.0, confirming proximity to other values found in the literature for cashew cake dry [17,33]. Thus, bagasse pH characterized can promote the pectinases production without requiring adjustment using buffer solution. Moreover, acid pH favors the storage at room temperature without contamination problems.

Bagasse was dried to below 15% (w.b), it became necessary to store a reasonable amount, noting that below 15%, the organic materials retain their properties over time and makes it difficult to contamination by bacterias.

Water amount in fermentation medium is a limiting factor and it directly affects the microorganism needs, and the final product type. To use this residue, it will be necessary to adjust the moisture content, since some microorganisms that produce pectinases require higher levels. This quantity of water is related with the environment via two variables: moisture and water activity.

Moisture regards percentage of water in the total mass of medium. And determination of this value in process is closely related to substrate nature, the requirements of microorganism used and the type of end product desired [11]. Water activity indicates that the organism may grow by fermentation, ensuring product quality.

Microorganism growth depends on the water activity, due to influence of osmotic pressure by on exchange membranes. And it can be related to moisture in the substrate used in fermentation using sorption isotherms for a given temperature [32].

Some authors [8,31] cite the use of various substrates for pectinase production by *Aspergillus niger* with water activity above 0.93. Importantly, low levels of water activity means low availability of water molecules near the cell, making exchange of solutes in solid phase, reducing metabolism and generating lower rates of growth or synthesis of metabolites. In con-

trast, high levels of water activity hinder the diffusion of air through solid particles, leading to a reduction in microbial growth [35]

Crude protein value is approximately 8% and it is close to the value observed by some authors [36,37]. This value is important for characterization as serve as a nitrogen source for microorganism. And from that data, it can be observed the necessity of supplementation with alternative sources of nitrogen such as urea or ammonium sulphate.

Physical characteristics with respect to substrate morphology are essential, particularly, in size and porosity, as these properties governing the accessible surface area of microorganism [17].

For granulometric distribution, 80% of bagasse was retained in sieves 20, 24 and 35 mesh, corresponding to particle size of 0.85, 0.70 and 0.42 mm, respectively. This particle size can be used in a solid state fermentation process by *Aspergillus niger*, which was already described in literature, particle sizes for pectinases production from 0.5 to 0.7 mm [39,40].

Average particle size of residual fermentation media must be obtained so that there have been no particles large or small. Particles of small size promote greater surface area and consequently a higher degree of processing. However, the process itself needs to have a particle size allowing the circulation of air through the mass and waste gases and heat produced, which could harm the efficiency of process [11]. Particles larger interparticles promote more space, reducing the efficiency of nutrients absorption for microorganisms. Furthermore, particle size analysis is important in enzyme complex extraction, since finely divided solid carriers facilitate access by the solvent [29].

It is important to remember that, in general, the crops characterization crops can vary dramatically depending on time of harvest, agricultural practices and phenomena related to planting. Is then very important to characterize the substrate for solid state fermentation process and therefore the adjustment of certain parameters, which is also an additional challenge is to be considered in using this process.

3.2. Adjust of adsorption isotherms from cashew apple dry bagasse

Data water activity (aw) and average equilibrium moisture (xeq) of material at temperatures studied (25, 30, 35 and 40°C) was adjusted BET model.

Table 3 are the values of equation BET parameters, average deviation percentage (P) and correlation coefficient (R^2) for each temperature.

From Table 3, there is the BET model appropriately fit to experimental data because P value indicates a good fit when it is less than 10% and R^2 must be as close to unity [38].

When comparing the monolayer humidity values of humidity (Xm) by BET equation, it is noted that the range of 25-30°C to 35-40°C, Xm increased by approximately 0.4%. This is not a common behaviour, but can be explained by two mechanisms: (1) increase of temperature may cause changes in product physical structure, providing a larger number of active sites with affinity for water molecules, (2) or may cause an increase in intrinsic solubility of solute to the product, causing a greater number of water molecules is retained on monolayer [39].

Observed that the curves 25 and 30°C overlap, indicating a similar behaviour as regards the adsorption of water to these their temperatures, in which case this difference (5°C) showed no influence of temperature on these isotherms, unlike curves 35 and 40°C, in which there is small, but clearly influence of this variable. There is also a greater effect of temperature interval 25-30°C to 35-40°C. An analysis of the equilibrium moisture behaviour in relation to water activity shows similarity to temperature behaviour.

Can be found in the literature several studies that relate initial content of water activity with development of various microorganisms responsible for synthesis products obtained in solid state fermentation process.

In particular, *Aspergillus niger* is described as the organism that best fits to the fermentation process, being around 0.7 the value of minimum water activity for development of their metabolic activities [11].

Thus, minimum value being 0.7 from water activity to grow *Aspergillus niger*, when using cashew apple dry bagasse as substrate in solid state fermentation process and adsorption isotherms obtained (Figure 4), has an indicative that moisture in substrate at intervals of 25-30°C and 35-40°C should not be less than 17.5 and 19.0%(d.b), respectively.

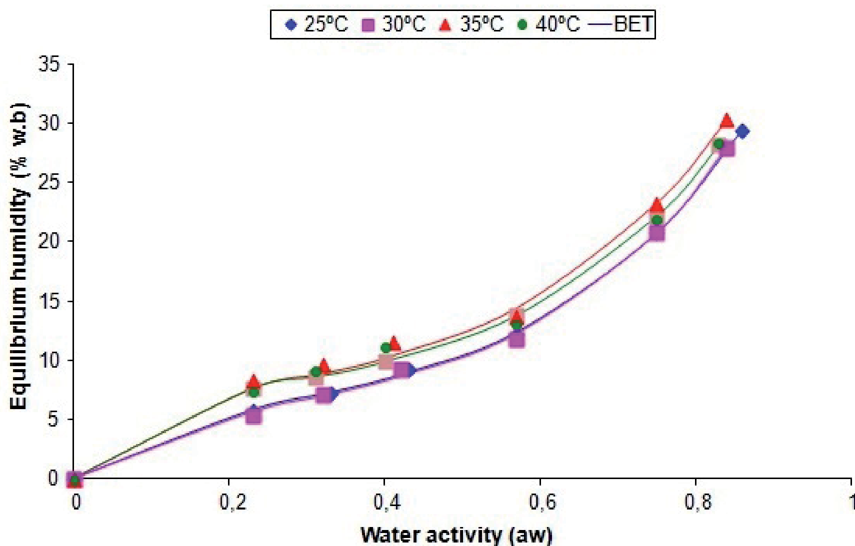

Figure 4. BET model to adsorption isotherms of dry cashew apple bagasse

Parameters	Temperature (°C)			
25	30	35	40	
Xm	5.64	5.61	6.33	6.07
C	12.59	11.28	39.72	49.85
n	14.39	15.02	13.35	13.18
R^2	0.9998	0.9986	0.9948	0.9941
P (%)	0.86	2.70	4.79	4.11

Table 3. Adsorption isotherms fitting parameters of cashew peduncle dry bagasse to the BET model

For pectinases production by *Aspergillus niger* in solid state fermentation process, several authors describe fermentation processes in which it can be seen that water activity which best favourable to synthesis of product is above 0.90. This implies that substrate moisture must be greater than 35%(d.b).

It is therefore extremely important to understand the hygroscopic behaviour of semisolid product used as substrate in a fermentation process, since the quantity of available water in through the microorganism to grow and synthesizing reactions during the production process is a limiting factor.

3.3. Most influential factor in solid state fermentation process on laboratory scale

As previously noted, there are many factors that affect a solid state fermentation process: amount of water available to microorganism can be quantified by moisture of medium (U), amount of inoculum necessary to overcome the adaptation phase and microorganism to produce the desired products (E), addition of nitrogen source such as ammonium sulphate (N) and fermentation temperature (T_f). These are variables that will be studied as previously described. It was conducted an experimental design 2^4, taking an answer the polygalacturonase activity. Objective was to determine which variable most affects the process and maximize the amount of enzyme produced.

The highest activities found for each assay and the fermentation time it was noted, are available in the Table 4. The greatest polygalacturonase activity (33.27 U/g) found, during the execution of experimental design, was obtained under the initial conditions: 50% (w.b) initial moisture, 10^6 spores/g, 1.5% (w/w) ammonium sulfate and 35°C at 29 hours of fermentation.

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Test	PPG (U/g)	t (h)
1	1.06	21
2	14.93	54
3	0.42	70
4	15.91	46
5	0.59	5
6	15.38	54
7	5.00	46
8	10.78	21
9	0	---
10	24.23	46
11	0	---
12	7.69	29
13	1.66	54
14	33.27	29
15	0	---
16	11.31	70
17	2.01	70
18	5.07	29
19	10.28	46
20	14.29	70
21	5.53	78
22	11.37	21
23	8.30	46

Table 4. Highest polygacturonase activities (PG) observed for which solid state fermentation assay.

From regression of polygalacturonase activity data and values of factors studied, it was constructed a first order model with 95% confidence for peaks of enzymatic activities.

$$PPG = \mathbf{8.66 + 7.80U - 2.50E} + 0.86N + 0.88T_f \mathbf{- 2.76UE} + 0.14UN + 1.56UT_f - 0.48EN \mathbf{- 2.52ET_f} + 0.93NT_f \quad (4)$$

Model validation was done using Test F. This test shows the ratio between calculated value of F and F tabulated, knowing that the latter was equal to 2.75. When this ratio is greater than 1, regression is significant. Not only to a statistically significant regression, but also predictive value of ratio between this two F's must be greater than four [40].

Regarding the determination coefficient (R^2), it is known that the maximum value is 1, meaning that between the experimental data and curve, there is no waste and any variation about the mean is explained by regression.

In Equation 4, calculated F was equal to 7.99. Thus, the ratio between calculated F and tabulated is equal to 2.91, meaning that this equation is statistically significant. The R^2 was equal to 0.8695, showing a good fit of experimental data to this equation.

Figure 5 indicates the profile of curve representing synergistic effect of studied factors on peaks of polygalacturonase activity response. These figures show the influence of initial moisture content (U), concentration of spores in inoculation medium (E), ammonium sulfate concentration (N) and fermentation temperature (T_f) on polygalacturonase activity response (PPG).

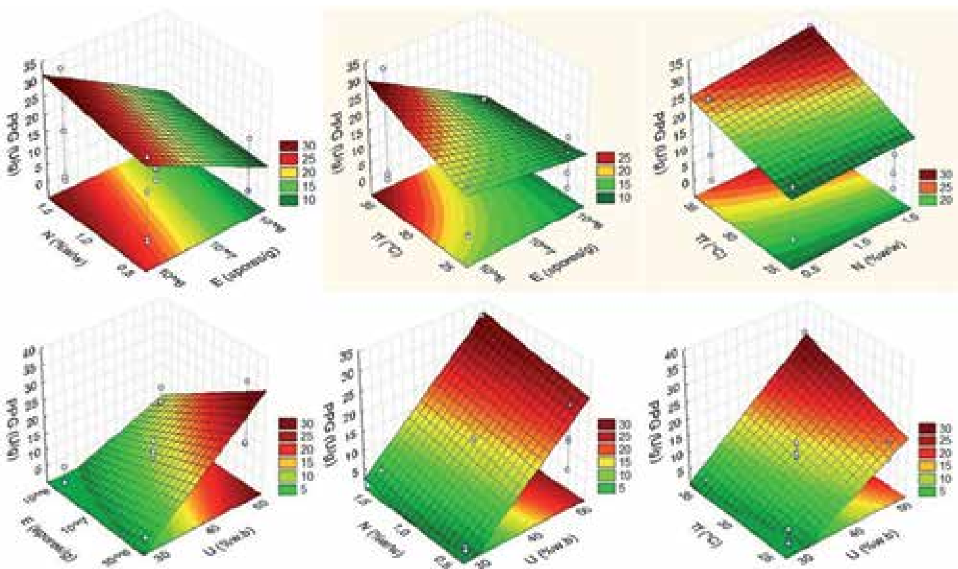

Figure 5. Response surface for polygalacturonase activity.

The highest peak of polygalacturonase activity calculated by the model (30 U/g) was obtained at higher levels for initial moisture content, for the lower concentration of inoculum and higher for fermentation temperature, observing that ammonium sulfate concentration did not influence significant on the response.

According to Pareto graphic (Figure 6), variable that most influenced fermentation process was initial moisture content of medium (U), and its interaction with variable concentration of inoculums (E), confirming claims reported in literature about the amount of water is thus a limiting factor [11,12].

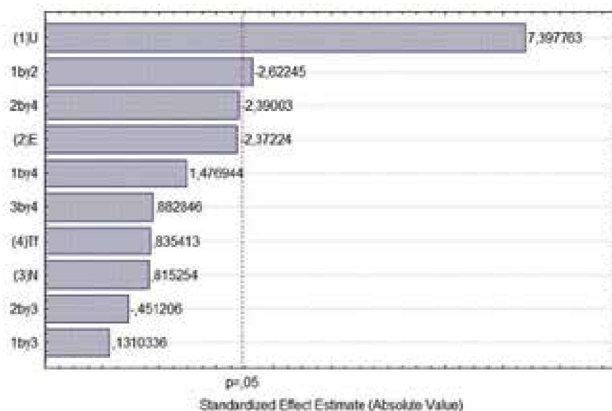

Figure 6. Pareto graphic

Authors [41] examined the effect of temperature on a solid state fermentation process using *Aspergillus niger* 163 and apple pomace as a substrate in a bioreactor rotary drum with 15L of solid medium. Temperature was studied within the range 22-60°C, observing their influence on polygalacturonase activity. Inoculated spore concentration was equal to $5x10^8$ spores/ml. Temperature of 35°C was found to be more susceptible to production of polygalacturonases enzymes.

Similarly to what was described in this paper, authors [42] studied the influence of ammonium sulfate concentration (from 0.25 to 0.45%), pH (4.82 to 6.12) and fermentation time (50-90h) on endopectinase enzyme production in a solid state fermentation process, using as substrate apple pomace and *Aspergillus niger* PC5. It was observed that ammonium sulfate concentration have positive effect on enzymatic activity. However, the effect was insignificant compared to fermentation time.

3.4. Scaling-up of solid state fermentation process using tray reactor

It was evaluated the scale-up process using a tray reactor, setting the mass of humid medium in 500 g. Spores concentration, moisture content and ammonium sulphate concentration were determined based on experiments performed on laboratory scale. Thus the conditions of fermentation process was 50%(w.b) of initial moisture content, 10^6 spores/g of inoculum

concentration, 1.5%(w/w) of ammonium sulphate concentration, 40 mm of substrate thickness [15] and 23°C of fermentation temperature [16].

In Figure 7, there are the behavior of polygalacturonase activity (PG) and process productivity (Prod) as function of fermentation time.

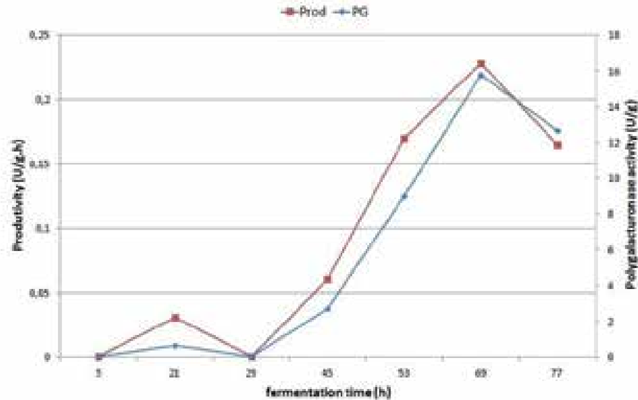

Figure 7. Polygalacturonase activity and productivity versus fermentation time.

Under conditions described previously, it was observed a peak of polygalacturonase activity of 15.76 U/g at 69 hours of fermentation, corresponding to the highest productivity.

Comparing the maximum activity obtained with reactor tray, and maximum activity obtained at laboratory scale, it is clear that production was 45 times lower. In this regard, the first fact to note is the internal temperature of medium. When using tray, there is an increase of at least 10°C above of initial value. Thus, there may be denatured enzyme.

Other factors can change during fermentation. One is the medium moisture, because with increase of temperature, there is an increased in evaporation rate of water, reducing the amount available to microorganism, which complicates the production process.

It can be observed that the time for adaptation of microorganism is greatly increased. Remembering, the greatest polygalacturonase activity in laboratorial scale was obtained at 29 h of fermentation. In the fermentation with tray, the highest activity obtained was reached at 69h of fermentation.

When the subject is solid state fermentation process, a large number of studies were conducted in a laboratory scale. It is relatively easy to control certain parameters for enzyme production. On this scale, many productions show great promise. However, beyond this process to pilot-scale bioreactors, which contain greater amounts of substrate, difficulties in carrying out the fermentation are revealed. Thus, it is characterized one of difficulties in scaling-up solid state fermentation processes [28].

3.5. Enzymatic stability as temperature and pH

In summary, the enzyme is maintained by a delicate balance of noncovalent forces such as hydrogen bonds, ion pairing, hydrophobic interactions and van der Waals force [45]. Thus, variations in temperature and pH are important in the analysis of enzyme activity.

Temperature has the activation and deactivation effect on enzyme activity. Continued increases of temperature beyond the maximum or optimum for enzyme activity leads to protein denaturation, which involves the deployment of large segments of polypeptide chain [45]. At the other extreme, enzymes inactivation by cooling can occur when nonpolar forces are involved and association of polypeptides. Low temperature reduces the strength of such interactions can promote the dissociation of subunits and compromise enzyme activity [7].

With respect to pH, all of ionisable groups of proteins undergo transitions pH dependent on the basis of intrinsic pKa values of amino acid residues. Many of these transitions will cause impacts on the stability of the enzyme and, in a narrow range of pH, can act together to destabilize it completely.

Knowing the pH and temperature stability is important for selection of enzymes compatible with the prevailing conditions for application potential so that the enzyme persist long enough to fulfil the expected function [7].

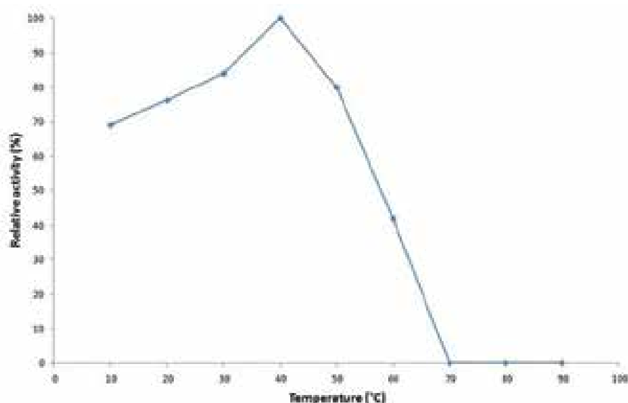

Figure 8. Thermostability of polygalacturonase enzyme

It can be observed (Figure 8) that temperatures of between 30 and 50°C, polygalacturonase activity remained with up to 80% of its maximum activity, and its optimum temperature at 40°C. After 50°C, relative enzymatic activity falls abruptly, reaching zero at 70°C.

Considering the greater use of pectic enzymes is in fruit juices industry, the processing of juices is normally done at 30-50°C. Thus, produced enzymes are been active during the process. For inactivation, the binomial time versus temperature must be considered, as well as chemical characteristics of juices. Usually, enzymatic inactivation is made between 70-90°C [7].

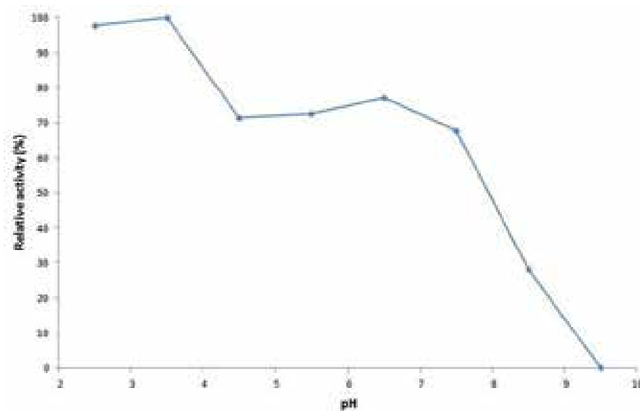

Figure 9. Stability of polygalacturonase enzyme in relation to pH

It is observed that the pH's of 2.5 and 3.5 (Figure 9), polygalacturonase activity was highest, almost 100%, characterizing the enzyme as acidic. Relative activity is equal to zero only at pH 9.5.

In literature some authors have reported the viability of various types of waste from processed fruits (apple, cranberry and strawberry), as substrates for polygalacturonases production, with *Lentinus edodes* as microorganism, using solid state fermentation process. These authors also observed the effects of temperature and pH on the enzymatic extract. Poligalactorunase produced has good thermal stability up to 50°C and high tolerance between pH 3.0 and 6.5 [46].

Other authors have studied the polygalacturonases production, using submerged fermentation with orange peel and passion fruit as substrate and *Aspergillus niveus* as microorganism. In terms of stability, polygalacturonase produced showed the highest activity at 40°C and pH between 3.0 and 4.5 [48].

In general, polygalacturonase enzyme activity has maximum pH ranges between 3.5 - 6.0 and temperature between 40 - 55°C [7].

For most industrial uses, fungal polygalacturonase is useful for high activity and optimal activity at low pH range, serving for most applications in the food industry [16]. Thus, enzyme extract studied can be applied in fruit juice processes, such as Barbados cherry (pH 3.3), orange (pH 3.0), apple (pH 3.6), passion (pH 3.4), peach (pH 3.3) and grapes (pH 3.1) [47].

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Chapter 19

Effect of Mucilage Extraction on the Functional Properties of Chia Meals

Marianela I. Capitani, Susana M. Nolasco and
Mabel C. Tomás

Additional information is available at the end of the chapter

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1. Introduction

Chia (*Salvia hispanica* L.) is an annual herbaceous plant that belongs to the *Lamiaceae* family, which is native to southern Mexico and northern Guatemala. The *Salvia hispanica* fruit consists of four nutlets, similar to an indehiscent achene, which contain a single seed. These nutlets are commonly called "seeds" [1]. Chia seed, together with corn, beans, and amaranth were important crops for pre-Columbian civilizations in America, including the Mayan and Aztec populations [2, 3]. With time its use was abandoned, but by at the end of the last century there was a resurgence of interest in chia due to its nutritional value [4]. Chia is considered an alternative crop to diversify and stabilize the economy of Northwestern Argentina [5]. The plant produces numerous small white and dark seeds that mature in autumn [6]. These seeds contain about 30% oil, and they mainly consist of unsaturated fatty acids [4, 7]. Chia seeds are a natural source of omega-3 fatty acids, antioxidants, proteins, vitamins, minerals and dietary fiber [5, 7, 8].

Chia meal (residue of the seeds after oil extraction) is a good source of proteins (19-23%) [9], dietary fiber (33.9-39.9%) [10], and compounds with antioxidant activity [7]. It also exhibits some interesting functional properties for its use in the food industry [11]. Functional properties are generally associated with the presence of proteins [12] and also of dietary fiber [13-16].

Dietary fiber (DF) consists of a heterogeneous mixture of compounds that are classified according to their physical properties and effects of their intake into: soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) [17], referring to the solubility of fibers in water. Plant secretions such as pectins and gums, components such as mucilage, and chelating

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agents such as phytates are sources of SDF; whereas cellulose, lignin, some fractions of hemicellulose, phenolic compounds such as tannins and lipid structures such as waxes, suberins and cutins constitute IDF [18].

The functional properties of food components can be defined as any physicochemical property that affects and/or modifies some of its characteristics and that contributes to the quality of the final product. Knowledge about of functional properties such as color, particle size, water holding, absorption and adsorption capacity, as well as those linked to the affinity for lipid components is very useful for the food industry, because during the processing some modifications can occur that must be taken into account according to the usage of the final product and its marketing conditions [19]. For example, water-holding capacity (WHC) is related to the freshness and softness effect present in bakery products, and the oil-holding capacity (OHC) is related to the un-fatty effect in fried food when it is low and to the juiciness and texture in meat products when it is high [18, 20, 21].

In addition to the characteristics mentioned above, it is important to consider the physiological effects of the DF intake. Given the capacity of SDF to form gels, it increases the viscosity of the bolus in the gastrointestinal tract, slowing the intestinal transit, making digestion and the absorption of nutrients more efficient, providing more of a feeling of satiety. Soluble fiber are fermentable fibers that can be microbiologically decomposed in the colon, producing gases such as carbon dioxide, hydrogen and methane, and short-chain fatty acids (acetic, propionic and butyric) which are absorbed and used as energy sources. Some of the most important beneficial effects of SDF is that it regulates blood sugar and lower cholesterol levels. On the other hand, IDF is responsible for adding bulk to the stool, speeding the passage of stool through the intestine by promoting peristalsis, alleviating constipation and other gastrointestinal disorders [22, 23]. Both types of fiber may also reduce the risk of obesity, hypertension, appendicitis, and other disorders [24]. The beneficial effects noted above show the important role that DF play in human intake, and that is why a daily intake of 25-30 g is recommended, with a good SDF/IDF balance (a minimum of 30% SDF and 70% IDF, optimum 50/50 ratio) in order to benefit from both fractions of fiber [25, 26].

Chia mucilage (SDF), a complex carbohydrate of high molecular weight, is an important component of the seed due to its physiological role. The mucilage is secreted when the seed comes into contact with water, generating high-viscosity solutions [27, 28]. Many studies have examined the functional properties of different types of gums (*Linux usitatissimum*, *Opuntia Picus indica, Alyssum homolocarpum, Psyllium plantago)* [29-32]. However, little information has been reported on the functionality of chia seed mucilage as a stabilizing or thickening agent of food products.

The objective of the present work was to perform a comparative evaluation of the functional properties of chia meals (*Salvia hispanica* L.) obtained from seeds with and without mucilage.

2. Materials and methods

2.1. Seeds

Chia seeds were obtained from commercial sources in Salta, Argentina (25º S and 65.5º W). They were cleaned manually by removing the foreign matter such as stones, dirt and broken seeds. They were packed in hermetic plastic vessels and stored at 5ºC until further use.

2.2. Meal without mucilage (Msm)

Meal without mucilage refers to the residue obtained after the oil extraction process using a Soxhlet apparatus following the IUPAC Standard Method [33] (*n*-hexane under reflux, 8 h, 90 °C,) of seeds that had previously had the mucilage extracted.

2.2.1. Mucilage extraction

The mucilage was extracted of chia seeds previously soaked in water (1:4) for 4 hours at room temperature. This mixture was distributed into plastic trays and covered with aluminum foil and frozen at -80°C, lyophilized, and the mucilage was removed by a sieving process (20 sieve mesh ASTM, 840 μm) (3 sections of 15 min each).

2.3. Meal with mucilage (Ms)

The data corresponding to the meal with mucilage that was considered in the analysis of the results corresponds to that reported by Capitani *et al*. [11]. The meal was obtained after oil solvent extraction (*n*-hexane) in a Soxhlet apparatus (Buenos Aires, Argentina) by thermal cycles at 80ºC for 8 h, following the IUPAC Standard Method [33], of chia seeds previously ground in a laboratory grinder (Moulinex, horizontal blade grinder, Buenos Aires, Argentina).

Meals (Msm and Ms) were homogenized and stored in plastic vessels at 5ºC until further use.

2.4. Scanning Electron Microscopy (SEM)

The whole seeds and seeds after mucilage removal were adhered to a cover slip, coated with a thin gold film (600 Å) in a sputter coater (Pelco 91000) and observed in a scanning electron microscope (LEO model EVO 40) at 5 kV. Longitudinal sections were sliced with a razor blade, after being plunged into liquid nitrogen to ensure the maintenance of their internal structure, and analyzed by microscopy using the same procedure and magnification ranges between x136 and x5000.

2.5. Characterization of meals

2.5.1. Proximate composition

Moisture, crude fiber and ash content were determined according to AOCS recommended practices Ba 2ª-38, Ba 6-84 and Ba 5ª-49, respectively [34]. Oil and nitrogen content (N) were

determined following IUPAC Standard Method [33] and AOAC Method [35], respectively. Protein content was calculated as nitrogen x 6.25. Carbohydrate content was estimated by calculating the nitrogen-free extract (NFE) by difference using Eq. (1).

$$\text{NFE:}100-\left(\text{oil}+\text{protein}+\text{crude fiber}+\text{ash}\right) \tag{1}$$

2.5.2. Total, soluble and insoluble dietary fiber

Total dietary fiber (TDF), soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) were determined according the enzymatic gravimetric method [36].

2.5.3. Neutral Detergent fiber (NDF), Acid Detergent fiber (ADF), lignin, cellulose and hemicellulose

The vegetable cell was separated into two parts (Van Soest method): cell content (highly digestible) and cell wall (partially digestible). The cell wall was analyzed and its components (cellulose, hemicellulose and lignin) were determined. The technique makes use of acidic and neutral detergent [35, 37].

2.5.4. Antioxidant activity

The extraction of phenolic compounds was carried out according to the method of Re *et al.* [38]. Ten mL ethanol were added to 1 g sample, then it was homogenized in Vortex for 2 min, decanted and filtered (0.45 μm nylon paper). The supernatant was transferred into a flask and evaporated using a rotavapor apparatus (BUCHI R124, Germany) to concentrate the sample. It was then redissolved in 1000 μL ethanol.

A spectrophotometric method was used to determine the antioxidant activity using a Hitachi U-1900 UVeVIS spectrophotometer (Japan). The antioxidant activity was quantified by a dying assay of the radical cation $ABTS^+$ measuring $ABTS^+$ reduction as the percentage of absorption inhibition at 734 nm, just 6 min later. The radical cation ABTS and potassium persulfate were obtained from Sigma Aldrich. Chlorogenic acid was used as standard antioxidant. Results were expressed as μmol/L Trolox g/sample, considering that chlorogenic acid diminishes twice the amount of absorption than Trolox [39].

2.5.5. Functional properties

2.5.5.1. Water-Holding (WHC) and Oil-Holding Capacity (OHC)

Water and oil holding capacities were determined according to the method of Chau *et al.* [40]. Briefly, 1 g (dry base (d.b.)) sample was weighed and then stirred into 10 mL distilled water or corn oil (density 0.92 g/mL, Arcor). These suspensions were centrifuged at 2200 x g for 30 min (Rolco Centrifuge Refrigerate, Model CR-5850, 22 cm radius, Buenos Aires, Argentina) and the supernatant volumes were measured. Water-holding capacity was expressed as gram water held per gram sample, and oil-holding capacity as gram oil held per gram sample.

2.5.5.2. Water Absorption Capacity (WA_bC)

This property was determined according to the AACC method 88-04 [41]. Approximate water absorption capacity was first determined by weighing out 2 g (d.b.) sample, adding water until saturation (approx. 35 mL) and centrifuging at 2000 x g for 10 min in a Rolco Model CR-5850, 22-cm radius centrifuge (Buenos Aires, Argentina). Approximate water absorption capacity was calculated by dividing the increase in sample weight (g), by initial weight, quantifying the water needed to complete the original sample weight (2 g d.b.) to 15 g. Water absorption capacity (WAbC) was then determined by placing samples in four tubes, adding different quantities of water (1.5 and 0.5 mL water above original weight, and 1.5 and 0.5 mL water below; one in each tube), agitating vigorously, and centrifuging the samples at 2000 x g for 10 min in a Rolco Model CR 5850. The supernatant was discarded and the residue weighed. Average water absorbed was calculated, and WA_bC was determined and expressed as gram water absorbed per gram sample.

2.5.5.3. Organic Molecule Absorption Capacity (OMAC)

This capacity was determined according to the method of Zambrano *et al*. [19]. A three gram (d b.) sample was placed in excess quantity corn oil (approx. 25 mL) for 24 h at room temperature, and then centrifuged at 2000 x g for 15 min in a Rolco Model CR-5850. OMAC was expressed as the absorbed hydrophobic component and calculated in terms of sample weight gain (g oil/sample g).

2.5.5.4. Emulsifying Activity (EA) and Emulsion Stability (ES)

These properties were evaluated according to the method [36] of Chau *et al*. [40]. Briefly, 100 mL 2 g/100 mL suspension was homogenized using an Ultra-Turrax T25 disperser (Janke & Kunkel, IKA-Labortechnik, Germany) at 7800 rpm for 2 min. Then, 100 mL corn oil (density 0.92 g/mL, Arcor) were added and homogenized at 15,000 rpm for 2 min. Emulsions were centrifuged in a 15 mL graduated centrifuge tube at 455 x g for 10 min, and then emulsion volume was measured. The EA was expressed as the remaining volume of the centrifuged emulsion corresponding to 100 mL of initial emulsion. The emulsion stability was determined by heating the emulsions to 80ºC for 30 min, cooling them to room temperature and then centrifuging the samples at 455 g for 10 min. ES was expressed as the remaining volume of the centrifuged emulsion corresponding to 100 mL of initial emulsion. On the other hand, all emulsions were evaluated by optical characterization using a Vertical Scan Analyzer (QuickSCAN, Beckman Coulter, Fullerton, USA). The QuickSCAN head scans the entire length of the sample (approximately 65 mm), collecting backscattering (BS) data every 40μm. Thus, it is possible to obtain curves showing the percentage of backscattering light flux, relative to external standards, as a function of the sample height in mm [42]. Coalescence kinetics were determined by measuring the mean values of BS as a function of time in the 25-30 mm zone (Backscattering % 25-30 mm).

2.6. Statistical analysis

The results obtained were analyzed using ANOVA and Tukey's test ($p \leq 0.05$), using Infostat software [43].

3. Results and discussion

The nutlet of Salvia hispanica consists of the seed and a pericarp surrounding the seed. The true seed, in turn, consists of a coat (testa), the endosperm and the embryo, consisting mainly of two cotyledons [1]. Basically, the pericarp of the chia seed is similar to that other Nepetoideaes because it shows cuticle, exocarp, mesocarp, layers of sclereids and endocarp. The cells of the mesocarp and exocarp are parenchimatic. Figure 1 shows a scanning electron microscopy of a *Salvia hispanica* nutlet. In the exocarp there often are cells that produce mucilage when the nutlets get wet (Figure 1D).

Figure 2 shows SEM microscopy of *Salvia hispanica* L. seeds after mucilage extraction. In these images it can be observed that the mixocarpy phenomenon occurs in the outer layers (cuticle and exocarp). After removing the mucilage, the nutlet surface is characterized by small hill-like eminences, spaced, that cover the entire surface, corresponding to the mesocarp cells. Chia seeds presented a similar structure to that of two mucilaginous species (*Carrichtera annua* and *Anastatica hierochuntica),* which could be associated with the presence of concentric aggregates of glucuronic acid [44]. The retention of the mucilage close to the seed can be due to the association of the mucilage with the columella (a secondary wall cell produced after mucilage secretion) and portions of the cell wall [45].

The proximate composition of chia meals with and without mucilage is presented in Table 1. Both meals were characterized by a high protein content, higher than that reported for sunflower meals of different origin (20.6-23.1%) [46] and canola meals (36.1-40.0%) [47, 48], and within the range of the values reported for linseed meals (38.9-43.3%) [48, 49].

On the other hand, both types of meals presented a high crude fiber content, with values higher than those reported for sesame, soybean, linseed and canola meals, 5.8%, 3.5%, 5.27% and 11.54%, respectively [50, 48].

In Table 2 it is possible to observe that both types of meals presented a high content of TDF, consisting mainly of IDF. Even though the value of SDF was relatively low, this could be attributed to the fact that, during the determination of this fiber, some components were not quantified because they cannot precipitate during the treatment with ethanol, and thus SDF was underestimated [26]. It is worth noting that the meal obtained from seeds that previously had their mucilage extracted (Msm) exhibited a statistically higher content of IDF ($p<0.05$) than that for Ms, at the expense of a significant decrease in its SDF content. These results are consistent with the data obtained from the analysis of NDF, consisting of cellulose, hemicellulose and lignin (structural polysaccharides that contribute to the IDF fraction), which was statistically higher ($p<0.05$) in Msm (Table 3). Regarding Ms, it presented a better IDF/SDF balance, with a 89/11 ratio.

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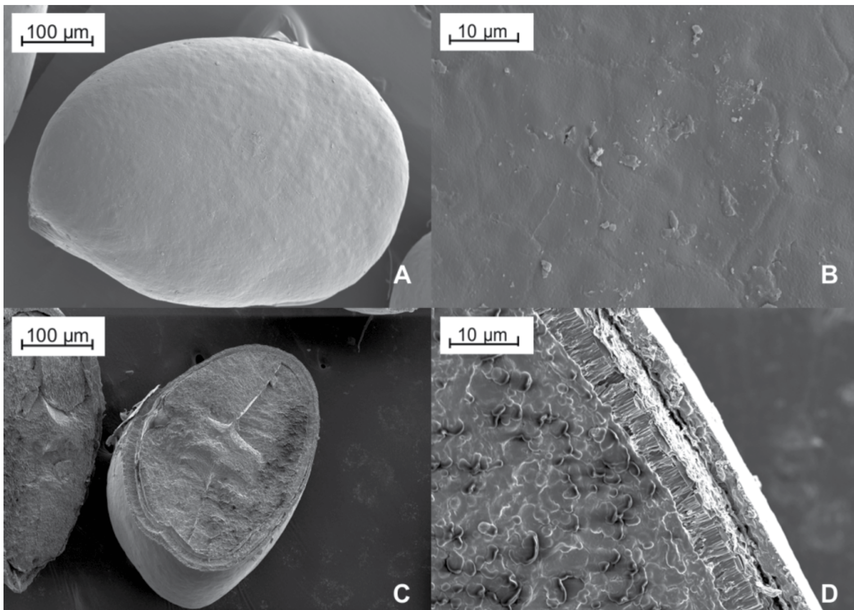

Figure 1. SEM microscopy of *Salvia hispanica* nutlets. (A) Whole nutlet, lateral view (x150), (B) pericarp surface (x5000), (C) broken nutlet, longitudinal section (x149), (D) broken nutlet (x1500).

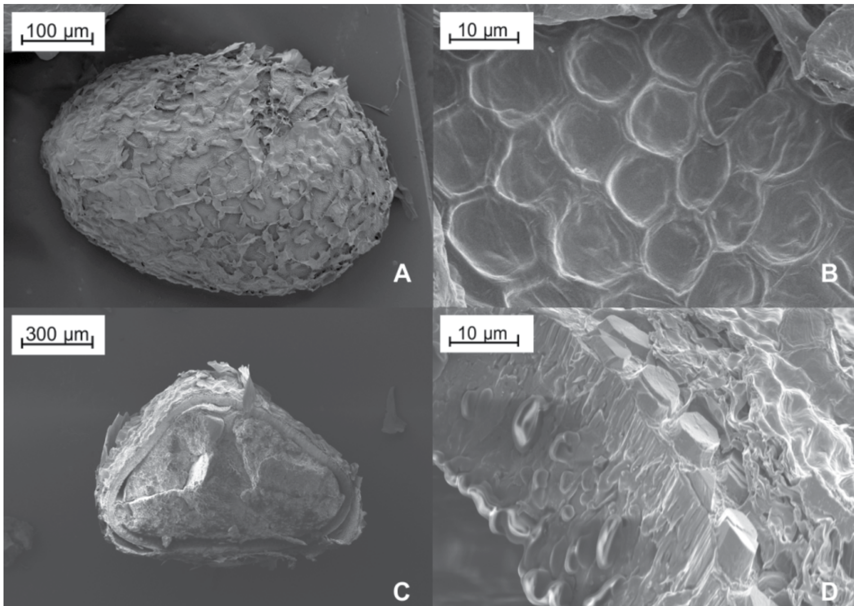

Figure 2. SEM microscopy of *Salvia hispanica* L. nutlets after mucilage extraction. (A) Whole nutlet (x136), (B) nutlet surface (x5000), (C) broken nutlet (x150), (D) broken nutlet (x3500).

Component	Msm	Ms #
Moisture	10.66 ± 0.04 [a]	10.47 ± 0.16 [a]
Protein*	42.43 ± 0.71 [a]	41.36 ± 0.28 [a]
Crude fiber	27.75 ± 0.97 [a]	27.57 ± 0.07 [a]
Ash	7.82 ± 0.13 [a]	7.24 ± 0.15 [a]
Oil	0.22 ± 0.25 [a]	0.21 ±0.08 [a]
NFE	24.17 ± 0.76 [a]	23.62 ± 0.94 [a]

Capitani *et al*. [11]

Mean value (n = 3)

Values followed by different letters differ significantly (p ≤ 0.05), according to Tukey's test.

*Factor: 6.25; NFE: nitrogen-free extract.

Table 1. Proximate composition of chia (*Salvia hispanica* L.) meals (% d.b.)

Component	Msm	Ms #
TDF	47.13 ± 0.17 [a]	46.06 ± 0.86 [a]
IDF	45.62 ± 0.37 [b]	41.13 ± 0.47 [a]
SDF	1.51 ± 0.24 [a]	4.93 ± 0.65 [b]

Capitani *et al*. [11]

Mean value (n = 3)

Values followed by different letters differ significantly (p ≤ 0.05), according to Tukey's test.

Table 2. Total (TDF), soluble (SDF) and insoluble (IDF) dietary fiber of chia (*Salvia hispanica* L.) meals (% d.b.)

Component	Msm	Ms #
NDF	63.6 ± 2.1 [b]	53.9 ± 0.3 [a]
ADF	30.8 ± 1.2 [a]	38.1 ± 1.2 [b]
Lignin	6.9 ± 0.5 [a]	4.5 ± 0.7 [a]
Cellulose	23.1 ± 0.9 [a]	34.6 ± 1.3 [b]
Hemicellulose	33.6 ± 1.0 [b]	14.8 ± 1.2 [a]

Capitani *et al*. [11]

Mean value (n = 3)

Values followed by different letters differ significantly (p ≤ 0.05), according to Tukey's test.

Table 3. Fiber composition of chia meals analyzed according to the method of Van Soest (% d.b.)

The antioxidant activity of the two chia meals compared with other types of meals is shown in Table 4. Both for Ms and Msm, the activity was high, without a significant difference between them (p>0.05). These values were higher than those found for wheat bran and sorghum and barley whole grain meals. But they were significantly lower than those found for chia meal obtained as a byproduct of cold-pressing oil extraction. The latter could be attributed to the fact that the meal obtained by pressing shows a higher percentage of residual oil (11.39% d.b.), which contains tocopherols, a class of compound with natural antioxidant activity [11].

Sample	Trolox equivalent antioxidant coefficient (TEAC, µmol/g)
Msm	187.4 ± 33.21 [a]
Ms[1]	226.6 ± 4.13 [a]
Chia meal from oil pressing extraction[1]	557.2 ± 28.18 [b]
Wheat bran [2]	48.5
Sorghum meal [3]	51.7
Barley meal [3]	14.9

[1] Capitani *et al*., [11]

[2] Iqbal *et al*. [57]

[3] Ragaee *et at*. [58]

Mean value (n = 3)

Values followed by different letters differ significantly (p ≤ 0.05), according to Tukey's test.

Table 4. Antioxidant activity of chia (*Salvia hispanica* L.) meals compared with other meals

Regarding the functional properties, the meal with mucilage (Ms) exhibited a statistically higher absorption and water holding capacity (p<0.05) than that of the meal without mucilage (Msm) (Table 5). This behavior can be associated with the presence of mucilage in Ms, which acts as soluble dietary fiber, capable of holding water inside its matrix [51]. The WAbC of both meals was higher than that observed for canola and soybean meals (3.90 g/g and 3.28 g/g, respectively) and similar to that of linseed meal (6.03 g/g) [48].

Both types of chia meals presented a low absorption of organic molecules and oil-holding capacity, being significantly higher in Msm. These differences could be explained in terms of the particle size and the cellulose content of the meal [52, 53]. The determination of OHC is

important because it is related to the capacity that food components have to hold oil, affecting their flavor and mouthfeel [54]. The OMAC is associated with the interaction of the fiber with fats, bile acids, cholesterol, drugs, and toxic and carcinogenic compounds at the intestinal level. Due to their low OHC levels, both types of chia meals could be considered an important ingredient in the manufacture of fried products due to their low fatty mouthfeel contribution. It is noteworthy that the OHC values were higher than those reported for the *Jessenia polycarpa* fruit meal [55] and similar to those of the fibrous residue of *Canavalia ensiformis* and barley [53, 56] and those reported by Khattab and Arntfield [48] for linseed and canola meals (2.01 g/g and 2.09 g/g, respectively).

Property	Msm	Ms #
WHC (g/g)	5.25 ± 0.39 [a]	10.64 ± 0.60 [b]
WA_bC (g/g)	4.79 ± 0.49 [a]	6.45 ± 0.41 [b]
OHC (g/g)	2.94 ± 0.14 [b]	2.03 ± 0.08 [a]
OMAC (g/g)	2.22 ± 0.01 [b]	1.64 ± 0.02 [a]

[#] Capitani *et al.* [11]

Mean value (n = 3)

Values followed by different letters differ significantly (p ≤ 0.05), according to Tukey's test.

Table 5. Functional properties of chia (*Salvia hispanica* L.) meals

In Figure 3 it can be noted that the differences observed in the emulsifying activity were not significant, although the stability of the emulsion prepared with Ms was statistically higher than that with Msm ($p<0.05$). This effect can be associated with the capacity of mucilage to act as a thickening agent due to its ability to increase the viscosity of the aqueous phase in an O/W emulsion, thus hindering movement of the oil droplets of the dispersed phase [59]. This property is similar in the linseed mucilage, which has a strong thickening capacity, favorably affecting the water-holding capacity and the emulsifying properties of defatted linseed flour [60].

The behavior of the meals studied with respect to emulsion stability, examined by their optical characterization with a vertical scan analyzer (QuickScan), is shown in Figure 4. Both meals presented a high initial emulsifying capacity (66.3 and 62.2 %BS. for Ms and Msm, respectively), which remained approximately constant for all the time span studied (60 min) for Ms. However, the emulsion stability of the meal without mucilage decreased markedly by the end of the 60 min (50.4 %BS).

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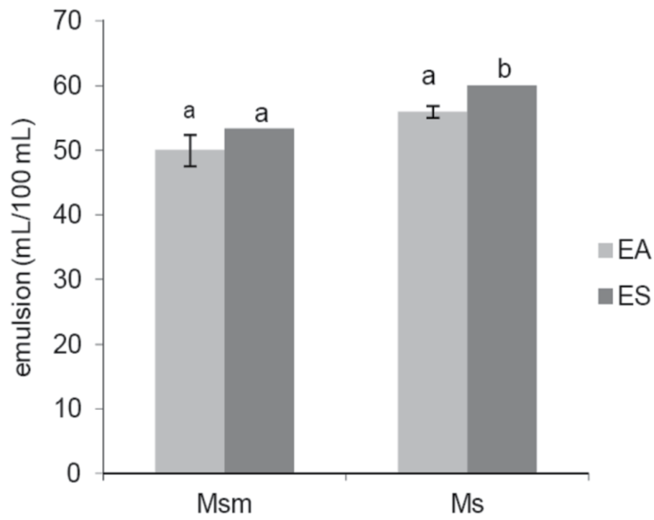

Figure 3. Activity and stability of O/W emulsions (50:50 p/p) with chia meal with and without mucilage. Values followed by different letters differ significantly (Tukey's test, p ≤0,05)

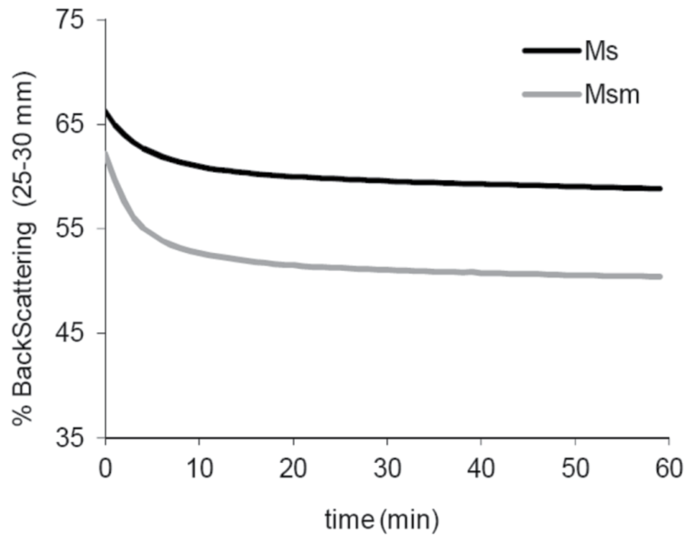

Figure 4. Destabilization kinetics of O/W emulsions (50:50 p/p) with chia meal

4. Conclusions

The results obtained show that both types of chia meals (with and without mucilage) present interesting functional properties for the food industry, which could be applied for example in the manufacture of bakery products, powdered beverages, yogurts, ice-creams, sauces and creams. They also suggest the potential use of two chia byproducts: the residual meal obtained after the oil extraction of whole chia seeds, and the use of a byproduct of the mucilage extraction. As regards the formulation of stable emulsions, the meal with mucilage is recommended for use given the role of mucilage as a thickening agent. From a physiological point of view, the presence of mucilage becomes a potentially interesting food ingredient due to its health benefits, since it has the capacity to form high-viscosity gels, slowing the intestinal transit, providing more of a feeling of satiety, and helping to prevent diseases such as obesity, colon cancer, hypercholesterolemia and diabetes.

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Chapter 20

The Redesign of Processes' Development in Food Production Organizations Using Quality Engineering Methods and Tools

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Additional information is available at the end of the chapter

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1. Introduction

A number of trends and challenges such as increased competition, new technologies and regulations, quality and new consumer trends have been forcing the food industry to change [1, 2, 3]. In response to these new challenges, food companies are improving competitiveness by restructuring, redesigning existing processes, and intensifying the fight for market share through product differentiation and/or the development of new food products [1]. In order to improve, companies in the food industry must adopt: Restructure of their organizations and redesign their processes; Automation of production and other processes to decrease dependence on human resources and transfer activities to self-service facilities for customers and partners; Optimization of logistical infrastructure and systems; Energy saving measures through new technology and materials, new production methods and good-practice implementation; Political and regulatory developments (food safety and other regulations); Technological changes (biotechnology, ICT and RFID, robotics, sensors, e-business); Understand globalization, market developments and customer trends.

In this chapter process development is analyzed because of its impact on food quality, safety and sustainability [4, 5, 6]. A redesign of process development could be accomplished through many different approaches, techniques and tools [7, 8, 9, 10]. In this chapter Business Process Management (BPM) is used with accompanied quality engineering methods and tools. A number of important questions will be addressed concerning the redesign of process development in food production organizations using quality engineering tools and methods.

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The first question that will be raised is the quality of processes in the food industry. The analysis will start from a typical process map for companies in the food processing industry in Serbia. After that the production process, as one of the most important processes in food processing will be decomposed. For each sub process appropriate metrics will be defined as the road of evaluation for the quality of the sub processes and quality of the goal of the process itself. Redesign of process development will be analyzed by comparing different approaches and by consideration of process redesign as the process. Different quality engineering methods and tools in the food industry will be compared according to the frequency of their implementation in the Serbian food industry as well as a correlation between the application of different quality engineering methods and tools, and profit in the companies. As an extension of the general ranking idea presented on the ranking and definition of goals in the production process, a fuzzy approach for evaluation of the importance of entities in supply chains in the food industry is presented. The general idea is to present an application of a mathematical tool in a situation that is very common in the food industry where conditions have been constantly changing so the observed values could not be stochastically described and where there is not a sufficient amount of data for statistical analysis. In other words, the application of fuzzy sets on evaluation of the importance of entities in the supply chain will be presented. A strategic map as a strategic part of the BSC (Balanced Score Card) framework is presented as one of the quality engineering methods. The presented strategic map started from the Kaplan – Norton model but it was adjusted in order to meet the needs of food processing companies in Serbia. Relations between entities (from all four perspectives) are defined as the result of research among Serbian companies. In the final part of the chapter the process framework for food processing companies is presented. The questionnaire used for the research is presented as well as gathered data from 53 Serbian companies. The gathered data was the input in modeling and evaluations presented in previously discussed issues.

The main idea of the chapter is to provide an overview of the redesign of process development, starting from analysis (decomposition of processes), redesign, implementation of modern quality engineering tools and methods (frequency of usage and impact of different tools and methods and implantation of some of them) as well as theoretical and mathematical tools on ranking of quality goals (theory of fuzzy sets) and finally providing a process framework and, at the same time, the keeping a connection and solid ground in data gathered from the food industry.

2. Quality of processes in the food industry

The food industry contains a number of completely different processes which require a wide range of measuring instruments. On the one hand, the quality of processes has market goals in brand development, demand management and new product introduction, while embracing food security and quality requirements. On the other hand, quality of processes in the food industry depends on many factors such as customer demands, key performance indicators, the process map, technology level, management level etc. In order to provide quality

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analysis, and redesign and improvement of the process the first step is an analysis of the process map of a typical organization in the food industry. A typical process map for an organization in the food industry is presented in figure 1.

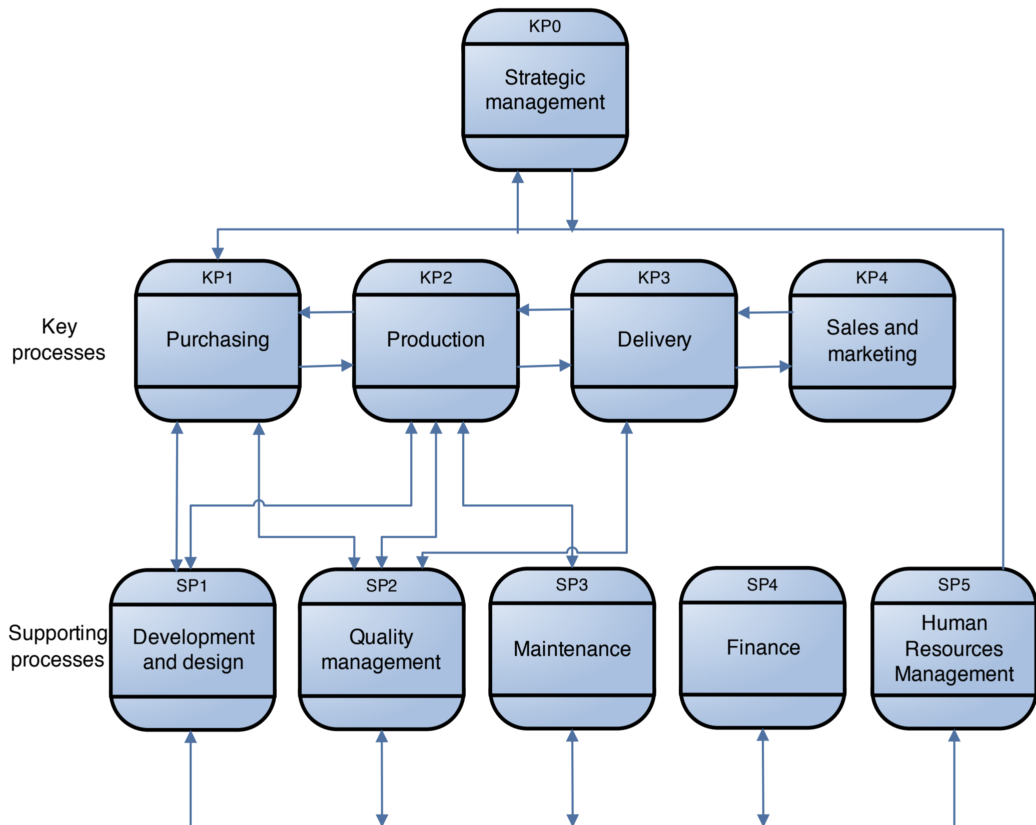

Figure 1. Typical process map for organizations in the food industry

Besides strategic management, each component process is functioning at a tactical and operative level and has quality metrics. In this chapter the major focus will be placed on the production process (KP2), all other decompositions could be performed using the same pattern, with appropriate quality metrics. In further analysis the production process could be decomposed into the sub processes presented in figure 2.

The production process (according to figure 2) consists of four sub processes: Logistic process, Process realization, Process planning and scheduling and Production process control.

In further analysis the logistic sub process (KP 2.1) could be decomposed into the following sub processes: Definitive logistic strategy; Plan inbound material flow; Operate outbound warehousing; Operate transportation and Manage reverse logistics.

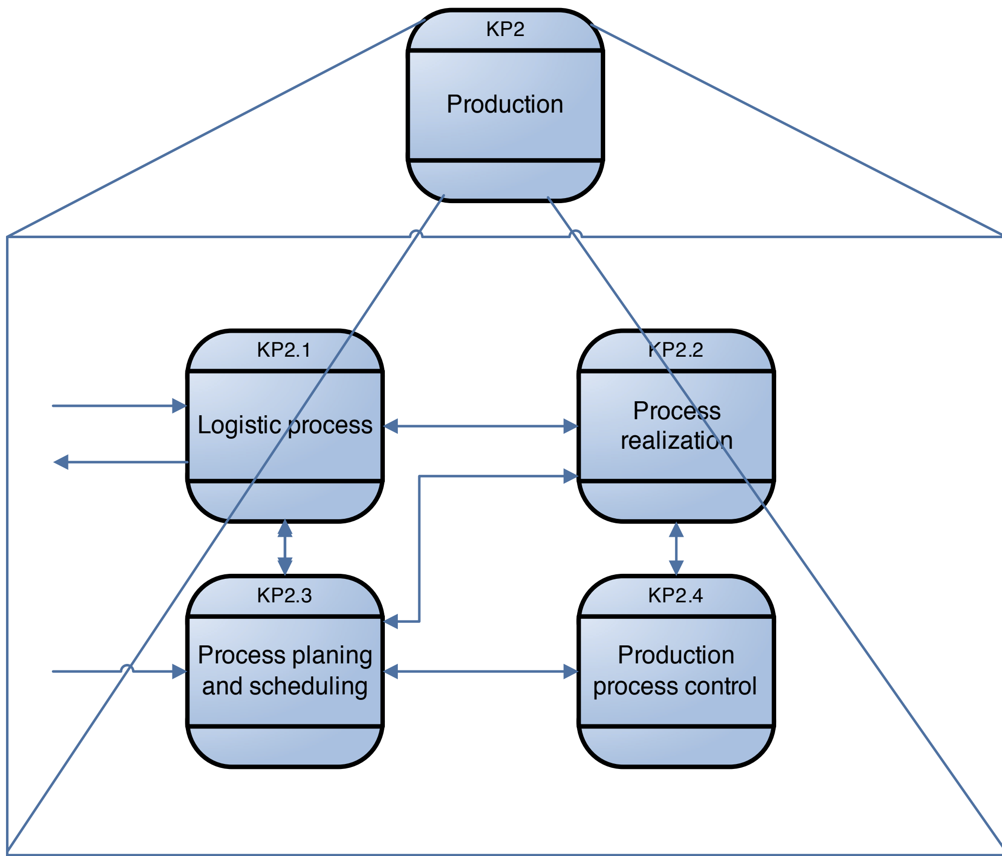

Figure 2. Decomposition of production process

The next important step is the definition of quality metrics. According to the definition a metric is a verifiable measure stated in either quantitative or qualitative terms. Quality metric data may be used to: spot trends in performance, compare alternatives and predict performance. Organizations need to collect information for a particular quality metric in order to evaluate and improve their processes. For further analysis of the logistic process the following quality metrics are presented in Table 1.

The second sub process of KP2, Production process realization (KP 2.2) is decomposed into sub processes: Preparing workers for obligatory measures; Preparing working places; Realization of working activities and Work reporting. The accompanied quality metrics are presented in table 2.

Production process planning and scheduling (KP 2.3) is decomposed into the following sub processes: Manage demand for products; Create material requirement plan (MRP) and Schedule production. The accompanied quality metrics are presented in table 3.

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Logistic strategy realization %	Realization of inbound plan of material flow	Costs of warehousing /plan *100	Realization of plan of outbound transportation	Score
95-100	>100	<50	>100	10
85-95	90-100	50-60	90-100	9
75-85	80-90	60-70	80-90	8
65-75	70-80	70-80	70-80	7
55-65	60-70	80-90	60-70	6
45-55	50-60	90-100	50-60	5
35-45	40-50	100-110	40-50	4
25-35	30-40	110-120	30-40	3
<25	<30	>120	<30	2
0.25	0.25	0.25	0.25	weight

Table 1. Quality metrics of "Logistic process" KP 2.1

Level of preparing workers	Flexibility of working plans	Level of plan fulfillment *100%	Quantitative Waste %	Value of waste %	Score
10	10	>100	<0.5	<0.5	10
9	9	90-100	0.5-1.0	0.5-1.0	9
8	8	80-90	1.0-2.0	1.0-2.0	8
7	7	70-80	2.0-3.0	2.0-3.0	7
6	6	60-70	3.0-4.0	3.0-4.0	6
5	5	50-60	4.0-5.0	4.0-5.0	5
4	4	40-50	5.0-6.0	5.0-6.0	4
3	3	30-40	6.0-7.0	6.0-7.0	3
2	2	20-30	7.0-8.0	7.0-8.0	2
1	1	<20	>8.0	>8.0	1
0.2	0.1	0.2	0.25	0.25	weight

Table 2. Quality metrics of "Production process realization" KP 2.2

Accuracy of demand %	Accuracy of MRP %	Work in progress/ production %	On time delivery (OTD) %	Score
85-95	85-95	5-10	90-95	9
75-85	75-85	10-15	85-90	8
65-75	65-75	15-20	80-85	7
55-65	55-65	20-25	75-80	6
45-55	45-55	25-30	70-75	5
35-45	35-45	30-35	65-70	4
25-35	25-35	35-40	60-65	3
15-25	15-25	40-45	55-60	2
<15	<15	>45	<55	1
0.15	0.25	0.3	0.3	weight

Table 3. Quality metrics of "Production process planning and scheduling" KP 2.3

Effectiveness of control of inputs %	Effectiveness of process control %	Effectiveness of control of outputs %	Level of control of measurement devices %	Score
>95	>95	>95	10	10
85-95	85-95	90-95	9	9
75-85	75-85	85-90	8	8
65-75	65-75	80-85	7	7
55-65	55-65	75-80	6	6
45-55	45-55	70-75	5	5
35-45	35-45	65-70	4	4
25-35	25-35	60-65	3	3
15-25	15-25	55-60	2	2
<15	<15	<55	1	1
0.25	0.25	0.3	0.2	weight

Table 4. Quality metrics of "Production process control" KP 2.4

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Production process control (KP 2.4) could be decomposed into the following sub processes: Control of inputs; Process control, Control of outputs, Non conformance product control and Control of measurement devices. The accompanied quality metrics are presented in table 4.

Quality systems focus on the quality of what the organization in the food industry produces, the factors which will cause the organization to achieve its goals, the factors which might prevent it satisfying customers and the factors which might prevent it from being productive, innovative and profitable. To control, assure and improve quality there is a need to focus on certain goals, in the case of goals of the production process (KP2) of a typical company in the food industry we can define goals as the constituent of the previous sub process goals (figure 3).

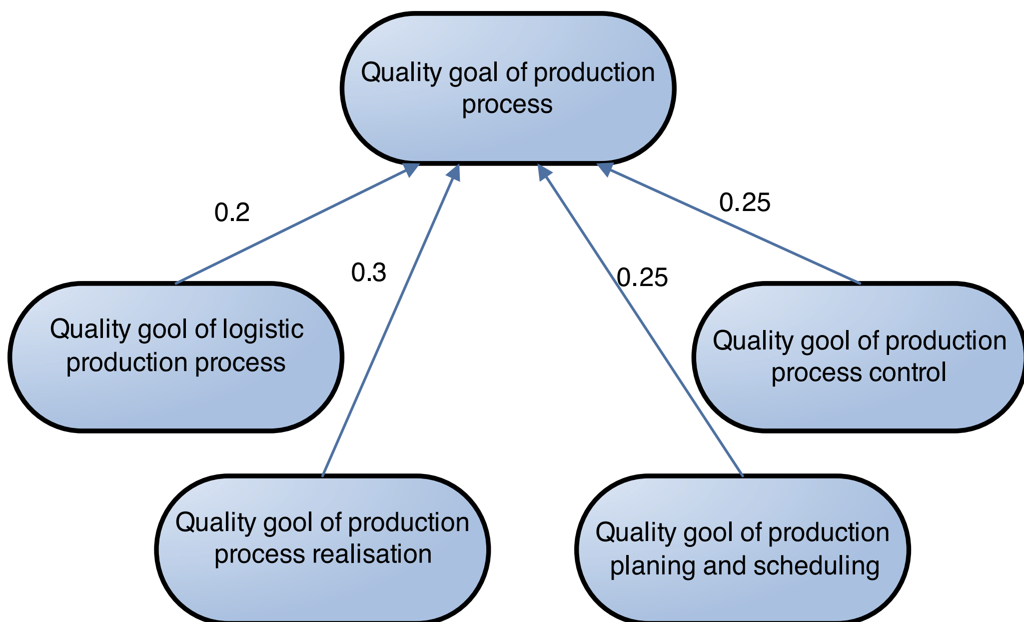

Figure 3. Quality goals of Production process KP2

By decomposition of the production process, analysis of sub processes and definition of metrics for each sub process it is possible to control, assure and improve the quality of a process in companies from the food industry sector. Definition of scores and weights in the metrics of each sub process is performed according to the authors' experience and available literature. In order to clearly demonstrate the idea all values are presented as deterministic ones. Of course some of them could be expressed by linguistic expressions rather than precious numbers but that issue will be elaborated in further text. The key processes in the food industry were analyzed and compared, and the results of research

are presented in figure 4., depicting the existence of the gap between the quality of development and design process, and other processes. The analysis was performed on selected companies from Serbia.

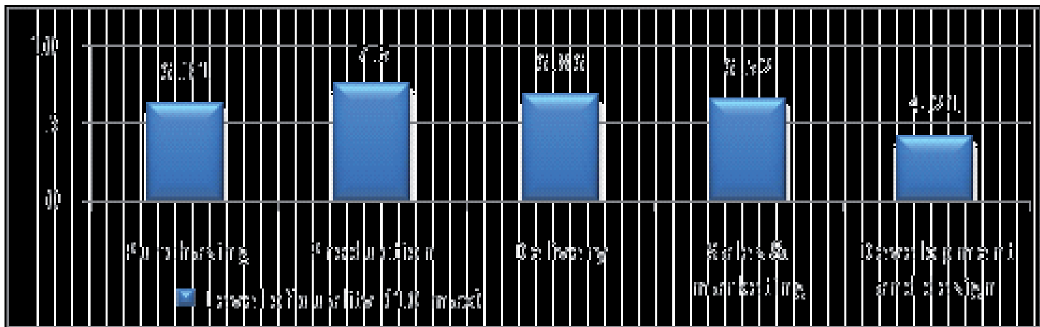

Figure 4. Quality of development and design process compared to quality of key processes

The gap between quality of the development and design process and other processes indicates that the quality of the development and design process is lower than the quality of the other processes so the focus in process redesign and improvement should be on the redesign of process development and design.

3. Redesign of process development

3.1. Redesign of process development: Different approaches

The redesign of process development is connected to different methodologies of process change. In the redesign of process development and in the process of business process redesign itself there are a number of methodologies which cover the different amount of changes, results, used tools and probability of success. According to [11], and presented in table 5 a comparison of possible methodologies indicating whether they are applicable in the food industry.

According to table 5 it is clear that Continuous Process Improvement (CPI) as a never ending effort to discover, and eliminate the main causes of problems, is most likely to succeed in companies from the food industry.

Process improvement and BPR & lean are less likely to succeed but they cover a larger number of changes in the companies. Beside a redesign of process development can be viewed as a process. According to [11], the redesign of process development is divided into 5 phases (4 different entities) (Fig.5) with 10 steps.

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No.	Change methodology	Amount of change	Score of change	Used tools	Probability of success	Applicable in food industry
1	BPR & lean	-reduction more than 50% of time, costs and quality	-cross func. teams or functional teams	-process maps -design principles -benchmarking -best practices -lean tools	-less than 40%	yes
2	Process improvement	-reduction more than 20% of time, costs and quality	-cross teams or functional teams	-process maps -design principles -LE -six sigma -lean tools	-more than 70%	yes
3	Continuous process improvement	-small reduction more than 20% of time, costs and quality	-one person or one sub process		-more than 90%	yes

Table 5. Comparison of change methodologies (adapted from [11])

There are five phases in this model:

- Analysis Phase — Identify areas of opportunity and target specific problems.
- Design Phase — Generate solutions and identify the required resources to implement the chosen solution with approval of senior management.
- Development Phase — Formulate a detailed procedure for implementing the approved solution with staff and customers.
- Implementation Phase — Execution of the solution and implementation of the redesign.
- Evaluation Phase — Build metrics, measurement tools, monitor implementation, and evaluate measurements for continuous improvement.

Process redesign as a process could be presented in the 10 steps, according to figure 5. The first five steps are common for most companies and the other steps could be defined according to the specific problem and in some cases using different quality methods and tools.

The first step covers:

- Meeting with senior management for the purpose of discussing barriers to process,
- Improvement, problem of eventual job losses and crafting of the kick-off-speech,
- Meeting with affected process managers and employees.

The second step is very important for success of the complete process. It is realized according to the team engineering approach [12, 13] with specified roles of team members: project manager, project principal, process improvement team, facilitator, and expert in ICT.

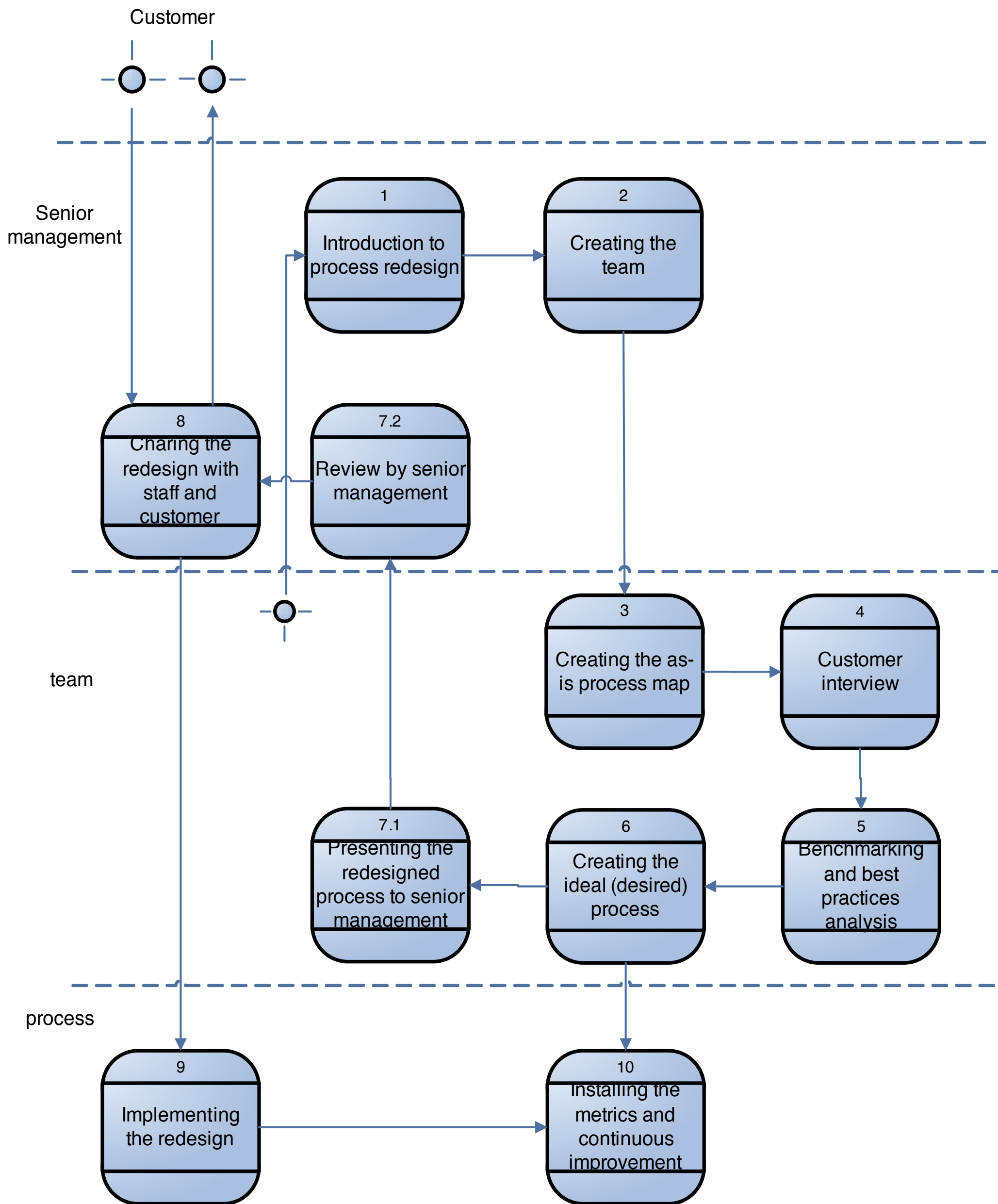

Figure 5. Process redesign as process

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Senior management has the important task of directing team work in the first meetings and by the definition of statements and roles which will define the procedures for the team work.

The third step is performed by team work, starting from analysis of the existing state of processes, best practice and creating the as-is state of processes.

The fourth step covers the interviews with customers, according to: Customer request and needs; Ranking the criteria; Needed performance according to each criteria and Competitor position according to ranking and criteria.

The use of benchmarks and the development of new forms of benchmarking for best practice is well established in the food industry, although less so in other areas of the food chain. Benchmarking and best practice analysis is performed in step five through analysis of food industry competitions, using information from: industry trade associations (trade chambers etc.), industry studies, consultants' reviews, distributors, former employees, competitors themselves, published documents, indirect information sources from competitors, government sources, customers, supplies, and reverse – engineering products.

Other steps are performed according to appropriate redesign methodology adapted for each specific organization from the food industry. In addition, different quality engineering tools and methods could be employed in the following steps. It is important to emphasize that ICT could be used as a support during process redesign on the one hand, and on the other hand ICT and Business Process Management cover various aspects such as process control and supply chain management.

3.2. Quality engineering methods and tools in the food industry

Quality engineering methods and tools have an important role in the food industry because customers and markets demand proven high quality products and protection against low quality and unsafe products. On the other hand, the food industry needs to attain the quality that meets international standards for quality products. As it was mentioned the food industry needs to cope with the challenge of modern technological production methods, know them and assimilate them in quality assurance areas using new innovative hi-tech sensory and measurement instruments, supervise the production process, so that the designated quality level is always met. In order to perform all of these tasks and in order to meet all of the existing challenges the food industry has a number of engineering methods and tools available.

We analyzed companies from Serbia (listed in the following text) and compared results with world practice. The following quality engineering methods and tools used by the food industry have been analyzed according to their frequency and success rate: (1) Ichicawa's seven basic tools for quality (flow chart, check sheet, histograms, scatter plots, control chart, cause-effect diagram, Pareto analysis); (2) The seven new tools for improvement (affinity diagram, interrelationship diagram, tree diagram, prioritization grid, matrix diagram, process decision program chart, activity network diagram) ; (3) Cost of Quality (CoQ) ; (4) Project management; (5) Simultaneous engineering; (6) Statistical process control; (7) Reliability and

risk engineering; (8) World class manufacturing (WCM) ; (9) Six-sigma; (10) Lean six-sigma; (11) Taguchi method; (12) Zero defect (ZD) ; (13) Design of experiments; (14) Quality Function Deployment (QFD) ; (15) FMEA; (16) FMECA; (17) Just in Time (JiT) ; (18) Business Process Reengineering; (19) Balance Score Cards (BSC) ; and (20) other.

The results of the usage and frequency of the specific quality engineering methods and tools listed above, in the case of the Serbian food industry, is presented in Figure 6.

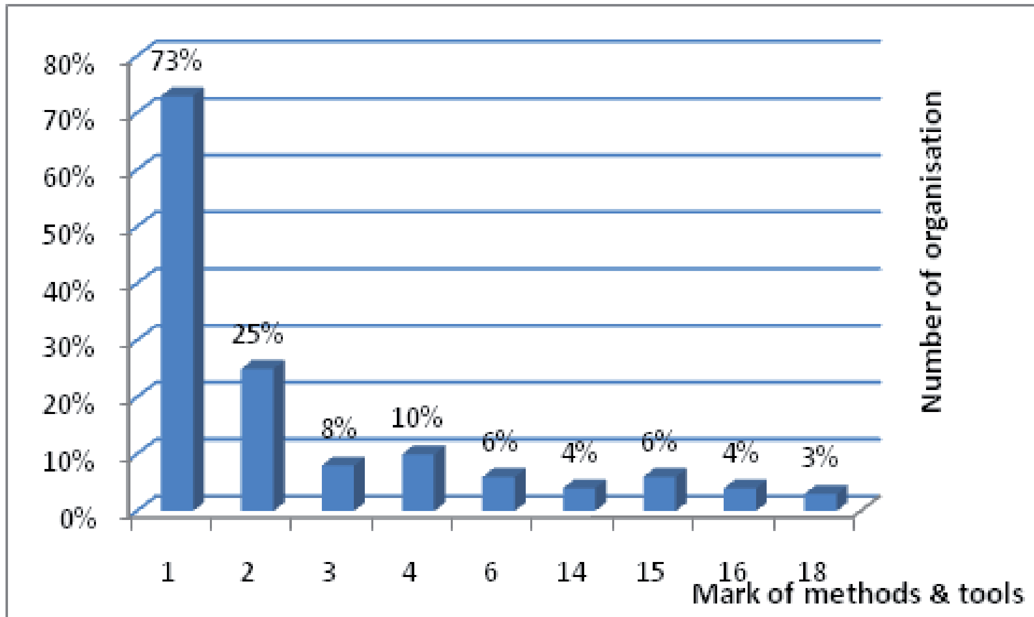

Figure 6. Distribution of quality methods' & tools' application

According to the results of the research, presented in figure 6, the dominant method or tool in food companies in Serbia position have Ichicawa's seven basic tools for quality (flow chart, check sheet, histograms, scatter plots, control chart, cause-effect diagram, Pareto analysis). It is also obvious that Serbian companies in general do not employ (at sufficient level) more advanced, new methods needed for higher quality process improvement.

Project management and FMEA are also popular in Serbian food processing companies. The second question is an analysis of profit compared with the quality tools and methods. According to research there is a high positive correlation between an increase of profit and application of quality tools and methods and it is presented in figure 7.

According to figure 7 the level of profit (per employee) increases with implementation of different quality methods and tools. The largest increase in profit can be seen in the application of: Cost of Quality (CoQ), Statistical process control, Business Process Reengineering. The increase in the profit with the application of seven new tools is larger than with the application of the basic seven tools for quality. It is interesting that the basic seven tools for

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quality are the most commonly used but their contribution to profit increase is the lowest compared with other frequently used quality methods and tools. Another result is that the seven new tools do not contribute more significantly to the increase of profit due to the lower level of knowledge of employees connected with the new approaches.

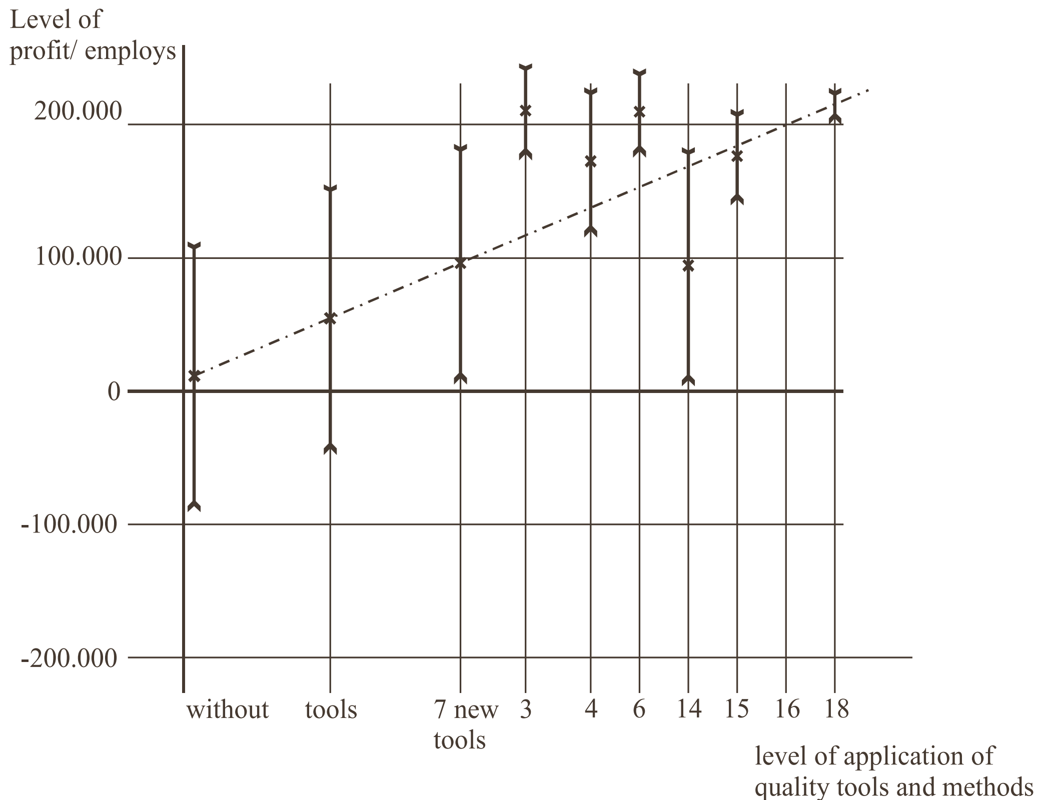

Figure 7. Impact of level of application of quality tools and methods on profit/employee

Finally, although rather "old" QFD proved itself as a very useful and efficient tool. Generally, with an increase of the level of application of quality tools and methods profit/employees increases in the analyzed organizations.

3.3. Fuzzy approach for evaluation of the importance of entities in supply chains in the food industry

The process of logistics and the food supply chain is very important for all companies from the food industry. In food supply chains, definition of weight / importance of different entities (processes, sub processes and goals) in the presence of uncertainties is one of the important goals for the management team. A similar problem has been met and emphasized in the definition of quality metrics and evaluation of scores and weights in section 2. The solution

to this problem must be placed on the whole of the supply chain because of its critical effect on efficiency.

It is realistic to assume, that decision makers express their evaluations more easy and more precisely by using linguistic expressions than numbers. The number and the type of linguistic expression used for a description of importance are defined by the management team and depend on the size of supply chain in food companies. Different mathematical approaches such as probability theory, fuzzy sets, rough set theory and others enable quantification of linguistic expressions. The development of fuzzy set theory enables the elimination of uncertainties and imprecision caused by lack of good evidence. In the fuzzy approach the uncertainties and imprecision caused are described by linguistic variables. They can be modeled by fuzzy sets with a different shape (triangle, trapezoid, but in some cases with Gaussian distribution, discrete fuzzy numbers) of membership functions.

The fuzzy approach has been used in cases: (1) where conditions have been constantly changing so the observed value could not be stochastically described (2) where there is no sufficient amount of data for statistical analysis. In other words, fuzzy sets theory could simulate human way of thinking in the process of decision making under imprecise, approximative and unclear data.

According to [14] the advantages of fuzzy sets theory can be presented in the following: it is conceptually clear, flexible, covers different non-linear functions of different complexity; tolerant on imprecise data; includes expert opinions and viewpoints; based on natural language; enables better communication between experts and managers.

Estimation of the relative importance of the processes and sub processes on the level of the supply chain p ∈ P is defined by a pair-wise comparison matrix whose elements are defined as the relative importance of process/sub-process i/j compared to process/sub-process $i^{'} / j^{'}, \; i, \; i, \; '=1, .., I; \; j, \; j^{'}=1, .., J_i$ where I and, J_i are the total number of analyzed processes and sub processes i, i (1,..,I, respectively). A number of authors consider this approach better compared to direct estimation. Elements of the pair-wise comparison matrix are linguistic expressions which are modeled by triangular fuzzy numbers [14, 15]. The domains of these fuzzy numbers are defined on interval [1-5]. Value 1, or value 5 define that analyzed entity i/j compared to entity $i^{'} / j^{'}, \; i, \; i, \; '=1, .., I; \; j, \; j^{'}=1, .., J_i$ has the same or extremely higher importance retrospectively. These triangular fuzzy numbers are defined as: Very low importance - $\tilde{R}_1=(x; 1, 1, 2)$ Low importance - $\tilde{R}_2=(x; 1, 1, 3)$, Medium importance – $\tilde{R}_3=(x; 1, 3, 5)$, High importance -$\tilde{R}_4=(x; 3, 5, 5)$ and Very high importance-$\tilde{R}_5=(x; 4, 5, 5)$.

The weights vector can be calculated by applying fuzzy extent analysis [16]. The weights vector of processes is denoted as $W=(w_1, .., w_i, ..., w_I)$and sub processes $W_i=(w_{i1}, .., w_{ij}, ..., w_{iJ^i})$. The relative importance of processes w_i and sub-processes $w$are ordinal numbers. In literature, there are many papers in which the weights vector is given by applying extent analysis [17].

The importance of the goals which are defined on the level of each sub process are defined by direct estimation made by a management team. According to literature, it can be concluded that this approach of estimation of importance of an entity is justified when the number of entities is less than five.

The importance of each goal k, k=1,,.,K_{ij} on the level of sub process j, j=1,.., J_i could be described using five linguistic expressions which are modeled by triangular fuzzy numbers $\tilde{v}_{ijk} = (y; l_{ijk}, m_{ijk}, u_{ijk})$. The total number of goals on the level of each sub process is denoted as K_{ij}. The value of domains is defined on a standard measurement scale [18]. These triangular fuzzy numbers are defined in the following way: very low value-$(y; 1, 1, 3)$, low value-$(y; 1, 3, 5)$, medium value-$(y; 3, 5, 7)$, high value-$(y; 5, 7, 9)$ and very high value-$(y; 8, 9, 9)$. Since the goals could be beneficial or costly it is necessary to perform normalization of fuzzy values $\tilde{v}_{ijk}, \; i=1, .., I; \; j=1, .., J_i; k=1, .., K_{ij}$. In this case the procedure of linear normalization is used [16]. Normalized values of goals weights are marked as $\tilde{r}_{ijk}, \; i=1, .., I; \; j=1, .., J^i, k=1, .., K_{ij}$. In further analysis only one goal with a critical effect on the management of sub-process j, j=1,.., J_i is considered.

For the management team carrying out the analysis, the following tasks are important: (1) to determine the rank of the process in a company (2) to determine the rank of a sub process on the process level in a company, (3) to determine the rank of sub processes with respect to the importance of goals and the importance of the considered sub process (4) to determine the rank of processes with respect to the importance of the goals, the relative importance of sub-processes of process i, i=1,..I_m and the relative importance of process i, i=1,.,I, and (5) calculate the degree of belief that the sub process, or process which is on second place in the rank could be on first place; answers to these questions are given by comparing triangular fuzzy numbers $\tilde{c}_{ij}, \; \tilde{d}_i, \; i=1, .., I; \; j=1, .., J_i$, respectively.

The algorithm for analysis of the relative importance of processes, sub-processes and goals in a company p ∈ P is formally given as follows.

Step 1. Input fuzzy matrix $\tilde{W} = [\tilde{w}_{ii'}], i, i' = 1, .., I; i \neq i'; p = 1, ..., P$

Step 2. Calculate weight vector$W = (w_1, .., w_i, ..., w_I)$; rank the processes by placing on first place the process with highest w_i.

Step 3. Calculate weight vector$W_i = (w_{i1}, .., w_{ij}, ..., w_{iJ^i})$; rank sub processes on the level of each process i, i=1,..,I by placing on the first place the sub process with the highest value w_{ij}.

Step 4. Transform all linguistic expressions which are modeled by triangular fuzzy numbers $\tilde{v}_{ijk}$ into $\tilde{r}_{ijk} = (z; L_{ijk}, M_{ijk}, U_{ijk})$by applying linear normalization procedure [16].

Step 5. Calculate the weighted normalized aggregated relative importance of sub-process j:

$$\tilde{c}_{ij} = \frac{1}{K_{ij}} \cdot \sum_{k=1}^{K_{ij}} w_{ij} \cdot \tilde{r}_{ijk}\ i=1,\ ..,\ I;\ j=1,\ ..,\ J^{i},\ k=1,\ ..,\ K_{ij},$$

Step 6. Calculate the weighted normalized aggregated relative importance of process i:

$$\tilde{c}_{i} = \frac{1}{J_{i}} \cdot \sum_{j=1}^{J_{i}} w_{i} \cdot \tilde{c}_{ij},\ i=1,\ ..,\ I;\ j=1,\ ..,\ J^{i}$$

Step 7. Rank sub-processes and processes according to decreasing order, $\tilde{c}_{ij}$ and $\tilde{d}_{i}$, respectively and define level of belief that sub-process j, j=1,...,J_i, or process i, i=1,..,I could have the highest importance with respect to the importance of all goals and the importance of sub process j, j=1,...,J_i, or process i, i=1,..,I [19].

The developed procedure is illustrated with an example with real-life data from the authors' research.

The pair-wise comparison matrix of relative importance of processes is:

$$\begin{bmatrix} 1,1,1 & 1/\tilde{R}_3 & \tilde{R}_2 \\ \tilde{R}_3 & 1,1,1 & \tilde{R}_3 \\ 1/\tilde{R}_2 & 1/\tilde{R}_3 & 1,1,1 \end{bmatrix} \tag{1}$$

The weight vector of processes is:

$W = (0.015 \quad 0.072 \quad 0.114 \quad 0.114 \quad 0.171 \quad 0.171 \quad 0.171 \quad 0.171)$

The most important processes in considered food company are: Project execution (i=5), Process execution (i=6), Quality assurance (i=7) and Support processes (i=8).

The pairwise comparison matrix of relative importance of subprocesses under process i=1 is:

$$\begin{bmatrix} 1,1,1 & 1/\tilde{R}_1 & 1/\tilde{R}_3 & 1/\tilde{R}_3 & 1/\tilde{R}_4 & 1/\tilde{R}_4 & 1/\tilde{R}_4 & 1/\tilde{R}_4 \\ \tilde{R}_1 & 1,1,1 & 1/\tilde{R}_2 & 1/\tilde{R}_2 & 1/\tilde{R}_3 & 1/\tilde{R}_3 & 1/\tilde{R}_3 & 1/\tilde{R}_3 \\ \tilde{R}_3 & \tilde{R}_2 & 1,1,1 & 1,1,1 & 1/\tilde{R}_3 & 1/\tilde{R}_3 & 1/\tilde{R}_3 & 1/\tilde{R}_3 \\ \tilde{R}_3 & \tilde{R}_2 & 1,1,1 & 1,1,1 & 1/\tilde{R}_3 & 1/\tilde{R}_3 & 1/\tilde{R}_3 & 1/\tilde{R}_3 \\ \tilde{R}_4 & \tilde{R}_3 & \tilde{R}_3 & \tilde{R}_3 & 1,1,1 & 1,1,1 & 1,1,1 & 1,1,1 \\ \tilde{R}_4 & \tilde{R}_3 & \tilde{R}_3 & \tilde{R}_3 & 1,1,1 & 1,1,1 & 1,1,1 & 1,1,1 \\ \tilde{R}_4 & \tilde{R}_3 & \tilde{R}_3 & \tilde{R}_3 & 1,1,1 & 1,1,1 & 1,1,1 & 1,1,1 \\ \tilde{R}_4 & \tilde{R}_3 & \tilde{R}_3 & \tilde{R}_3 & 1,1,1 & 1,1,1 & 1,1,1 & 1,1,1 \end{bmatrix} \tag{2}$$

The weight vector of sub-processes on level process i=1 is:

$$W_1 = (0.295 \quad 0.497 \quad 0.208)$$

Sub process under process is "Strategic choice" (j=2).

The pairwise comparison matrix of relative importance of subprocesses under process i=2 is:

$$\begin{bmatrix} 1,1,1 & 1/\tilde{R}_2 & 1/\tilde{R}_2 & 1/\tilde{R}_3 & 1/\tilde{R}_2 & 1,1,1 & 1,1,1 \\ \tilde{R}_2 & 1,1,1 & 1,1,1 & 1/\tilde{R}_2 & 1,1,1 & \tilde{R}_2 & \tilde{R}_3 \\ \tilde{R}_2 & 1,1,1 & 1,1,1 & \tilde{R}_2 & 1,1,1 & \tilde{R}_2 & \tilde{R}_3 \\ \tilde{R}_3 & \tilde{R}_2 & \tilde{R}_2 & 1,1,1 & \tilde{R}_2 & \tilde{R}_3 & \tilde{R}_4 \\ \tilde{R}_2 & 1,1,1 & 1,1,1 & 1/\tilde{R}_2 & 1,1,1 & \tilde{R}_2 & \tilde{R}_3 \\ 1,1,1 & 1/\tilde{R}_2 & 1/\tilde{R}_2 & 1/\tilde{R}_3 & 1/\tilde{R}_2 & 1,1,1 & \tilde{R}_2 \\ 1/\tilde{R}_1 & 1/\tilde{R}_3 & 1/\tilde{R}_3 & 1/\tilde{R}_4 & 1/\tilde{R}_3 & 1/\tilde{R}_2 & 1,1,1 \end{bmatrix} \tag{3}$$

The weight vector of sub-processes on level process i=2 is:

$$W_2 = (0.095 \quad 0.165 \quad 0.178 \quad 0.229 \quad 0.165 \quad 0.107 \quad 0.061)$$

Sub process under process is "Level of leadership transformation" (j=4).

The pairwise comparison matrix of relative importance of subprocesses under process i=3 is:

$$\begin{bmatrix} 1,1,1 & \tilde{R}_2 & \tilde{R}_3 & \tilde{R}_3 \\ 1/\tilde{R}_2 & 1,1,1 & \tilde{R}_2 & \tilde{R}_2 \\ 1/\tilde{R}_3 & 1/\tilde{R}_2 & 1,1,1 & 1,1,1 \\ 1/\tilde{R}_3 & 1/\tilde{R}_2 & 1,1,1 & 1,1,1 \end{bmatrix} \tag{4}$$

The weight vector of sub-processes on level process i=3 is:

$$W_3 = (0.39 \quad 0.275 \quad 0.168 \quad 0.168)$$

Subprocess under process is "Roles and responsibilities" (j=1).

The pairwise comparison matrix of relative importance of subprocesses under process i=4 is:

$$\begin{bmatrix} 1,1,1 & 1/\tilde{R}_2 & 1,1,1 & 1/\tilde{R}_2 & 1,1,1 & 1/\tilde{R}_2 & 1/\tilde{R}_3 \\ \tilde{R}_2 & 1,1,1 & \tilde{R}_2 & 1,1,1 & \tilde{R}_2 & 1,1,1 & 1/\tilde{R}_2 \\ 1,1,1 & 1/\tilde{R}_2 & 1,1,1 & 1/\tilde{R}_2 & 1,1,1 & 1/\tilde{R}_2 & 1/\tilde{R}_3 \\ \tilde{R}_2 & 1/\tilde{R}_2 & \tilde{R}_2 & 1,1,1 & 1/\tilde{R}_2 & 1,1,1 & 1/\tilde{R}_2 \\ 1,1,1 & 1/\tilde{R}_2 & 1,1,1 & \tilde{R}_2 & 1,1,1 & 1/\tilde{R}_2 & 1/\tilde{R}_3 \\ \tilde{R}_2 & 1,1,1 & \tilde{R}_2 & 1,1,1 & \tilde{R}_2 & 1,1,1 & 1/\tilde{R}_2 \\ \tilde{R}_3 & \tilde{R}_2 & \tilde{R}_3 & \tilde{R}_2 & \tilde{R}_3 & \tilde{R}_2 & 1,1,1 \end{bmatrix} \quad (5)$$

The weight vector of sub-processes on level process i=4 is:

$W_4 = (0.1 \quad 0.155 \quad 0.1 \quad 0.144 \quad 0.123 \quad 0.155 \quad 0.222)$

Sub-process under process i=4 is "Process significance". (j=7).

Processes i=5 and i=6 could be decomposed on four sub processes each. The relative importance of sub processes under process i=5 and i=6 are equal. Such that $w_{5j} = w_{6j} = 0.25, \quad j = 1, 2, 3, 4.$

The pairwise comparison matrix of relative importance of subprocesses under process i=7 is:

$$\begin{bmatrix} 1,1,1 & 1/\tilde{R}_3 & 1/\tilde{R}_4 & 1/\tilde{R}_4 & 1/\tilde{R}_3 \\ \tilde{R}_3 & 1,1,1 & 1/\tilde{R}_3 & 1/\tilde{R}_3 & 1,1,1 \\ \tilde{R}_4 & \tilde{R}_3 & 1,1,1 & 1,1,1 & \tilde{R}_2 \\ \tilde{R}_4 & \tilde{R}_3 & 1,1,1 & 1,1,1 & \tilde{R}_2 \\ \tilde{R}_3 & 1,1,1 & 1/\tilde{R}_2 & 1/\tilde{R}_2 & 1,1,1 \end{bmatrix} \quad (6)$$

The weight vector of sub-processes on level process i=7 is:

$W_7 = (0.027 \quad 0.186 \quad 0.29 \quad 0.29 \quad 0.205)$

Sub process under process i=7 is "Level of accomplishment of quality goals".(j=3).

Process i=8 could be decomposed on eight sub processes. According to the fuzzy rating of the management team, all sub processes have equal of the relative importance, so that $w_{8j} = 0.143, \quad j = 1, ..., 8.$

Estimation of the importance of goals with critical effect management of the sub processes are given in Table 6. By applying the Algorithm (Step 5 to Step 7) rank of sub processes with respect to the relative importance of the defined goals and the relative importance of the sub

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processes and the rank of the processes with respect to the relative importance of the sub processes and the relative importance of processes is determined. The calculated ranks are presented in Table 7 and Table 8.

According to the calculated values of importance of the sub processes with respect to their relative importance and the relative importance of the goals of each sub process (see Table 2) the following analysis can be made: The sub process which has the highest importance: (1) Strategic alignment (i=1) is Strategic choice (j=2), (2) Process development (i=4) is Process significance (j=7) and (3) Quality assurance of goals (j=3) and documentation of the process (j=4). Based on the calculated degree of belief it is clear that the other sub processes, i=1, i=4 and i=7 have very low importance compared to the first ranked sub process.

ijk, i=1,..,I; j=1,...,J_i k=1,.., K_{ij}	$\tilde{v}_{ijk}$	$\tilde{r}_{ijk}$	ijk, i=1,..,I; j=1,...,J_i k=1,.., K_{ij}	$\tilde{v}_{ijk}$	$\tilde{r}_{ijk}$
111	*low value*	(0.11,0.11,0.33)	511	*high value*	(0.56,0.78,1)
122	*medium value*	(0.33,0.56,0.78)	522	*high value*	(0.56,0.78,1)
133	*very low value*	(0.11,0.11,0.33)	533	*high value*	(0.56,0.78,1)
222	*medium value*	(0.33,0.56,0.78)	544	*high value*	(0.56,0.78,1)
233	*high value*	(0.56,0.78,1)	611	*high value*	(0.56,0.78,1)
244	*medium value*	(0.33,0.56,0.78)	622	*very high value*	(0.89,1,1)
255	*medium value*	(0.33,0.56,0.78)	633	*very high value*	(0.89,1,1)
266	*high value*	(0.56,0.78,1)	644	*very high value*	(0.89,1,1)
277	*medium value*	(0.33,0.56,0.78)	711	*high value*	(0.56,0.78,1)
311	*medium value*	(0.33,0.56,0.78)	722	*very high value*	(0.89,1,1)
322	*medium value*	(0.33,0.56,0.78)	733	*very high value*	(0.89,1,1)
333	*high value*	(0.56,0.78,1)	744	*very high value*	(0.89,1,1)
344	*very high value*	(0.89,1,1)	755	*very high value*	(0.11,0.11,0.12)
411	*high value*	(0.56,0.78,1)	811	*high value*	(0.56,0.78,1)
422	*high value*	(0.56,0.78,1)	822	*high value*	(0.56,0.78,1)
433	*very high value*	(0.89,1,1)	833	*high value*	(0.56,0.78,1)
444	*high value*	(0.56,0.78,1)	844	*high value*	(0.56,0.78,1)
455	*high value*	(0.56,0.78,1)	855	*high value*	(0.56,0.78,1)
466	*high value*	(0.56,0.78,1)	866	*medium value*	(0.33,0.56,0.78)
477	*very high value*	(0.89,1,1)	877	*very high value*	(0.89,1,1)

Table 6. Importance of goals on the level of each sub process with the critical effect on management of those sub processes

ij, i=1,..,I; j=1,.J_i	$\tilde{c}_{ij}$	Rank	Degree of belief	ij, i=1,..,I; j=1,..J_i	$\tilde{c}_{ij}$	Rank	Degree of belief
11	(0.032,0.097,0.165)	**2**	0.005	51	(0.14,0.195,0.25)	**1**	
12	(0.164,0.278,0.388)	**1**		52	(0.14,0.195,0.25)	**1**	
13	(0.023,0.023,0.068)	**3**		53	(0.14,0.195,0.25)	**1**	
21	(0.031,0.053,0.074)	**5**		54	(0.14,0.195,0.25)	**1**	
22	(0.092,0.129,0.165)	**1**		61	(0.14,0.195,0.25)	**2**	0.34
23	(0.059,0.1,0.139)	**3**		62	(0.222,0.25,0.25)	**1**	
24	(0.076,0.128,0.179)	**2**	0.99	63	(0.222,0.25,0.25)	**1**	
25	(0.092,0.129,0.165)	**1**		64	(0.222,0.25,0.25)	**1**	
26	(0.035,0.05,0.083)	**4**		71	(0.015,0.021,0.027)	**4**	
27	(0.02,0.034,0.048)	**6**		72	(0.165,0.185,0.185)	**2**	0.00
31	(0.129,0.218,0.304)	**1**		73	(0.258,0.29,0.29)	**1**	
32	(0.091,0.154,0.214)	**3**		74	(0.258,0.29,0.29)	**1**	
33	(0.094,0.131,0.168)	**4**		75	(0.023,0.023,0.026)	**3**	
34	(0.15,0.168,0.168)	**2**	0.44	81	(0.08,0.112,0.143)	**2**	0.34
41	(0.056,0.078,0.1)	**6**		82	(0.08,0.112,0.143)	**2**	0.34
42	(0.087,0.121,0.155)	**2**		83	(0.08,0.112,0.143)	**2**	0.34
43	(0.089,0.1,0.1)	**4**		84	(0.08,0.112,0.143)	**2**	0.34
44	(0.081,0.112,0.144)	**3**		85	(0.08,0.112,0.143)	**2**	0.34
45	(0.069,0.096,0.123)	**5**		86	(0.055,0.08,0.112)	**3**	
46	(0.087,0.121,0.155)	**2**	0.00	87	(0.127,0.143,0.143)	**1**	
47	(0.196,0.22,0.23)	**1**					

Table 7. Rank of sub-process with respect to the importance and goals of the sub process

Under the following processes, the sub processes which have the most importance are: (1) Process governance (i=3)- Rules and responsibilities (j=1), (2) Process execution (i=6)- Planned and achieved goals (j=2), Resource utilization (j=3), and Human resources (j=4), and (3) Support processes (i=8)- ICT support (j=7). Based on the degree of beliefs, the management team can conclude that the sub processes which are placed on second place in the rank under treated processes have less importance compared to the sub processes which are placed on first place. However, in making operational decisions, the importance of the sub processes which are on second place should be considered. The most important sub-process under Process leadership (i=2) is Level of leadership transformation (j=5). Since the degree of belief for the sub process Level of trust and communication in an organization (j=4) is 0.99, it is clear that these two sub-processes have equal relative importance.

Process execution is the most important process in the analyzed food processing companies according to analysis of the importance of all sub processes, the importance of the defined goals of the sub process and the importance of the process. The degree of belief that the process which is denoted as Project execution has the highest importance of 0.8. According to the calculated result, the mentioned process has high importance for the specific food company, so the management team must have this in mind in making strategic decisions.

i i=1,..,I	$\tilde{d}_i$	Rank	Degree of belief that process has the highest importance
1	(0.001,0.0.002,0.003)	4	
2	(0.004,0.006,0.009)	7	
3	(0.013,0.019,0.024)	5	
4	(0.011,0.014,0.016)	6	
5	(0.024,0.033,0.043)	2	0.8
6	(0.1015,0.04,0.043)	1	
7	(0.025,0.028,0.028)	3	
8	(0.014,0.019,0.024)	5	

Table 8. Rank of process with respect to its importance and importance of goals of the sub-process of each process

The presented model is used for the development of a very usable software solution that enables calculation of the importance of each goal, process and sub process [20, 21].

3.4. Strategy map

A strategy map describes how an organization can create sustained value for its shareholders, customers and communities.

The strategy map is developed based on the Kaplan and Norton model. A Strategy map describes how the organization creates value by connecting strategy objectives in an explicit cause and effect relationship in the four BSC objectives (financial, customer, processes, learning and growth). Strategy map is a strategic part of the BSC (Balanced Score Card) framework to describe strategies for value creation.

- Financial perspective is recognized in competitiveness.
- Customer perspective is identified: (1) quality, (2) safety, and (3) image.
- Internal perspective's eight processes: (P1) strategy alignment, (P2) process leadership, (P3) process governance, (P4) process development, (P5) project execution, (P6) process execution, (P7) quality assurance process, and (P8) supporting processes.
- Perspective or growth and learning : (1) technology and (2) people capability.

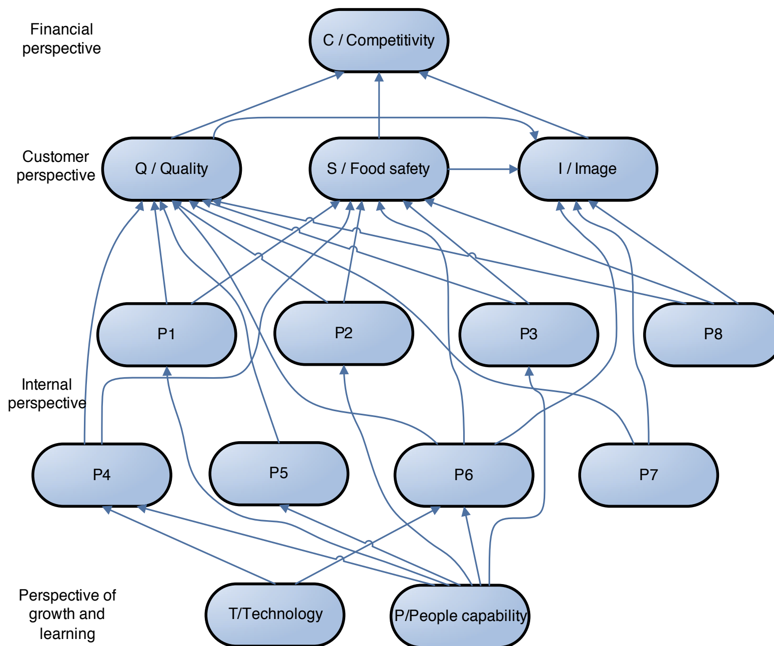

Figure 8. Strategy map

A strategy map for the food industry is presented in figure 8. The level of each component process is identified using a questioner for companies in the Serbian food industry. Relations among processes are defined using a method with 3 iterations.

A strategic map should describe a strategy and present the strategy to management and employees, and in the same way connect stakeholders, customer management, process management, quality management, core capabilities, innovations, human resources, ICT, organizational design / redesign and learning.

4. Research results: Case study of the serbian food industry

4.1. Proposed model: Process framework for companies in the food industry

In this section we will provide the process framework for companies from the food industry sector. All processes are divided into the following categories: leadership processes, core processes and support processes. As it was shown in the section above "Project execution" process (P5) has the highest importance.

The support processes contain the following: CRM (Customer Relation Management), Supply management, Human resources management, ICT support, Maintenance, Marketing, Sales, Finances, Quality management and Other management systems.

Process Pi	Estimation of process importance	Estimation of importance of sub process in the frame of specific process							Estimation of importance of sub process goals						
		PP1	PP2	PP3	PP4	PP5	PP6	PP7	PP1	PP2	PP3	PP4	PP5	PP6	PP7
P1	5	7	8	6					5	6	4				
P2	6	6	7	7	8	7	6	5	5	6	7	6	6	7	6
P3	7	8	7	6	6				6	6	7	8			
P4	7	6	7	6	7	6	7	8	7	7	8	7	7	7	8
P5	8	7	7	7	7				7	7	7	7			
P6	8	7	6	7	6				7	8	8	8			
P7	8	7	8	9	9	8			7	8	9	9	8		
P8	8	5	6	6	6	5	5	6	7	7	7	7	7	6	8

Table 9. Cross reference of processes, their importance and importance of their sub processes and goals

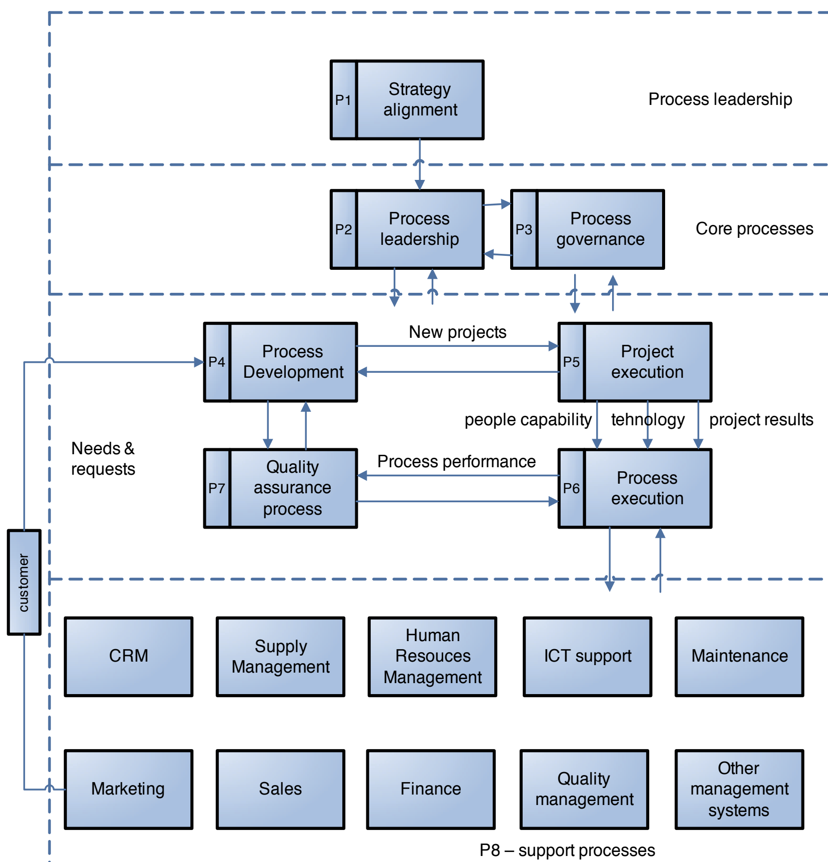

Figure 9. Process framework for companies in the food industry

Each process presented in figure 9 has its own importance as a whole. It is clear that each process could be decomposed on the accompanied sub processes.

Questionnaire							
Questionnaire			**M**	**Questionnaire**			M
1	Questionnaire for strategy alignment			6	Questionnaire for process execution		
	1	Estimation of strategic alignment of processes;			1	Level of achieving of process goals	
	2	Estimation of strategic choices;			2	Gap between planned and achieved goals	
	3	Estimation of process architecture.			3	Level of resource utilization (for process needs):	
2	Questionnaire for Process Leadership				4	Human;	
	1	Level of transformational leadership;			5	Equipment;	
	2	Level of transactional leadership;			6	ICT;	
	3	Level of trust in leadership;			7	Knowledge.	
	4	Level of trust and communication in organization;			6.1	Questionnaire for process performance	
	5	Level of business process awareness;				Evaluation of increase of process awareness;	
	6	Level of process innovation;				Evaluation of definition and establishment of rewards;	
	7	Level of promotion of manager success.				Evaluation of understanding or responsibilities for the process;	
3	Questionnaire for Process governance					Evaluation of process metrics;	
	1	Estimation of roles selection and responsibilities;				Evaluation of performance monitor;	
	2	Estimation of roles selection;				Evaluation of management of the process;	
	3	Estimation of evaluation and control of management including estimation of the risk of process;				Evaluation of continuous improvement;	
	4	Evaluation of implementation of contemporary methods and tools in business processes.				Evaluation of communications.	
4	Questionnaire for Process development			7	Questionnaire for Quality assurance of processes		
	1	Evaluation of concept of desired process;			1	Level of effectiveness of processes – On time Delivery of products	

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Questionnaire					
Questionnaire		**M**	**Questionnaire**		M
	2	Evaluation of relevance of process goals and indicators;		2	Level of quality of inputs in process
	3	Evaluation of achievement of the process goals;		3	Level of quality of working procedures
	4	Evaluation of documentation of processes;		4	Level of achieving the quality goals
	5	Evaluation of processes investigation;		5	Level of process coast/planned process costs
	6	Evaluation of analysis of processes;	8	Evaluation of support processes	
	7	Evaluation of process significance;		1	Evaluation of marketing process;
	8	Evaluation of process flexibility;		2	Evaluation of sale;.
	9	Evaluation of Process agility;		3	Evaluation of customer relations management (CRM);
	10	Level of inclusion and complexity of process demands .		4	Evaluation of supply chain management (SCM);
5	Questionnaire for Project execution			5	Evaluation of finances;
	1	Evaluation of ,,right" projects;		6	Evaluation of human resources management;
	2	Evaluation of establishment of project organization;		7	Evaluation of ICT support;
	3	Evaluation of portfolio management and control;		8	Evaluation of implementation of quality management;
	4	Evaluation of project management frameworks.		9	Evaluation of maintenance;
		Evaluation – Technology		10	Evaluation of other management systems
		Evaluation of technology level of production	9	Evaluation of entities in customers perspective	
		Evaluation of level of quality approach (WCM, Lean, 6 sigma, IMS)		1	Evaluation of product quality
		Evaluation of implementation of system and process approach (reengineering, time, electivity)		2	Evaluation of quality of organization
		Evaluation of business decision making, business intelligence		3	Evaluation of product safety

Questionnaire			
Questionnaire	**M**	**Questionnaire**	M
Evaluation of human resources	4	Evaluation of the brand, image of organization	
Evaluation of internal capability.	10	Evaluation of entities in financial perspective	
Evaluation of Center of Business Innovation (CBI)	1	Level of competitiveness compared to EU market	
Evaluation of CBI engagement model.			

Table 10. Questionnaire for Serbian companies

Total number of 53 companies were analyzed and results are presented in table 10.

Each sub process has importance in the frame of the specific process as well as the importance of its goal. The overall data gathered as the result of research in Serbian companies in the food industry is presented in table 9. The presented data could be combined with linguistically expressed opinion and used for ranking and simulation of quality goals according to the approach presented using fuzzy sets. Data were gathered using the questionnaire presented as table 10.

No.	Company	Stand.	1	2	3	4	5	5.1	5.2	6	6.1	7	8	9	10
	Company 01	22000	8	6	7	7	5	8	8	1	7	9	8	6	9
	Company 02	22000	5	8	5	4	7	6	4	1	6	7	7	6	5
	Company 03	22000	7	6	7	5	8	6	4	1	7	5	9	7	2
	Company 04	22000	7	6	7	4	5	6	5	1	6	6	7	5	6
	Company 05	22000	6	8	5	5	7	8	7	1	7	6	9	8	6
	Company 06	CAC	4	7	2	2	5	5	1	1	5	2	6	4	3
	Company 07	CAC	5	7	4	4	6	8	4	1	6	5	8	8	3
	Company 08	CAC	7	8	6	4	6	8	5	1	8	7	9	7	5
	Company 09	CAC	3	6	2	2	6	4	1	1	3	2	4	4	2
	Company 10	CAC	5	6	5	3	5	4	4	1	4	4	6	4	5
	Company 11	CAC	6	7	6	5	5	6	6	1	5	7	7	5	8
	Company 12	CAC	4	7	4	3	5	6	2	1	5	5	5	5	4
	Company 13	CAC	4	5	4	2	5	6	5	1	5	4	6	6	4
	Company 14	CAC	4	5	3	3	7	5	4	1	6	6	6	6	2
	Company 15	CAC	5	6	5	3	6	8	1	1	5	5	6	7	4
	Company 16	CAC	4	4	3	3	4	4	3	1	5	4	4	3	4

No.	Company	Stand.	1	2	3	4	5	5.1	5.2	6	6.1	7	8	9	10
	Company 17	CAC	4	3	2	2	4	3	1	1	4	1	3	3	3
	Company 18		5	4	5	4	5	8	6	0	7	6	7	8	5
	Company 19		5	3	5	3	5	8	6	1	7	6	7	8	2
	Company 20		5	3	5	3	5	8	6	1	8	5	8	8	4
	Company 21		4	2	4	3	4	9	7	0	6	5	6	7	7
	Company 22		6	5	5	4	4	8	6	0	5	7	6	5	9
	Company 23		4	3	4	2	6	7	5	0	5	4	5	5	3
	Company 24		4	6	4	2	6	4	4	0	4	2	6	5	2
	Company 25		4	4	4	2	6	4	4	0	5	2	7	7	2
	Company 26		5	5	5	4	4	6	5	0	5	4	6	6	4
	Company 27		5	5	5	4	6	7	5	0	6	6	5	5	6
	Company 28		4	5	4	2	5	7	4	0	4	5	5	5	4
	Company 29		4	2	4	3	5	5	4	0	5	4	6	7	5
	Company 30		6	2	7	7	5	3	7	0	5	8	7	5	3
	Company 31		4	5	4	3	4	5	5	0	4	4	5	5	3
	Company 32		4	5	4	3	4	4	4	0	5	4	4	4	3
	Company 33		5	5	4	3	4	5	4	0	4	5	6	6	4
	Company 34		5	5	4	3	5	4	5	0	5	4	6	6	3
	Company 35		4	5	4	3	4	4	4	0	5	4	5	5	3
	Company 36		5	4	5	3	5	6	5	0	6	6	7	5	3
	Company 37		4	4	3	2	4	4	5	0	6	5	6	5	3
	Company 38		4	4	4	2	4	5	4	0	4	4	7	8	2
	Company 39		5	5	5	2	5	3	4	0	4	4	5	6	4
	Company 40		4	4	3	2	5	3	4	0	3	5	4	5	3
	Company 41		4	4	2	1	6	5	4	0	5	4	5	5	3
	Company 42		3	5	4	2	4	4	4	0	4	3	6	5	3
	Company 43		4	4	4	2	4	5	4	0	6	4	6	5	4
	Company 44		5	4	6	6	5	6	6	0	6	6	6	5	4
	Company 45		2	6	1	1	2	2	1	0	2	1	4	6	2
	Company 46		5	5	5	2	4	5	6	0	4	2	4	6	4
	Company 47		3	5	3	2	3	6	2	0	3	2	5	6	3
	Company 48		4	5	2	2	3	5	4	0	4	1	4	7	2
	Company 49		8	4	6	7	4	4	3	0	3	6	7	6	8

No.	Company	Stand.	1	2	3	4	5	5.1	5.2	6	6.1	7	8	9	10
	Company 50		6	5	6	6	5	6	7	1	6	5	7	8	5
	Company 51		5	7	7	7	7	7	7	1	7	6	7	7	4
	Company 52		4	6	7	7	6	6	5	1	7	6	8	7	4
	Company 53		6	4	6	6	5	7	3	0	5	5	8	8	7

Legend: CAC – Codex Alimentarius – Food Hygiene, Recommended International Code of Practice General Principles of Food Hygiene CAC/RCP – 1969, Rev. 4-2003.

Table 11. Gathered data from Serbian companies

In table 11, the numbers in the columns correspond to the Questionnaire presented in table 10 (The names of companies are left and replaced by “company x” due to protection of companies data.). Structure of the sample is: 25 organizations with less than 10 employees, 21 organizations with 10-50 employees, 3 organizations with 50-250 employees, 3 organizations with 250-500 employees and 1 organization with more than 500 employees.

Based on the expert opinion of consultants working with organizations in the sample the relation among processes and other entities are determined. The form of relation is: $R_{I,O}$ where I - goal (performance) of entity and O – goal (performance source entity) of destination entity

RT, P4: $GP4= GPot + 0.11*\Delta T$
RT, P6: $GP6= GPop + 0.15*\Delta P$
RP1, P: $GP1= GP01 + 0.25*\Delta P$
RP2, P: $GP2= GP02 + 0.28*\Delta P$
RP3, P: $GP3= GP03 + 0.24*\Delta P$
RP4, P: $GP4= GP04 + 0.27*\Delta P$
RP5, P: $GP5= GP05 + 0.18*\Delta P$
RP6, P: $GP6= GP06 + 0.16*\Delta P$
RP1, Q: $GQ= GQ01 + 0.30*\Delta P1$
RP2, Q: $GQ= GQ02 + 0.32*\Delta P2$
RP3, Q: $GQ= GQ03 + 0.29*\Delta P3$
RP4, Q: $GQ= GQ04 + 0.35*\Delta P4$
RP5, Q: $GQ= GQ05 + 0.18*\Delta P5$
RP6, Q: $GQ= GQ06 + 0.17*\Delta P6$
RP7, Q: $GQ= GQ07 + 0.24*\Delta P7$
RP8, Q: $GQ= GQ08 + 0.17*\Delta P8$

RP1, S: $GS= GS01 + 0.03*\Delta P1$
RP2, S: $GS= GS02 + 0.04*\Delta P2$
RP3, S: $GS= GS03 + 0.08*\Delta P3$
RP4, S: $GS= GS04 + 0.18*\Delta P4$
RP6, S: $GS= GS06 + 0.28*\Delta P6$
RP7, S: $GS= GS07 + 0.15*\Delta P7$
RP8, S: $GS= GS08 + 0.20*\Delta P8$

R S, I: $GI= GS0 + 0.38*\Delta I$
RP6, I: $GI= GI06 + 0.32*\Delta P6$
RP7, I: $GI= GI07 + 0.30*\Delta P7$
RP8, I: $GI= GI08 + 0.35*\Delta P8$

RQ, C: $GC= GQ0 + 0.39*\Delta Q$
RS, C : $GC= GS0 + 0.28*\Delta S$
RI, C : $GC= GI0 + 0.15*\Delta I$

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Starting values of constants are determined by investigation of the organization in the sample. Presented relations describe the importance of relations presented in the strategy map.

5. Conclusion

The food industry is a sector that is involved in rapid, multidimensional changes. At the same time this is an industrial sector that is emerging and increasing its importance in relation to different trends and challenges: growth of population, increased need for healthy food, food safety regulations, and different customers' demands.

It is completely clear that the food industry must respond in many different directions in order to avoid challenges, reduce threats and explore its strengths using available opportunities. Some of the changes in the food industry will go in the direction of automation of the production process and technological changes, employing the full potential of biotechnology, information and communication technology, RFID, robotics, sensors and even e-business. Other changes will be directed in the optimization of logistic infrastructure and energy savings. But companies in the food industry will also need to understand globalization trends, market developments and chanciness and swings in customers' needs. All these changes will increase the success of the food industry on the global markets. Although all of these changes could have only limited, partial results that will not fulfill the complete potential of the suggested changes if companies do not restructure their organizations and redesign their processes. Changing and adopting the changes of organization as well as restructure and redesign of processes are a pre-condition for all other changes. On the other hand, companies usually do not pay much attention to organizational challenges and process redesign, compared with other directions of changes such as the implementation of new and emerging technologies.

In this chapter we addressed different questions and issues in the redesign of process development in food production organizations using quality engineering tools and methods. The first step is analysis of existing processes, its decomposition and introduction of quality metrics for evaluation of the quality of processes and quality of goals. A typical process map for a company in the food processing industry in Serbia is presented and the production process is decomposed on sub processes. All indicators for quality metrics were proposed as numeric values in order to clearly demonstrate the concept.

After decomposition another step is redesign of process development. Different changing methodologies are compared according to the amount of change, score of change, used tools, probability of success and their applicability in food industry. The concept of continuous process improvement is presented and redesign of the process has 5 phases with 10 steps. Different quality engineering methods and tools used in the food industry were compared according to the frequency of their usage and correlation to increase of profit in Serbian companies. The general conclusion was that companies are using older, established methods and tools even if they do not have that large impact on profit increase compared to the modern tools and methods. A total number of 20 different quality engineering methods

and tools were analyzed among 53 Serbian companies from the food industry sector. With many different problems, starting from the ranking of importance of quality goals, ranking of importance of processes or entities, up to ranking the methods or tools, there is a need for an approach that will solve these issues. All these problems could be solved by usage of the fuzzy approach. As an extension of the general ranking idea presented on the ranking and definition of goals in the production process, the fuzzy approach for evaluation of the importance of entities in supply chains in the food industry is presented. A strategic map as a strategic part of the BSC (Balanced Score Card) framework is presented as the role model for food processing companies. The special contributions of this map are the relations between entities (from all four perspectives) that are defined as the result of the research in Serbian companies. In addition, the framework of processes for the companies from the food industry was presented. Finally, the general contribution of all the presented issues, decomposition, redesign, evaluation of quality tools and methods, fuzzy ranking and the strategic map, is based on results of research in 53 Serbian companies. The questionnaire for Serbian companies is presented as well as the results of research. A very important contribution presented is the fact that all decompositions, redesigns, modeling, simulations and calculations were performed using real life data acquired from Serbian companies.

A possible and very useful extension of this research would be a comparison of data with data from the EU but according to the literature the variation would not be significant.

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Chapter 21

Calculus Elements for Mechanical Presses in Oil Industry

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Valentin Vladut

Additional information is available at the end of the chapter

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1. Introduction

Oil products industry produces edible and inedible oils. About 2/3 of total oil products are the edible oils, which are used directly in foods or in the manufacture of margarine, mayonnaise, bakery and pastry products, cooking fats, preserves etc. The remaining 1/3 of the total volume of produced oil are the technical oils, used in the production of various products, such as: detergents, paint, glycerin, fatty acids, varnish, pharmaceuticals or cosmetics (Banu, 1999).

Vegetable oils are one of the oldest classes of known chemical compounds. There are multiple references and clues on the use of these oils during Stone Age and Bronze Age (Willems, 2007).

Raw material for vegetable oil industry are oilseeds, an important component of modern agriculture. Oilseeds provide easily highly nutritious human food and oil crops and their products represent one of the most important commerce commodity. Vegetable oils are a source of vitamins, calories and essential fatty acids for human diet, at a relatively low cost. After the processing of oilseeds remains the cake, or the solid part which is a valuable source of protein for animal feeds (Bargale, 1997).

Fats are found in plant and animal tissues, and in secretions of animal body glands (i.e. in milk). Fatty matter of plants is concentrated only in seeds, kernels, germs, fruits and tubers, and they reserve substances that the plant uses as energy source. Fat content in these parts of the plant is highly variable (below 5% in most plants).

There are a wide range of raw materials for oils industry. In the vegetable reign for example are more than 100 oleaginous plants, but only 40 of them can be are used for oil expression.

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The other plants are unprofitable, as they have low oil content of the seeds or as they require a difficult expression process. The most important oleaginous plants are: sunflower, soya, rape, cotton, poppy, almond, sesame, nut, palm, coconut, olive, flax, castor (Banu, 1999).

Separation of oil from oilseeds is an important processing operation. The process employed has a direct effect on the quality and quantity of protein and oil obtained from oilseeds (Bargale, 1997). The terms “expression” and “extraction” are used frequently when discussing about vegetable oil separation. Expression is the process of mechanically pressing liquid out of liquid-containing solids. Extraction is the process of separating a liquid from a liquid-solid system with the use of a solvent (Khan & Hanna, 1983). There has been some confusion in the literature between the operations of “expression” and “extraction”. The latter word has been used quite loosely to designate either operation (Gurnham & Mason, 1946). This tendency has been so extensive that the distinction between the two terms appears to be disappearing from the literature. The term ”extraction” is also used for mechanical oil expression (Biris et al., 2009/a).

Worldwide, for extraction of oil from seeds, fruits and nuts, four basic methods are used, as shown in the following figure.

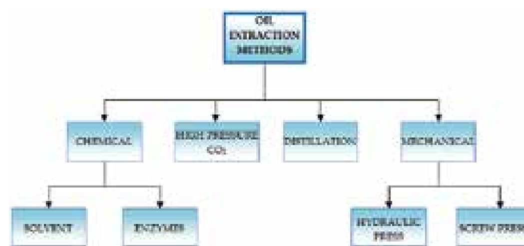

Figure 1. Oil extraction methods (Sari, 2006)

Chemical extraction method is based on the use of enzymes or solvent to extract the oil from the raw material. Solvent extraction method uses a solvent (which is, in generally, a hexane, meaning a petroleum distillate) mixed with ground seeds. Seeds are grounded to maximize the contact area of the seed with the solvent; thus the oil yield is higher. After the mixing process, the obtained mixture is heated up to 100°C to separate the oil from the solvent. The other chemical extraction, enzymatic extraction, is adopted by powerfull vegetable oil companies, as the process produces many high value products. For this extraction methods, seeds are cooked and put into water. Next, enzymes are added as they digest the solid material. The basic difference of this type of extraction method from the solvent type is that the residual enzymes in the oil are separated by the use of a liquid-liquid centrifuge (Sari, 2006).

The extraction using high pressure carbon dioxide (i.e. supercritical fluid extraction, SFE) embodies several features of conventional solvent extraction, but it has important features of its own (Bulley et al., 1984). This extraction method consists in seeds mixing with a liquid form of high pressure carbon dioxide. Oil is dissolved in the carbon dioxide and when the pressure is released, the carbon dioxide becomes a gas, leaving the oil behind.

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Steam distillation is the method used for the extraction of 93% from the essential oils, the rest of 7% being extracted with other method (Masango, 2005). Hot steam releases the aromatic molecules from the plant material, by forcing the open of the pockets in which the oils are kept in the plant material. The molecules of these volatile oils will exit from the plant material and evaporate into the steam. The steam mixed with the essential oil is furter passed through a cooling system to condense the steam, which forms a liquid from which the essential oil and water is then separated.

The mechanical process is another method for oil extraction. Mechanical expression of oil requires the application of pressure to force oil out of the oil bearing material (Ogunsina et al., 2008). Various types of machines can be used for compression: screw presses, hydraulic presses, roll presses and mills, collapsible-plate and frame-filter presses, disc mills, interlocking-finger juice extractors, juice reamers (Khan & Hanna, 1983). From this variety of machines, there are available for expression processing two of them, the hydraulic press and screw press mechanisms. Hydraulic press, based on the principle of the hydraulic ram, originates in England and it was first patented in 1795 by Joseph Bromah (Dunning, 1953). The first screw press was developed by V.D. Anderson in the United States, in 1900. Due to the advantages it presents (continuous operation, high working capacity, run without high shocks and vibrations, working pressures which can be easily adjusted, etc.) screw presses quickly replaced hydraulic presses (Biris et al., 2009/b). In the figure below is presented a model of screw press developed by De Smet Rosedowns company, from United Kingdom.

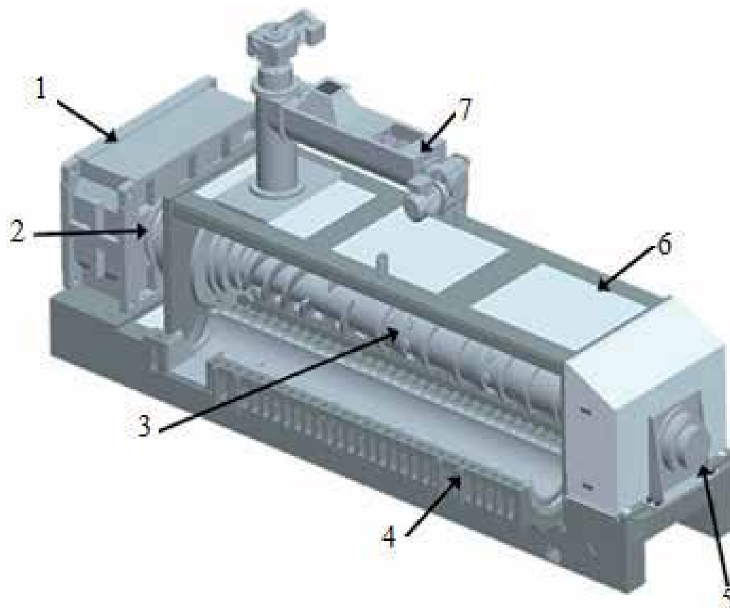

Figure 2. De Smet Rosedowns screw oil press 1 – gearbox; 2 – thrust housing; 3 – worm assembly; 4 – drainage cage; 5 – discharge end bearing; 6 – frame; 7 – feeder.

Mechanical pressing and solvent extraction are the most commonly used methods for commercial oil extraction. Oils obtained by mechanical pressing has high quality, but it can be recovered up to 90-95% of available oil. Solvent extraction has the advantage of the high yield that can be obtained (up to 99%), and as for disadvantages, oil quality is lower (Karaj & Müller, 2009). This quality reduction is produced by the extensive solvent recovery processes that are necessary and the fact that the solvent co-extracts undesired components from the cell walls (Willems et al., 2008).

Pressing technology of oleaginous material meal occurs under the influence of compression forces in mechanical presses. First, the oil from particles surface is separated – retained by surface forces of the molecular field, by the channels formed between particles. For a certain pressure, deformation and strong compression of particles begin, producing the elimination of oil from the capilars of particles. At a certain point, the space between particles gets so small that the oil film is subjected to the retaining forces exerted by particles surfaces, the oil can not be removed, the particle breaks in several places, the particle surfaces are in contact, and the briquetting begins, namely the forming of broken (cakes).

Increasing the pressure on meal particles must be done gradually, as for the harsh increase, fine meal particles will block the outler of oil from the capilaries, thus reducing the general pressing yield.

Pressing process cand be assimilated to the process of capillary filtration (fluid flow throuth capillary), expressed by the equation:

$$V = \frac{\pi \cdot p \cdot d \cdot t}{128 \cdot \eta \cdot l} \left[\mathrm{m}^3\right] \tag{1}$$

where: V – volume of separated liquid (passing through capillaries), [m3]; p – applied pressure, [N/m^2]; d – diameter of cappilary channel, [m]; η - dinamic viscosity of oil, [Pa s]; l – lenght of capillary channel which must be passed by the separated oil, [m]; t – time of applied pressure, [s].

From the above equation it results that the process of oil separation can be positively influenced if the values of p, d and t are increasing and if values of l and η will decrease.

Pressure p in mechanical presses is created by an helical conveyer (worm) which rotates in a closed space (pressing chamber). Gradual increase of pressure is done by the decrease of free volume of pressing chamber from one stage to another (by increasing the shaft diameter and decreasing the chamber diameter) and by reducing the pitch of worm. Pressing force is influenced by the resistance at the exit of material from the pressing chamber, as the amterial is forced to pass through a space with variable section.

Pressing time t must be high enough to allow the proper oil flow. A prolongation of the pressing time does not lead to significant increase of oil extraction efficiency, but leads to the sensitive decrease of press productivity.

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Pressing time can be determined as the sum of the pressing times in each section (compression stage) of the press:

$$t = \sum_{i=1}^{n} t_{si} \tag{2}$$

where: n is the number of pressing stages, and the pressing time in a certain section is given by:

$$t_s = \frac{V_{ls} \cdot c_s}{Q_v \cdot (1 - \beta_s)} \tag{3}$$

where: V_{ls} – volume of free space in the pressing section, [m^3]; c_s – pressing degree of meal in the respective section ; Q_v – volumic feed flow of the press with meal, [m^3/s]; β_s – correction coefficient related to the quantity of meal removed from the press with the oil, until the analyzed section.

Pressing time also depends on the design and functional characteristics of the press and it ranges between 40-200 seconds. Pressing time depends on the shaft speed, cake (brocken) thickness at the press exit, and physico-chemical characteristics of the meal. Pressing time is inversely proportional to shaft speed and to cake thichness. As cake thickness is greater, the pressure in pressing chamber decreases, and pressing time decreases due to the fact that the material passes easier through the pressing chamber. At high pressure presses, by reducing the thickness of sunflower brocken from 11 mm to 4 mm the pressing time increases from 93 s to 106 s.

Parameters η, l and d are influenced by the preparation oparations of meal thus:

- oil viscosity η decreases by meal heating in roasting operation;
- capillars lenght l can be lowered by advanced destruction of cellular structure by grinding and, partially, during roasting, as well as by the reduction of passing distance of oil to the outlet hole from the pressing chamber (material layer in the pressing chamber must have small thickness).

In the technological diagram of pressing followed by solvent extraction, the presses can be of various types:

- for moderate preliminary pressing, which ensure the separation of 75-80 % oil and 18-22 % remaining oil in brocken;
- for advanced preliminary pressing, which ensure 12-14 % remaining oil in brocken.

To obtain oil just by pressing, without solvent extraction, there are:

- presses with a single pressing stage, mechanical presses for final pressing, which realize separation with maximum 3-6 % remaining oil in brocken;
- presses with two pressing stages, the first is used for moderate pressing, and the second stage for final pressing.

Depending on the press type, pressure applied on the meal gets to 250-280 daN/cm^2 – for preliminary pressing, respectively 400-2000 daN/cm^2 – for final or single pressing.

In Romanian oil factories, processing oleaginous seeds with high oil content is done after the diagram of pressing-extraction, so by using preliminary pressing equipments (moderate or advanced).

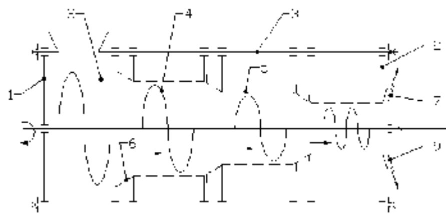

Figure 3. The operating principle of the continuous mechanical press 1 –front plate; 2 –back plate; 3 –clamping columns; 4 –screw; 5 –cylindrical strainer (barrel); 6 –tapered strainer (barrel); 7 –tapered exhaust end for pressure adjustment of pressing chamber; 8 –feed area of meal; 9 – evacuation area of cake from the pressing chamber.

Mechanical oil expression involves the release of oil from the seed interior into the interparticle voids on application of pressure. Filling of the interparticle voids leads to a buildup of pore pressure, thereby to the development of pressure gradient in the voids. As a result, oil flows through the porous medium and is finally expressed through the porous retaining envelope (Ajibola et al., 2002). The efficiency of expression is influenced by: the porosity of the cake, yield stress of the solid phase, the compressive force applied and viscosity of the expressed liquid (Clifford, 1973). The pressing process has been studied by several authors and they have found the following parameters influencing oil expression: applied pressure, moisture content, heating temperature and heating time, particle size (Adeeko & Ajibola, 1990; Khan & Hanna, 1983). Thus, increasing parameters such as heating temperature, heating time and applied pressure while reducing oilseed moisture to a certain degree will result in the increase of oil yield. A significant influence on oil yield has the postheating moisture content of some oilseeds (Ajibola et al., 1993). Effects of oilseeds heat treatment are: rupture of the oil bearing cells of the seed, coagulate the protein in the meal, adjust the moisture level of the meal to optimum level for oil expression. Lower the viscosity and increase the fluidity of the oil to be expelled and destroy mould and bacteria thereby facilitating oil expression from material (Adeeko & Ajibola, 1990). Optimum heating temperature for oilseeds is found in the range of 90-110°C for an average retention time of 20 minutes (FAO, 1989).

Oil expression is accompanied by compression and consolidation process brought about by the reduction in the volume of the compressed material (figure 4).

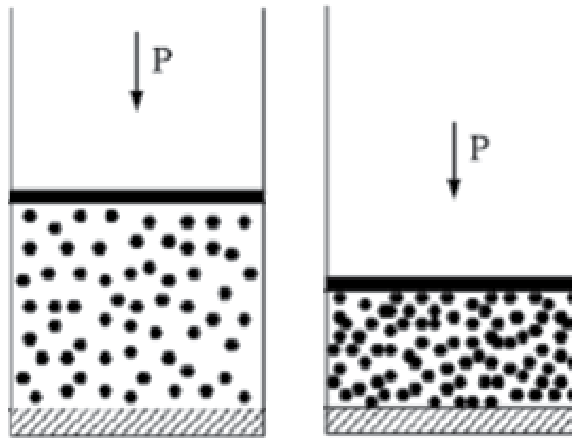

Figure 4. Volume reduction of the compressed material (Venter et al., 2007)

Thus, reduction of the total void space ocurrs, causing oil elimination (Sivalla et al., 1991). Value of the applied pressure at the point that oil leaves the interparticle voids is viewed as the oil point pressure, namely the minimum pressure that must be applied before oil expression begins. Applied pressures below this point are regarded as effort required to mobilize oil from the seed cells to the surface (Sukumaran & Singh, 1989; Mrema, 1979).

The general theoretical description of expression is based on consolidation theories originally developed for soil mechanics (Terzaghi, 1954). There are several studies on the modelling of oilseed expression, resulting in the development of empirical models, Terzaghi-type models and models mased on the nature of the cell structure of the oilseeds (Venter, 2007).

Processes and phenomena that occur during the pressing process of oleaginous materials are very complex.This paper contains a theoretical model on the power necessary to operate an oil press. The necessary components to power the press are: the power required for material transport along the pressing chamber, the power required to press the oleaginous material, the power required to overcome the friction between the screw spire and the material, the power required to push the material from the press through the exhaust cylinder head. The paper presents some diagrams showing the influence of various constructive and functional parameters on the pressing process.

Theoretical elements of a functional calculus, and of the power necessary to operate the press are rather poor and based especially on simple formulas containing some correction coefficients, whose value is empirically obtained from experiments. This is due to the complexity of the processes and phenomena taking place during pressing, such as: material transport, proper pressing, overcoming the frictions between auger and material, pushing the material through the slot at the end of the pressing chamber.

Mechanical work for material pressing it results from the expression of the equivalent stress, which occurs in the pressing chamber after applying on the cross surface of the pressing

chamber an equivalent pressing force, so that it may be produced a reduction of the volume occupied by the material, from the initial value to the final value.

Based on the mathematical model developed in this study, was found the variation of the component parts of the power necessary for operation in the case of a press from the oil industry.

The main data taken into consideration for modelling are: the variable auger rotational speed ($n=15\text{-}40\ min^{-1}$), the variable pressure inside the pressing chamber ($p=50\ 10^5\text{-}200\ 10^5$ Pa), the diameter of the pressing chamber (D=200 mm), the diameter of the auger shaft (d=100 mm).

The mathematical model which was created in this paper permits the high precision determination of the functional parameters and of the necessary power for operating the presses in food industry.

From the figures presented in this paper it can be observed that the necessary power for proper pressing is the highest, being followed by the necessary power for overcoming the frictions between the auger spire and the material subdued to pressing. The values for the power necessary to push the material through the exhaust space and for the material transport through the pressing chamber are much lower than those for the presses, being possible to even be neglected.

This paper can be useful to students undertaking batchellor studies, to professors and researchers in design and development of mechanical oil presses.

2. Theoretical elements

2.1. Functional calculus elements

Pressure ratio is the reduction of the material subjected to pressing and it can be calculated using equation [4], where V_i is the initial volume of the material, [m^3] and V_f is the final volume, [m^3].

$$\varepsilon = \frac{V_i - V_f}{V_i} \tag{4}$$

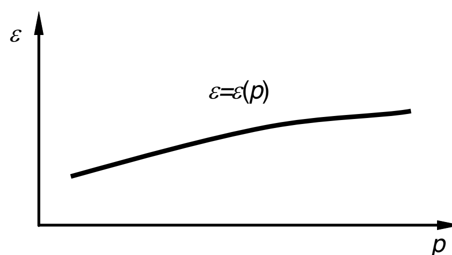

Figure 5. Variation of the pressure ratio with the pressure

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The value of pressure ratio is directly proportional to the working pressure of the press, and its variation is shown in figure 5.

Press volume flow rate can be evaluated by using the relation [5]:

$$Q_v = V_{te} \cdot (1-\varepsilon) \cdot n \cdot k \cdot 60 \quad \left[\mathrm{m}^3\right] \tag{5}$$

where: V_{te} – is the theoretical volume of the material displaced by the auger spire during a complete rotation, in the exhaust area [cu.m.]; n – auger rotative speed, [rpm]; k – coefficient taking into account the material flowing back through the spire extremities, as well as the incomplete feed with material, (k=0,2 ▪ 0,35).

The theoretical volume of the material displaced by the auger spire is calculated using the following equation:

$$V_{te} = \frac{\pi}{4} \cdot (D^2 - d^2) \cdot (s - \delta) \quad \left[\mathrm{m}^3\right] \tag{6}$$

where: s – the auger spire pitch [m]; δ – thickness of the auger spire, [m]; D – outer diameter of the auger spire, [m]; d – inner diameter of the auger spire (of the auger shaft), [m].

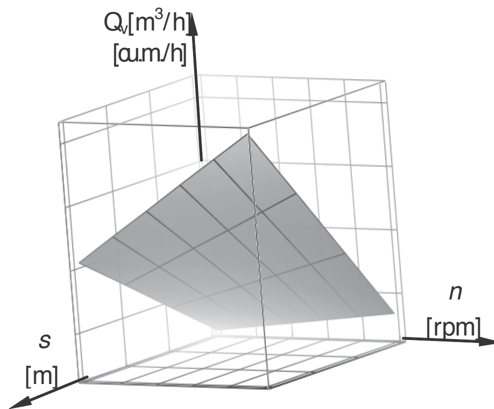

Figure 6. Variation of the press flow rate depending on the auger rotative speed and the spire pitch

By replacing in equation (5) the expression of the theoretical volume given by equation (6), it results the expression of the press volume flow rate under the form (see Figure 6):

$$Q_v = \frac{\pi}{4} \cdot (D^2 - d^2) \cdot (s - \delta) \cdot (1-\varepsilon) \cdot n \cdot k \cdot 60 \quad \left[\mathrm{m}^3/\mathrm{h}\right] \tag{7}$$

2.2. Calculus of the power necessary to operate the press

- *The power necessary to operate the press* can be evaluated by using the equation:

$$P_p = \frac{P_{tr} + P_{pres} + P_{fr} + P_{cap}}{\eta_{tm}} \quad [\text{kW}] \tag{8}$$

where: P_{tr} – represents the necessary power to transport the material from feeding chamber to exhaust head, [kW]; P_{pres} – necessary power for pressing the material, [kW]; P_{fr} – necessary power for overcoming the frictions between the auger spire and the material, [kW]; P_{cap} – necessary power for pushing the material through the exhaust space in the press, [kW]; η_{tm} – mechanical transmission yield (output).

- Necessary power for material transport

Taking into account the calculus equations of the slow helical conveyors, it can be written the expression of the necessary power for proper transport of the material along the auger:

$$P_{tr} = \frac{F_r \cdot v}{1000} \quad [\text{kW}] \tag{9}$$

where: F_r – represents the resistant force to the material advancing along the press auger, [N]; v – mean speed by which the material moves along the press auger, [m/s].

The resistant force F_r is given, on one part, by the phenomenon of outer friction between the material and the walls of the pressing chamber, and, on the other part, by the phenomenon of outer friction of the material subdued to pressing. The value of this force can be calculated by the expression:

$$F_r = q \cdot l \cdot g \quad [\text{N}] \tag{10}$$

where: g – represents the gravity acceleration, [m/sq.s]; q – the linear load (mass per linear meter of material) in the press, [kg/m]; l – length of pressing chamber, [m].

The expression of the linear load, q, can be written:

$$q = S \cdot \psi \cdot \gamma = \frac{\pi \cdot (D^2 - d^2)}{4} \cdot \psi \cdot \gamma \quad [\text{kg/m}] \tag{11}$$

where: ψ – represents the coefficient of admission for the press section; S – area of the cross section of the pressing chamber, [sq.m.].

By replacing into the relation (10) it results:

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$$F_r = \frac{\pi \cdot (D^2 - d^2)}{4} \cdot \psi \cdot \gamma \cdot l \cdot g \quad [\mathrm{N}] \tag{12}$$

It results the necessary power for transporting the material along the pressing chamber:

$$P_{tr} = \frac{F_r \cdot v}{1000} = \frac{\pi \cdot (D^2 - d^2) \cdot \Psi \cdot \gamma \cdot l \cdot g \cdot v}{4 \cdot 1000} = \frac{\pi \cdot (D^2 - d^2) \cdot \Psi \cdot \gamma \cdot l \cdot g \cdot s \cdot n}{4 \cdot 1000 \cdot 60} \quad [\mathrm{kW}] \tag{13}$$

- Necessary power for pressing the material

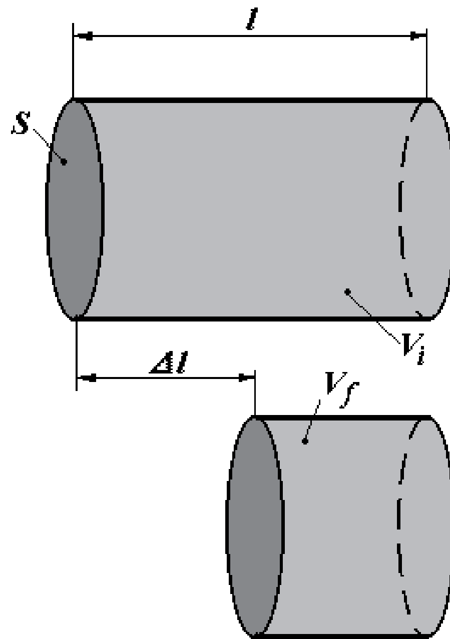

Figure 7. Variation of the material volume in the pressing process

The mechanical work for material pressing (L_{pres}) results from the expression of the equivalent tension (stress) σ, which appears inside the pressing chamber as a result of applying on the cross surface (S) of the pressing chamber an equivalent pressing force (F_{pres}), so that it may be produced a reduction of the volume occupied by the material, from the initial value, V_i to the final value, V_f, as it is also shown in figure 7. Hence, the equivalent tension (stress) in the pressing chamber can be written as:

$$\sigma = \frac{F_{pres}}{S} = \frac{F_{pres} \cdot \Delta l}{S \cdot \Delta l} = \frac{L_{pres}}{\Delta V} = \frac{L_{pres}}{V_i - V_f} \quad [\mathrm{Pa}] \tag{14}$$

Taking into account equation (4), it results:

$$V_f = V_i \cdot (1 - \varepsilon) \tag{15}$$

and from the equations (14) and (15) is obtained the expression of the mechanical work for the material pressing:

$$L_{pres} = \sigma \cdot (V_i - V_f) = \sigma \cdot [V_i - V_i \cdot (1 - \varepsilon)] = \sigma \cdot \varepsilon \cdot V_i \quad [\mathrm{J}] \tag{16}$$

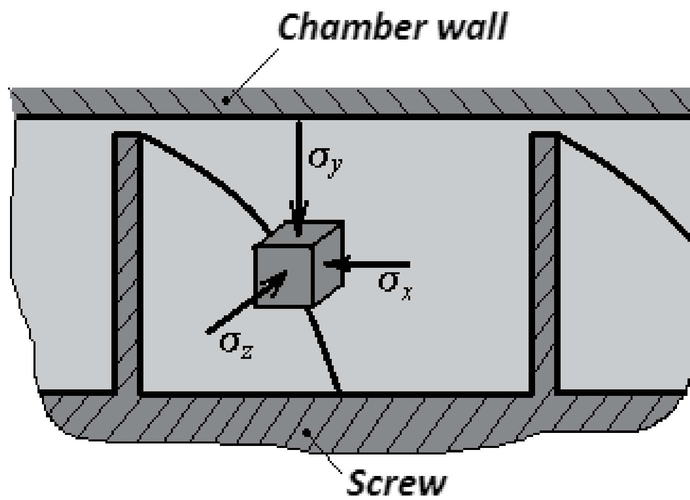

Figure 8. Elementary volume of material subdued to the pressing process

To determine the value of equivalent tension (stress) σ, it is considered an elementary volume of material subjected to pressing, uniformly loaded on each section, as shown in figure 8, which, during the pressing process will move only on the longitudinal direction of the press (direction x). Under these conditions it can by written:

$$\begin{cases} \sigma_y = \sigma_z \\ \sigma_x = p \end{cases} \tag{17}$$

where: p [Pa] - represents the pressure performed by the auger, which is exerted on the material.

It is considered that the tensions (stresses) on the direction y and z occur due to the pressure oriented to the direction of material displacement, respectively:

$$\sigma_y = \sigma_z = \beta \cdot \sigma_x \tag{18}$$

where: β - represents the coefficient of the side pressure.

Taking into account equation (18), it results:

$$\sigma_x + \sigma_y + \sigma_z = p \cdot (1 + 2 \cdot \beta) \tag{19}$$

As the materials subjected to the pressing process in the food industry also contains a certain percentage of liquid substance (oil, must, etc.), it can be considered that the hydrostatic pressure law remains valid, respectively:

$$\sigma = \frac{\sigma_x + \sigma_y + \sigma_z}{3} = \frac{p \cdot (1 + 2 \cdot \beta)}{3} \tag{20}$$

Thus, it results the expression of the mechanical work necessary for pressing the material:

$$L_{pres} = \frac{1 + 2 \cdot \beta}{3} \cdot p \cdot \varepsilon \cdot V_i \quad [\mathrm{J}] \tag{21}$$

respectively, the expression of the necessary power for pressing the material:

$$P_{pres} = \frac{F_{pres} \cdot v_{pres}}{1000} = \frac{F_{pres} \cdot \frac{\Delta l}{\Delta t}}{1000} = \frac{F_{pres} \cdot \Delta l}{1000 \cdot \Delta t} = \frac{L_{pres}}{1000 \cdot \Delta t} \quad [\mathrm{kW}] \tag{22}$$

where: F_{pres} – represents the pressing force [N]; v_{pres} – the pressing speed, [m/s]; Δt – the time interval when the reducing of the material volume is performed from the initial value V_i to the final value V_f, [s]. The value of this time interval can be calculated depending on the rotative speed [rpm] of the press auger, respectively:

$$\Delta t = \frac{60}{n} \tag{23}$$

Taking into account equations (21), (22) and (23), the expression of the necessary power for pressing the material is obtained:

$$P_{pres} = \frac{L_{pres} \cdot n}{1000 \cdot 60} = \frac{(1 + 2 \cdot \beta) \cdot p \cdot \varepsilon \cdot V_i \cdot n}{3 \cdot 1000 \cdot 60} \quad [\mathrm{kW}] \tag{24}$$

In reality, for presses in food industry, the value of the pressure, p, is not kept constant along the auger, having a variation which can be as that seen in figure 9.

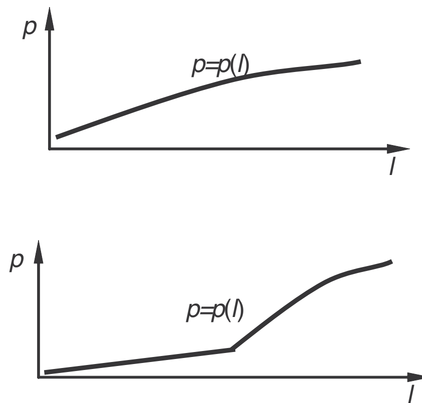

Figure 9. Pressure variation along the pressing chamber

- Power necessary to overcome the frictions between the auger spire and material

To calculate the necessary power for overcoming the frictions between the auger spire and the material, first it must be calculated the friction torque (moment), which occurs on the spire surface when it comes into contact with the material. For the calculus of this friction torque (moment) it is first taken into consideration an elementary ring, dr, situated on the auger spire on the radius r (Figure 10) and for the auger length suitable to a pitch, s it is determined *the normal force* exerted on the elementary ring:

$$dN = p \cdot dS = p \cdot 2 \cdot \pi \cdot r \cdot dr \tag{25}$$

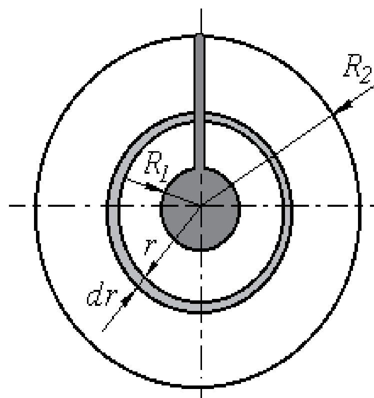

Figure 10. Elementary ring on the auger spire

The value of *the friction force* which occurs on the surface of the elementary ring is calculated by the following equation:

$$dF_f = \mu \cdot dN = \mu \cdot p \cdot 2 \cdot \pi \cdot r \cdot dr \tag{26}$$

The expression of the friction torque (moment) at the surface of the elementary ring is:

$$dM_f = r \cdot dF_f = \mu \cdot p \cdot 2 \cdot \pi \cdot r^2 \cdot dr \tag{27}$$

which, by integration, for the whole active cross surface of the auger spire, suitable to a length equal to a pitch, *s*, leads to the equation:

$$M_f = \int_{R_1}^{R_2} \mu \cdot p \cdot 2 \cdot \pi \cdot r^2 \cdot dr = 2 \cdot \mu \cdot p \cdot \pi \cdot \int_{R_1}^{R_2} r^2 \cdot dr = 2 \cdot \pi \cdot \mu \cdot p \cdot \frac{r^3}{3}\Big|_{R_1}^{R_2} \tag{28}$$

respectively:

$$M_f = 2 \cdot \pi \cdot \mu \cdot p \cdot \frac{R_2^3 - R_1^3}{3} \quad [\mathrm{Nm}] \tag{29}$$

It results the expression of the necessary power for overcoming the friction between the auger spire and the material:

$$P_{fr} = \frac{M_f \cdot n}{9550} = \frac{2 \cdot \pi \cdot \mu \cdot p \cdot \left(R_2^3 - R_1^3\right) \cdot n}{3 \cdot 9550} \quad [\mathrm{kW}] \tag{30}$$

- Power necessary for pushing the material through the exhaust space

For pushing the material through the exhaust space from the end of the pressing chamber the power consumed is given by:

$$P_{cap} = \frac{F_c \cdot v_{cap}}{1000} = \frac{F_c \cdot \frac{\Delta l_c}{\Delta t}}{1000} = \frac{F_c \cdot \Delta l_c}{1000 \cdot \Delta t} = \frac{L_c}{1000 \cdot \Delta t} = \frac{L_c \cdot n}{1000 \cdot 60} \quad [\mathrm{kW}] \tag{31}$$

where: F_c – resistant force to material pushing through, the head of the pressing chamber, [N]; v_{cap} – the material speed through the head of the pressing chamber, [m/s]; l_c – length of exhaust canal, [m].

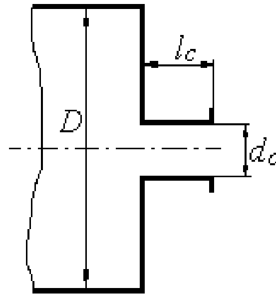

Figure 11. End of the pressing chamber

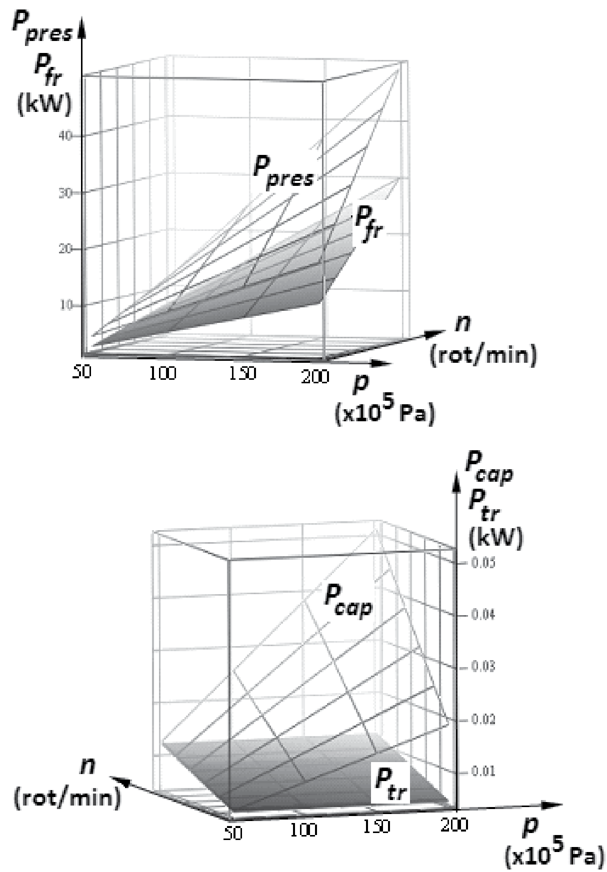

Figure 12. Power variation depending on pressure and auger rotational speed

The necessary mechanical work for pushing the material through the exhaust space (Fig. 11) for the end of the pressing chamber L_c is calculated using the following equation:

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$$L_c = F_c \cdot l_c = p \cdot A_c \cdot l_c = p \cdot \frac{\pi \cdot d_c^2}{4} \cdot l_c \quad [\mathrm{J}] \tag{32}$$

It results the expression for the calculus of power P_{cap}:

$$P_{cap} = \frac{p \cdot \pi \cdot d_c^2 \cdot l_c \cdot n}{4 \cdot 1000 \cdot 60} \quad [\mathrm{kW}] \tag{33}$$

3. Application

Using the mathematical model developed in this study, figure 12 shows the variation of the component parts of the power necessary for operation of a press from the oil industry.

The main data taken into consideration for modelling are: the variable auger rotational speed (n=1540 min^{-1}), the variable pressure inside the pressing chamber (p=50 10^{5}200 10^5 Pa), the diameter of the pressing chamber (D=200 mm), the diameter of the auger shaft (d=100 mm).

4. Conclusions

The mathematical model which was created in this paper allows the high precision determination of the functional parameters and of the necessary power for operating the presses in food industry.

In figure 12 it can by observed that the necessary power for the proper pressing P_{pres} is the highest, being followed by the necessary power to overcome the frictions between the auger spire and the material subdued to pressing P_{fr}. The values for the power required to push the material through the exhaust space P_{cap} and for the material transport through the pressing chamber P_{tr} are much lower than those for the presses P_{pres} and P_{fr}, so it is possible to neglect them.

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Chapter 22

Gastrointestinal Immunoregulation and the Challenges of Nanotechnology in Foods

MaryAnn Principato

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/53287

1. Introduction

1.1. Nanoparticles: Physicochemical characteristics and applications in foods

Nanoparticles are elemental three dimensional structures that are typically between 1-100 nanometers (nm) in size that exhibit unique physiochemical characteristics that provide the basis for their utilization, and present unique challenges associated with the development of new applications [1, 2]. Because of their size, nanoparticles provide the opportunity to interact with human physiology at the subcellular level, affording many potential uses in nutrient and drug delivery, vaccination therapies, and tissue repair. Specific physiologic applications can be achieved by chemical modification of the nanoparticle to achieve increased blood circulation parameters thus increasing their residence time in the tissues, or by the specific targeting of tissues using ligands. The uses of nanotechnology in foods are as complex and varied as the types of formulations that can be created with this technology. Current technological applications that impact foods include the manufacture of food packaging, including packaging that incorporates antimicrobial agents such as silver, [2] or detection particles (gold), flavor enhancement, and delivery of dietary supplements and nutraceuticals [2-5]. While their potential or actual application present strong advantages, it is imperative that there be a thorough understanding regarding the physiology of nanoparticle absorption, or the consequences of their containment or integration within the mammalian physiological and cellular environment.

1.2. Food packaging

Historically, food packaging has typically consisted of conventional materials such as paper or metal-based materials. The use of polymeric formulations improved the ability to retain

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moisture and provided a gas barrier, thus extending food shelf life. Typically, the Food and Drug Administration requires that the manufacturer of food contact material comply with the regulatory requirements for each individual substance that comprises the entire formulation of the food contact material [6]. These food contact materials have typically included paper, metallic-based items, and polymeric compounds such as polyethylene terephthalate (PET), polypropylene, polyethylene, polystyrene, and others. Recently the formulation of nanocomposites has improved the ability to produce food contact surfaces that are superior with respect to their heating and gas barrier resistance characteristics [2, 7, 8]. Typically, a combination of previously approved compounds and nano-material has been used for the construction of the newer nanocomposite materials that strive to enhance the storage and preservation of foods. Nanocomposites are described as a combination of inorganic nanomaterial and a continuous phase consisting of synthetic polymers [9]. Nanoclay composites consist of magnesium aluminum silicate nanoparticles (bentonite or montmorillonite), and have proven to be a superior gas barrier for the preservation of foods [7]. The production of sustainable, biodegradable polylactide (PLA)-based polymers present the potential to reduce the dependence upon petrochemical based polymers by using alternative renewable sources to produce packaging materials with qualities comparable to presently used products. The combination of PLA with montmorillonite (MMT) nanocomposite [10-12] has been reported to produce a short term packaging material with good O_2 gas permeability, and can be converted into CO_2 and H_2O through decomposition by microorganisms [10].

Silver, which has long been recognized for its antimicrobial characteristics [13], has been among the inorganic constituents incorporated into nanocomposite materials. Prior to the advent of nanotechnology, silver had long been used as an ingredient within dental composite material [14], integrated into wound dressings [15-17] and other medical devices approved by the FDA, and is recognized as a biocide by the EPA [18]. Analysis of the antimicrobial effects of silver ion on gram positive and gram negative cell walled microorganisms demonstrated similar effects [19, 20]. Exposure of microbial organisms to silver results in the retraction of the cytoplasm from the cell wall, condensation of the DNA into electron-dense granules, and there is an accumulation of silver ions into the cytoplasm. The damage, as inferred in these studies, is due to the inability to replicate at the DNA level [19]. Additional denaturant effects attributed to the silver ion include its ability to attach to sulfhydryl groups, amino groups, and the terminal phosphate and carboxyl groups of bacterial proteins [13], essentially inactivating the enzymes involved with electron transport and metabolism. Of the electron transfer functions, cytochrome reductase and cytochrome oxidase are targeted [21]. While interest in silver's use as an antimicrobial has increased due to the observed rise in hospital and community-acquired antibiotic resistances, it is important to note that a growing microbial resistance to silver has also been reported [22]. Antimicrobial properties are similarly attributed to silver (Ag) nanoparticles [20, 23-27], and this property has spurred the inclusion of this material into a wide array of products within the foods sector including packaging and service containers, and bottles, or used as a measure to prevent or control surface contamination by *Escherichia coli* and *Staphylococcus aureus* [28]. Indeed, the incorporation of silver into MMT composite preparations was shown to inhibit the

growth of *Escherichia coli* 0157:H7, *Staphylococcus aureus*, and *Klebsiella pneumonia* on agar at levels that were 35% of the levels achieved by cefotaxime and chloramphenicol [29].

1.3. Dietary supplements and nutraceutical delivery

The encapsulation of dietary vitamins and other nutritional supplements as a nanoparticle has gained considerable interest as a means to increase the shelf life of such materials, and to improve delivery and release within the body. The engineered particles provide potential strategies with which to overcome the impermeability of the mucosal epithelium, and offer a possible means of circumventing the degradation of the nutrient by harsh degradative gastrointestinal conditions. Several candidate materials, used successfully for the delivery of drugs and vaccines, have been examined for their ability to encapsulate nutrients. Finally, compounds such as polysaccharides and proteins that are already in use within commercial food applications are attractive candidates for the production of new nanocomposite packaging and encapsulation material, as several are generally regarded as safe and are biodegradable.

Poly (D,L)-lactic co glycolic acid (PLGA) nanoparticles are widely used for the encapsulation and delivery of drugs due to their reported biocompatibility and lack of overt toxicity. The physicochemical properties of the PLGA particles are affected by specific formulation and processing parameters, such as drug and polymer concentration, solvent volume, polymer molecular weight, the type of emulsifier used in the processing and its concentration, and the aqueous-to-organic phase ratio [30, 31]. Thus, PLGA nanoparticles have been shown to adequately encapsulate hydrophobic and hydrophilic molecules albeit the latter present some challenges with respect to a lowered load efficiency, and many PLGA-encapsulated delivery systems have been designed for a wide variety of macromolecules including drugs, biologically active cytokines, and peptides [31-33].

Chitosan, an N-deacetylated derivative of chitin, has been analyzed for use in nutrient delivery due to its wide acceptance in drug delivery, and is generally regarded as non-toxic and biocompatible. Chitosan ((1 ▪ 4)-2-amino-2-deoxy-β-D-glucan) is a naturally occurring cationic polysaccharide found in the shells of shrimp, lobsters, and crab that has an intrinsic ability to bind mucin. The bioadhesive property of chitosan permits organ-specific delivery, and surface modification of the polysaccharide particle has been successfully used to alter organ delivery [34]. Chitosan has been demonstrated to induce increased permeability in Caco-2 monolayers across tight junctions as measured by changes in the measured transepithelial electrical resistance and in a ^{14}C-mannose absorption assay [35, 36]. The improved absorption across cell layers due to the opening of tight junctions is thought to be the result of ionic interactions between the cell membrane and chitosan polysaccharide. While these characteristics favor the polysaccharide's use as a delivery method for a variety of compounds, it is necessary to incorporate anionic alginate to prevent burst release of the encapsulated material due to protonation in an acidic environment. The results obtained by encapsulation of Vitamin A within dual layered chitosan-alginate nanospheres have been reported to be successful [37, 38]. In this instance, a high encapsulation efficiency and improved storage stability was achieved using double-layered microcapsules that incorporated

chitosan, alginate, calcium chloride and Tween 20. The production of combined Chitosan/ PLGA spherical particles have also been reported for the encapsulation of Vitamin A [38].With this construct, the microspheres (averaged 283 nm) demonstrated stability within an acidic environment and a lowered release rate into the gastric environment when compared to particles composed solely of PLGA. Thus, release of the target nutrient would be mainly in the small intestine where the vitamin would be absorbed. Interestingly, the material was visualized in the intestinal villi, and in the endothelium of rabbit GI.

Whey protein, derived from dairy, is recognized for its natural ability to form films and gels [7]. Whey nanospheres containing alginate have demonstrated the controlled release of an encapsulated nutrient, riboflavin, when tested in simulated gastric juices [39]. In this instance, 94 nm whey nanoparticles were constructed using an emulsification and cold gelation method, which averts the use of toxic solvents, and modification of the alginate concentration provides some control over degradation of the particle by pepsin in their assay. The encapsulation of viable probiotic yeast cells has been reported using whey –alginate microspheres produced by a cold gelation extrusion technique [40]. The encapsulation of a hydrophobic, fat-soluble nutrient can be achieved using casein maltodextrin nanoparticles produced by the Maillard reaction. In this reaction, the ε-amine groups found on the protein's lysine residues are covalently bonded to the aldehyde of reducing sugars. Particles produced in this manner consist of an exterior composed by the bulky hydrophilic domains of casein. The result of this design is a particle with increased curvature, i.e., a smaller diameter, containing an outermost saccharide layer and a hydrophobic inner core. Once the optimal casein: maltodextrin ratios were determined for the formation of the conjugates, incorporation of oil-soluble vitamin D resulted in particles that were 30 nm in diameter and demonstrated significant protection of the vitamin at low pH values that simulated gastric juices [4].

Liposomes composed of polar lipids such as lecithin have been used as delivery systems for antimicrobials, colors, and antioxidants. However, best results have been reported incorporating an additional layer of material such as the cationic polysaccharide, chitosan. Liposomes composed of soy lecithin and prepared by homogenization, and combined with chitosan with stirring and sonication, were used to encapsulate grape seed extract [3]. In this instance, the particle size increased with the addition of the grape seed extract due to surface incorporation of the grape seed extract into the liposomes' layer. This was rectified by production of particles containing multiple polymer layers composed of chitosan and citrus pectin; grape seed polyphenols were no longer exposed to the matrix. Finally, microspheres with a mineral composition have also been developed for the encapsulation of nutrients. In this case, the encapsulation of water soluble polyphenols extracted from green tea has been accomplished using calcium carbonate salt solutions containing phosphate and carbonate[41].

2. The Gut-Associated Lymphoid Tissue

The proposed and anticipated uses of orally-delivered nanoparticles, the use of nanoparticles on food-contact surfaces, and the introduction of microencapsulated nutrients, necessi-

tate an understanding of the events within the mucosal immune compartment known as the Gut-Associated Lymphoid Tissue (GALT), which is critically involved in the formation and maintenance of oral tolerance to introduced nutrient-derived antigens, and the generation of mucosal immune responsiveness to ingested pathogens and their toxins. The gastrointestinal tract is responsible for the digestion and absorption of ingested nutrients. This function is aided by the intestine's mucosal lining, whose absorptive surface is greatly increased by villi which project into the lumen and are composed of a single layer of epithelial cells and a rich network of capillaries and lymphatics. While the gastrointestinal tract is responsible for the absorption of nutrients, it is also the site of ongoing immune surveillance. The intestinal lumen normally contains dietary degraded products, commensal microbial flora, and any ingested contaminants including pathogenic bacteria and their products, viruses, fungi, or parasites. The resident gastrointestinal immune system must: 1) generate immunologic tolerance towards nutrients and the resident microflora, and 2) recognize and remove infectious agents and their toxins [42-44]. Oral tolerance is driven by prior administration of antigen by the oral route, generating suppressive regulatory T cells, but is also dependent upon the maintenance of an effective epithelial barrier. The role of the resident gastrointestinal $CD4^+$ T cell population for the establishment and maintenance of the tolerant state is critical [45, 46]. Investigators have reported the formation of exosome-like structures, designated as "tolerosomes, " which are assembled in and released from small intestinal epithelial cells, that seem to play a crucial role for the induction of tolerance [47]. Breakdown of oral tolerance is thought to lead to the development of food allergy and some autoimmune diseases, including inflammatory bowel diseases (Crohn's disease and ulcerative colitis) and celiac disease.

The GALT of the gastrointestinal tract consists of Peyer's patches (PP) containing B cells, dendritics, and T cells (Figure 1), appendix, draining mesenteric lymph nodes, and lymphatic follicles distributed throughout the length of the intestinal tract. The first line of immunologic defense is the provided by antibodies of the secretory IgA type found in the mucosal secretions of the gut [48]. This is supported by the observation that individuals with IgA deficiencies demonstrate circulating immune complexes to bovine and milk proteins [49]. In this case, the lack of IgA permits the entrance of food-derived antigens into the peripheral circulation, resulting in immune complex formation. The production of IgA is now known to be induced by regulatory T cells that have been activated by CD11+ dendritic cells [50]. Additionally, lymphocytes are scattered within the columnar epithelial layer (Intraepithelial lymphocytes, or IEL) and throughout the lamina propria.

2.1. T cells of the Gut-associated lymphoid tissue

Beneath the epithelial layer of the mammalian gastrointestinal tract lies a rich source of immunocompetent cells within the submucosal lymphoid follicles known as the intraepithelial lymphocytes (IEL) that comprise a significant portion of the body's T cells. The peripheral immune system contains effector T lineage cells bearing the $\alpha\beta$ T cell receptor (TCR) which are either class II-restricted $CD4^+$ T cells or class I-restricted $CD8^+$T cells. Intraepithelial cells are distinguished by the predominant presence of homodimeric $CD8\alpha\alpha^+$ T cells and T line-

age cells containing the $\gamma\delta$ TCR [51]; interestingly, the TCR$\gamma\delta$ lineage and TCR$\alpha\beta^{+}$CD8$\alpha\alpha$ populations do not retain immunologic memory of infection. However, the $\gamma\delta$-T cell enriched IEL function as a surveillance system for damaged or infected epithelial cells, and may modulate local immune responses by controlling cellular traffic and limiting mucosal access of inflammatory cells [52]. The $\gamma\delta$ T cells are thought to play an important role in the pathophysiologic response to infections including Staphylococcal infection. In mice, 45% of the IEL present in the small intestine are estimated to be conventional thymus-derived lymphocytes that coexpress TCR-$\alpha\beta$ and classical CD8-$\alpha\beta$. These cells primarily exhibit a cytolytic function and are recognized residents of the lamina propria, yet retain the ability to dessiminate to various anatomical sites including the gut epithelium following an antigen priming [53]. However, there are also TCR bearing $\alpha\beta$ T cells in the lamina propria, the majority of which exhibit the activated/memory phenotype; the major histocompatibility (MHC) class II-restricted CD4^{+} T helper (Th) cells.

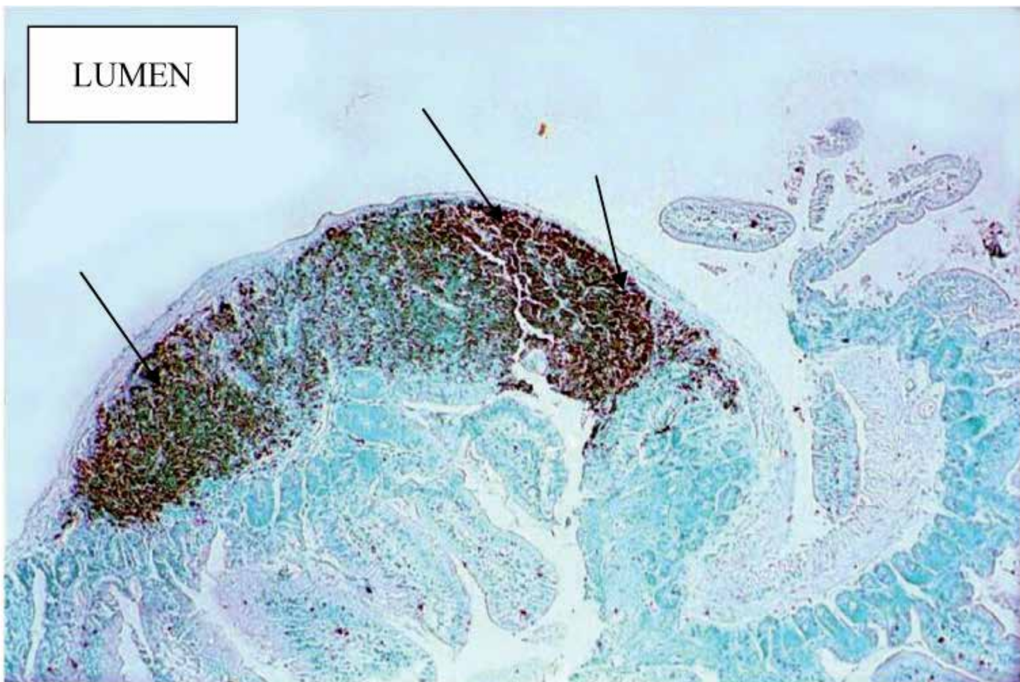

Figure 1. B220^{+} lymphocyte localization (indicated by arrows) in Peyer's Patch derived from normal C57Bl/10J mice. Formalin fixed tissue section [10X magnification) was stained using a monoclonal directed against B220 (RA3-6B2) and a horse-radish peroxidase conjugated antibody. B220 staining demonstrates a predominance of B cells in the unstimulated Peyer's Patch.

2.2. T cell immune activity in the GALT

During a gastrointestinal immune response, ingested antigens in the lumen enter the Peyer's Patches via the specialized epithelial cells known as M cells present in the epithelial layer

overlaying the PP. The M cells can take up particulate antigen by endocytosis and transport the antigen into the interior of the PP where the dendritic cells process the antigen and present antigen to the T cell areas of the PP and MLN, initiating T cell activation and differentiation into effector cells, that will either mediate tolerance or immunologic responsiveness [54, 55]. In experiments using genetically-defined mice, ingestion of the superantigenic food toxin, Staphylococcal entertoxin B (SEB), has been demonstrated to increase the TCR-$\alpha\beta$ populations in PP (Figure 2) such that the predominant response is generated as a result of the binding between the toxin, target T cell receptor-bearing populations containing the defined Vβ-8 sequence, and antigen presenting cells [56, 57].The result of this interaction is the receptor-mediated induction of cytokine-driven T cell proliferation, resulting in a proliferation and expansion of the SEB-reactive Vβ-8$^+$ T cells. As shown in Figure 2, normal PP contain an abundance of B220$^+$ B cells. However, the distribution of B220$^+$ B cells becomes dramatically altered following oral administration of SEB in quantities sufficient to induce illness in humans to genetically-defined C57Bl/10J mice. The B220$^+$ populations become sequestered, and the interior of the node becomes predominantly B220 negative. In this case, the PP lymph node becomes enriched for Vβ-8$^+$ T cells as determined by flow cytometric analysis (Principato, unpublished). $\gamma\delta$-T lymphocyte populations in PP, lamina propria, and epithelium have also been observed to increase following SE treatment [58].

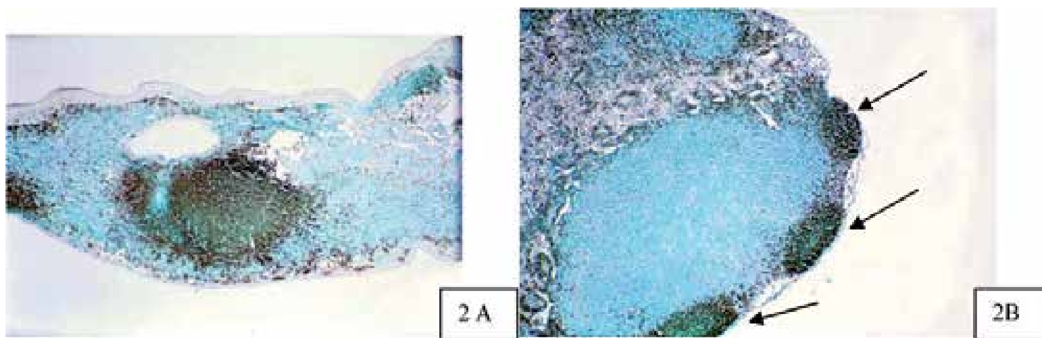

Figure 2. A. Distribution of B220$^+$ B cells in normal C57Bl/10J mice Peyer's Patch. Formalin fixed tissue section was stained using a monoclonal antibody directed against murine B220 (RA3-6B2) and a horse-radish peroxidase conjugated antibody [10X magnification). B200 staining (brown areas) demonstrates a diffuse presence of B220$^+$ B cells in the unstimulated Peyer's Patch. B. Expansion of non-B220+ (i.e., T cells) within Peyer's Patches 6 days following ingestion of Staphlyococcal enterotoxin B. A redistribution of the B220+ cells into aggregates forming below the PP epithelial capsule is indicated by arrows. [40X magnification).

2.3. T helper subsets of the GALT

The intestinal mucosa harbors all of the major T helper (Th) cell subsets (Th1, Th2, Treg (immunoregulatory), Th17) that are defined by their lineage-specific transcription factor expression, cytokine production, and immune function. The Th1 subset is critical for immune responses generated against intracellular pathogens, and provides cytokine-mediated "help" to the cytotoxic T lymphocytes. It is characterized by the production of interferon-gamma

(IFN-γ) which is controlled by the transcription factor T-bet [59]. The Th2 subset provides help for B cells and is also implicated in allergic sensitization, including those attributable to foods [60]. The specific transcription factor for Th2 cells is GATA-3, which drives the synthesis of IL-4, IL-5, and IL-13 [61]. Tregs that have arisen from antigen-specific induction of $CD4^{+}Foxp3^{+}$ T cells are critical for the induction of oral tolerance[62]. The Th17 cells express retinoic acid-related orphan receptors (RORγt and RORα) that are needed for the transcription and synthesis of IL-17 [63, 64], and provide important protection of mucosal surfaces against extracellular bacteria.

2.4. Innate immunity in the GALT

The cells of the innate immune system include macrophages, dendritic cells, and Langerhan's cells, and are involved in critical activities pertaining to the initiation and support of T cell-mediated, antigen-specific immunity. Significantly, the distribution of these cell types includes the skin and epithelia that line the internal organs including the gastrointestinal tract. Macrophages and dendritic cells are situated below the single layer of epithelial cells that lines the Peyer's Patches and lamina propria [65]. Macrophages have long been identified as components of the reticuloendothelial system and are recognized for their ability to ingest extracellular matter including proteins, cellular fragments, and debris that is foreign to the body in the process known as phagocytosis. They are widely distributed within the tissues of the body, and are crucial components of immune responsiveness and inflammation. Initial binding of the target occurs on the surface of the cell, utilizing receptors with specific capabilities. Receptors identified include surface Fc receptors that bind the Fc portion of IgG immunoglobulin, complement C3b and C3d receptors, MHC Class I and Class II, Toll like receptors (TLR), cytokine receptors, and other membrane receptors such as the C-type lectins [66] that provide additional innate functionality which supports the binding and internalization of a wide variety of targets. Opsonization of target by plasma proteins is known to improve phagocytosis, and the endocytosing vesicles have been demonstrated to consist of clathrin structures [67, 68]. Interestingly, the endosome exhibits plasticity, and its shape has been demonstrated to change depending on the material that is engulfed [69]. In an early examination of macrophage activity, Unanue and coworkers demonstrated distinct differences in macrophage effector function based on the anatomical source of the macrophage. These investigators compared the ability of alveolar and peritoneal-derived macrophages to bind and present antigen, the intracellular pathogen *Listeria monocytogenes*, to previously sensitized T cells [70]. While alveolar and peritoneal macrophages both expressed class II Ia antigen, alveolar macrophages were less efficient with respect to the uptake and presentation of antigen to sensitized T cells as compared to the peritoneal macrophages. However, opsonizing *Listeria* using an anti-Listeria antiserum to coat the bacterium enhanced the alveolar macrophages' ability to engulf the bacterium and effectively present the antigen to sensitized T cells. Once internalized, the ingested antigen undergoes intracellular metabolic and proteolytic degradation, and modification. The resulting fragment [71, 72], is transported to the surface of the cell where it is presented in conjunction with the major histocompatibility (MHC) gene molecule. This structural relationship is critical for the activation of the appropriate responding T cell, which contains a great variability

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of gene sequences which must be rearranged to configure a mature, functional, TCR. This permits the specific recognition of the presented peptide sequence by the TCR of the responding T cell, and provides for the development of the adaptive immune response, which will also generate an immunologic memory of the peptide target.

Innate immunity through the Toll like receptors (TLR) is conferred with the task of recognizing a broad range of repetitive antigenic specificities that are found on a wide array of pathogens. With this type of recognition, pathogen detection is based on the ability to recognize pathogen-associated molecular patterns using evolutionarily conserved, germline encoded recognition receptors, the TLR [73-75]. Thus, while a strict sequence-dependent antigenic specificity is not required as with antigen-specific immune responsiveness, what is required is an ability to bind carbohydrate residues in a Ca^{++} dependent manner, and the recognition of conserved molecular patterns such as in bacterial cell wall components. Thus, LPS is the ligand for TLR 4, and targeted mutation of the TLR4 locus in mice results in LPS non-responsiveness [76]. TLR2 recognizes ligands found on yeast cell walls, bacterial lipoproteins [77], and lipoteichoic acid found in the cell walls of gram positive bacteria [78]; other TLR recognize bacterial DNA, or double stranded viral RNA. In humans and mice, there are now at least 10 such TLR identified. Unlike the sequence-specific receptors found on the antigen-binding T cells of the adaptive immune response, TLR are non-clonal and do not require gene rearrangement in order to become functionally mature.

Upon contact with a pathogen, the cells of the innate immune system become activated, the binding of their receptors initiating signaling cascades that turn on required transcription of target genes for the production of inflammatory cytokines, and the upregulation of costimulatory and MHC molecules necessary for the direct elimination of the infection or for the recruitment of adaptive immune responses. The binding of TLR with their target ligand induce costimulatory molecules that were first identified as the B7.1 and B7.2, or now referred to as CD80 and CD86. Thus, the responding T cell must recognize the modified target ligand which is expressed on the surface of the macrophage or dendritic cells in the context of both MHC and costimulator molecules with its sequence-specific TCR. It is clear that the cells of the innate immune system are critical to the establishment of an effective immune responsiveness against pathogens and for the recruitment of an efficient adaptive immune response. The extremely successful yellow fever vaccine, YF-17D, which induces both Th1/Th2 responses and generates powerful neutralizing antibodies in vaccine recipients, was shown to induce such a strong protective immunity as a result of its ability to stimulate multiple subsets of human dendritic cells and multiple TLRs [79]. Vaccine designs utilizing synthetic 300 nm PLGA nanoparticles containing antigen and ligands that bind TLR 4 and TLR 7 on the surface of dendritic cells, have successfully induced enhanced antigen-specific antibody responses against the immunizing antigen when injected into experimental mice[80]. The immunization protocol induced long-lived, high avidity antibody that was dependent upon the expression of the targeted TLR on both B cells and dendritics. The B cell response indicated the generation of memory-type B cells.

Macrophage and dendritic cells have been documented with respect to the striking specializations of the subsets. For instance, $CD11b^+$ dendritic cells of the lamina propria can sample

luminal microbes by extending their dendrites to interdigitate between neighboring intestinal epithelial cells [81], and have been reported to promote the differentiation of Th17+ regulatory T cells following activation of TLR 5 due to exposure to bacterial flagellin [82]. Interestingly, as previously observed by Unanue and coworkers [70], anatomic localization can denote distinctions in the functional effector function within subsets of cells. Thus, CD11b+CD103+ dendritic cells of the lamina propia are found preferentially in the duodenum and rarely in the colon during the steady state, but accumulate in the lamina propria of the colon along with Th17 cells during intestinal inflammation [83]. Macrophages of the lamina propria have been demonstrated be hyporesponsive to certain inflammatory stimuli, secrete IL-10, promote the differentiation of FoxP3+ regulatory T cells [84] and are able to dampen some immune responses and intestinal inflammation. For instance, a severe dextran sulfate-associated experimental colitis can be induced in a macrophage-depleted transgenic mouse or in clodronate-treated normal C57BL/6 or Babl/c mice [85]. Finally, CD103+ dendritic cells are known to assist in the antigen specific induction of FoxP3+ T regs necessary for tolerance induction[86]. Thus, the cells of the innate immune system maintain a balance between a normal state of tolerance, and inflammatory and autoimmune responses.

3. Ingestion of nanoparticles

The ingestion of nutrients with subsequent transit throughout the lumen of the gastrointestinal tract leads to the translocation of the material across the mucosa via the M cells of the epithelial layer. M cells are specialized cells that exhibit endocytic activity, and are known to transport antigens into the interior of the PP where the dendritic cells process the antigen and present antigen to the T cell areas of the PP and MLN, initiating T cell activation and differentiation into effector cells, that will either mediate tolerance or immunologic responsiveness [54, 55]. Multiple physiochemical properties, including size and surface charge, have been shown to influence nanoparticle uptake and absorption in the gut, and the extent and rate at which the particles are removed from the circulation and their ultimate biodistribution. Thus, orally-administered non-ionic nanoparticles of 100 nm or less have demonstrated preferential absorption in the Peyer's Patch and the small intestine. Focused, engineered targeting of particles to the GALT has reported success with respect to the induction of measurable antibody responses. However, the specific immunologic mechanisms inherent to nanoparticle intake and absorption within the gastrointestinal tract have not been adequately identified, and the effector pathways that generate the immune responses measured have not been characterized.

3.1. Influence of nanoparticle size and charge

Desai and coworkers demonstrated that 100 nm nanoparticles underwent a preferential uptake in the gastrointestinal tract [87] using an *in situ* rat ileal loop model. Polylactic polyglycolic acid (PLGA) nano- and microparticles were synthesized with averaged diameters of 100 nm, 500 nm, 1 μm, and 10 μm and infused into the tissue. Tissue uptake was quantified as weight of the nanoparticles (μg) (taking into account the density of the polymer and the

diameter of the microparticle) per square mm area of rat intestinal tissue. Infusion of the particles into gastrointestinal tissue demonstrated 100 nm particle uptake by both duodenal and ileal tissue. However, the ileum's Peyer's Patch and non-Peyer's Patch tissue demonstrated a higher uptake of 100 nm size particles. This observation was repeated using surrogate-loaded microparticles. Histologic examination of the tissue using fluorescent microscopy confirmed a greater retention of the 100 nm nanoparticles, with a concentration below the epithelial layer.

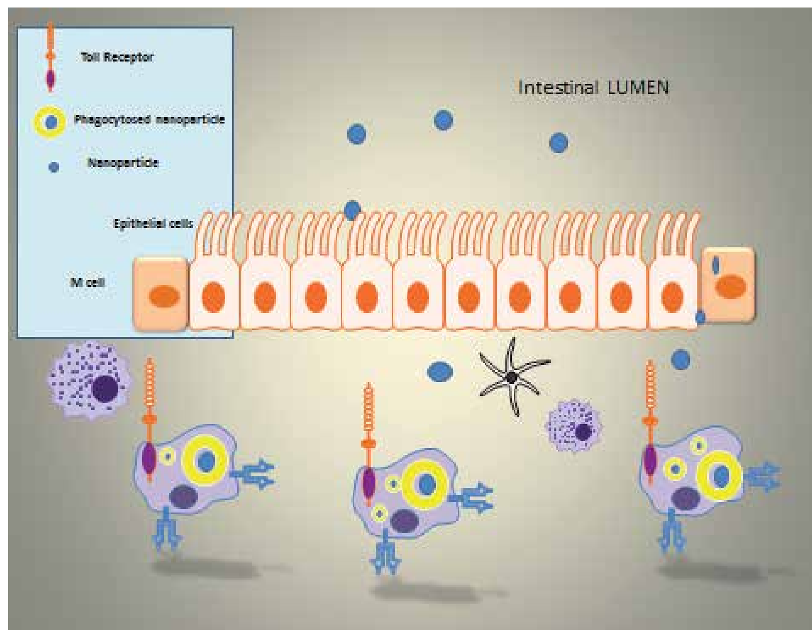

Figure 3. Schematic representation of the ingestion of nanoparticles. Macrophages and dendritic cells can be found beneath the eptihelial layer of the GALT. Ingested nonionic or targetted nanoparticles distribute preferentially below the intestinal epithelium, and can meet macrophages bearing class II and TLR molecules, and are phagocytosed by the macrophage. Actively phagocytosing macrophages are represented in the foreground; engorged macrophages are represented containing multiple particles.

The authors' observations recollect those of an earlier study utilizing latex particles [88]. In that study, Jani and coworkers conducted a 10-day feeding study in which non-ionic latex particles ranging in size from 100 nm, 500 nm, 1 micron, and 3 microns were fed to Sprague-Dawley rats. Their histologic and radiologic examination provided unequivocal evidence of a preferential tissue distribution of 100nm particles in which the Peyer's Patches, liver, and spleen demonstrated significant uptake. Significantly, their result confirmed the potential transport of particles from the gastrointestinal tract to the periphery via the lymphatics.

A separate 5 day feeding study in rats demonstrated the effects of a hydrophilic charge upon the tissue distribution of normally hydrophobic polystyrene particles [89]. In this study, commercial non-ionized polystyrene particles with a mean diameter of 60 nm were compared to similarly-sized particles coated with poloxamer 407. Their results confirmed

the earlier observations by Jani and coworkers: a preferential uptake of uncharged polystyrene was noted in the small intestines and Peyer's Patches as measured using gel permeation chromatography to quantify polystyrene in the tissue, and by microscopy. Further, a smaller concentration of particles was observed to be in the mesenteric lymphatic tissue and liver. Collectively, these data indicate a movement of the particles from the lumen of the intestinal tract to the peripheral circulation with subsequent residence in other tissues. However, charged particles demonstrated a significant reduction in uptake, 1.5%- 2% of the total administered dose of particles were absorbed as opposed to 10% uptake using uncharged particles. Interestingly, the tissue distribution was altered as a result of the poloxamer coating: the particles were particularly concentrated within the tissues of the large intestine. Taken together, these results demonstrate the importance of particle size in determining the tissue range of ingested neutrally charged particles, and the critical role of charge as a particularly strong determinant of distribution within the body.

3.2. Biodistribution

The macrophage is most often implicated in the uptake of nanoparticles and opsonization will influence nanoparticle uptake into the cells. Nevertheless, final biodistribution and disposition is likely determined by the transport of particles by phagocytic and endocytotic cells. The intraperitoneal injection of 40 nm gold nanoparticles in mice has been demonstrated to result in the localization of particles in the Kupffer cells of the liver. In this research, commercially-produced colloidal gold nanoparticles containing a negative surface charge, in sizes of either 2 nm or 40 nm, were injected either intraperitoneally (ip) or intravenously (iv) into C57Bl/6 mice, and detected within cryostat sections of liver and other organs by autometallography, which amplifies the detection of gold. Interestingly, a preferential uptake of the 40 nm particles by Kupffer cells of the liver was observed 24 hours after ip injection. Very little uptake was observed 1 hour after injection. Animals who received the particles by ip injection also demonstrated particle uptake within the walls of the small intestine, mesenteric lymph node, and in the spleen illustrating that transit of the administered particles had occurred, most likely via the phagocytic cells [90]. Using a rabbit model, orally administered chitosan/PLGA spherical particles (averaged 283 nm) for the encapsulation of Vitamin A were found along the mucosal epithelium of the lumen, and within the intestinal epithelial cells, presumably due to endocytosis. Macrophages in the lamina propia showed evidence of particles as did the endothelial cells [38].

Variations to the particle, such as addition of a polymeric coating, will alter the biodistribution. Thus, coating polystyrene 60 nm and 5.25 μm particles with poloxamer polymers will decrease uptake by liver and spleen macrophages. Importantly, increasing the thickness of the coating will alter uptake as well; in this case it has resulted in a reduction of uptake by the peritoneal macrophage [91]. Experiments in which hydrophilic negatively-charged alginate-coated chitosan nanoparticles were passively absorbed into gastrointestinal tissue demonstrated localization beneath the follicle associated epithelium of the Peyer's Patches and agglomeration of the particles intracellularly, although the specific nature of the cell was not described [92].

Significant patterns in organ compartmentalization have also been described for metallic nanoparticles.

The fate of ingested silver salt and silver nanoparticle was examined in a feeding study in which separate groups of female Wistar rats were administered 9 mg silver acetate or 12.6 mg silver nanoparticle per kg of body weight daily for 28 days. It was estimated that 63% of the daily ingested dose was excreted in the feces. The overall accumulation of either silver ion or nanoparticle was similar, and appeared greatest in the small intestine while also detectable in liver, kidney, and stomach. Autometallographic staining (AMG) detects the presence of either silver acetate or nanoparticle; thus, silver was localized to the lamina propia and submucosa in the ileum. Interestingly, silver was concentrated around the veins and portal circulation of the liver and was not preferentially taken up by the Kupffer cells of the liver as was reported with injected gold [90]. Transmission electron microscopy displayed similar localizations for both the silver nanoparticles and silver acetate; the material was found within the lysosomes of the macrophages within the lamina propia of the ileum [93].

4. Immune responses and the ingested nanoparticle/microparticle

Particulate antigens are known to induce stronger immune responsiveness to the antigen when compared to an immune response generated with the soluble form of antigen. Thus, vaccine design has recently emphasized the use of nanoparticles to maximize induction of the protective immune responsiveness. Mucosal immunizations have been viewed increasingly as an alternative to parenteral administration of vaccines, and features such as carbohydrate residue targeting by lectins has been examined by many groups [94-96]. Nevertheless, nanoparticle absorption within the GALT is still not well understood, and the effector cellular interactions involved in the generation of the induced immune response have not been fully defined.

Immunoglobulin production as a function of particle size, was measured by Gutierro and coworkers [97]. Bovine Serum Albumin (BSA) –loaded PLGA microspheres of 200 nm, 500 nm, and 1000 nm were constructed using a double emulsion technique; size was determined by laser diffractometry using a CoulterCounter® particle size analyzer. PLGA microspheres containing BSA target antigen was administered by each of three routes: subcutaneously, intranasally, or orally into 6-8 week old Balbc/J mice and the elicited immune response was measured by assaying IgG immunoglobulin production. Their results showed that IgG antibodies were elicited using each of the three sizes of microspheres when administered subcutaneously; one size did not elicit greater antibody production than the others. Further, all three sizes elicited antibody responses that were greater than that elicited using either soluble antigen or conventional adjuvant approaches. The oral immunization protocol consisted of orally feeding each of the three sizes of microspheres, each containing 500 ∝g BSA, on three successive days. Interestingly, oral administration of the loaded microspheres showed that the 200 nm and 500 nm sized particles elicited fewer antibodies than an administration of antigen with either alum or Freund's adjuvant. The greatest production of serum IgG was

demonstrated using the 1000 um size particle and this group contained the higher percentage of individual responders. Analysis of serums at weeks 3 and 5 following immunization did not reveal differences in IgG2a/IgG1 isotype profiles, the latter being indicative of Th1/Th2 subset immunity and antigen-presenting differences. Ultimately, no differences were found among the various sized particles, suggesting that the method of antigen presentation was the same for all of the sizes tested. Again, the larger particles provided the higher immunoglobulin production, regardless of the mode of immunization. These results are interesting from the perspective of what has been reported [87, 88] regarding the effect of size and nanoparticle biodistribution, and what is known about the distribution of effector cells within the GALT. The present experiments used particles that were larger than those previously published; it is likely that the biodistribution affected the manner in which particulate antigen was presented for the induction of an immune response.

4.1. Targeting M cells in the GALT

Directed PLGA nanoparticles, using lectins to bind onto target sugar residues, has been shown to be a means to achieve organ targeting for the induction of a systemic immune response. In one study, PLGA nanoparticles were created by the double emulsion method and loaded with hepatitis B surface antigen (HBsAg) [95]. Lectin directed to α-L- fucose residues, *Tetragonolobus purpureas,* was bound to the nanoparticle using 1-ethyl-3-(3 dimethylaminopropyl) carbodiimide to produce TLA lectin-PLGA-HBsAg nanoparticles that were measured to be 270 + 23nm in size. Confocal microscopy confirmed binding of the particles to the M cells of the Peyer's Patches within immunized mice. Further, lectinized particles were stabilized by the addition of hydrophilic trehalose, which improves the release of antigen. Therefore, nanoparticles stabilized with trehalose demonstrated an increased antigen release of 43.2+2.7% after 35 days, as opposed to a release of 32.4 +2.3% by the non-stabilized equivalent. In this investigation, 10 mg of encapsulated antigen per dose was used for the oral immunization of 8 week old Balb/c mice, followed with a booster 2 weeks following the primary immunization. Thus, HBsAg -loaded PLGA nanoparticles, TLA lectin-PLGA-HBsAg nanoparticles, trehalose-stabilized HBsAg - loaded PLGA nanoparticles, and trehalose-stabilized TLA lectin-PLGA-HBsAg nanoparticles were compared for the induction of antibody. Significantly, this study demonstrated the successful induction of antigen-specific IgG antibody by each of the engineered nanoparticles as determined by ELISA assay of the immune seras, when compared to the levels of antibody produced by the animals immunized with an alum based antigen. Isotyping of the antibodies demonstrated induction of IgG1 antibody, indicative of a Th2 response, at levels that were twice those attained by IgG2a which is indicative of a Th1 response. While demonstrable levels of the Th1 cytokines, IL-2 and γ-IFN, were detected in the spleens of all nanoparticle-treated animals, greater levels of γ-IFN were obtained with TLA lectin-PLGA-HBsAg, with or without stabilization by trehalose. It is not known whether the engineered particle could have induced a greater γ-IFN response as PLGA nanoparticles without antigen were not used for comparison in this study.

A directed approach has been extremely successful using chitosan alginate microparticles [94]. In this instance, chitosan nanoparticles (CNP) prepared by the ionic gelation method were

loaded with BSA test antigen, and coated with alginate. Thus, alginate was modified by using 1-ethyl-3-(3 dimethylaminopropyl) carbodiimide to form amide linkages between the carboxylate residues on alginate and the amino group of the lectin *Ulex europaeus* agglutinin (UEA-1). The lectin *Ulex europaeus agglutinin* (UEA-1) was used to direct the microparticles towards the α-L- fucose residues found on the surface of M cells. Confocal microscopy confirmed the targeting; punctate staining was visualized using the lectin-modified microspheres. The conjugation and loading resulted in a particle shift in size: the particle size of the original CNP particle is reported as 257+ 55.17 nm, while the lectin-modified antigen carrier CNP particle size increased to 1485 + 214.3 nm. Oral immunization of 6-8 week old Balb/c mice with each of the preparations and control antigen provided striking differences in the antibody responses against BSA antigen. The highest IgG titers were obtained using alum-absorbed BSA as the immunogen's positive control, and the lowest titers were obtained using BSA loaded CNP. In contrast, antigen encapsulated in lectin-modified alginate chitosan particles (LACNP) consistently generated IgG titers that were greater than those obtained with CNP or ACNP formulations. Demonstrable levels of antigen-specific IgG2a/IgG1 were detected with all three formulations. Significantly, the highest titers of antigen-specific IgG were obtained with lectin-modified microspheres, and the results seem to indicate that there was a greater IgG2a, or Th1 response, to antigen (BSA) with that particle. The original CNP particle and the alginate chitosan particles seemed to have induced a greater Th2 response.

Together, these studies demonstrate the induction of a Th1/Th2-induced immunity using engineered particles as do others [93]. However, it is not known whether α- fucose residues are found on macrophages and dendritic cells present at other body sites, possibly resulting in multiple pathways of immune responsiveness. As discussed earlier, Unanue and coworkers demonstrated distinct differences in macrophage effector function based on the anatomical source of the macrophage [69]. Further, while TLA lectin-PLGA-HBsAg nanoparticles induced the production of sIgA in saliva and gastrointestinal fluids, it was not reported whether the engineered particles in these reports ultimately interacted with $CD11^+$ dendritic cells. IgA has been reported to be induced by regulatory T cells that have been activated by $CD11^+$ dendritic cells [50]. Finally, the directed attachment of the particles to the endocytotic M cells of the epithelial layer presents the possibility that the particles were transcytosed by the M cells towards $CD103^+$ dendritic cells, found beneath the epithelial layer. In that case, the possibility exists for the induction of tolerance [86]. Normal exposure to ingested, digested antigen results in the production of regulatory T cells that suppress an immune response in an antigen specific manner, resulting in tolerance and preventing food allergy. However, the targeted microparticles document the induction of Th1and Th2 responses.

5. Future consideration: Ingested nanoparticle and immune allergic dysfunction to foods

Proteins used in commercial food applications include casein, whey protein, collagen, egg white, and fish myofibrillar protein, and popular plant-based proteins including soybean protein and wheat gluten [7]. Compounds such as polysaccharides and proteins that are al-

ready in use within commercial food applications are attractive candidates for the production of new nanocomposite packaging and encapsulation material, as several are generally regarded as safe and are biodegradable. However, food allergy has emerged as a growing health problem throughout modern society, and current research efforts towards the identification and characterization of clinically relevant food allergens are critical to our understanding of their role in the immunopathogenic mechanisms involved in hypersensitivity reactions, and the safety of novel and proposed food-oriented nanotechnology. Thus, the characterization and identification of the proteins responsible for immune-mediated food allergies is critical.

In view of reported differences with respect to nanoparticle size and organ biodistribution, it is interesting to note that particles are often detected below the epithelium of the gastrointestinal tract. Since the Intraepithelial lymphocytes (IEL) reside below the epithelial layer of the mammalian gastrointestinal tract, an understanding of the interactions between particle and resident IEL is crucial. Following the ingestion of food, digested protein fragments, or antigens, cross the epithelium to be processed and presented on the surface of class II molecule- bearing antigen presenting cells for recognition by specific TCR-bearing T cells. Allergic sensitization in the presence of IL-4 results in the generation of Th2 cells that will assist the development of IgE^+ B cells. A repeat encounter with the antigen will result in a food allergic response. This event generates a skewed Th2 response, and will occur when luminal antigen is introduced to IgE bound onto IgE Fc receptor on the surface of mast cells. Thus, crossing the epithelial barrier to reach the mast cells is a critical step. The binding of antigen to the receptor-bound complex will result in the release of histamine, serotonin and prostaglandins in anaphylactic reactions including those generated by food.

Recent studies suggest that intestinal epithelial cells play a central regulatory role in determining the rate and pattern of uptake of ingested antigens. This is particularly critical in food allergy within the antigen-sensitized gastrointestinal tract. Studies using rats sensitized to horseradish peroxidase (HRP) showed that intestinal antigen transport is keenly affected by antigen-specific sensitization and is composed of 2 phases. The first phase consists of the rapid transepithelial transport of specific antigen from the lumen, via endocytosis, into the lamina propia. This phase is antigen specific, implying the existence of an antigen -specific receptor on the surface of the epithelial cells, and occurs within 2 minutes in sensitized rats as compared to a transit time of 20 minutes in non-sensitized, normal control animals. This is followed by a flow of the antigen in tight junctions resulting in an increase of antigen across the tissue. The second phase of antigen transport is not antigen specific, but is markedly increased by antigen challenge in sensitized rats compared with non-sensitized controls [98], indicative of the paracellular penetration through the epithelium by antigen. These studies clearly demonstrate that the kinetics of transport of antigen during IgE-mediated reactions in the gastrointestinal tract is markedly increased across the epithelium. The result of this transport is the generation of a Th2 response.

Finally, a feeding study using mice orally sensitized to the known milk allergens, casein, β-lactalbumin, and β-lactoglobulin, provided compelling evidence regarding the importance of the form of the antigen (soluble vs. particulate) for the induction of anaphylaxis [99]. The

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soluble proteins, β-lactalbumin, and β-lactoglobulin, resulted in anaphylactic reactions when administered orally. Interestingly, the soluble proteins were detected in the lamina propria of the small intestine of sensitized mice indicating that these proteins were able to transcytose through the enterocytes *in vivo.* This observation was confirmed *in vitro* using Caco-2 cells. Further, the challenge with sensitizing antigen resulted in significant levels of serum IgG1, and low, but detectable levels, of serum IgE and IgG2a. Casein, normally present within micelles, demonstrated a significant difference in anaphylactic induction. Oral administration did not induce anaphylaxis. Instead, casein required a systemic administration (i.p. injection) in order to induce anaphylaxis; and it induced significantly higher serum IgE and IgG1 (Th2) allergic responses as compared to the soluble milk allergens. Further, transcytosis by casein through Caco2 monolayers was poor compared to the soluble milk allergens. When the tissue was examined by fluorescence microscopy, the casein was detectable in the Peyer's patches. Thus, these data indicated that the form of the sensitizing antigen was critical to the induction of an anaphylactic response. Next, the soluble allergens, β− lactoglobulin and soluble α-lactalbumin, were next converted into particulate aggregates by pasteurization; the process reportedly abolishes the monomeric form and supports the formation of aggregates of approximately 670 kDa. Pasteurization does not alter casein, and it exists in two predominant types as it would in its natural state: 180 kDa and 670 kDa. The conversion of soluble β− lactoglobulin and soluble α-lactalbumin into particulate aggregates by pasteurization altered the immunogenicity of the proteins such that they now required a systemic administration to induce anaphylaxis. Oral administration of either protein aggregate in sensitized mice did not induce anaphylaxis. The magnitude of the elicited serum IgG1 and IgE immunoglobulin production was much greater than that induced by their soluble forms. Further, the proteins were now detectable in association with the Peyer's Patches. Casein's induction was not altered by the process. Taken together, these results present the critical role of antigenic structure and its uptake across the epithelium as critical factors contributing to the allergic state.

The allergic state presents serious challenges to the incorporation of nanoparticles in food and food-associated products, particularly when considering the composition and ultimate biodistribution of the particles. The engineering of nanoparticle containing materials implicated, related, or identified as allergens raises concern for the initiation of alternate allergy-inducing pathways in the host. For instance, while casein is incorporated in a variety of foods and is generally regarded as safe, it is also known to elicit strong allergic responses in afflicted individuals with dairy intolerance. Disruption of the epithelial barrier is known to result in gastrointestinal illness [100]. Infection and inflammation are conditions associated with a disruption of the epithelial layer leading to the increased paracellular transport of luminal antigen. Cytokines such as IFN-γ and TNF-α directly affect barrier function of the epithelium, the latter being implicated in milk allergy [101-103]. Thus, the transit of nanoparticle through a sensitized gastrointestinal system might result in a more complicated scenario, depending upon the sensitizing antigen and the composition of the nanoparticle itself. Thus, casein nanoparticle constructs, with or without targeting lectins, might not be advisable for individuals with casein sensitivity. In this instance, the transit of the nanoparticle might be hastened across the layer, due to the pre-existing sensitivity, resulting in in-

creased transit through the layer, perhaps overwhelming the resident macrophage phagocytic activity (Figure 4), leading to exacerbation of the allergic state or the generation of alternative immunologic reactions.

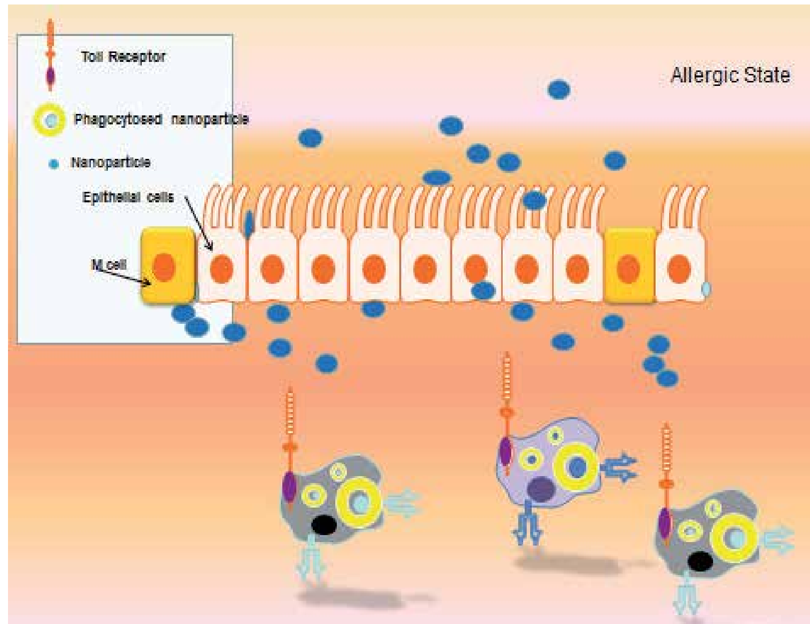

Figure 4. Schematic representation of nanoparticle transit through the epithelial layer and the allergic state. An increased rate of transit by the nanoparticles is theorized as a result of the induction or presence of an allergic state.

6. Conclusions

The choice of material used in the formulation of nanoparticles and spheres during the formulation of encapsulated nutrients or supplements intended for ingestion can be critical to the possible outcomes in mucosal immunity. A crucial consideration is whether the material will influence the induction of either tolerance or active immunity to the introduced nutrient as a result of its deposition within the gastrointestinal tract and possible interaction with resident effector cells.

The specific targeting of the nanoparticles and spheres using specific ligand interactions provides an advantage in this respect. While polymers containing natural biodegradable materials such as chitosan, PLGA, whey, casein, and others offer great advantages within this technology, they also present further challenges towards an understanding of the mechanism involved in the maintenance of gastrointestinal immune homeostasis, and preventing the induction or potentiation of immune dysfunction.

Abbreviations

GALT, gut-associated lymphoid tissue; Ig, immunoglobulin; M cell, microfold/membranous cell; CD, Cluster designation; sIgA, surface IgA; MHC, major histocompatibility complex; BSA, bovine serum albumin; PP, Peyer's patch: MLN, mesenteric lymph node

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Yeast: World's Finest *Chef*

Fábio Faria-Oliveira, Sónia Puga and Célia Ferreira

Additional information is available at the end of the chapter

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1. Introduction

Yeast is the simplest eukaryotic organism of our days. They are unicellular microorganisms classified in the kingdom Fungi. Nevertheless, yeasts were probably the first microorganism to be domesticated and since early in human history have been used on a daily basis in bread making and in alcoholic beverages. Nowadays, yeast has become a key microorganism for many types of industrial and food processing manufactures, including the production of beer, wine, cheese and bread. In particular, its use in baking industry is quite relevant due to the central role of bread as a dietary product all over the world. Moreover, yeasts are regarded with reasonably interest as nutrients and health provider sources both for humans as well as for animals. We dare to appoint yeast as the one of the world finest chefs.

Yeasts are found in diverse natural environments; colonizing from terrestrial, to aerial and aquatic environments. They can be found on decomposing fruit, on soils, as opportunistic pathogens in human beings, in the gut of the fish and free living in the sea. In general they contribute to the decay of organic material, but their successful colonization is intimately related to their capacity of physiologically adapt at diverse milieus. Hitherto, it has been described approximately 1500 species [1].

This chapter aims at contribute to a comprehensible analysis of the role of yeasts on the actual feed lifestyle, mainly in what regards the yeast *Saccharomyces cerevisiae*. This yeast is known with by many appellations: "Baker's yeast" in baking and confectionery fields, "Brewer's Yeast" by all beer industrial and artisanal producers, and perhaps less familiar "Wine's Yeast" by wine-like alcoholic beverages producers. We will first go over several physiologic aspects of this yeast metabolism, specifically associated with glucose catabolism, under anaerobic environments (fermentation) as well as aerobic conditions. Most of our attention is given to glycolysis pathway and to alcoholic fermentation in order to prepare the reader for the issues discussed later. Considerable notice will be paid to the intervention of

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yeast in alcoholic beverages, in particular beer and wine, important economical industries of our times. The particular role of *S. cerevisiae* in baking industry, interactions with lactic acid bacteria (LAB) in sourdoughs, and also the scientific approaches/advances for sustainability were exhaustively reviewed, recently by us in [2] but also by [3], therefore we will focus preferentially on the production of commercial yeast for baking. In the end, we will briefly review the other applications of *S. cerevisiae* in less familiar products, including animals and fish feeding rations and biotic supplements.

1.1. Yeast metabolism

Yeasts, resembling other heterotrophic organisms, have the energy and carbon metabolism operating in concert, *i.e.*, anabolism is coupled with catabolism. Their chemical energy, in the form of ATP, results from the oxidation of organic molecules and is used as energy resource by the cell. On the other hand, those organic molecules can also operate as carbon sources for biosynthesis. Yeast environmental diversity leads to a vast metabolic complexity, due to the multiplicity of carbon and the energy sources available in nature. This includes polyols, alcohols, organic acids and amino acids yet, yeasts preferentially metabolize sugars.

1.1.1. Greedy yeasts – Sugar metabolism

The yeast metabolize diverse sugars, hexoses such as glucose, fructose, galactose or mannose, some can use pentoses like xylose or arabinose, disaccharides as maltose or sucrose; yet, glucose and fructose are the preferred substrates. The metabolic routes for the dissimulation of hexoses and disaccharides share the same pathways, with the great majority of the metabolic elements arising from intermediaries of glycolysis, the tricarboxylic acid cycle (TCA) and the pentose phosphate pathway, and differ only in the initial basic steps of metabolism.

The sugar dissimilation may occur in anaerobic or in aerobic environment. In the first case is called **fermentation** and in the presence of oxygen is named **respiration**. The most common process is the glucose dissimilation, generally known as **alcoholic fermentation**, which occurs anaerobically and yields as final products: ethanol and CO_2.

For the sugar utilization, yeast has primarily to sense the presence of glucose in the environment and then to transport it across the plasma membrane [4, 5]. The presence and levels of glucose sensed by the yeast can influence the enzyme levels through several processes, alteration of mRNA translation rates; mRNA stability or protein degradation, but also the concentration of intracellular metabolites (for a review see [6]). Yet, the major outcome is the extensive transcriptional regulation of a large number of genes leading to the adaptation to fermentative metabolism (alcoholic fermentation). These encompasses the induction of genes required for the utilization of glucose, such as genes encoding glycolytic pathway enzymes (discussed below), whereas genes required for the metabolism of alternative substrates, and those encoding proteins in the gluconeogenic and respiratory pathways are repressed by glucose (for reviews see [6] and [7]).

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The gene family of hexose transporters in *S. cerevisiae* consists of more than 20 members: i) 18 genes encoding transporters (*HXT*1-*HXT*17, *GAL*2), being the most relevant Hxt1p and Hxt3p, with a low affinity for glucose and high transport capacity, and Hxt2p, Hxt4p and Hxt7p, with a high affinity and low transport capacity and; ii) at least two genes encoding sensors (*SNF*3, *RGT*2), although several points of evidence suggest that *GPR*1 and *HXK*2 also sense and signal glucose levels [6, 8]. All these, sensors and transporters are therefore the primary interveners on sugar metabolism. After glucose uptake, it enters in the glycolytic pathway (Figure 1 – Steps from glucose to pyruvate) in order to be metabolized to pyruvate, whereby production of energy in form of ATP is coupled to the generation of intermediates and reducing power in form of NADH for biosynthetic pathways (for reviews see [5, 6, 9]) [10].

The first step of the glycolytic pathway consists on the phosphorylation of glucose to glucose 6-phosphate by the action of the hexokinases (Hxkp) and the glucokinase (Glkp); which are linked to high-affinity glucose uptake. Then glucose-6-phosphate is isomerized by the phosphoglucose isomerase, encoded by *PGI* gene, to fructose-6-phosphate. The next step, done by the phosphofructokinase (Pfkp) requires energy, in the form of ATP, to convert fructose-6-phosphate into fructose 1,6-biphosphate.

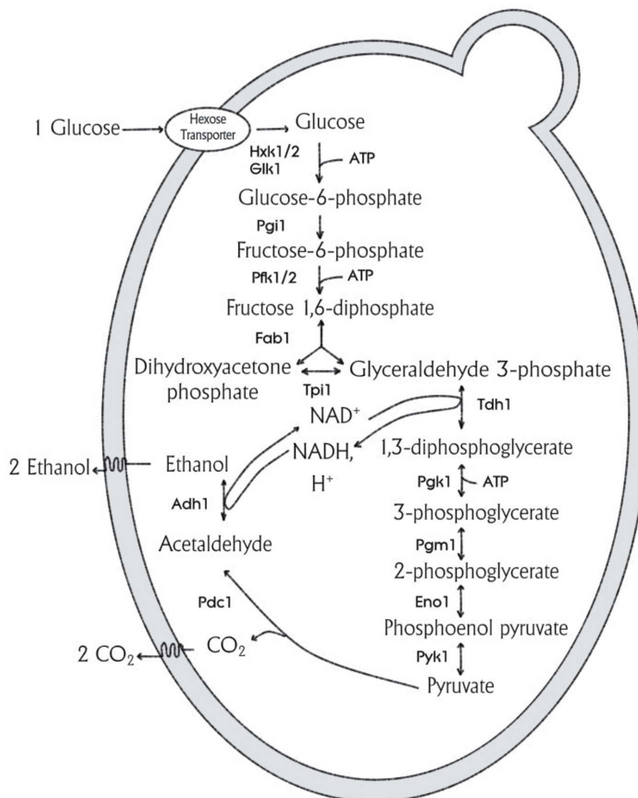

Figure 1. Alcoholic fermentation - enzymatic steps on *S. cerevisiae* (adapted from [11]).

Yeast phosphofructokinase, Pfkp, is a heterooctameric enzyme subject to a complex allosteric regulation. Aldolase (fructose 1,6-bisphosphate aldolase- Fbap) in turn, catalyses the reversible cleavage of fructose 1,6-bisphosphate to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. These two compounds can be converted one into another, again in a reversible way, by the triosephosphate isomerase (Tpip). Subsequently, glyceraldehyde 3-phosphate yields the pyruvate by the action of a series of acting enzymes, whereas some of the dihydroxyacetone phosphate follows gluconeogenesis. Glyceraldehyde 3-phosphate is firstly oxidised by NAD^+ (with the production of a reducing equivalent, which will take part in the latter steps of glycolysis when acetaldehyde gives ethanol (Figure 1)) and then phosphorylated, under the catalysis of the 3-phosphate dehydrogenase (Tdhp). The resulting 1,3-diphosphoglycerate, by the action of phosphoglycerate kinase (Pgkp), donates a phosphate group to an ADP molecule originating the 3 phosphoglycerate and releasing 1 molecule of energy (ATP). The next step is just a relocation of the phosphate group on position 2, done by the phosphoglycerate mutase (Pgmp); preparing this way the following reaction, the dehydration by the enolase (Enop) and from which results the phosphoenol pyruvate, a high energetic molecule. This is then phosphorylated by the pyruvate kinase (Pykp) giving the pyruvate and also releasing another molecule of ATP.

At this point, pyruvate can follow distinguished metabolic routes (Figure 2) depending on the environmental conditions, which in turn regulate the enzymes involved as well as their kinetics properties, but also of the yeast species [12]. Conversely, the carbon flux gets to a branching point in which may be divided among the respiratory and the fermentative pathways.

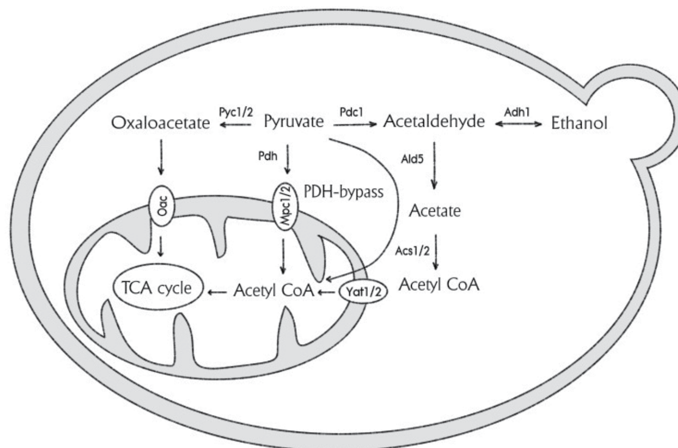

Figure 2. Pyruvate formed in glycolysis alternative metabolic routes. Pyruvate can be converted into 2 intermediates of TCA cycle: acetyl–CoA by the pyruvate dehydrogenase complex (Pdhp) and transported to the mitochondria by mitochondrial oxaloacetate carrier (Oacp); and/or oxaloacetate by pyruvate carboxylase (Pyc1p/2p) whose mitochondrial carrier is (Mpc1p/2p). Pyruvate can also be decarboxylated to give acetaldehyde by the pyruvate decarboxylase (Pdc1p). Adh1p - alcohol dehydrogenase; Ald5p - acetaldehyde dehydrogenase; Acs1p/2p - acetyl–CoA synthase; Yat1p/2p - carnitine acetyltransferase (adapted from [9]).

In alcoholic fermentation, pyruvate is decarboxylated to give acetaldehyde and CO_2, by the pyruvate decarboxylase (Pdc1p). In the final reaction, catalysed by the alcohol dehydrogenase (Adhp), acetaldehyde is reduced yielding the ethanol and promoting the re-oxidation of NADH to NAD^+. At the same time, and in addition to the 2 molecules of CO_2 and of ethanol, formed per molecule of glucose, the sugar is incorporated into other by-products such as yeast biomass, acids (pyruvic, acetaldehyde, ketoglutaric, lactic) and also importantly glycerol. This is generated from dihydroxyacetone-phosphate and is, to a certain extent, very desired by the wine producers in order to get fuller bodied wines (discussed below). Furthermore, alcoholic fermentation is a redox-neutral process; given that the NADH produced during the oxidation of glyceraldehyde 3-phosphate is afterwards reoxidized in the reduction of acetaldehyde to ethanol [13]. Yet, one must keep in mind that with fermentation is associated culture growth and, biomass composition is more oxidized than glucose, consequently an excess of reducing equivalents may be attained. The way yeast circumvent this problem, under anaerobic conditions, consists on the production of glycerol by reduction of the glycolytic intermediate dihydroxyacetone phosphate to glycerol 3-phosphate catalysed by NAD^+-dependent glycerol 3-phosphate dehydrogenase (encoded by the two isogenes *GPD*1 and *GPD*2), and its subsequent dephosphorylation due to the action of glycerol 3-phosphatase (encoded by *GPP*1 and *GPP*2) [14-16].

Although fermentation usually happens in the absence of oxygen, this is not a strict rule. Even in the presence of high levels of oxygen, if the sugars are fully accessible to be metabolized, yeasts choose to ferment instead of respire. This phenomenon is called the Crabtree effect [17], defined as the inhibition of aerobic metabolism when glucose is available, which occurs both in the presence or absence of oxygen. For instance, *S. cerevisiae* is known as Crabtree negative yeast, since is able to produce ethanol aerobically in the presence of high external glucose concentrations. These high concentrations promote the acceleration of glycolysis, producing appreciable amounts of ATP through substrate-level phosphorylation. Simultaneously, it reduces the need of oxidative phosphorylation done by the TCA cycle via the electron transport chain, inhibits respiration and ATP synthesis, and therefore decreases oxygen consumption. Conversely, Crabtree negative yeasts produce biomass via TCA cycle, but these should not be mystified with obligate aerobes: Crabtree-negative yeasts are able to ferment, yet usually only ferment under anaerobic conditions, since there is no inhibition of aerobic respiration in the presence of glucose and this is a more efficient form of energy metabolism. Obligate aerobe yeasts, on the other hand, cannot ferment and only respire aerobically, providing another category of metabolic diversity. Moreover, Crabtree effect is not specific to yeasts: many mammalian tumour cells display a Crabtree effect as well [18-20].

In **aerobic respiration** (Figure 3), the pyruvate is converted to Acetyl-CoA due to an oxidative decarboxylation, catalysed by the pyruvate dehydrogenase multi enzyme complex. In this way starts TCA cycle, which major issue is to supply the respiratory chain with reducing equivalents (in form of NADH and $FADH_2$) obtained from the oxidative decarboxylation of Acetyl-CoA, which is then used to generate energy through the highly conserved electron transport chain. Moreover TCA cycle also has anabolic functions, almost all intermediates

are utilized in other metabolic reactions; exception is made to isocitrate, including the synthesis of amino acids and nucleotides (for reviews see [9, 21]).

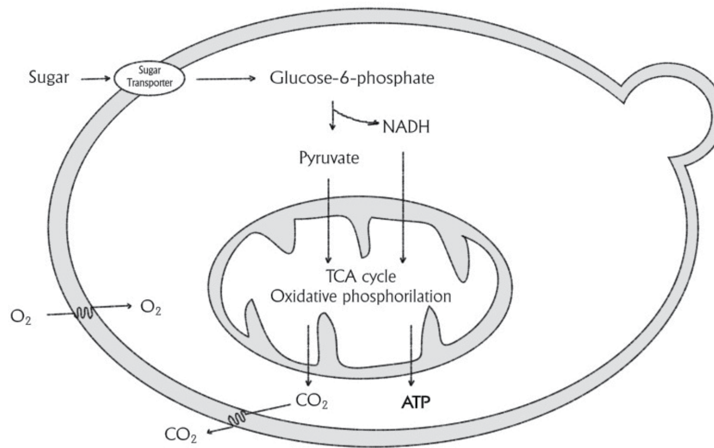

Figure 3. Aerobic respiration in *S. cerevisiae* (adapted from [22]).

2. *S. cerevisiae*, the party starter

Beverages with an alcoholic content are largely consumed by mankind since ancient times. Such beverages made from fermentation of sugar-rich goods, namely cereals and fruits, are present in oldest records [23]. Beer, made from germinated barley, and wine, produced from grapes, are among the most popular and their worldwide consumption is second only to non-alcoholic drinks as water, tea and coffee [24].

Wine and beer history is hand to hand with human civilization history, as most likely only the agriculture advent and the establishment of permanent settlements provided the conditions for its production. Nevertheless, wine and beer are most probably result of an "accident", as some harvested grapes were not consumed rapidly enough or some cereal wet pulp was left aside, and *S. cerevisiae* took advantage of the sugary free meal. The result should have been pleasant enough, especially the mild psychotropic effect, therefore the early farmers must have tried to repeat such "accident". The high ethyl alcohol content of this beverage and its analgesic, disinfectant and conservative properties contributed to a widespread utilization as a drug. Hence, our successful partnership with this yeast began.

In fact, our relationship with *S. cerevisiae* can be traced back as far as 7,000 years ago in China, with the first fermented beverages similar to beer, and Mesopotamia, with the first wines as well as domesticated vines [11, 25]. Conversely, the oldest known written account on the production of beer was found in Sumeria, modern day Iraq, in a stone tablet dating from 2,000 BC. This tablet, called "Hymn to Ninkasi", describes the production of beer and its es-

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pecially relevant role in religious ceremonies to Ninkasi, the Sumerian Goddess of beer [26]. Babylonians succeeded the Sumerians and kept producing the fermented beverage. They became so skilful at this art that at least 20 different brews were produced and exported, to as far as Egypt, at the zenith of Babylonian empire. Egyptians were so adept of this "imported beer" that they started producing their own from unbaked dough and even created a special hieroglyph for this new craft. Furthermore, records show that pyramid workers were paid in beer, a readily storable merchandise, and Pharaohs were entombed with model breweries to ensure an afterlife beer supply [26, 27]. Beer popularity grew and this beverage spread for the entire Europe, especially in the Mediterranean region [28].

Wine was also very popular in the ancient cultures, with references to this beverage in religious ceremonies of Egypt and Phoenicia. Pharaohs tombs were frequently adorned with vintage scenes and jars filled with wine accompanied the Kings afterlife [11, 23]. Wine consumption spread with the rise of the Greek and Roman Empires. Under the Greek and Roman influence, wine earned the status of "Civilized" drink, becoming very popular with the Empire upper classes, and beer was labelled as a "Barbarian" drink. Wine production and vine cultivation spread across Europe and replaced beer as the main drink in many countries. Some of these are still nowadays associated with wine production like Portugal, Spain and France. The production and consumption of beer continued mainly in northern borders of the Roman Empire, where Germanic tribes ruled and Roman influence was weaker [26, 28].

In Middle Ages, wine and beer production gained a new impetus with the shift from the familiar production to a more centralized production in monasteries [28]. Such happened because, at the time, water was frequently polluted, so alcoholic beverages were safer than water for the monks' consumption. Additionally, during the long fasting periods that the monks subjected themselves, the drinking of these highly nutritious beverages became common to satisfy hunger. This happened because wine and beer were considered similar to water and didn't constitute a breach of fast. In fact, in some monasteries monks were allowed to drink up to 5 litres of beer per day [28]. Southern monasteries produced mainly wine, as the weather was warmer and suitable for vines, but in the north the colder weather was more fitting barley and wheat growth and therefore Northern monasteries were more devoted to beer production. Each monastery developed its own methodologies for wine and beer making, leading to new wines and new brews and to a great technical improvement. Later these products become a source of income for the monasteries.

From the sixteenth century, with the discovery of the New World by Portuguese and the Spanish explorers, wine and beer spread to new territories. Vines were introduced in Brazil by the Portuguese around 1500 [29], and in Africa by the Dutch around 1650. In the Australian continent and North America this happened later, around 1800.

In the nineteenth century, wine and beer making suffered probably the major scientific advances. Around 1860 Louis Pasteur, a name forever associated with wine and beer production, developed studies on the conservation of wine through a heating-cooling process later known as "pasteurization", showing that wine could be stored for longer periods after such treatment. Moreover, in 1870 Pasteur made known to the world the role of *S. cerevisiae* in the fermentation process. Later, in 1876 Pasteur conducted similar studies in beer in his work

"Études sur la bière". Such works were based in the first observations of yeast and bacteria by Antonie Van Leeuwenhoek in late seventeenth century [11, 26, 27]. Only almost a decade after it was isolated a pure yeast culture resulting from a single cell by Emil Christian Hansen at the Carlsberg brewery, Denmark and to that followed the name of several yeast species [30]. A few years later, based on Hansen's work, Hermann Müller-Thurgau introduced the notion of inoculating wine fermentations with pure yeast starter cultures [11].

The intensive study of this amazing microorganism and its role in fermentation showed the specificities of each yeast species and strains. The necessity of consistent properties and quality in different fermentations, both in brewing and winemaking, paved the way for the selection of the right yeast for the job. The quest for stable and improved yeast began.

2.1. The "Right" yeast for the job

S. cerevisiae, known as "Wine's Yeast" or "Brewer's Yeast" (and also as "Baker Yeast", see below), is the main responsible for some of the world's most important fermented beverages. However, brewing and winemaking have inherent differences: i) the culture medium, ii) the bioreactor and iii) the yeast starter culture.

The final product, either wine or beer, is greatly influenced by the sugar-rich fermentable broth, grape juice or malted cereals, with different composition in fermentable sugars and nitrogen sources. The progression of the fermentation is another very important aspect of winemaking and brewing, *e.g.,* the oxygen available, the temperature and pH variations during substrate consumption and ethanol and CO_2 production. But the most significant difference is the yeast starter culture, its physiological state, whether it is dried yeast or a fresh inoculum, how well it ferments the available sugars and resists to fermentation by-products and its ability to flocculate at the right moment.

All *S. cerevisiae* strains described so far are capable of fermenting sugars to ethanol, but centuries of partnership with mankind directed the yeast evolution. Such evolutionary pressure resulted in a selection of distinct yeast strains for different applications, to produce wine and beer you need "Wine's Yeast" and "Brewer's Yeast", respectively.

2.1.1. Yeast physiology

Beer is the denomination commonly attributed to a carbonated alcoholic beverage produced by fermentation of malted barley, while wine is made of the fermented juice of any of several types of grapes. However, there are as many different wines and beers as there are different producers, all with their unique character and flavour influenced by the selected ingredients, kind of fermentation and yeast selected.

As said, the choice of the ingredients greatly impacts the fermentation final product; usually beer is the product of malt, hops, water and yeast. Malt is the result of germinating and drying (kilning) barley, yet other cereals besides barley can be used to produce beer, as wheat and rye. Malt extract will provide the entirety of the carbohydrates and nitrogen to the fermentation process and as such, it will influence the final ethanol concentration as well as

colour and flavour development. Conversely, another important aspect is the intervention of hop, the female flower cluster of *Humulus lupulus*, which acts as bacteriostatic agent against Gram-positive bacteria, helping to control unwanted microorganism during brewing. It also functions as bittering agent, disguising beer natural sweet taste [31, 32]. In winemaking, the maceration of grapes is the starting point to wine production. The variety of grapevines, as well as the weather and cultivation/soil conditions, greatly influences the wine final properties. In fact, the environment has such influence that some type of wines can only be produced in certain regions, like the Porto wine in Douro region, Portugal, and Champagne wine in Champagne region, France.

Brewer's yeast can be distinguished in top fermenting and bottom fermenting yeasts, based in the position at which the fermentation occurs. This division accounts with the yeast flocculation behaviour, and it is such an important element of brewing that defines the two main classes: **ale beers** (top fermenting) and **lager beers** (bottom fermenting). Such categories were devised as soon as the first pure yeast culture was isolated. Hansen was able to purify two different species, a top fermenting appropriate for ale brewing, *S. cerevisiae*, and a bottom fermenting, *S. carlsbergensis*, suitable for lager beer [26]. Such taxonomic classification was reviewed several times [1], and the top fermenting yeasts are now included in the *S. cerevisiae* and *S. bayanus* species and the bottom fermenting yeasts fit to the *S. pastorianus* species, all belonging to the *Saccharomyces sensu stricto* genus [27, 33].

These brewer's yeasts present several differences in their genomes. **Lager yeast** strains present complex polyploidy genomes, with evidences of contribution from distinct *Saccharomyces* species [33]. Usually these complex genomes are tetraploid, which may result from the fusion of diploid parental strains or from duplication of the genetic information after the original cell fusion. Analysis on lager yeast genomes revealed other changes such as chromosome loss and/or duplications, likely due to human selection of relevant phenotypes [33, 34]. In reference [34] lager yeast genomes were analysed and classified in two groups. In group I, cells present one *S. cerevisiae* genome equivalent and, in group II, cells present two *S. cerevisiae* genome equivalents. Both groups exhibit one *S. bayanus* genome equivalent and the remaining genome was mostly hybrid chromosomes from both species. These different yeast must be related with the conditions they are exposed, meaning for instance to lager beer fermentation, yeast has to react to conditions inherent to beer production, as cropping and pitching, and to bottom fermentation specificities, *e.g.*, temperature of reaction [35].

As for **ale yeasts**, studies revealed that these strains are closely to *S. cerevisiae* [34, 36]. In fact, a genotype analysis of 651 *S. cerevisiae* strains revealed that ale strains were more closely related to wine and bread strains (above referred as Baker's yeast), than to lager brewer's yeast strains [36]. Reports of hybrids in ale yeasts showed that strains traditionally classified as *S. cerevisiae* may indeed be the result of hybridization events [37]. Ale beer has less representation in worldwide markets, and as a consequence less studies and information are available on the corresponding yeasts. As such, the considerations on beer yeast physiology will be focused on lager strains.

In winemaking, most wine yeasts belong to *S. cerevisiae* species, but *S. bayanus* has also been detected. Yet, wine fermentations also present yeast, derived from vineyard environment,

belonging to the genera *Candida*, *Debaryomyces* and *Brettanomyces*. But, yeast is mainly selected for its resistance to ethanol, favouring *S. cerevisiae*. There is also selection for capacity to float or to flocculate, important for some specific wines. While in most wines the ability to flocculate is important to improve the filtration, in some wines such as sherry wine, the formation of a floating film is vital. This *vellum* is formed at the surface of the wine and promotes oxidative metabolism. Sherry wine is characterized by high ethanol content and low aldehyde. Its featured nutty flavour can be ascribed to partial oxidation of ethanol to acetaldehyde [11].

The utilization of dried yeast as a starter culture is very common in the wine industry. Cells are dehydrated through a cycle of filtrations and centrifugation to remove external water and then submitted to streams of dehumidified hot air. Such procedure can reduce yeast cells' content in water to as low as 6%. However, even though yeast cells can survive such treatment, it causes cellular damage. Damages to cell wall and plasma membranes caused by the changes on cell size and shape, as well as damages to proteins produced by free radicals were reported [35].

One of beer brewing specificities is the utilization of a freshly grown starter culture. In one hand, it ensures a healthy population fully adapted to growth medium. Cells are usually collected at the late exponential phase, preventing a large percentage of aged cells, and at the same time ensuring metabolic fitness. On the other hand, it meets the requirement for flavour consistency of the final product, even though it is more expensive than the alternatives. The pattern of metabolic products of yeast is highly dependent on its growth conditions, and cells fully adapted to *wort* produce a more consistent flavour. The first batches inoculated with dried yeast are often of inferior quality, with by-products of fermentation conferring off-flavours, which compromises the regularity of a brand product [30]. An alternative that meets the requirements of fresh grown cells and flavour consistency, but at the same time reduces the process duration and costs, is the fed-batch technology. Cells are kept in late exponential phase by leaving a certain amount of yeast in the reactor and adding fresh *wort*, shortening the fermentation time and maintaining the beers properties [38].

During fermentation, yeast is constantly facing new pressures. The high osmotic stress due to the sugar high content of *wort* and *must* is just the beginning. *Wort* is a rich and complex medium, composed of carbohydrates (90%), nitrogen sources (5%) and small amounts of inorganic ions, lipids and polyphenols. *Wort* composition, being highly dependable of the quality of the cereal and the process used to malt it, is usually enriched in fermentable sucrose (5%), monosaccharides (10%), maltotriose (15%) and maltose (50%). About 20-30% of total carbohydrates are non-fermentable dextrins, polysaccharides result from starch degradation [35]. On the other hand, grape juice is rich in fructose and glucose, presenting small amounts of sucrose. Grape variety influence the ratio glucose/fructose, Chardonnay is a high fructose variety, whereas Zinfandel is regarded as high glucose variety. Such high-gravity *wort*s, 12-18 g of extract per 100 mL, subject cells to high osmotic pressure. The production of compatible solutes, as glycerol and trehalose, and a "robust" plasma membrane composition seem to be the main adaptations to withstand such stress. The cells fully adapted to *wort* used to inoculate (pitch) fermentations are important to avoid extended lag phases where

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cells are adapting its physiology. In wine the use of active dried yeast (ADY) is common and no effect on fermentation time was reported [39].

Nitrogen assimilation is especially important in flavour development. The main sources of nitrogen are free amino acids and ammonium ions, which are used by the cell for protein formation [35, 40]. Such amino acids are also relevant for the production of alcohols and esters, important in these beverages flavour. During fermentation, amino acids are always used following a certain order, independent from the fermentation conditions. Group A, including arginine, asparagine, aspartate, glutamate, glutamine, lysine, serine and threonine, are used first. Group B amino acids are utilized slowly and include histidine, isoleucine, leucine, methionine and valine. Group C is composed by alanine, glycine, phenylalanine, tyrosine, tryptophan, and are only absorbed after the complete exhaustion of group A. Group D is composed of proline, which require an aerobic metabolism for its uptake and it is poorly used during fermentations [40].

In winemaking, fermentations are usually developed under anaerobic conditions, but it is common in brewing to oxygenate the *wort*. So another important stressor is the dissolved oxygen, which may lead to the formation of reactive oxygen species (ROS). ROS, as hydrogen peroxide or superoxide radical, can promote damages in cell main constituents, DNA, proteins and lipids. However, oxygen is very important for the synthesis of sterols and unsaturated fatty acids ensuring the physiological fitness for cell replication. The control of dissolved oxygen is vital to ensure a healthy population. An important problem is the excessive growth of yeast cells when exposed to high amounts of dissolved oxygen, at the expenses of ethanol production [41].

The inorganic ions are necessary, but at nanomolar concentrations. These trace elements, as calcium, zinc or copper, are mainly required as cofactors of enzymes or in the flocculation process. For instance, the response to oxidative stress is dependent on enzymes such as the different superoxide dismutase isoforms that require manganese, zinc or copper [32]. On the other hand, calcium is vital for the flocculation advance [42]. Conversely, insufficient amounts of such elements can lead to cellular damage and stress, and consequent stuck fermentations.

The use of antimicrobials in vineyards is common to control fungi that spoil grapes. But, when grapes are macerated these compounds are incorporated into the juice. Even though they may help to prevent the wine oxidation and microbial spoilage, a concentration to high may lead to off-flavours and in worst case, yeast death. So antimicrobials, especially sulphur dioxide, are an important stress to yeast during fermentation. Commercially available yeast also has to deal with toxins produced by wild yeasts derived from the vineyards. These toxins are produced to give those wild yeast advantages over others species in accessing to the nutrients. Isolation of strains resistant to both antimicrobials and natural toxins is an important research field [11, 43].

Certain compounds are extremely important for brewing and wine making not as substrates but as by-products. Such metabolites greatly influence the final product's colour and flavour, as well as its stability. In fact, the importance of these compounds is such that lager

beers are usually stored from several days to weeks, lagering, solely to remove diacetyl, an off-flavour causing metabolite. This time consuming maturation phase consists in a second fermentation at low temperature to eliminate the butter-like flavour caused by this vicinal diketone. Studies are being conducted in order to minimize this metabolite formation and reduce the maturation time [30]. Sulphur containing compounds are other family of by-products receiving great attention. Such group comprises sulphite, sulphide and dimethyl sulphide, and while sulphite is a beneficial and flavour stabilizing metabolite, the remaining compounds are responsible for off-flavours. The equilibrium of such compounds formation could lead to better wine and beer and shorter fermentations [44].

Ethanol is one of the most important by-products of beer fermentation. Nevertheless, it represents an important stressor for yeast cells due to its high toxicity. Ethanol concentration can reach 10% in higher gravity fermentations, and acts especially upon biological membranes [35]. Reports showed ethanol effects in growth inhibition [45], lipid modification and loss of proton motive force across the membrane and increased membrane permeability/ fluidity [46]. Yet, cells exposed to oxygen, with high levels of sterols in membranes, and adequate levels of nutrients, amino acids and trace elements in the fermentation broth are able to respond efficiently to such effects [35].

Nutritional stress occurs at the end of fermentation and cells enter stationary phase. This occurs because fermentable carbon sources tend to be depleted, and cells have to change their metabolism from fermentative to respiratory (explained in section 1), entering in a quiescent state [30]. Such phenomenon induces flocculation, a cell-cell interaction process dependent of lectins and calcium that promotes sedimentation. Flocculation in turn is influenced by several other factors besides nutrient depletion. Reports showed the influence of ethanol content, calcium concentration, pH changes, oxygen concentration and temperature [47]. The onset of flocculation is an important area of interest in brewing. If flocculation occurs to soon, stuck fermentation may occur, which results in a high sugar and low ethanol content. If, on the other hand, happens in a later stage, it has a high impact in beer filtration as most cells tend to be kept in suspension.

Fermentation is the most yeast-dependent phase of these alcoholic beverages production, but yeast also interferes with others proceedings. The metabolic fitness of the starter culture, the storage and maintenance of both dried and fresh yeasts, and the storage of the products submit yeast to different conditions to which they have to respond/adapt. To obtain detailed information on such processes please see reviews [35] and [43].

2.2. Old beverages, new solutions

Wine and beer industrial production led to a demand for better and more efficient yeast. Yeasts with improved utilization of substrates, carbohydrates and nitrogen, and consistent flocculent behaviour, as well as high fermentative capacity and high ethanol production are the industry goal. Enhancing beer and wine flavour through modification of by-products formation is another field of intensive research [30, 39]. As it is the improvement of the fermentation process, through encapsulation/immobilization of yeast [48].

Large collections of yeast were assembled, as the Centraalbureau voor Schimmelcultures (CBS) collection, in The Netherlands, and the Carlsberg collection, in Denmark. Manipulation of these strains to improve wine and beer properties has been performed in several ways, from spores manoeuvring and natural mutants' survey to genetic engineering (GE). A rather recent and extensive review in strategies for the improvement of *S. cerevisiae* industrial strains can be found in [2]. As referred above, an efficient utilization of substrates by yeast during fermentation is extremely important for wine and beer industries. Such efficiency will yield higher amounts of by-products, as ethanol, and reduce the fermentation time. Furthermore, glucose repression on other sugars and consumption of unusual nitrogen sources are vital research areas [30].

The presence of glucose, even in small amounts, represses the simultaneous uptake and consumption of several sugars, namely maltose and galactose. Maltose (50%), maltotriose (15%) and sucrose (5%) are the main sugars in *wort* under glucose repression. Such repression leads to late fermentation of these sugars and slower fermentations, as their utilization is dependent on glucose depletion. This process is controlled at the transcriptional level, through the action of the proteins Mig1p, Ssn6p and Tup1p. Mig1p, a zinc finger protein, binds to specific sequences in the promoter region of the glucose-repressed genes and recruits the SSN6-TUP1 complex, the responsible for the actual repression [49]. Mig1p binding sites were found in genes associated with the utilization of sucrose (*SUC*2), maltose (*MALR*, *MALS* and *MALT*) and galactose (*GAL*1-5) [50]. Therefore, *MIG1* presents itself as a potential target to improve yeast sugar consumption.

Conversely, in sucrose metabolism, glucose repression addresses the sucrose conversion in fructose and glucose under the action of Suc2p. Studies showed that the disruption of *MIG*1 lessen the glucose repression on the transcription of this excreted invertase in both lab and industrial strains. Therefore, the lag in sucrose utilization was greatly diminished. Besides Mig1p, another zinc finger protein, Mig2p, was associated with glucose repression of *SUC*2 [51]. The interruption of both *MIG*1 and *MIG*2, in *S. cerevisiae* strains led a high sucrose metabolism in the presence of high glucose concentrations [52].

Maltose metabolism is more complex than sucrose, as it responds to both glucose repression and maltose induction. Maltose induction is under the influence of the locus *MAL*, a closely integrated group of genes. This sugar presence induces *MALR*, a transcription factor, which in turn will induce *MALT*, coding for a maltose permease, and *MALS*, coding for a maltase [30]. Up to 5 different *loci* have been detected in *S. cerevisiae* industrial strains; still haploid lab strains present a single *locus*. In both situations these *loci* are under repression of Mig1p [53]. However, disruption of *MIG*1 in industrial strains only alleviated the sucrose metabolism [50], but didn't cause any effect regarding maltose metabolism. Even though, in haploid lab strains *MIG*1 disruption lifted the glucose repression; the same has not happen in the polyploid strains, presenting multiple *loci*. Complex regulation between the different genes must be under way and most probably not solely controlled by Mig1p [53].

Finally, maltotriose, a glucose tri-saccharide, is the second most abundant sugar in *wort* (15%). This is under similar regulation by the presence of glucose and maltose, therefore most studies have focused in an efficient uptake of this carbohydrate [54]. Those works

showed that overexpression of maltotriose transporters lead to positive effects on its metabolism [54, 55].

In winemaking and brewing, where flavour has such importance, amino acids metabolism has a notorious place. As said, amino acids are involved in formation of higher alcohols and esters that significantly contribute to beer and wine flavour. Since yeast cannot hydrolyse *must* and *wort* proteins, it depends on the available ammonium and amino acids in solution [11, 30]. However, the predominant amino acid in both *must* and *wort*, proline, is the less assimilated [40]. As such, improvements in yeast ability to uptake this amino acid has been attempted. Efficient proline uptake was reported in lager beer yeast expressing a mutagenized proline permease, Put4p. The site-directed mutagenesis stabilized the permease and enhanced amino acid utilization without affecting the beer quality [56]. The study of the same problematic in winemaking led to the disruption of *URE*1, a repressor of permease encoding *PUT*1 and pyrroline-5-carboxylate dehydrogenase *PUT*2, with significant improvements in fermentation rate and vigour described [57].

Flocculation is a phenotype of industrial interest. It facilitates the filtration process in the end of fermentation, saving both time and money. In the case of brewing, it also serves the cropping (recover of part of the yeast population of the fermentation to pitch the next). Flocculation is a reversible aggregation of cells, where lectins recognize sugar residues in neighbour cells. Two industrially relevant flocculation phenotypes are well-known, Flo1 and NewFlo. Both are under the control of *FLO* genes, Flo1 phenotype is repressed by mannose and NewFlo by mannose, glucose and sucrose. Almost every industrial strain is NewFlo, associated with *FLO*10 [30]. The main approach to improve these phenotypes is to put *FLO* genes under a promoter active only in stationary phase. Promoters of *HSP26* and *HSP30* were proven as the most suitable for induction at this late growth phase in lab strains [30].

The control of by-products production in order to improve wine and beer organoleptic properties is an expanding research area. The production of glycerol, to improve wine and beer's fullness, as well as sulphite, to improve stability, and the reduction in diacetyl and sulphide content are the main targets. Glycerol, as the second fermentation metabolite, is rather important to wine and beer; the increase of its concentration to improve these beverages sensory character is an active field. Overexpression of *GPD*1, encoding glycerol-3-phophate dehydrogenase, is the main approach. However, this change resulted in a redox imbalance with increased production of unwanted metabolites [39]. This point has been fully discussed in [2].

The presence of sulphite, an antioxidant and flavour stabilizer, and reduction of off-flavour producing sulphide is another important problem addressed by the industry. Both these goals can be achieved at the same time with the directed mutagenesis of NADPH-dependent sulphite reductase, an important enzyme in sulphur–containing amino acids synthesis. This strategy lowered this enzyme activity and increased the amount of sulphite in wine while reducing the sulphide presence in wine [58].

The reduction of diacetyl has special importance, as the maturation time (lagering) is directly dependent on this compound concentration. The expression of the bacterial enzyme ace-

tolactate decarboxylase (ALDC) in yeast is the main approach to reduce the amounts of this compound. ALDC catalyses the reaction of α-acetolactate to acetoin, preventing the formation of diacetyl. However, after heterologous expression of ALDC, the yeast became auxotrophic for some amino acids and the growth rate was very low in *wort*. An alternative approach was the interruption of *ILV*2, encoding acetolactate synthase. Such strategy also resulted in an auxotrophic strain with slow growth. The search for natural mutants in *ILV*2 with an appropriated growth rate is now the major strategy [30].

Improvement of yeast to render fermentations faster and cheaper is an industry goal, but the enhancing of the fermentation process itself is another alternative. The fed-batch technology has already proved its benefits [38], and improvements of such process with yeast immobilization/encapsulation are now under the spotlight. This results in much faster fermentation rates as compared to the existing free cell fermentations. However, it has some disadvantages, such as: i) complexity of production process including the choice of the suitable carrier materials, ii) bioreactors design, iii) fine-tuning of the flavour formation during fermentation processes, and iv) cost constraints [59].

3. Baker's yeast – Magic on bread making

The process of bread making relies on the fermentation carried out by a mixture of yeast and bacteria. Even when all this was unknown and the flour leavening seen as "magic", bread was already produced and extensively consumed. On those ancient times, the leavening resulted presumably due to the action (fermentation) of the natural microbial contaminants of flour or dough ingredients. This was obviously not a controlled process, yet with the practice of maintaining a fresh inoculum from one preparation to the next, promoted the selection of yeast and bacteria biodiversity. Nowadays, some types of bread are still prepared in this fashion, sourdoughs are one example (for a review see [2]), but the baking industry moved for the use of commercially baker's yeast, typically the strain *S. cerevisiae*, for the bread production. And, while the flour types, geographical origin and mixtures introduce organoleptic differences in bread, the globalization of commercial baker's yeast market decreased worldwide bread diversity (for a review see [2]).

3.1. Commercial baker's yeast production – The break of spell

Commercial baker's yeast is produced in several forms in order to meet specific requirements of climate, technology, methodology, transportation, storage and final product. As with all biotechnology processes, this is in constant development/undergoing research not only to optimize the process technology and its components, but as to produce faster growing strains with the characteristics to deliver better quality end products.

Molasses (beet and cane molasses), the common carbon and energy source used in the production of baker's yeast, is a by-product of sugar refining industries, therefore cheaper than the formerly used cereals grain. Furthermore the sugars present on those molasses (around 50%), consisting on a mixture of sucrose, fructose and glucose, are ready to be

fermented by the yeast. In order to obtain the proper broth for the optimum yeast biomass yield; the mixture of molasses has to be supplemented with nitrogen sources, minerals, salts and vitamins [60, 61].

After the preparation and sterilization of the broth, the production of baker yeast can take place. It begins by the inoculation of a small closed test flask containing the prepared sterilized broth with a pure yeast culture. The growth is allowed and careful screened, and when the culture reaches an elevated density, it is transferred to larger vessels and supplied with more broth, fed-batch reactors. This scale-up process continues until a desirable biomass quantity is attained, the so-called commercial starter, able to inoculate industrial fermenters/ reactors, which production ranges from 40,000 to 200,000L [62].

The entire fermentation process of baker's yeast has to be directed towards maximum biomass production; by-products such as ethanol are not desired. As we saw in section 1.1.1, in anaerobic dissimilation of sugars (alcoholic fermentation) the ATP yield is quite low comparing with respiratory dissimilation, affecting drastically the biomass yield. The way to avoid anaerobic ethanol production is the use of the mentioned fed-batch reactors, in which is possible to control the specific growth rate and sugar concentration by controlling the fed of reactors with fresh broth [63].

Nowadays, during the industrial large reactors the addition of nutrients and regulation of pH, temperature and airflow are carefully monitored and controlled by computer systems during the entire production process. In this way, the tones of baker's yeast obtained in the end of the fermentation have the same quality/characteristics/properties as the original pure yeast culture that started the process. These tones of yeast are suspended in a large amount of water, resulting in a creamy suspension of active yeast, being necessary the so-called **downstream processes** to obtain the concentrated yeast [64, 65]. At the end of the fermentation, the yeast culture is concentrated using a series of combined centrifugation and washing steps, into a yeast cream with concentration of approximately 20%. The yeast is then cooled to approximately 4°C, and stored. It can be sold in this form – **Cream Yeast**- however is quite expensive due to the manipulations required because of high content in water. Cream yeast can be further processed, compressed or dried originating the **Granular Yeast** or **Instant Dried Yeast**, if then converted in small granules, or **Cake Yeast** or **Active Dry Yeast**, if as an alternative the dried yeast is extruded or cut into blocks/cakes. All these yeast types are then packaged, typically vacuum packed to reduce the risk of contamination, and distributed to wholesalers or traders. The shelf life of Active Dry/Cake Yeast and Instant Dry/ Granular Yeast at ambient temperature is 1 to 2 years.

3.2. Idol baker's yeast

Yeast has a significant role on bread making, greatly influencing the final product properties. The most important contribution is in the leavening phase; after the dough has been kneaded and the gluten network start to develop, yeast starts to consume the available fermentable sugars and to produce ethanol and CO_2, as mentioned in section 1. As fermentation occurs, the dough is gradually depleted of O_2 present in the air bubbles trapped in the dough, leaving small bubble *nuclei* full of N_2. As the metabolism is more and more stimulat-

ed, with incubation at optimal temperatures, the CO_2 starts to saturate the liquid phase of the dough and starts to accumulate in the bubble *nuclei* [66]. This leads to dough rising provided that a mature gluten network, capable of ensure the dough foam-like structure, is formed. The amount of time such process occurs will influence dough gumminess and rheology, as well as crust colour, crumb texture, and firmness of the bread (reviewed by [2, 3]). The amount of sugars present in the dough that are actually fermented, as well as the efficient secretion of enzymes as invertase, responsible for the conversion of sucrose to glucose and fructose, has a great impact in the flavour characteristics of the bread. The action of several enzymes, namely proteases, lecithinases, lipases α-glucosidase and β-fructosidase, leads to different utilization of the dough substrates. In bread making, the flavour is also greatly influenced by the metabolic by-products of the yeast fermentation, and while the most important by-product of yeast metabolism is certainly the CO_2, the production of metabolites such as alcohols, esters, and carbonyl compounds have also a deep impact. More than 300 volatile compounds associated with bread's flavour and aroma, are produced by yeast. Although, some are dependent more on the substrates, the vast majority of such compounds are yeast dependent, introducing an important variability to bread [67].

As mentioned, the common procedure of bread making today, at least in developed countries, consists of using this commercial baker's yeast. Its quality/individuality depends on storage stability, osmotolerance and freeze-thaw resistance. Considerable efforts have been made to obtain the Idol Yeast, including evolutionary engineering, genetic engineering (mainly to provide yeast with high capacity to tolerate freeze-thaw treatments). Yet, there is still considerable space for improvement. Those several strategies to achieve the Idol Yeast has been thoroughly revised and discussed in a previous work from the beginning of this year [2] as well as on [3, 68].

4. Yeast à la Carte

Fresh *S. cerevisiae* consists of approximately 30–33% of dry materials, 6.5–9.3% of nitrogen, 40.6–58.0% of proteins, 35.0–45.0% of carbohydrates, 4.0–6.0% of lipids, 5.0–7.5% of minerals and various amounts of vitamins, depending on its growth conditions [69]. So, today yeasts are acquiring increasingly more attention for other uses, besides the production of alcoholic beverages and the baling industry. The products of modern yeast biotechnology form the backbone of many commercially important sectors, including functional foods (for animals, fish and humans), health food supplements, including additives, conditioners and flavouring agents, as pharmaceutical products, for the production of microbiology media and extracts, as well as livestock feed or even as agents of detoxifying effluents containing heavy metals [70, 71].

4.1. Yeast treats for animals

The pioneering research conducted almost a century ago by Max Delbrück and his colleagues was the first to highlight the value of surplus brewer's yeast as a feeding supplement

for animals [72]. Yeasts have been fed to animals for more than a hundred years, either in the form of yeast fermented mash produced on the farm, yeast by-products from breweries or distilleries, or commercial yeast products specifically produced for animal feeding. In animals, including pets, this practice is used to compensate for the amino acid and vitamin deficiencies of cereals [73, 74], and in fish as a substitute for other ingredients [71].

Brewer's yeast biomass, as described above, which results from the cultivation of *S. cerevisiae* on malted barley, separated after the *wort* fermentation, debittered and then dried, is the second major by-product from brewing industry, just after the brewer spent grain [73]. This biomass is an excellent source of proteins, peptides and amino acids, vitamins (especially of B-group: B1, B2, PP, B5, B6, B8, B12), minerals and trace elements (calcium, phosphorus, potassium, magnesium, copper, iron, zinc, manganese, chromium, selenium), carbohydrates (glucans and mannans), as well as phospholipids [75]. The winemaking industry also generates a huge amount of microbial biomass - **leeds**. Yet, this incorporates the yeasts (that die due to nutrient depletion) but also other microorganisms, suspended solids, colloids, and organic matter, and have been shown to display quite low nutritional value to be considered for use as a supplement in animal feed [76].

Those yeast used for monogastrics food or feeding rations is generally inactivated because feeding of live yeast might cause avitaminosis due to the depletion of B-vitamins in the intestine [77]. They can also cause adverse fermentation in the digestive tract of swine leading to diarrhoea and bloating [75]. Yeasts can be killed through application of heat or using chemicals. High temperature destroys the yeast membrane, but does not necessarily inactivate all yeast enzymes, unless quite elevated temperatures are applied. Alternatively, there are the chemical treatments with propionic acid or formic acid, which also act as a preservatives for yeast, and contribute to the feed value of the yeast [73].

Yeasts have been used in diets of numerous species with varying levels of success. Yeast for swines is sold for feed applications as wet slurry, as dried brewer's yeast, or in mixtures with other brewery by-products [73]. It is ideal for their feed as a good protein source, it contains most of the essential amino acids in adequate quantities, and numerous vitamins, selenium, copper, and phosphorus. Selenium concentrations are much higher in yeast than in soybean meal, and deficiency of this compound in the swine's alimentation has been the cause of higher swine mortality [78, 79]. Additionally, dried brewer's yeast contains mannan oligosaccharides, which have been reported to increase the growth performance and intestinal health of pigs [80]. Benefits have been described as well for nursing and weanling pigs [81].

Either live or inactivated brewer's yeast have been used as well in ruminants diets, consequently it was observed an increase in productivity of animal meat or milk [73, 82], but also live yeast cultures have been used. These are prepared by inoculating wet cereal grains or grain by-products with live yeast, partially fermenting the mash, and then drying the entire medium without killing yeast or destroying vitamins and enzymes [73]. Live yeast is reported to stimulate fermentation in the rumen through its ability to stimulate the development of anaerobic, cellulolytic and acid lactic bacteria fermentations. In addition, the ingestion of yeast offers continuous supply of vitamins, dicarboxylic acids, removal of oxygen, buffering effect, and reduction in the number of protozoa. As a result, there is an improvement of the

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digestion of the fibrous and cellulolytic portion of the diet, which leads to a greater intake of food and better performance [83, 84].

Dried yeast was used traditionally in poultry diets in the past as a source of aminoacids and micronutrients, and though the broiler growth was improving this practice was largely discontinued for economic reasons [77]. On the other hand, brewer's yeast appears to be especially beneficial for breeder turkeys and laying hens [85]. Reproductive improvements are attributed to the high level of dietary biotin and selenium in yeast, which is more beneficial than inorganic selenium added to poultry diets [86] and it contributes for the prevention of biotin deficiency in poultry diets, which may result in reduced feed conversion, low egg production, and poor hatchability [87]. Brewer's yeast is also very rich in folic acid, an important vitamin for turkeys [73].

Brewer's yeast has been recognized to have potential as well as a substitute for live food in the production of certain fish or as a potential replacement for fishmeal [88-90]. In addition, it has low content in phosphorous, meaning less water and environmental contamination than common fish meal and other plant-based alternate protein sources [91]. Multiple studies have demonstrated the immunostimulant properties of yeasts, such as their ability to enhance non-specific immune activity [92]. That reaction can be related to β-glucans, nucleic acids as well as mannan oligosaccharides [93]. Brewer's yeast may serve as an excellent health promoter for fish culture as even when administered for relatively long periods is able to enhance immune responses as well as growth of various fish species, without causing immunosuppression [94, 95]. Furthermore, the relative high levels of nucleic acid nitrogen present (mostly in the form of RNA) that in humans and most monogastric animals can became toxic if taken in excess, as the capacity of excretion of the uric acid formed is limited [96], in fish does not happen due to their very active liver uricase [97].

4.2. Human little treats, big benefits

In a world of rapidly increasing population and low agricultural production, yeasts are relatively cheap and easily produced on an industrial scale representing a sustainable alternate protein source to cover the population nutritional demands. The first time that yeast was cultivated in large scale for human nutritional use was in Germany during both World Wars [72]. The yeasts *S. cerevisiae* as baker yeast, *Candida utilis* as torula yeast, and *Kluyveromyces fragilis* as whey yeast, when produced on suitable, food grade substrates (*e.g.*, sugars, ethanol, and lactose), are permitted in many foods around the world [70]. Besides the alcoholic beverages and baking products referred above, yeast are used in the health food industry; as additives, conditioners, and flavouring agents; as sources of high nutritional value proteins, enzymes, nucleic acids, nucleotides, and cell wall polysaccharides [98] as well as for the production of food-grade yeast extracts and autolysates [69, 99]. Yeast extract from dried brewer's yeast cells can be used by enzymatic treatment in a wide variety of foods (*e.g.*, meat products, sauces and gravies, soups, chips and crackers) as flavours enhancers or potentiators [73, 100]. β-Glucan obtained from brewer's yeast can be used in food products as a thickening, water-holding, or oil-binding agent and emulsifying stabilizer [101]. The probiotic activity is an additional role of some yeast that is attracting increasing interest [102].

S. cerevisiae has been studied extensively for its medicinal properties and several beneficial/probiotic effects on human health and well-being have been reported, including prevention and treatment of intestinal diseases and immunomodulatory actions, are the most well-known. Probiotics are viable microorganisms that are beneficial to the host when consumed in appropriate quantities [103]. The probiotic properties of yeasts reported refer the ability to survive through the gastrointestinal tract and interact antagonistically with gastrointestinal pathogens. As described above, *S. cerevisiae* have been used as supplements to animal and fish feeds with reported improvements on the growth and health of the hosts [104]. Regarding humans, *S. cerevisiae* var. *boulardii* has been successfully used as an oral biotherapeutic agent to treat patients with severe cases of diarrhoea (*e.g.*, antibiotic-associated diarrhoea and traveller's diarrhoea) and other gastrointestinal disorders (*e.g.*, irritable bowel syndrome and Crohn's disease) [105]. Several studies have shown that *S. cerevisiae* var. *boulardii* confer beneficial effects against various enteric pathogens, involving different mechanisms as: i) prevention of bacterial adherence and translocation in the intestinal epithelial cells, ii) production of factors that neutralize bacterial toxins and iii) modulation of the host cell signalling pathway associated with pro-inflammatory response during bacterial infection [106]. As reviewed in detail in [106] prevention of bacterial adherence and translocation in the intestinal epithelial cells is due to the fact that the cell wall of *S. cerevisiae* var. *boulardii* has the ability to bind enteropathogens, which results in a decrease of their adherence to host epithelial cells. This yeast also produces proteins that are responsible for degradation, neutralisation or dephosphorylation of bacterial toxins. Moreover, the mechanism by which *S. cerevisiae* var. *boulardii* modifies host cell signalling pathways associated with pro-inflammatory response is based on blocking activation of nuclear factor-kappa B (NF-κB) and mitogen activated protein kinase (MAPK) which decreases the expression of inflammation-associated cytokines such as interleukin 8 (IL-8), tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ). Conversely, *S. cerevisiae* var. *boulardii* also stimulates the peroxisome proliferator-activated receptor gamma (PPAR-γ) expression in human colonocytes and reduces the response of human colon cells to pro-inflammatory cytokines. There are several studies indicating the stimulation of the host cell immunity, both innate and adaptive immunity, by yeast in response to pathogen infections. Furthermore, it has been shown that *S. cerevisiae* var. *boulardii* also has a role in the maintenance of epithelial barrier integrity; during bacterial infection the tight junctions are disrupted and this yeast enhances the ability of intestinal epithelial cells to restore the tight-junction structure and the barrier permeability [106].

The benefits from ingesting yeasts do not stop here, many other have been reported as: selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon [107], *e.g.*, fructooligosaccharides [108]; decreasing the serum cholesterol levels [109], in the treatment of diabetes (regulation of insulin levels) and chronic acne, reducing the appetite, for healthy hair and nails [70, 110, 111] and also for promoting the bioavailability of minerals through the hydrolysis of phytate, folate biofortification and detoxification of mycotoxins due to surface binding to the yeast cell wall [106]. Yet, still are some concerns about public health safety as cause of crescent reports associating the intake of yeast with cases of fungaemia [112, 113].

4.3. What the future holds

Since *S. cerevisiae* var. *boulardii* is recognised as a member of the species *S. cerevisiae*, it is most likely that also other strains within *S. cerevisiae* might show probiotics properties. So far, great efforts have been placed on utilising the probiotic effects of especially LAB, whereas rather limited emphasis has been placed on the beneficial effects offered by yeast. However, yeasts offer several advantages compared to LAB. They have a more diverse enzymatic profile and appear to have a more versatile effect on the immune system. They also provide protection against pathogenic bacteria and toxic compounds by surface binding and appear to be better suited for nutritional enrichment and delivery of bio-active molecules. Besides, yeast is much more robust than LAB and therefore easier to produce and to distribute, especially in less developed areas [106]. Furthermore and though there is still much room for improvement, also the encapsulation technology applied to probiotics has shown benefits, *e.g.*, *S. cerevisiae* var. *boulardii* in microspheres protect the yeast from destruction in the gastrointestinal tract and therefore increase intestinal delivery of the viable probiotic [114].

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Chapter 24

Valorisation of Cheese Whey, a By-Product from the Dairy Industry

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Additional information is available at the end of the chapter

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1. Introduction

Whey is the by-product of cheese or casein production, it is of relative importance in the dairy industry due to the large volumes produced and the nutritional composition. Worldwide whey production is estimated at around 180 to 190×10^6 ton/year; of this amount only 50% is processed [1]. Approximately 50% of worldwide cheese-whey (CW) production is treated and transformed into various food and feed products. About half of this amount is used directly in liquid form, 30% as powdered cheese-whey, 15% as lactose and its byproducts and the rest as cheese whey- protein concentrates [2].

A total of 40×10^6 tons/year of whey is produced in the European Union [3]; the annual surplus of whey is 13×10^6 tons, containing about 619,250 tons of lactose. Nowadays this surplus is not utilized for further production of lactose; consequently, whey disposal represents a serious problem from both an economical and an environmental point of view. On the contrary, recovery of whey components and/or use of whey as fermentation medium may be advantageous not only for the environment but also for a sustainable economy [4,5].

Whey contains more than half of the solids present in the original whole milk, including whey proteins (20% of the total protein) and most of the lactose, water-soluble vitamins and minerals. Consequently, whey can be considered a valuable by-product with several applications in the food and pharmaceutical industries.

From a valorization point of view, two different options in CW management can be considered: the first one is based on the application of technologies to recover valuable compounds such as proteins and lactose. Currently, valorization processes applied to CW constitute the preferential option to treat this by-product, only exceeded by the production of powdered CW. The second option relies on the application of fermentation processes to obtain value

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added products [6] such as: organic acids (e.g. lactic, succinic and propionic), single cell proteins and oils, biopolymers (enzymes, polyhydroxyalkanoates, exopolysaccharides) and bacteriocins. Sometimes whey permeate, obtained from ultrafiltration step, has been used as fermentation medium; in this case, both the management options are applied.

The ultrafiltration process produces a whey permeate rich in lactose (about 80% of the original lactose in milk) new technologies have been developed (using nanofiltration or reverse osmosis) for concentration of the lactose which can be applied in the sweet industry or in pharmaceutical fermentation procedures [7]. In addition to lactose, whey permeate containing other nutrients essential for microbial growth; so the possibility to use it as a fermentation medium to obtain high value products represents an interesting opportunity [4] which must not be neglected. Moreover, whey permeate is an attractive source of oligosaccharides for potential application in human nutrition [8].

Among the different possibility of whey valorization, reported in Figure 1, individual whey protein purification and application of fermentation technology on whey permeate will be discussed.

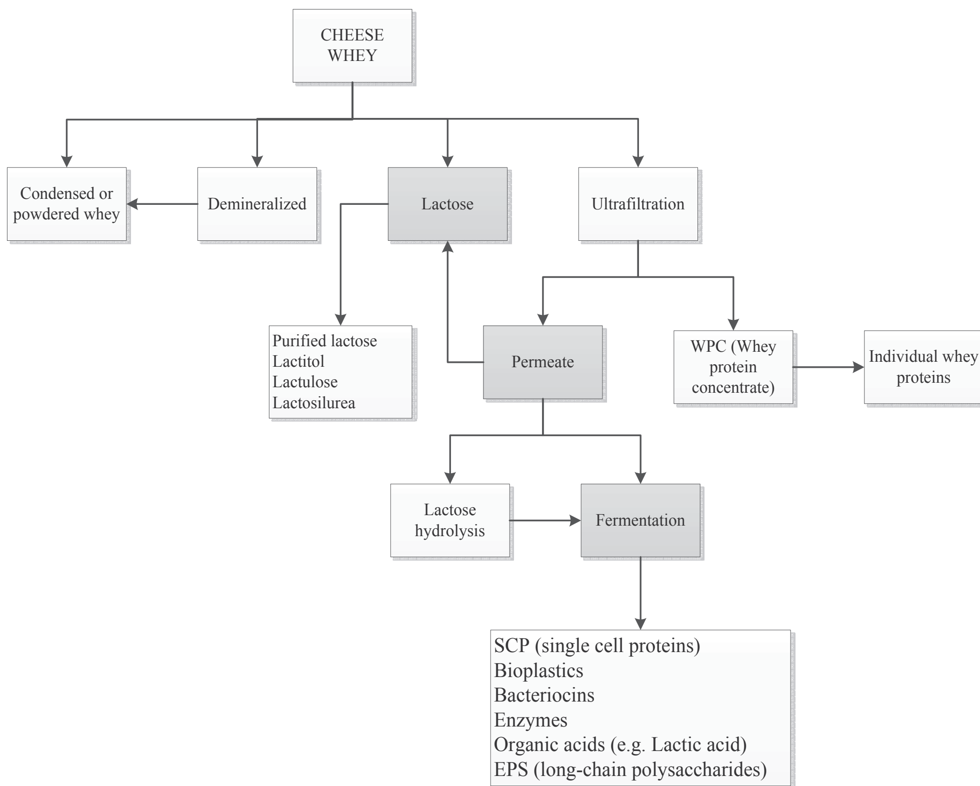

Figure 1. Scheme of current possibility of whey valorization.

2. Whey proteins

Today, whey is a popular dietary protein supplement purported to provide antimicrobial activity, immune modulation, and to prevent cardiovascular disease and osteoporosis. In addition, whey has the ability to act as an antioxidant, antihypertensive, antitumoral, hypolipidemic, antiviral, antibacterial, and chelating agent [9].

Advances in processing technology, including ultrafiltration, microfiltration, reverse osmosis, and ion-exchange, have resulted in development of several different finished whey products: whey protein concentrates (WPC) containing between 50 - 85% protein on a dry basis, whey protein isolate (WPI) containing between 90-98% protein and very small amounts of lactose and fat, reduced lactose whey, demineralized whey and hydrolyzed whey [10]. Each whey product varies in the amount of protein, carbohydrates, immunoglobulins, lactose, minerals, and fat in the finished product [9].

Nowadays whey ultrafiltration (UF) and diafiltration (DF) are standard operations in the dairy industry that allow protein recovery without significant loss of their functional properties and with a low salt content, making it suitable for human consumption [11,12].

The recovery of proteins by UF and DF represents the first step in whey valorisation. UF has been used in the dairy industry to produce WPC, because this technology allows the selective concentration of the proteins in relation to the retention of protein and selective permeation of lactose, minerals, water and compounds of low molecular weight [10]. DF is used for the production of WPC with a high protein content and purification grade. WPC, which are obtained by whey UF and DF are available in great variety according to protein content and functional properties [7,9].

Whey proteins have a high nutritional value, due to the high content of essential amino acids, especially sulfur-containing ones [13].They are high quality proteins with a protein efficiency ratio (PER) of 3.4, higher than casein (2.8) and similar to egg albumin [14].

Moreover whey proteins have functional properties (e.g. high solubility, water absorption, gelatinization and emulsifying capacities) essential in food application [15].

Thanks to the excellent nutritional and functional properties, commercial value of WPC is from 3 to 40 times greater than that of whey powder [1].

Moreover, the possibility of a different use of whey proteins is taken into account to obtain the so-called "functional foods". Individual whey proteins have their own unique nutritional, functional and biological characteristics that are unrealised in whey protein concentrates. WPC micro-, submicro- and nanocapsules have been applied in the encapsulation of bioactives of interest in the development of novel functional foods (e.g. the antioxidant β-carotene) [16].

The major components among whey proteins are β-lactoglobulin (β-LG), α-lactalbumin (α-LA), bovine serum albumin (BSA) and immunoglobulin (IG), representing 50%, 20%, 10% and 10% of the whey fraction, respectively. All of these major proteins, except for BSA and

IG, are synthesized by epithelial cells in the mammary gland. Besides these, whey contains also numerous minor proteins, called low abundance proteins, such as lactoferrin (LF), lactoperoxidase (LP), proteose peptone (PP), osteopontin (OPN), lisozyme (LZ), among others; LF and LP are the most abundant minor proteins [17,18].

The concentration of whey proteins depends on the type of whey (acid or sweet), the source of milk (bovine, caprine or ovine), the time of the year, the type of feed, the stage of lactation, and the quality of processing. Whey proteins are globular molecules with a substantial content of α-helix motifs, in which the acidic/basic and hydrophobic/hydrophilic amino acids are distributed in a fairly balanced way along their polypeptide chains [19]. Major characteristics of whey proteins are summarized in Table 1.

Protein	Molecular mass (Kg/mol)	Isoelectric point	Concentration (g/l)	Number of amino acids
β-LG	18	5.4	3.2	162
α-LA	14	4.4	1.2	123
Immunoglobulin G	150	5 ÷ 8	0.7	*
BSA	66	5.1	0.4	582
LF	77	7.9	0.1	700
LP	78	9.6	0.03	612

*variable values

Table 1. Major characteristics of whey proteins [20,18,21].

The three major forms in which whey protein products are available, such as concentrates (WPC), isolates (WPI), and hydrolysates (WPH), have limited acceptance by the food processing industry because of the lack of consistency in the gross composition and functionality. Whereas each whey protein has unique attributes for nutritional, biological and food ingredient applications; otherwise individual milk proteins exhibit better functionality than in their native protein mixtures [22,9].

As a matter of fact whey represents a rich mixture of proteins with wide-ranging chemical, physical and functional properties. These proteins play an important role in nutrition and, in a number of instances, also appear to have specific physiological actions, such as: ability to bind metals, functions related to the immune or digestive systems, source of essential amino acids also branched chain amino acids (leucine, isoleucine, and valine) which are thought to play a role as metabolic regulators in protein and glucose homoeostasis, involvement in lipid metabolism, etc. [23,24].

By this way these bioactive compounds are able to reduce disease risk and/or to prevent disease development and have been reported to have utility in many different applications ranging from effects on bone, muscle, blood, brain, pancreas, immune, cancer, infection, me-

tabolism, wound healing, learning, and aging [25]. Moreover, these proteins, once partially digested, serve as a source of bioactive peptides with further physiological activities.

All of these biological and physiological activities offer to the food industry several opportunities; in particular they provide the basis for development of valuable whey protein-based food ingredients targeted to the functional food sector [26].

The term functional food was first introduced in Japan in 1980's: it refers to healthy food similar in appearance to conventional food, consumed as part of a usual diet, and claimed to have physiological benefits like health-promoting or disease-preventing properties beyond the basic function of supplying nutrients. Various definitions of functional food, proposed by authorities, academic bodies and industries, exist worldwide; the difficulty to give a unique definition depends on the fact that foods consumed perform some functions in one way or the other, in particular depending on the state of health of the consumer. Moreover various different terms, listed in Table 2, are sometimes linked or interchanged with the term functional foods [13,27,28]. Considering on one hand the ambiguity among these definitions and on the other the wide set of functions of whey proteins and related peptides, it is quite difficult to establish whether they fall within a definition or into another.

Terms	Definition
Bioactive compounds:	they are chemical compounds derived from a plant, animal, or marine source, that exert the desired health/wellness benefit.
Dietary supplements:	they act as a supplement to the diet in which the active ingredient is added to the food or it can be consumed in the form of pills, powders, or in liquid forms; they do not replace the complete food or meal.
Functional ingredients:	they are preparations, fractions or extracts containing bioactive compounds of varying purity, that are used as ingredients by manufacturers in the food.
Medical foods:	they are formulated to be administered under the supervision of a physician, for the specific dietary management of a disease or condition for which distinctive nutritional requirements are established.
Natural health products (NHP):	they include homeopathic preparations, substances used in traditional medicines, minerals or trace elements, vitamins, amino acid, essential fatty acids, or other botanical, animal, or microorganism derived substances. They are generally sold in medicinal to diagnose, treat, or prevent disease, restore or correct function, or to maintain or promote health. NHP also include nutraceuticals.
Nutraceuticals:	they are substances, either a food or part of a food, that provide medical or health benefits, including the prevention and treatment of disease. They are derived from foods and can be used in the form of pills, capsules, potions, and liquids.

Table 2. Different terms linked or interchanged with functional foods [29,28].

2.1. Whey proteins separation

Protein functions are related to their native structure, which depends on pH, temperature, pressure and solvent effects. Changes in native structure affect functional properties, by this way in the last years there has been a great interest in developing efficient separation and purification processes that prevent denaturation and loss of biological activity. On the other hand fractionation can emphasize the functional and nutritional properties of the individual proteins [30,31].

Separation processes tend to exploit to the maximum extent different molecular masses, concentrations, and isoelectric points of whey proteins. The main available processes for commercial-scale production of whey protein fractions belong to four categories: selective precipitation, membrane filtration, selective adsorption, and selective elution [20].

A brief description and some examples for each separation category are after-presented.

- Selective precipitation involves adjusting the solution physical properties to promote insolubility. Proteins are typically least soluble at a pH near the isoelectric point (pI) and in low ionic strength solutions, and most likely to aggregate under these conditions. While β-LG precipitates rapidly and selectively at high temperature (70-120 °C) and pH near neutral (pH 8), α-LA precipitates and aggregates better at acidic pH (3.5-5.5) and moderate temperature (50-65 °C) with long reaction times, usually accompanied by the precipitation of bovine serum albumin, immunoglobulins and lactoferrin, while β-LG remain soluble [32,33].
- Membrane filtration is commonly used to make whey protein concentrate, a mixture of all the proteins in whey, with the aid of membranes with a 5,000 to 10,000 g/mol molecular mass rating. As regard fractionation, traditionally it has been based solely on differences in molecular mass; by this way in the past fractionation was possible only for proteins with great differences in molecular mass (e.g. α-lactalbumin vs. IGG) or for proteins with a great combined difference in charge and size. Nowadays it is possible to achieve separation also when proteins have little or no difference in molecular mass together thanks to a careful adjustment of the solution pH and ionic strength [34,35]. The electrostatic rejection by the membrane, due to their slight residual charge can be enhanced or reduced by adjusting the solution pH. Furthermore separation is enhanced by operating near the pI of the smaller protein and far from the pI of the larger protein to maximize the difference in effective hydrodynamic size: this because the effective diameter of a protein increases with decreasing ionic strength. Low salt concentrations (1–20 mmol/L) increase electrostatic and steric rejection by the membrane: by this way a multi-step adjustment of the pH and ionic strength of the whey may allow fractionation of proteins using a sequence of membrane separation processes [20]. As an example reference [36] is reported; in this experimental study, the effect of working pH was evaluated, employing a 300 kDa tubular ceramic membrane in a continuous diafiltration mode, by measuring the flux-time profiles and the retentate and permeate yields of α-LA, β-LG, BSA, IgG and LF. It was found that a 300 kDa membrane could be employed to fractionate the original array of whey proteins in two parts: α-LA and β-LG in the permeate and BSA, IGG and LF in the reten-

tate. As a matter of fact important protein yields for α-LA and β-LG were obtained in the permeate (except for pH 4 and 5), while for the rest of the proteins studied, there was no significant diffusion through the membrane.

- In selective adsorption, a single purified protein is produced in conjunction with a treated whey solution depleted in that protein; the cost of manufacture must be borne by the income generated from that single purified protein product and the depleted whey solution [20,36]. There are many examples of selective adsorption processes for whey proteins: the immobilized hexapeptide ligand affinity resin or immobilized phenyl groups were used for α-LA, while for β-LG immobilized retinal or ceramic hydroxyapatite chromatography with sodium fluoride as a displacer were applied [37-40].

- In selective elution all the proteins in a mixture are trapped simultaneously onto the adsorbent, rinsed free of contaminants, and then eluted one by-one to obtain different purified proteins. The process uses an adsorbent and buffers that are inexpensive and food-grade, and it is operated at a high flow rate. Further on the cost of manufacture is spread among many purified protein products with the possibility to manufacture different products simply by using different elution buffers; by this way it is considered an attractive alternative to selective adsorption [41]. Differently from precipitation and membrane separation processes, which are volume-dependent separation methods, selective adsorption and selective elution processes are less volume dependent because adsorbent capacity depends mostly on the mass of protein recovered, not the volume of liquid processed [42]. Various studies about the development of ion exchange or affinity chromatography processes to separate whey proteins exist; referring as a case in point [43] various superparamagnetic anion-exchangers and their use together with cation-exchangers in the fractionation of bovine whey proteins (LF, LP, IG, and β-LG) were studied. While in reference [41] all the positively charged proteins in whey were bound simultaneously to a cation exchange column, rinsed free of contaminants and then eluted selectively to produce different fractions: with a single piece of equipment they were able to manufacture WPI, or α-LA and WPI depleted in α-LA or LF and LP only.

2.2. Biological properties of individual whey proteins

2.2.1. β-lactoglobulin (β-LG)

β-lactoglobulin, a member of the lipocalins family, is the major whey protein of ruminant species, 58% (w/w). It is present in many mammalian species but absent in human milk. Genetic variants of a single gene, of which β-LG A is the most common, have been widely reported particularly in the cow [44,21].

The lipocalins family presents various kind of features, most of which involve some ligand-binding functions; these last must be the physiological reason for the significant quantities of β-LG found in milk (the domestic cow produces 2 to 3g L^{-1}) [45].

In isolated form, despite its globular nature, it exhibits a low solubility and a low ionic strength. It contains normally 162 aminoacidic residues and has a molecular weight of

18,362 g/mol. The secondary structure of this protein comprises nine strands of β structure, a short α helix segment and three helicoidal turns [46].

Its quaternary structure depends on the medium pH: at pH values between 7 and 5.2 it is a stable dimer (molecular weight of 36,700 kDa); at pH values between 5.2 and 3.5, an octamer (molecular weight of ca. 140,000 kDa); and below pH 3.0 and above 8.0, a monomer, with two-cysteine residues per monomer [47].

2.2.1.1. β-LG biological functions

The endogenous function of β-LG is not clear, but it is known that it is a source of amino acids. Indeed β-LG, over-expressed in the lactating mammary gland of many species, is primarily an important source of amino acids for the offspring of those animals that produce it [45]. The amino acid content fuels muscle growth and it is a source rich in cysteine, which is important for the synthesis of glutathione [13].

It participates in the digestion of milk lipids in the neonate: β-LG binds to free fatty acids as they are released by pregastric lipases, by this way the digestion of milk fat is facilitated [48].

β-LG is the major allergen in cow's milk, responsible for causing milk allergy [25]. It was evidenced that milk's major allergen can be rendered non-allergenic and it can also be modified or administered inhibiting rather than stimulating the allergic process. β-LG has been conjugated with acidic oligosaccharides to reduce its antigenicity. Immunization of mice with the conjugates leads to a reduced T cell response, predominantly Th1-mediated, suggesting that the conjugates may have utility in preventing Th2-mediated allergy [49]. Oral administration of recombinant *Lactococcus lactis* expressing bovine β-LG induces a specific Th1 response, suggesting that probiotics expressing β-LG could be useful in the management of food allergy [50]. Intranasal co-administration of live lactococci expressing IL-12 and β-LG produces a protective Th1 response that inhibites allergic airway disease in mice [51]. Acidic β-LG-derived peptides hydrolyzed with *Lactobacillus paracasei* peptidases represse lymphocyte stimulation, upregulate IL-10 production, and downregulate IFN-γ and IL-4 secretion [52].

β-LG structure comprise a ligand-binding site. The ligands that bind to β-LG tend to be hydrophobic and include long-chain fatty acids, triglyceride, retinoids, cholesterol and, more weakly, hydrocarbon molecules [53].

β-LG can also be a source of peptides with different functions:

- Lactokinins, Tyr-Leu (f102–103) and Ala-Leu-Pro-Met-His-Ile-Arg (f142–148), are inhibitors of angiotensin-I-converting enzyme (ACE) and represent potential nutraceuticals/functional food ingredients for the prevention and/or treatment of high blood pressure [54].
- β-lactorphin (f102–105) has ACE-inhibitory activity, improved vascular relaxation in spontaneously hypertensive rats, and it is an opioid receptor agonist suggesting that it can modulate absorption processes in the intestinal tract [55].
- Beta-lactotensin, His-Ile-Arg-Leu (f146 –149), is an ileum-contracting peptide, which exhibits hypertensive activity. It is a natural ligand for neurotensin NT2 receptors, has an

anti-stress effect, promotes the abolition of fear memory, reduces sensitivity to painful stimuli, and consolidates memory. It is able to reduce blood cholesterol levels, but only when given parenterally, and not orally [56-58].

2.2.2. α-lactalbumin (α-LA)

α-lactalbumin is one of the most studied proteins: it is an important component of milk and it contributes significantly to its physical, biological and nutritional characteristics [59]. It is biosynthesized in the mammary gland during lactogenesis, and participates actively in the synthesis of lactose [60].

In human whey, α-LA is a major protein (1.7 mg/mL); however, in bovine whey it is ranked second with respect to protein content after β-LG. The bovine protein is characterized by its relatively low molecular mass (14.2 kDa) and its acidity (isoelectric point of 4.8); α-LA possesses a high mineral content and a balanced amino acid composition indeed its sequence is composed of 123 amino acids, that includes four residues of tryptophan and a high proportion of essential amino acids, Cys, Ile, Leu, Lys [61,62].

The structure of bovine α-LA is highly stabilized by four disulphide bonds and by the association of Ca^{2+} at the binding loop that joins together two domains of the protein: a large α-helical and a small β-sheet domains [63,64].

In addition to Ca^{2+} ion, α-LA is able to bind several other cations such as Mg^{2+}, Mn^{2+}, Na^{+} and K^{+} and also contains various Zn^{2+} binding sites [65]. Nevertheless, only the binding of Ca^{2+} ion is fundamental for the maintenance of the native conformation of the protein [66,67].

2.2.2.1. α-LA biological functions

α-LA and its biological and functional peptides are characterized by potential health benefits; as regard of that there is a growing scientific interest in the context of health-promoting functional foods. In particular, α-LA and α-LA-peptides can be used as supplements of essential amino acids in food to improve/maintain the immune system, to reduce the stress, for opioid activity, antihypertensive action, regulation of cell growth, immunomodulation etc. [68].

α-LA is cytotoxic and this property can be exploited for therapeutic uses. α-LA has protective properties against mucosal injury[69,70].

α-LA is particularly rich in essential amino acids. It has a high content of lysine and cysteine and a particularly high content of tryptophan (5.9% of the total amino acid content) [61].

The high content in tryptophane makes α-LA a nutraceutical; in particular it may help improve mood, sleep, and cognitive performance [71,72]. On the other hand the content of cysteine is valuable in boosting the immune system and promoting wound healing. As a general consideration, thanks to the high content in essential amino acids, α-LA is an invaluable supplement for infant formulas [68].

The protein may possess bactericidal or antitumor activity. The active form of the protein is called "human α-LA made lethal to tumor cells" (HAMLET), a complex formed by which

induces apoptosis in tumor cells but spares mature cells and has received much attention due to its potential use as a new therapeutic agent against tumor cells [73].

The digestion of α-LA with trypsin and chymotrypsin creates some polypeptides with bactericidal properties, mostly against Gram-positive bacteria and a weak bactericidal activity against Gram-negative strains. The peptide α-lactorphin behaves like opioid receptor agonists [74,75].

α-LA has a marked suppressive effect against the increased release of proinflammatory cytokines and tumor necrosis factor-α, from the D-galactosamine induced liver injury rat model or ischemia/reperfusion induced intestinal injury rat model [76]. Two synthetic peptides corresponding to the sequences f50-51 (Tyr-Gly) and f18-20 (Tyr-Gly-Gly) of α-LA also enhance both the in vitro proliferation and protein synthesis of concanavalin A-stimulated human peripheral blood lymphocytes [77].

2.2.3. Immunoglobulins (IG)

Immunoglobulins constitute a complex group of elements produced by B-lymphocytes and their concentration in whey is 0.7 g/l. IG are divided into three basic classes: IGG, IGA and IGM. IGG is often sub-divided into two subclasses, IGG1 and IGG2. IGG represents up to 80% (w/w) of all IG in milk or whey. Qualitatively the family of IG found in bovine whey and colostrum include IGA, secretory IGA, IGG1, IGG2, IGG fragments, IGM, IGE, J-chain or component, and free secretor component [78,21].

IG make a significant contribution to the whey protein content and they exert an important immunological function (especially in colostrum). IG are subject to postnatal transfer via colostrum because the placenta does not permit passage of macromolecules. These proteins are present in the serum and physiological fluids of all mammals; some of them attach to surfaces, where they behave as receptors, whereas others function as antibodies, which are released in the blood and lymph [79].

In terms of quaternary structure, IG are either monomers or polymers of a four-chain molecule, consisting of two light polypeptide chains (with a molecular weight in the range 25,000 kDa) and two heavy chains (with molecular weight of 50,000–70,000 kDa) [80].

2.2.3.1. IG biological functions

Milk immunoglobulins normally provide passive immunity for the neonate, but they are also potentially powerful agents that could be incorporated into diets to remove toxic, or undesirable dietary factors. As an example, naturally occurring antibody in milk can be extracted which binds to cholesterol in the human digestive tract and prevents its absorption into the bloodstream. This anti-cholesterol antibody can be a useful food supplement for the functional food market [25].

Concerning IG antimicrobial and antiviral properties it is known that concentration of colostrum whey antibodies against a particular pathogen can be raised by immunising cows with the pathogen or its antigens. Antibody concentrates derived from immune milk collected

from cows immunised with inactivated human rotavirus possess preventive/treatment features in enteric disease caused by said viruses in therapeutics of child infections [81]. The hyperimmune whey that results can potentially provide prophylactic protection against various infectious gut microbes including rotavirus and *Helicobacter pylori* [82]. Infant gastritis originated by *H. pylori* is well fought via a diet including immune milk containing specific anti-*H. pylori* antibodies [83]. There is also evidence of protection via bovine antibodies against dental caries caused by cariogenic streptococci [84].

IG can also act in the immune system modulation; they are recognised to provide protection against diseases in the newborn through passive immunity. Systemic immunisation of pregnant cows increases the levels of antibodies against immunising bacteria, and also reduces susceptibility to disease [85]. Vaccination of pregnant cows originates colostrum characterized by high concentrations of specific antibodies against the antigens of the vaccine used [86].

Other methabolic features of IG are also known. Immune milk is suggested to lower blood pressure. In reference [87] a clinical-trial study regard the effects on reduction of cholesterol and blood pressure of immune milk, produced by dairy cows previously hyper-immunised with a multivalent bacterial vaccine, was described. The involved human hypercholesterolemic subjects consumed 90 g of immune milk daily: this was a useful adjunct in the dietary management of hypercholesterolemia.

2.2.4. Bovine Serum Albumin (BSA)

Bovine serum albumin represents the 5% of the whey proteins and its concentration in whey is about 0.4 g/l. It appears in milk following passive leakage from the blood stream indeed it is not synthesized in the mammary gland [21,25]. The most outstanding property of BSA is its ability to bind reversibly various ligands; in particular it is the principal carrier of fatty acids [88].

It contains 582 amino acid residues, which lead to a molecular weight of 66,267 kDa; it also possesses 17 intermolecular disulphide bridges and one thiol group at residue 34 [89]. BSA molecule is heart-shaped; it consists of three homologous α-helical domains and each domain contains two sub-domains that share common structural motifs [88].

Thanks to its size and higher levels of structure, BSA can bind to free fatty acids and other lipids, as well as flavor compounds, this feature is severely injured upon denaturation [90].

Its heat-induced gelation at pH 6.5 is initiated by an intermolecular thiol-disulphide interchange, similar to what happens with β-LG [78].

2.2.4.1. BSA biological functions

BSA has the ability to inhibit tumor growth thanks to the modulation of activities of the autocrine growth regulatory factors; this was evidenced by *in vitro* incubation with human breast cancer cell line MCF-7 [91]. In relation to this activity [92] the delivery system MTO-BSA-NS (mitoxantrone nanoparticles loaded with bovine serum albumin) was studied; human MCF-7 breast cancer in nude mice and animal model of P388 lymphnode metastases in

Kunming mice were applied to investigate the therapeutic efficiency. The inhibition rate of the nanospheres against breast cancer was high and lymphnode metastases were efficiently inhibited.

BSA is able to bind fatty acids stored in the human body as fat; this allows BSA to participate in synthesis of lipids [93]. BSA has also antioxidant activities; *in vitro* it is able to protect lipids against phenolic-induced oxidation [94]. BSA is also a source of essential amino acids, whose therapeutic potential is largely unexplored.

Biological functions of some BSA-derived peptides have also been examined. The peptide serorphin (Tyr-Gly-Phe-Gln-Asn-Ala) (f399–404) has opioid agonist activity, while the peptide albutensin A (Ala-Leu-Lys-Ala-Trp-Ser-Val-Ala-Arg) (f208 –216) is an ACE inhibitor and is reported to have ileum contracting and relaxing activities [54].

2.2.5. Lactoferrin (LF)

Lactoferrin together with lactoperoxidase is one of the minor whey proteins. It belongs to the transferrin family which is composed by proteins capable of binding and transferring Fe^{3+} ions. LF is a glycoprotein characterized by a molecular weight of 80,000 kDa. It is synthesized by glandular epithelial cells and mature neutrophils, and can be found in milk, saliva, tears, nasal and intestinal secretions, pancreatic juice, seminal fluid, and in secondary granules of neutrophils. Bovine milk contains between 0.02 and 0.35 mg/ml of LF, depending on the period of lactation [25,95].

LF is a bilobal protein that contains two homologous metal-binding sites with high affinities for ferric iron. The iron-binding sites are situated in the inter-domain clefts. The requirement for an anion, bound synergistically with the Fe ion is a unique feature of LF. Iron release can occur at low pH and is associated with a large-scale conformational change in which the two domains that enclose each iron-binding site move wide apart [96].

LF possess a wide range of biological functions, many of which are not connected with its iron binding ability; it is the most valuable biomedical protein present in whey due to the various therapeutic properties it exhibits [97,25].

2.2.5.1. LF biological functions

LF has quite important role in iron metabolism. It may contribute to local iron accumulation at sites of inflammation and it has been known to be responsible for hypoferraemia through binding free iron and shuttling it back to macrophages [98,97]. LF might also have a control function in situations when increased amounts of iron are released from its depots [99]. Further on LF seems to affect intestinal iron absorption in infants depending on the organisms need for iron [100].

LF is a part of the innate immune system and it also takes part in specific immune reactions; it represents one of the first defense systems against microbial agents [101,95].

LF can have a bacteriostatic effect thanks to its ability to bind free iron, essential for the growth of bacteria (e.g. *E. coli*). LF, as an iron donor, supports the growth of *Lactobacillus* sp.

or *Bifidobacterium* sp., characterized by a lower iron demands and generally considered as beneficial [102,103]. LF owns also a bactericidal effect, which is not iron-dependent, against Gram-negative and Gram-positive bacteria. In the first case there is the disruption in the cell wall because the microorganisms have the receptors for the N-terminal region of LF; while for Gram-positives, changes in the permeability of the membrane occurs mediated by electrostatic interactions between the lipid layer and LF surface [104,105]. LF may contribute to defense against the invasion of facultative intracellular bacteria such as: *E. coli* HB101, *Yersinia enterocolica*, *Y. pseudotuberculosis*, *Listeria monocytogenes*, *Streptococcus pyogenes*, and *Staphylococcus aureus*. LF binds both target cell membrane glycoaminoglycans and bacterial invasins. The proteolytic activity of LF inhibits the growth of *Shigella flexneri* and *E.coli* through degrading proteins necessary for colonization [105,106].

The main contribution to antiviral defense of LF consists in its binding to glycosaminoglycans of cell membrane: viruses (Herpes simplex virus, cytomegaloviruses, human immunodeficiency virus) cannot enter cells and infection is stopped at an early stage [106]. LF is also capable of binding certain DNA and RNA viruses [107].

LF may support the proliferation, differentiation, and activation of immune system cells and strengthen the immune response; LF also acts as an anti-inflammatory factor. Thanks to its antimicrobial activity and capability of binding components of bacterial cell walls or their receptors, LF may prevent the development of inflammation and subsequent tissue damage caused by the release of pro-inflammatory cytokines and reactive oxygen species [101].

As regard the influence of LF on tumor growth, it is known that it is able to halt the growth of human mammary gland carcinoma cells between the G1 and S stage; this effect may be ascribed to the altered expression or activity of regulatory proteins [108]. The lactoferrin-dependent, cytokine-mediated stimulation of activity of NK cells and lymphocytes CD4+ and CD8+, represents an important factor in defense against tumor growth: after the oral administration of lactoferrin the number of these cells increases [109]. Even if the exact mechanism has to be discovered completely, it seems that LF-mediated inhibition of tumor growth might be related to apoptosis of these cells induced by the activation of the Fas signaling pathway [110].

LF is also able to act as a growth factor activator; it can have effect on small intestine epithelial cells and endometrium stroma cells [111]. It has also been identified as a transcription factor [112].

LF contributes to the stabilization of the osseous tissue; it may affect bone cells through the inhibition of osteolytic cytokines whose levels rise during inflammation. LF is a potent anabolic factor affecting osteocytes; it stimulates osteoblast proliferation, enhances thymidine incorporation into osteocytes, and reduces apoptosis of osteoblasts. By this way LF might be potentially useful in the treatment of diseases such as osteoporosis [113].

2.2.6. Lactoperoxidase (LP)

Lactoperoxidase is member of the family of mammalian peroxidases. It is present in a variety of animal secretions (e.g. tears, saliva and milk), it is one of the most abundant enzymes

in plain milk and represents 1% (w/w) of the total protein pool in whey; its concentration in whey corresponds to 0.03 g/l [114].

LP consists of a single polypeptide chain containing 612 amino acid residues and its molecular mass is about 80 kDa. It contains 15 half-cystine residues and carbohydrate moieties comprise about 10% of the weight of the molecule [115].

The complete LP system (i.e. enzyme plus substrate) was originally characterized in milk [116]; its activity depends on many factors (e.g. animal species, breed and lactation cycle). Other members of that group of oxidoreductases include myeloperoxidase (present in neutrophils and monocytes), eosinophil peroxidase and thyroid peroxidase; they are characterized by a close evolutionary relationship [21].

2.2.6.1. LP biological functions

LP is characterized by the antimicrobial activity related to the LP system, formed by LP, thiocyanate anion, and hydrogen peroxide, which is active only in the presence of all these three components: LP catalyses the oxidation of thiocyanate by H_2O_2 and generates intermediate products with a broad spectrum of antimicrobial effects against bacteria, fungi and viruses. The LP system is naturally occurring in milk and saliva and has been used in foods, cosmetics and in clinical applications because of its safety and broad spectrum of action; in particular it is fundamental in the dairy industry for the preservation of raw and pasteurized cheese, and yogurt [117,118].

The thiocyanate anion is significantly present in saliva, milk and airway secretions. The amount of the anion in cow's milk ranges from 0.1 to 15 mg/kg and its concentration varies according to animal species, breed, lactation cycle, season and composition of feed [114,21].

Hydrogen peroxide is not normally detected in raw milk. It may be generated endogenously by polymorphonuclear leucocytes or under aerobic conditions by many lactobacilli, lactococci, and streptococci. H_2O_2 is normally present to very small levels because its content is rapidly reduced by catalases and peroxidases that are adventitious in milk [119-121].

The major intermediary oxidation product, at physiological pH, is hypothiocyanate; it mediates bacterial killing, as it is cell-permeable, and can inhibit glycolysis, as well as (NADH)/(NADPH)-dependent reactions in bacteria. It is bactericidal for enteric pathogens including multiple antibiotic resistant strains of *E. coli*. Other reaction products of the LP system, such as cyanosulphurous acid and cyanosulphuric acid, are able to oxidise sulphidrile groups of bacterial proteins [114,21].

The LP system inhibit Gram-negative, catalase positive organisms, such as pseudomonas, coliforms, salmonellae and shigellae; these microorganisms can also be killed if H_2O_2 is supplied exogenously. Gram-positive, catalase negative bacteria, such as streptococci and lactobacilli are generally inhibited but not killed by the LP system [122,121].

The LP system exerts both bacteriostatic and bactericidal activities against strains of *Salmonella typhimurium*, *S. aureus*, and *L. monocytogenes*; the system is bactericidal against *Campylobacter jejuni*, *Brucella melitensis*. As regard *Bacillus cereus*, *Streptococcus mutans*, *Streptococcus*

sanguis, *Streptococcus mitis*, and *Streptococcus salivarius* the LP system has an inhibitory effect [123-127,121].

The antifungal activity of the LP system with glucose oxidase as H_2O_2 source has been reported against *Rhodutorula rubra*, *Saccharomyces cerevisiae*, *Mucor rouxii*, *Aspergillus niger*, and *Byssochlamys fulva* in salt solution and in apple juice. The LP-thiocyanate-H_2O_2 system was found to inhibit the growth and proliferation of many fungal species (e.g. *Aspergillus flavus*, *Trichoderma* spp.); the same system showed also antifungal activity against *Alternaria* spp., *Penicillium chrysogenum* and *Claviceps* spp. [128,121].

Finally, as regard the antiviral properties of the LP system, the ability to kill both poliovirus and vaccina virus with halides (I^-, Br^-) as electron donors has been reported [129]. In references [130,131] the LP- H_2O_2-halide system virucidal activity against HIV-1 was reported.

3. Whey fermentation products

During last 50 years, cheese whey was used in different bioconversions; for examples the microbial biomass production for animal feed supplement [132], biogas production using anaerobic methanogenic bacteria [14], bioethanol production by *Kluyveromyces marxianus* [133,134] or recombinant *Saccharomyces* cells [135,136], hydrolized lactose solution in sweeteners and dietary supplements production [14].

Therefore, at present, it can be very interesting and promising to consider again the possibility to use cheese whey and, particularly, glycidic component because of new demands, as bioplastic synthesis (poly-hydroxyalkanoates -PHA- and polylactate acid -PLA-), antimicrobial peptides (bacteriocins), enzymes and esopolisaccharides (EPS).

Just below, there are the information regarding the fermentative production of the molecules and macromolecules previously mentioned.

3.1. Polyhydroxyalcanoate

Polyhydroxyalkanoates (PHA) are aerobic bacteria synthesized macromolecules (polyesters); they are carbon and energy reserve, accumulated as intracellular granules. These microbial compounds play the same role as glycogen and starch in animal and plant cells, respectively [137].

Among biodegradable plastics, PHA are very interesting polymers because of their chemical, physical and mechanical properties comparable to petroleum-derived plastics (polyethylene and polypropylene); otherwise, PHA are different for their complete biodegradability, UV-resistence, oxygen-impermeability (fundamental property for food packaging) and biocompatibility (essential property for medical and surgery applications) [138,139].

Under unbalanced growth conditions (limitation of essential nutrients, e.g., nitrogen, phosphate, or oxygen), several microorganisms redirect the acetyl-CoA flux from biomass formation towards accumulation of PHA [140,141].

At present, these biopolymers are synthesized in pure cultures, using synthetic pure substrates (as monosaccharides and organic acids), in macronutrient limiting conditions (N, O, P). Because of substrate weights on whole process cost at 40%, it becomes necessary to search alternative cheaply available source materials for PHA production, as food by-products (e.g. cheese whey).

In the last two decades, a broad number of studies were related to the production of PHA from milk whey permeate using pure cultures of wild type microorganisms or recombinant ones. In several of these works, whey lactose was hydrolyzed by lactase: poly-(3HB-3HV) was produced with *Ralstonia eutropha* DSM545 on whey permeate and inverted sugars [142], poly-3-(hydroxybutyrate-co-hydroxyvalerate) was produced by *P. hydrogenovora* with hydrolyzed whey permeate and sodium valerate [143].

In [141] *Hydrogenophaga pseudoflava* DSM1034 was reported as unique example of wild type microorganism able to synthesize PHA directly from lactose, three possible routes from whey lactose to PHA have been suggested: direct conversion of lactose to PHA, hydrolysis of lactose (chemically or enzimatically) and conversion of glucose and galactose to PHA, lactose fermentation to lactic acid and then conversion of lactic acid to PHA. In reference [144] a recombinant *E. coli* strain GCSC 6576 and whey powder was used in a pH-stat fed-batch fermentation. After 47 h of fermentation, it was obtained a dry cell weight and P(3HB) concentration of 109 g L^{-1} and 50 g L^{-1}, respectively. Further, with the same organism and using pH-stat fed-batch culture and concentrated whey solution containing 210 g L^{-1} lactose as nutrient feed, a dry cell weight of 87 g L^{-1} and P(3HB) concentration of 69 g L^{-1} containing 87% dcw P(3HB) was achieved. Using the recombinant *E. coli* strain (K24K), the authors successfully produced 70.1 g L^{-1} biomass containing 51.1 g L^{-1} P(3HB) in a pH controlled fed-batch fermentation at pH 7.20 with whey and corn steep liquor as carbon and nitrogen sources, respectively[145]. Previously, PHB production by *Methylobacterium sp.* ZP24 on lactose and whey with similar values of biomass polymer content was described [146].

Poly-β-hydroxybutyrate production was also described in lactic acid bacteria belonging to *Lactococcus, Lactobacillus, Pediococcus* and *Streptococcus* genera [147].

Lactic acid producing bacteria such as *Lactobacillus lactis* [148], *Propionibacterium* [149], *L. delbrueckii* [150-152] and *C. necator* have been also used in a co-culture fermentation system: LAB converted sugars into lactic acid which was later taken up *by C. necator* to produce PHAs. Generally, it has been demonstrated that co-culture fermentations resulted in increased yield, improved control of product qualities. A further advantage in the application of co-cultures is the possibility of utilizing secondary products (e.g. whey) cheaper than glucose as substrates for production of PHAs [153]. Bacteria that have a "generally recognised as safe" (GRAS) status for PHA-production such as lactic acid bacteria and bacilli belonging to probiotic species [147,154] might constitute an added value to these biotechnological processes [155].

3.2. Lactic acid

Acetic, propionic, lactic, lactobionic, citric, gluconic and itaconic acids can be obtained from lactose/whey fermentation. Among organic acids that find applications in specialty chemicals, lactic acid is the most important from an economical point of view [156].

Lactic acid is used in food and chemical industries (pharmaceutical products, textiles, leather), primarily as a preservative and as acidulant [157-159]. Also it has applications as a biodegradable plastic component (polylactide, polymers, polyhydroxybutyrate) [159]. Notably, the industrial demand for lactic acid (LA) has been increasing considerably in recent years, owing to the promising applications of its polymer, the polylactic acid (PLA), as an environment-friendly alternative to plastics derived from petrochemicals [160].

Polylactic acid (PLA) is a biodegradable polyester made by condensation of lactic acid (LA) monomers [161]. It can be worked with conventional facilities and techniques to produce implant devices and internal sutures [162]. Due to its low toxicity, it is classified as GRAS and can be also used in food packaging [161,163], which is one of the widest fields of plastics market. PLA is completely biodegradable under compostable conditions: however, if disposed in landfills, it will last in the environment for years, likewise oil-based plastics [164].

Cheese whey effluents have been used in fermentation processes to produce lactic acid [165-168,158,169,159]. Microorganisms used in lactic acid production are *Lactobacillus casei* [166,157,158,169], *Lactobacillus helveticus* [170-172,168,156,159]; *Lactobacillus acidophilus* [167]; *Lactobacillus delbrueckii* [165,167,168]; *Lactobacillus salivarius* [169]; *Lactococcus lactis* [158]. *Streptococcus thermophilus* [167]; *K. marxianus* [168]; *Leuconostoc* and *Pediococcus* [4].

Among different lactobacilli species employed in lactic acid production, *L. helveticus* is the generally preferred organism, it is a homolactic LAB that produces LA in racemic mixture (DL) [173]. *L. helveticus* showed enhanced lactose utilisation and lactic acid production at 42°C and pH 5.8 [159].

Currently, a high fraction of generated CW is managed by membrane processes, mainly, ultrafiltration. The obtained permeate has a low protein content and an elevated lactose and mineral salts concentration, both aspects are advantageous in lactic acid fermentation. As a consequence, several works have been carried out aimed at obtaining lactic acid after ultrafiltration of CW [171,157,172,169]. Highest lactic acid production rate was obtained with *L. helveticus* cultivated in whey permeate, with corn steep liquor (CSL) as the nitrogen source [174]. Lactic acid productivity of 9.7 g/L/h using *L. helveticus* strain milano has been obtained in continuous fermentation of whey-yeast extract permeate medium [173,175]. In a work with *L. salivarum* YE supplementation was replaced by *in situ* treatment of fermentation medium with proteolytic microorganisms [169].

However, lactic acid production from cheese whey or its permeate obtained without nutrients supplementation [165,168,159,169,] is of limited application to industrial scale because of the low productivity, nutrients supplementation is a key factor limiting the process efficiency.

Some studies report the use of mixed cultures in lactic acid production with synergistic effects [168,158]. Other research groups tried to improve LA production using r*E.coli* harbouring an inducible expression plasmid containing D-lactate dehydrogenase encoding gene of *Lactobacillus plantarum* [176] or using metabolic engineering of LAB, fungal or yeast systems [177,178,179], but all of these strategies, if compared with the use of mixed cultures, involve higher costs due to genetic engineering studies and the need of sterilization.

LAB have been immobilised by several methods on different supports (calcium alginate, k-carragenane, agar and polyacrylamide gels) [4] and the immobilised systems have been investigated for lactic acid production from whey. A two-stage process was used for continuous fermentation of whey permeate medium with *L. helveticus* immobilised in k-carrageenan/ locust bean gum, which resulted in high lactic acid productivity (19–22 g L^{-1} h^{-1}) [172].

In [4] *L. casei* was immobilized in Ca pectate gel. A high lactose conversion (94.37%) to lactic acid (32.95 g L^{-1}) was achieved and the cell system was found highly stable, no decrease in lactose conversion to lactic acid was observed up to 16 batches.

3.3. Exopolysaccharides

Exopolysaccharides (EPS) are long-chain polysaccharides ($4*10^4$-$6*10^6$ Da). They are produced by bacteria and microalghe and can be divided in homopolysaccharides (Homo-EPS) and heteropolysaccharides (Hetero-EPS) [180]. Homo-EPS consist of either D-glucose (glucans) or D-fructose (fructans) residues, with different types of linkage and branching degree. Hetero-EPS are constructed from multiple copies of an oligosaccharide and show little structural similarity to one another: glucose, galactose, xylose, mannose, arabinose and rhamnose are the most represented sugars but also amino-sugars and polyols can be occasionally present as well as glucuronic acid. They are often highly branched with different binding types [181].

The physiological role of EPS is related to microbial cell protection from toxic compounds, phagocytosis, antibiotics effect and osmotic stress [182]. In the last years, their prebiotic role was identified and described [183,184]. In addition to role as food additives and prebiotics, LAB synthesized EPS have been implicated as anti-tumor agent, immuno-stimulator and blood cholesterol lowering agent [185,184].

On the basis of their rheological properties, EPS are applied as stabilizers and emulsifiers in food industry, particularly for yogurt, fermented milk and mozzarella production [186,187]. EPS used in food industry must be considered additives and, consequently, must ensure safety qualification. EPS synthesize by LAB, generally used in food production, possess this safety characteristics [184]. The GRAS and probiotic status of some lactobacilli give to them more preference for consumable EPS production. Main drawbacks limiting their industrial expansion are their low yields of production [188]. Approximately 30 species of lactobacilli are described as EPS producers, among them, the best known are *L. casei*, *L. acidophilus*, *L. brevis*, *L. curvatus*, *L. delbrueckii bulgaricus*, *L. helveticus*, *L. rhamnosus*, *L. plantarum*. They are principally cultivated between 30 and 37 °C on rich media as Man Rogosa Sharp (MRS), milk or milk derivatives [189,188]. But *Lactobacillus* sp. are not the best polysaccharide pro-

ducers compared to some soil bacteria, as *Xanthomonas campestris* [190,188]. It is known that different strains of *X. campestris* can produce xanthan gums of different composition, viscosity and yield. In reference [191], cheese whey was used as substrate in the production of xanthan gum with optimised high gum production in a bioreactor and at a wide range of viscosity values. With the strain *X. campestris* C7L, [192] a production level of 14.7 g kg^{-1} was obtained, in a 40 g L^{-1} whole milk whey-based medium (powdered cheese whey) with the addition of 0.1 g L^{-1} magnesium sulphate and 5 g L^{-1} potassium phosphate. In [193], the feasibility of using cheese whey as carbon source for xanthan gum production was investigated using two different strains: *Xanthomonas campestris* pv mangiferaeindicae 1230 and *X. campestris* pv manihotis 1182. At 72 h of fermentation, using cheese whey as sole carbon source, in presence of 0.1% (w/v) $MgSO_4$*$7H_2O$ and 2.0% (w/v) of K_2HPO_4, maximum xanthan gum productions were observed. Although the xanthan gum concentration was similar for the two strains, approximately 25 g L^{-1}, chemical composition and ionic strength presented several differences.

A modelling approach was used to describe the influence of temperature and pH on the kinetics of both growth and EPS production of *Streptococcus thermophilus* ST 111 in milk-based medium; addition of whey protein hydrolysate to milk medium resulted in an increased growth and EPS production [194].

Growth and EPS production during free and immobilized cell chemostat culture of *Lactobacillus rhamnosus* RW-9595M D was described in [195]. Whey permeate powder was used, both very high EPS production (1800 mg L^{-1}) and volumetric productivity (542 mg L^{-1} h^{-1}) were obtained during chemostat culture with free cells for a D of 0.3 h^{-1}.

3.4. Bacteriocins

Bacteriocins are antimicrobial peptides synthesized, at ribosomal level, by various Gram positive and and Gram negative bacteria, acting towards strictly correlated bacteria (growth inhibition). Generally, bacteriocins are produced at the end of the exponential growth-phase and their spectrum of action can vary, depending on the producing specie [196].

Positive effect of bacteriocins was spotlights in different types of food: dairy and bakery products, meat and fishing products, fruit and vegetables, beverage [197]. Moreover, further application are known, in medical, pharmaceutical and veterinary fields [198].

LAB are particularly prolific in bacteriocins production and can biosynthesize different types of antagonistic molecules. Many LAB are able to synthesize different classes of bacteriocins, currently, nysin is the only bacteriocin industrially produced and which use in food is allowed and authorized [199,200]. Over the last two decades, there has been an explosion of basic and applied research on lactic acid bacteria (LAB) bacteriocins, primarily due to their potential application as biopreservatives in food and food products to inhibit the growth of food-borne bacterial pathogens [196]. Different experimental models demonstrated efficiency on pathogens and spoilage microrganisms growth control, both adding bacteriocins in food [201,202] and using bacteriocins *in situ* synthesized [203].

Although bacteriocins can be produced in the food matrix during food fermentation (*in situ*), bacteriocins by LAB can be produced in much higher amounts during fermentations under optimal physical and chemical conditions [204-207].

A recent review [208] summarized information on nisin production by *L. lactis* in batch cultures utilizing skimmed milk or whey as inexpensive medium. Nisin biosynthesis occurs during the exponential growth phase, several cultural factors influence nisin production: producer strain, media composition, pH and temperature values, agitation and aeration. Nisin production with *Lactococcus lactis* ATCC 11454 was carried out in two different media with pasteurized milk whey, filtered or not. In filtered milk whey nisin titer was 11120.13 mgL^{-1} up to 1628-fold higher than the filtered milk whey. The higher nisin concentration was probably related to insoluble proteins released into the media [204]. In [209] the utilization of milk whey was studied in batch cultures, a higher nisin production was observed in diluted whey (mixed with wash waters), 22.9 BU mL^{-1} (BU – bacteriocin units) in relation to concentrated whey (liquid remaining after the first cheese pressing), 8.3 BU mL^{-1}.

In reference [205], the production of nisin by *L. lactis* UQ2 in a bioreactor using supplemented sweet whey (SW) was optimized by a statistical design of experiments and response surface methodology (RSM). A 2nd-order model built from a CCD experiment predicted a maximum nisin production of 178 IU/mL at 6 h of incubation in the bioreactor, leading to a productivity of 0.74 mg nisin/([L][h]), which increased 1.62 times when using controlled pH (6.5) fermentation.

Continuous production of nisin in whey permeate, supplemented with casein hydrolysate, was investigated using a packed-bed bioreactor. *Lactococcus lactis* subsp. *lactis* ATCC 11454 was immobilized by natural attachment to fiber surfaces and entrapment in the void volume within spiral wound fibrous matrix. Optimal conditions for continuous nisin production were pH5.5, 31°C, 10–20 g/l casein hydrolysate, and D 0.2 h^{-1}. A maximum nisin titer of 5.1×10^4 AU/ml was observed. The bioreactor was operated continuously for 6 months without encountering any clogging, degeneration, or contamination problems [206].

Previously, nisin-Z production was studied during repeated-cycle pH-controlled batch (RCB) cultures using *Lactococcus lactis* subsp. Lactis biovar. diacetylactis UL719 immobilized in k-carrageenan/locust bean gum gel beads in supplemented whey permeate [210,211].

Bacillus bacteriocins are increasingly becoming more important due to their sometimes broader spectra of inhibition (as compared with most LAB bacteriocins), which may include Gram-negative bacteria, yeasts or fungi, in addition to Gram-positive species, some of which are known to be pathogenic to humans and/ or animals. Bacteriocins from Bacillus species offer a much broader spectrum of potential applications compared with LAB bacteriocins. The use of Bacillus bacteriocins in food preservation is just starting to be investigated [212]. A BLIS with a broad spectrum of activity against pathogenic and spoilage bacteria (*L. monocytogenes*, *B. cereus* and clinical isolates of *Streptococcus* spp.) was produced by *B. licheniformis* P40 when cheese whey concentration in the growth medium was about 70 g L^{-1} [213].

Pediocin production on whey by *Pediococcus acidilactici* NRRL B-5627 was described in [214], pediocin was obtained both in batch and re-alkalized fed-batch fermentations on diluted whey supplemented with 2% (w/v) yeast extract.

3.5. Single cell protein and single cell oil

The term SCP refers to dried cells of microorganisms such as algae, actinomycetes, bacteria, yeast, molds, and higher fungi grown in large-scale culture systems for use as protein source in human food or animal feed. Among suitable microorganisms to be grown on whey lactose as a substrate for SCP production, more research work has been carried out on yeasts, in particular *K. marxianus* [215-217]. The use of lactose or whey as a carbon source for the production of yeast biomass is a simple treatment process for increasing the value of food industry co-products [14,218].

Biomass produced from both batch and continuous processes [219-221] is mostly used as animal feed supplement [215] but also in production of baker's yeast [217].

In reference [221] deproteinized sweet and sour cheese whey concentrates were investigated as substrates for the production of SCP with *Kluyveromyces marxianus* CBS6556 up to a 100-l scale. Biomass concentrations up to 50 g l^{-1} ($Y_{x/s}$ 0.52) for sweet whey and 65 g l^{-1} ($Y_{x/s}$ 0.48) for sour whey concentrates were obtained.

Use of whey or buttermilk supplemented with YE for the growth of thermophilic LAB was reported in [222]; cell yields and kinetic parameters obtained were comparable or better than those obtained from control media, which appear to be too expensive for growing LAB on an industrial scale.

High added value probiotic biomass from deproteinized and non-supplemented milk whey was reported in [223]. Growth kinetics of *Lactobacillus casei* in deproteinized goat milk whey was analyzed in batch, continuous and fed-batch conditions.

In [224] a technology for kefir SCP production using whey was developed, a three-step process to scale-up kefir biomass production at a semi industrial scale pilot plant (100- and 3,000-L bioreactors) has been described.

The biotechnological production of SCO has been focused on the ability of various oleaginous microorganisms to convert agro-industrial wastes or raw materials into specialty lipids [225], or equivalents of plant oils that contain poly-unsaturated fatty acids (PUFAs) [226]. Three Zygomycetes, *Mortierella isabellina*, *Thamnidium elegans* and *Mucor* sp., were tested for their ability of producing biomass and lipid-containing g-linolenic acid (GLA) during their cultivation on cheese whey. All the tested microorganisms presented appreciable microbial growth; GLA concentration presented differences related with the strains and the fermentation time. *M. isabellina* produced noticeable quantities of SCO resulted in a maximum GLA production of 301 mg/L [227].

3.6. Enzymes

β-D-Galactosidase most commonly known as lactase, is one of the most important enzymes used in food processing, which catalyses the hydrolysis of lactose to glucose and galactose. The enzyme has been isolated and purified from a wide range of microorganisms but most commonly used *β*-D-galactosidases are derived from yeasts and fungal sources [228,229].

Yeast strains have been grown successfully on whey based medium among different strains of *Kluyveromyces* spp., *K. marxianus* and *K. fragilis* strains showed the maximum enzyme yield [230,14]. *Streptococcus thermophiles* and *Bacillus stearothermophilus* can be considered as potential bacterial sources of lactase. *S. thermophilus* was grown in deproteinized cheese whey [231], supplementation of whey and whey permeate basal media resulted in enhancement of specific growth rate and enzyme activity in bacterial cultures [232].

A simple feeding strategies to obtain high-cell-density cultures of *K. marxianus* (35 g L^{-1}) maximizing β-galactosidase productivity using cheese whey as basic medium was reported in reference [233].

A fermentation process for the production of penicillin acylase by a recombinant *Escherichia coli* and using whey as unique carbon was developed in [234].

Serratia marcescens ATCC 25419 was used for production of secreted proteases on reconstituted whey. A major metallo-protease and a minor serine protease were produced during growth [235]. In reference [236], a mixed culture *Serratia marcescens*–*Kluyveromyces fragilis* was tested on whey, microorganisms showed a synergistic effect in protease production.

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Chapter 25

Antioxidant and Emulsifying Properties of Modified Sunflower Lecithin by Fractionation with Ethanol-Water Mixtures

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Additional information is available at the end of the chapter

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1. Introduction

Lecithins are a mixture of acetone insoluble phospholipids, containing mainly phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidic acid (PA), and other minor substances such as carbohydrates and triglycerides [1-3]. The production of sunflower oil in Argentina, is of utmost importance from an economic point of view [4]. In this country, sunflower lecithin could represent an alternative to soybean lecithin because it is considered a non-GMO product, which is in accordance with the preference of some consumers [5].

Lecithins, in native or modified state, are used in a wide range of industrial applications: dietetic, pharmaceutical, food, cosmetics, etc. This by-product of the oil industry represents a multifunctional additive for the manufacture of chocolate, bakery and instant products, margarine, mayonnaise, due to the characteristics of its phospholipids [6-8].

The introduction of changes in the relative concentration of the original phospholipid composition of lecithin can originate enriched fractions in certain phospholipids with different physicochemical and functional properties for diverse industrial purposes [9-11].

The fractionation process by ethanol or ethanol:water mixtures takes advantage of the different solubility of the phospholipids in this solvent. PC is readily soluble in ethanol whereas PI and PA are virtually insoluble. Phosphatidylethanolamine can be found in both fractions. This process can be carried out alone or in combination with other techniques such

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as chromatography as a further purification step, especially for pharmaceutical, cosmetic and dietetic industry [12-13].

The main application of lecithin at the food industry is associated with its rol as emulsifying agent for dispersions or emulsions [14]. Emulsions are thermodynamically unstable systems from a physicochemical point of view. In virtue of that, it is important to characterize their behaviour against different destabilization processes (flocculation, coalescence, creaming, etc.) [15]. PC enriched fraction, due to its high PC/PE ratio and the lamellar phase structure of the PC at the interface between oil in water, is recognized to be a good oil-in-water (O/W) emulsifier [12,16,17].

On the other hand, PLs can contribute to an improvement of the oxidative stability of fats and oils. Various antioxidative mechanisms have been proposed for the phospholipid actions. For example, the amino functions of PC, PS, or PE, or the sugar moiety of PI have been shown to have metal-chelating properties and PC and PE presented a synergistic effect, with phenolic antioxidants such as tocopherols and flavonoids [18, 19].

The objective of this work was to evaluate the antioxidant and emulsifying properties of modified sunflower lecithins by fractionation with ethanol-water mixtures. In this sense, this study seeks to contribute to the food industry with useful information about developing tailor-made surface-active emulsifiers.

2. Materials and methods

2.1. Materials

Native sunflower lecithin was provided by a local oil industry (Vicentin S.A.I.C.).. This lecithin present a phospholipid composition of 43.1% (PC 16.2%, PI 16.5%, PE 5.3%, minor PLs 5.1%), 23.5% other compounds (glycolipids, complex carbohydrates), 33.4% oil.

Sunflower lecithin was deoiled with acetone, according to AOCS Official Method Ja 4-46, procedures 1–5 [20], obtaining the deoiled sunflower lecithin (DSL) (Figure 1). Then, DSL was stored at 0 ºC. This modified lecithin was used as control sample. Deoiling procedure was performed in duplicate.

2.2. Sunflower lecithin fractionation

Fractionation process was performed to 30 g of native sunflower lecithin with the addition of extraction solvents with different ethanol/water ratio (96:4, 100:0) using an ethanol/lecithin ratio of 3:1. These samples were incubated in a water bath at 65 °C during 60 min with moderate agitation (60 rpm), and then centrifuged at 1880 g, 10 ºC during 10 min. Afterwards, the corresponding ethanolic extracts were obtained and ethanol was eliminated by evaporation under vacuum [17].

Ethanol soluble phases were further deoiled with acetone, according to AOCS Official Method Ja 4-46, procedures 1–5, obtaining the different PC enriched fractions (PCF 96, PCF 100)

(Figure 1). Then, both fractions were stored at 0 ºC. Fractionation and deoiling procedures were performed in duplicate.

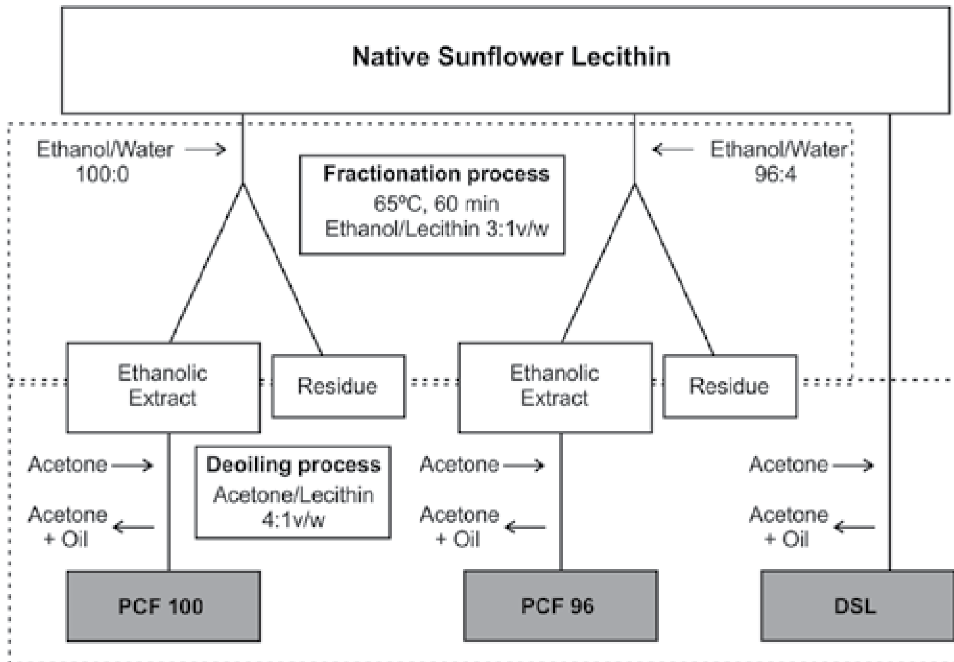

Figure 1. Flow diagram for deoiling and ethanol fractionation of native sunflower lecithin

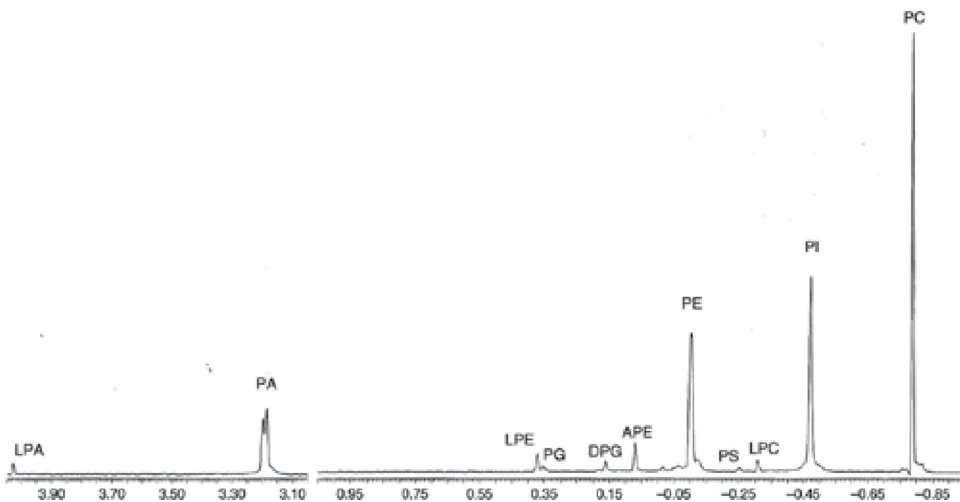

Figure 2. Spectrum of a phospholipidic sample

2.3. Phospholipid composition

Phospholipid composition of samples obtained after different modification processes was determined by ^{31}P NMR analysis in a Bruker Avance 600 MHz automatic spectrometer, using triphenyl phosphate as internal standard (Spectral Service GmbH, Köln, Germany) (Figure 2) [21-23]. For this purpose, 100 mg of each modified lecithin were diluted in 1 mL of deuterated chloroform, 1 mL of methanol and 1 mL of 0.2 M Cs-EDTA (pH 8.0). The organic layer was separated after 15 min shaking, and analyzed by the described spectroscopic technique. Phospholipid composition of the different modified sunflower lecithins (MSLs) was expressed in terms of molar concentration (mol / 100 mol lecithin) [17].

2.4. Antioxidant properties

The antioxidant properties of the MSLs were evaluated by the Rancimat (Mod 679, Metrohm) method. 5 g of sunflower oil were added with different concentration of the analyzed samples (500-2000 ppm), heated at 98 °C, air flow 20 L/h. Stability was expressed as the induction time, according to Gutiérrez [24].

Also, the highest antioxidant concentration was selected for each modified lecithin. The same procedure was carried out with previous thermal treatments at 120 and 160 °C for 1 h, adding the modified lecithins before heating. Refined sunflower oil with and without previous heat treatments were used as control samples.

Oil tocopherol content was determined by normal phase HPLC using a Hewlett Packard chromatography system (HPLC Hewlett Packard 1050 Series, Waldbronn, Germany) equipped with a fluorescence detector Agilent 1100 Series (Agilent Technology, Palo Alto, CA, USA) following the procedures described in IUPAC 2.432 [25] and AOCS Ce8-89 [20].

2.5. Oil-in-Water (O/W) emulsions preparation

Refined sunflower oil was used to prepare oil-in-water (O/W) emulsions with a formulation of 30:70 wt/wt. Emulsions were prepared at room temperature in an Ultra-Turrax T25 homogenizer using S 25 N–10 G dispersing tool (7.5 mm rotor diameter) at 10,000 rpm for 1 min, according to Cabezas et al. [26] with the addition of MSLs in a range of 0.1-2.0% (wt/wt).

2.6. Optical characterization of emulsions

The backscattering of light was measured using a QuickScan Vertical Scan Analyzer (Coulter Corp., Miami, FL). The backscattering of monochromatic light (λ = 850 nm) from the emulsions was determined as a function of the height of the sample tube (ca. 65 mm) in order to quantify the rate of different destabilization processes during 60 min. This methodology allowed to discriminate between particle migration (sedimentation, creaming) and particle size variation (flocculation, coalescence) processes [27]. The basis of the multiple light scattering theory has been exhaustively studied by Mengual et al. [28].

2.7. Statistical analysis

Data were evaluated by analysis of variance (ANOVA) using the software Systat® 12.0 [29]. For this purpose, differences were considered significant at $p < 0.05$.

3. Results

3.1. Phospholipid composition

The quantitative analysis of phospholipids was performed by ^{31}PNMR which represents a modern and the most sophisticated methodology for evaluating the composition of lecithins, since it is possible to obtain a separate signal for each phospholipid class [9, 21]. The results presented in Table 1, evidenced the high solubility of the phosphatidylcholine in the different ethanolic solvents assayed. ^{31}PNMR determinations of the different enriched PC fractions (PCF 96 and PCF 100) exhibited an important concentration of PC (>71.0 %) as well as low values of PI (<8%) in comparison with the native and deoiled sunflower lecithin assayed.

3.2. Rancimat analysis

The oxidative stability of the refined sunflower oil (control) and the activity of different modified lecithins were evaluated determining the induction time (t_i) by a Rancimat analysis. This methodology can be used to evaluate the efficiency of various synthetic or natural antioxidants to stabilize fats and oils against an accelerated oxidation test [30]. Figure 3 shows t_i for the modified sunflower lecithins used at different concentrations.

PL	DSL [a]	PCF 96 [a]	PCF 100 [a]
PC	36.7	71.2	76.3
1-LPC	< 0.1	< 0.1	< 0.1
2-LPC	1.5	3.3	2.8
PI	35.3	7.4	3.6
LPI	< 0.1	< 0.1	< 0.1
PE	15.1	12.2	10.6
LPE	< 0.1	0.8	0.4
PA	5.0	1.3	1.4
LPA	< 0.1	< 0.1	< 0.1
Others PL	6.4	3.8	4.9

[a] Values represent means (n = 2). The coefficient of variation was lower than 6%

Table 1. Percentage phospholipid composition (mol PL / mol total PL) of deoiled sunflower lecithin and PC-enriched fractions analyzed by ^{31}PNMR

The antioxidant addition increased the t_i of the control as a function of increasing concentration. MSLs did not show a marked difference of the respective t_i values at concentration of 500 ppm. However, at high concentrations (1000 - 2000 ppm), both PC fractions had a high significant effect (p <0.01) on the oxidative stability of the control system, in relation to the values recorded by DSL addition. In this sense, 2000 ppm of DSL increased the t_i value of the control oil of 49.1%, while similar concentrations of PCF 96 and PCF 100 enhanced this value 167.4% and 108.3%, respectively (Table 2).

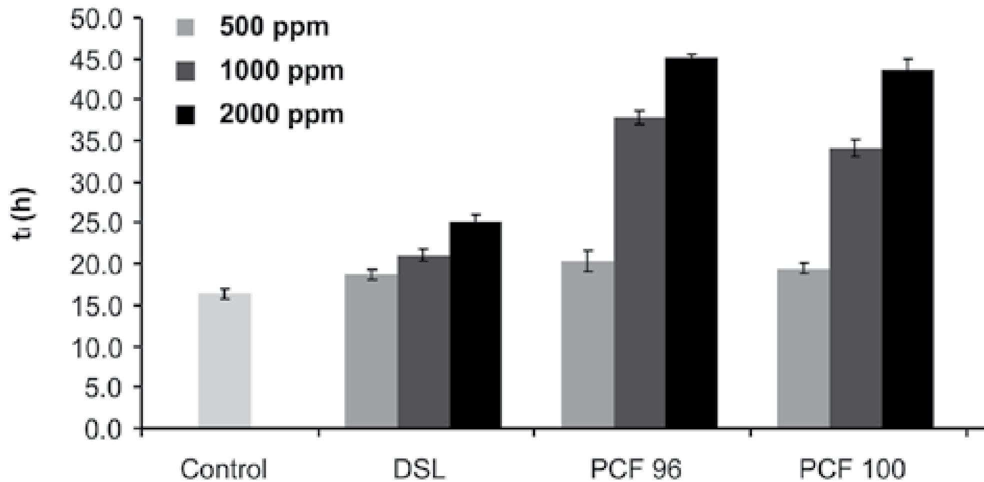

Figure 3. Induction times (ti) of refined sunflower oil (control) added with different concentrations of modified sunflower lecithins (Rancimat Mod 679, Metrohm). Mean values (n = 3) ± sd

	Δ ti WT / ti control WT (%)	**Δ ti T120 / ti control T120 (%)**	**Δ ti T160 / ti control T160 (%)**	**Δ ti T120 / ti control WT (%)**	**Δ ti T160 / ti control WT (%)**
Control	0.0	0.0	0.0	-26.6	-25.8
DSL	49.1	56.9	19.0	15.1	-11.7
PCF 96	167.4	211.9	113.9	128.8	58.6
PCF 100	157.7	177.5	95.4	103.6	44.9

control, refined sunflower oil; WT, samples without thermal pre-treatment; T120, samples with thermal pre-treatment 120°C, 1h; T160, samples with thermal pre-treatment 160°C, 1h

Table 2. Percentage induction times increase of refined sunflower oil added with different modified sunflower lecithins (MSLs) with or without thermal pre-treatment

Induction times of oils added with the different MSLs without thermal pre-treatments, showed higher values than those obtained by Pan et al. [31], who reported under similar test conditions that 2000 ppm of native sunflower lecithin produced a ti increase of 12%. The in-

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troduction of changes in the original phospholipids concentration of this native lecithin by different modification processes (deoiling, ethanol fractionation) allow to obtain modified lecithins with better physicochemical and functional properties with respect to the starting material.

A concentration of 2000 ppm of the different modified lecithins showed the highest antioxidant activity in this assay. Therefore, refined sunflower oil added with this concentration of MSLs was previously subjected to different heat treatments at 120 and 160 ° C for 1 h. The ti values of the oils added with PCF samples had a high significant difference (p <0.01) in relation to those added with DSL (Table 2). PC enriched fractions reduced the negative effect of the heat treatments over the oxidative stability of refined sunflower oil. These pre-treated samples showed higher ti values respect to the control system, even without thermal pre-treatment. In this sense, oils added with PCF 96 and PCF 100, subjected to a heat treatment at 160°C presented induction times of 58.6 and 44.9% higher than the untreated oil, respectively.

It is interesting to note that PCF 96 recorded the best antioxidant capacity producing an increase of 211.9% (120°C, 1h) and 113.9% (160°C, 1h) in relation to those recorded for the respective control oils. This fact indicates that the fractionation process carried out with ethanol 96 ° not only allows to obtain fractions enriched in PC with antioxidant activity, but also this activity is greater than those exhibited using DSL and the PCF obtained through fractionation with absolute ethanol (PCF 100).

The ability of phospholipids to inhibit lipid oxidation in bulk oils has been known for several decades, but the mechanism of stabilization still remains controversial [32]. However, many research works have proposed different antioxidant mechanisms for these compounds. Particularly, PC and PE have been shown to have metal-chelating and scavenging properties. Also, this type of phospholipids present a synergistic effect with the different tocopherols (α-tocopherol 512.84 μg/g; β-tocopherol 4.55 μg/g) regenerating the oxidized tocopherol molecule by donation of a hydrogen atom of their amino function [18, 33]. This fact and the high PC and PE concentrations are in correlation with the better antioxidants characteristics observed for both PCF assayed.

3.3. Optical characterization of O/W emulsions

Stability of the different O/W emulsions (30:70 wt/wt) was studied recording the backscattering (BS) profiles as a function of the cell length and time, by a vertical scan analyzer (QuickScan). These profiles were analysed in different zones of the emulsion: Zone I (10-20 mm) to visualize the destabilization process by migration of the oil droplets from the bottom towards the top of the tube (creaming), Zone II (40-45 mm) characterized by the accumulation of oil droplets after the creaming process (cream phase) and Zone III (50-60 mm), to analyze the destabilization of the cream phase [34]. For instance, Figure 4 shows a typical profile obtained for an O/W emulsion with the addition of 0.5% of DSL.

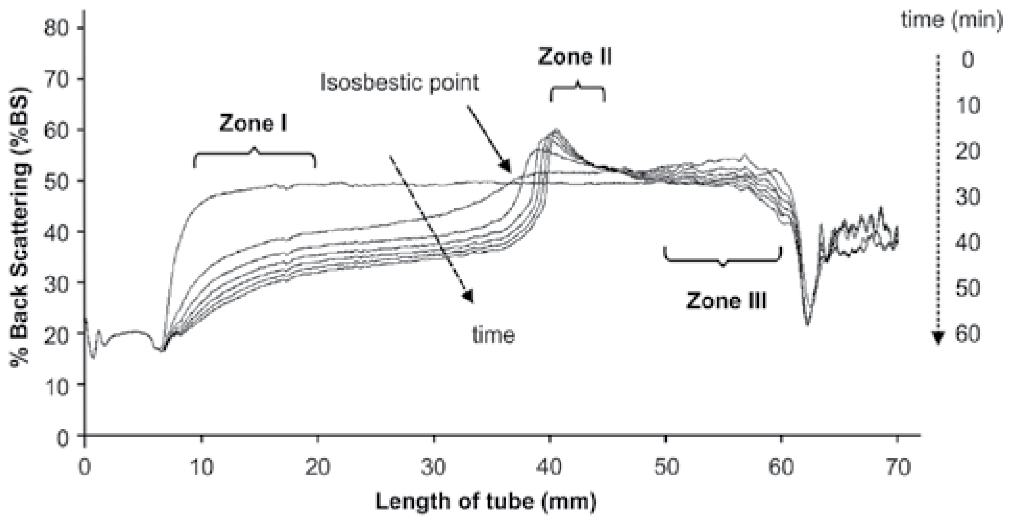

Figure 4. Backscattering (%BS) profile of a O/W emulsion (30:70 wt/wt) with the addition of 0.5% of DSL

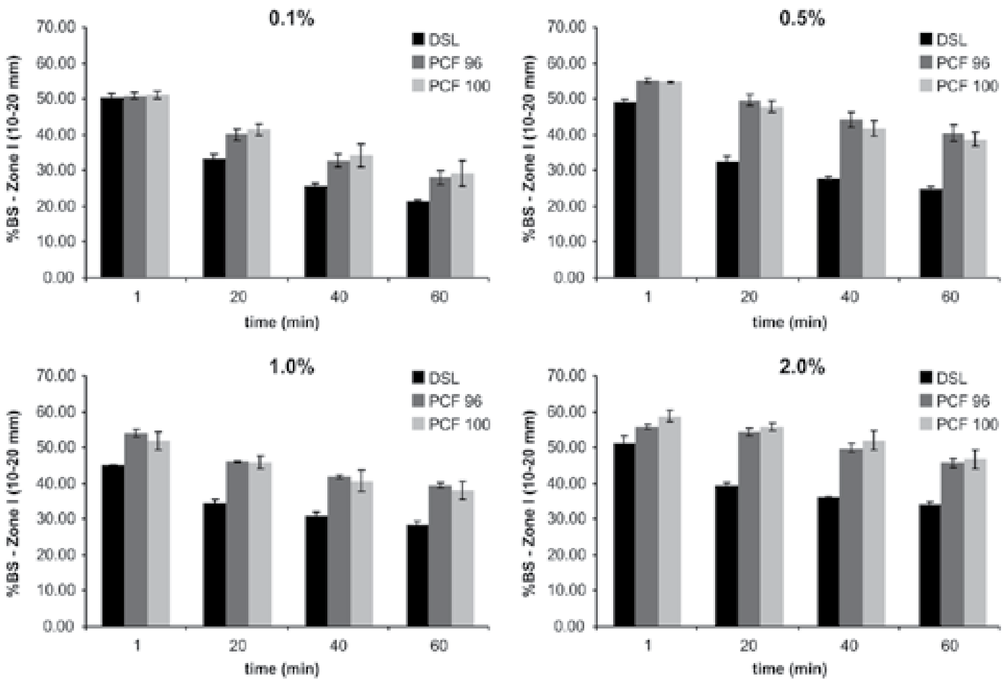

Figure 5. Backscattering (%BS) values of O/W emulsions (30:70 wt/wt) with the addition of different modified sunflower lecithins, Zone I (10-20 mm). Mean values (n = 3) ± sd

The creaming destabilization process (i.e. migration of oil particles to the upper portion of the tube) is evidenced by a decrease of %BS values at the bottom of the tube and the appearance of an isosbestic point [34]. This point separates the zones with lower (left) and higher (right) values than the initial %BS (Figure 3). The QuickScan profiles corresponding to the zone I (10-20 mm) showed an increase of the emulsion stability against the creaming process, as a function of increasing concentration of different modified lecithins (Figure 5). In particular, the PC enriched fractions (PCF 96 and PCF 100) produced a high stability in O/W emulsions than DSL, over the range of concentration studied. In this sense, it should be noted that QuickScan profiles of the different PCF did not show significant variations of %BS values for 2.0% during 40 minutes. These results are in concordance with those previously reported by Wu and Wang [12], related to the emulsifying activity of PC enriched fractions from soy lecithin.

Moreover, O/W emulsions with 0.1-0.5% of DSL showed a sharp decrease of %BS in the Zone I. This behaviour indicates a rapid destabilization of these emulsions by creaming.

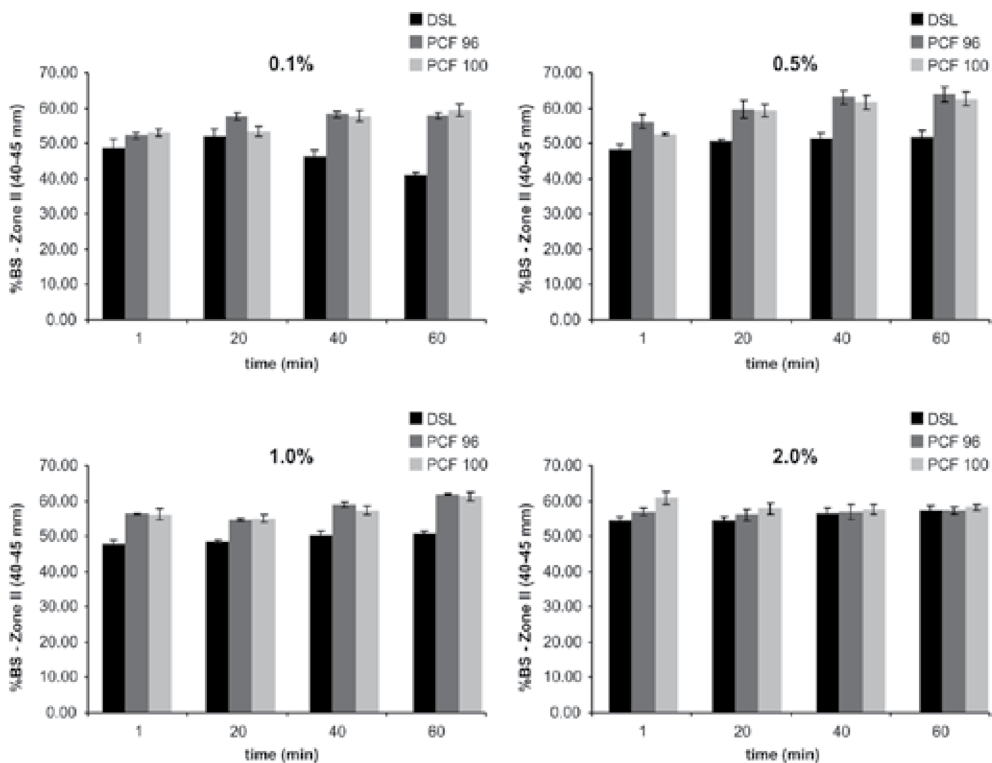

Figure 6. Backscattering (%BS) values of O/W emulsions (30:70 wt/wt) with the addition of different modified sunflower lecithins, Zone II (40-45 mm). Mean values (n = 3) ± sd

The tube zone between 40-45 mm (Zone II) is characterized by the accumulation of oil droplets after the creaming process (cream phase). Figure 6 shows the %BS values vs. time in Zone II. Emulsions formulated with PC enriched fractions presented higher %BS values than those obtained using DSL, for all concentrations studied. The higher levels of %BS and the greater stability of these emulsions formulated with a high concentration of phosphatidylcholine would be associated with the formation of dense cream phases with a lower proportion of continuous phase inside [26].

The stability of the different cream phases were analyzed by the evolution of the % BS values vs. time in Zone III (50-60 mm) (Figure 7). %BS values remained constant at concentrations of emulsifier above 0.1% of the different modified lecithins. This behaviour confirms the stability provided by these MSLs against the coalescence process, in these conditions.

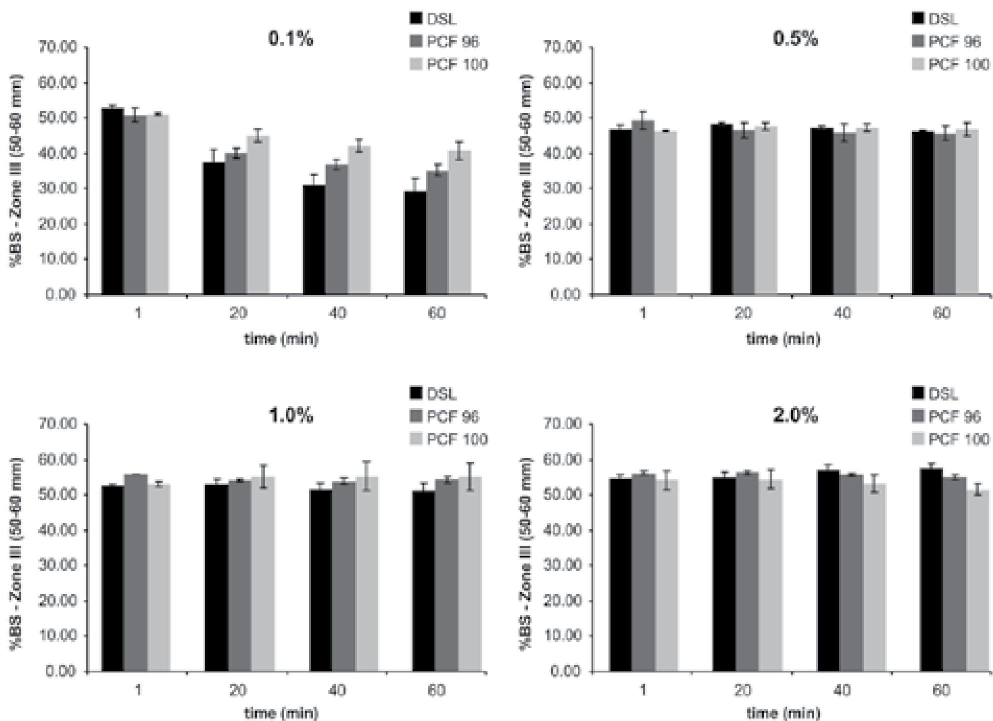

Figure 7. Backscattering (%BS) values of O/W emulsions (30:70 wt/wt) with the addition of different modified sunflower lecithins, Zone III (50-60 mm). Mean values (n = 3) ± sd

However, emulsion with 0.1 of DSL did not allow the formation of the cream phase. These results are related to the rapid decrease of %BS, the formation of an oil layer in the upper part of the tube and the absence of an isosbestic point (data not shown) ; suggesting the occurrence of a cream phase destabilization by coalescence [27].

The hydrophilic-lipophilic balance value (HLB) is often used in order to explain the performance of emulsifiers [15]. The high concentration of phosphatidylcholine (hydrophilic phospholipid) increases this empirical value in the PC enriched fractions. In this sense, the best properties of these modified lecithins as emulsifying agents in O/W emulsions was according with Carlsson [35], who has determined that lecithin with high HLB values present best O/W emulsifying properties.

Also, the phase structure at the interface of the different phospholipids influences the emulsion formation and stability [16]. PC forms a lamellar phase at the interface between oil and water with well ordered mono- and bi- layers. This structure has a great importance for the stabilisation of O/W emulsions.

4. Conclusions

The study of the induction times showed a highly significant difference ($p < 0.01$) between the antioxidant activity exhibited by the different PCF in relation to the addition of DSL. Also, these fractions allowed to obtain more stable O/W emulsions (30:70 wt/wt) in comparison with those added with DSL at different concentrations (0.1-2.0%) in terms of kinetic destabilization as a function of changes in the backscattering values vs. time. These results showed that PC enriched fractions (PCF 96 and PCF 100) constitute a potential alternative as emulsifier agent for the food industry.

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Section 5

Economic and Social Aspects

Chapter 26

Cabannina Cattle Breeding: An Agro-Ecological Challenge for Sustainable Rural Development in Northern Italy

Ricardo Communod, Carla Colombani,
Eleonora Munari and Daniele Vigo

Additional information is available at the end of the chapter

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1. Introduction

Intensive farming is an agricultural production system characterized by widely adopting external inputs - such as capital, mechanization, infrastructures, pesticides and chemical fertilizers intensively used - which affects the natural environment and rural societies. Since it allows to produce more food on a given land extension, such agricultural choice has been the predominant response to population growth so far. While permitting to raise many animals in limited areas, intensive animal farming practices require a large amount of food, water, medical treatments, capital intensive technology, energy, and fuel. Is being the selection of animals with rapid food conversion into milk and meat the aim of every industrial farm, a decline in, for example, the animal reproductive performances and in the product quality follows. Thus, nowadays problems in the dairy cattle scenario are easily highlighted. Just to name Friesian breed, its reproductive performances decreased worldwide with negative consequences on both cow robustness and longevity due to increased stress, udder health disturbances and locomotion disorders, which meant damages to the physiological parameters typical of healthy cows.

Despite all the above mentioned problems associated with conventional farming, many positive developments are creeping in. Several alternative initiatives are now flourishing all around the Italian peninsula to promote ecological agriculture; preservation of small farmers' livelihoods; production of healthy, safe and tradition-linked foods; localization of distribution, trade and marketing. These typologies of traditional agriculture offer promising models for marginal areas as they promote biodiversity, thrive without agrochemicals, and

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sustain year-round yields. Technological approaches are welcome provided, they promote yield improvements based on agro-ecological principles, emphasizing diversity, synergy, recycling and integration of animals, soil, and water; moreover, social processes involving and empowering a community are welcome, too. In the Italian north-western region of Liguria, Petramartina farm can be considered as a challenging pioneer thanks to its following agro-ecological principles in order to recreate a traditional and sustainable dairy breeding based on autochthonous cows, called Cabannina.

2. Conventional dairy farming in the "River Po Plain" and its critical points

The River Po Plain is characterized by an industrial breeding system whose bovine herds at present are fewer, larger, more specialized and more capital intensive than they were 60 years ago.

Revealing data are briefly shown in the following table (Table 1).

NATIONAL DATA			
YEAR	CONSISTENCY		COWS/HERD
	HERDS	COWS	
2002	15.106	1.088.178	72
2003	14.984	1.107.701	74
2004	14.823	1.100.543	74
2005	14.317	1.101.657	77
2006	14.069	1.102.655	78
2007	13.818	1.100.401	80
2008	13.510	1.101.868	82
2009	13.327	1.103.453	83
2010	13.164	1.113.859	85
2011	12.922	128.626	87

Table 1. Italian National Data about number of herds, total cows, and average number of cows per herd.

As clearly shown, since the last 10 years the Italian dairy situation has followed the international trend for which farmers, have to increase the number of animals in their herds to sustain their businesses.

Thus, in recent years the dairy system has been mainly concerned with both improving animal performances and with increasing the number of heads per farm in order to reduce

fixed costs that represent, together with raw materials necessary to compose animal feed formulations, the most important passive voice of a farmer's budget.

The following table (Table 2), gives an overview of Italian Friesian cow milk production and their reproductive situation in the last years.

Year	Milk Production	First Calving/ Total Cows	Days open	Number of Lactation/Cow
	(q/cow/year)	(%)	Mean	Mean
2004	85,9	34,8	147	2,48
2005	89,1	35,6	136	2,47
2006	89,4	36,1	141	2,45
2007	90,3	35,5	143	2,46
2008	89,8	36,2	143	2,46
2009	88,7	35,6	141	2,46
2010	91,3	36,1	141	2,44
2011	91,9	36,4	144	2,39

Table 2. An overview of Italian Friesian cow official production by AIA (Italian Breeders Association)

As shown, the increased number of heads and of milk production amount were not followed by any improvement in reproductive efficiency. In fact, Table 2 clearly demonstrates how the reproductive physiology of the animals was damaged. And mainly in Friesian breeds, cows' robustness and longevity negatively affected by increasing stress, udder health disturbances and locomotion disorders [1], are an issue of major concern to farmers. From a physiological point of view, Holstein cows reached a very critical situation having missed their good reproductive efficiency characters (e.g. calving interval and conception rate [2], excellent longevity in farm (only 2,39 lactations in their life), resistance to stress and diseases (metabolic syndromes, ketosis, mastitis and foot diseases) [1,3,4], whereas they dramatically increased "energy and financial voracity" (diet based on starch and protein meals, great health and structural investments due to several highly-recurring diseases [4-6]. A recent study conducted on an Italian dairy farm, located in the River Po Plain, highlighted the extreme financial voracity affecting a conventional farm of good quality in order to run it and make it survive [7]. The Authors showed that even an excellent business consisting of 208 productive cows, 70% of which are healthy animals, can lose more than 36000 €/year on including both direct and indirect costs due to animal diseases such as veterinary therapies, decrease in productive performances, milk removed, price-cutting for high milk quality, etc. These data are worrying in the consideration that in Italy milk production represents one of the most important sectors of its agro-industry,. In fact, in Europe Italy is the most important cheese producer and exporter, with its about 460,000 tons of products and almost 3 billion Euros (data from www.clal.it, www.ismea.it) derived from PDO (Protected Designation

Origin) and PGI (Protected Geographical Indication) production. Its most representative cheese is certainly *Parmigiano Reggiano* and *Grana Padano* that recently increased by 9.8% its export trend to Germany, the United States, France, Switzerland and the United Kingdom.

Italian breeders have to care about all the important milk quality parameters involved in cheese making processes such as milk fat, proteins and somatic cell count.Recently, Italian researchers demonstrated that milk whose somatic cell content is greater than 400,000 cells/ml evidences a scarce aptitude to rennet coagulation and cannot be generally considered suitable for cheese production, with particular reference to Grana cheese production [8].

Having in mind the difficulties characterizing the present background and in the aim to confirm Italian national data, some analytical studies were performed in five different Friesian herds bred in the River Po Plain.

The following parameters were investigated: milk production (herds A and B), reproduction and sanitary situation (herds C, D, E).

In herds A and B from an area south of Milan (Lodi) fat and protein in the milk yield as well as total cheese yield were monitored. Tables 3 and 4 show key results from this investigation.

YEAR	LACTATIONS CLOSED	MILK YIELD	FAT		PROTEIN		CHEESE YIELD
		Kg	%	Kg	%	Kg	Kg
2007	239	10.608	3,74	397	3,57	379	825
2008	243	10.639	3,68	392	3,48	370	811
2009	254	10.184	3,78	385	3,56	363	793
2010	232	9.991	3,87	387	3,56	356	785
2011	228	10.193	3,52	359	3,48	348	765

Table 3. Milk Production Data in Herd A

YEAR	LACTATIONS CLOSED	MILK YIELD	FAT		PROTEIN		CHEESE YIELD
		Kg	%	Kg	%	Kg	Kg
2007	54	10.691	3,37	360	3,3	353	768
2008	55	9.385	3,53	331	3,39	318	695
2009	48	9.344	3,68	344	3,29	307	690
2010	52	9.380	3,67	344	3,27	307	690
2011	55	8.536	3,45	294	3,28	280	616

Table 4. Milk Production Data in Herd B

Clearly, milk production and milk aptitude to produce cheese underwent a dramatic decrease causing economic losses to breeders.

In herds C, D, and E from an area west of Milan (Abbiategrasso), days open (DO), number of insemination for conception (AI), culling cows (CC) together with sanitary problems and treatments were monitored and key results from this investigation are represented in table 5.

HERD	COWS	DO	AI	CC
C	688	154	2,53	30%
D	438	147,5	2,09	36,50%
E	345	130,7	3,18	29,03%
TOTAL	1471			
AVERAGE		144,1	2,53	32%

C,D,E = herds in Abbiategrasso (MI); DO = days open; AI =number of artificial insemination per conception; CC= number of culling cows.

Table 5. Average data in Herds C, D, and E.

Such data broadly confirm official data related to Italian national situation. Among critical points to be underlined both nationally and internationally, we should consider that not only animal intensive breeding dramatically increase worldwide, but vegetal monoculture as well. Thus, while intensive farming and agriculture can be considered extremely competitive and the only way to feed a fast-growing human population, they also cause different economic, environmental and social problems [9]. In fact, from an ecological point of view, consequences of industrialization are manifold [10] as detailed below:

a. Cycles of nutrients, energy, water and wastes become more open rather than closed as occurring in a natural ecosystem;

b. The natural habitat of most wild creatures can be limited or destroyed by industrial agro-farm, and most soil can suffer erosion;

c. A large amount of energy input is needed to produce, transport, and apply chemical fertilizers;

d. Dwindling and ever more expensive fossil fuels are used to derive agrochemicals, and to work fuel-based mechanization and irrigation operations, the core of industrialized agriculture;

e. Air pollution can be prompted by chemical fertilizers that were recently implicated in the destruction of the ozone layer and in global warming;

f. Animal and vegetal biodiversity are severely affected as species more susceptible to diseases are selected.

Moreover, an excessive use of fertilizers is linked to acidification/salinization of soils and to a higher incidence of insect pests and diseases through mediation of negative nutritional changes in crop plants [11]. It also implies several harmful effects on the health of workers spreading them, of people living nearby or downstream/downwind an area of application, and of consumers swallowing leftover pesticides from food.

Last, but not least, a genetic selection is required in both vegetal and animal conventional production to maximize production, which implies biodiversity decrease. Thus, the amount of crop diversity per unit of arable land decreased and croplands tended towards concentration. In fact, in order to serve as many markets as possible some political and economic forces influence a general trend to devote larger and larger areas to monoculture [10].

3. Agro-ecological principles as a potential solution to extremely intensive breeding

Nonetheless, excessive intensive breeding can be successfully contrasted and new ecological concepts and principles can be applied to design, development and management of sustainable agricultural systems. In fact, agro-ecology can be a sensible solution.

Agro-ecology is the science that studies the relationship between agricultural production, land and traditions regarding a given territory [11]. It is a theoretical and practical discipline derives from different experiences related to organic agricultural production, characterizing the last and present centuries.

It investigates the elements of an agricultural ecosystem and their interactions carefully and provides principles and methods of work. Principles and methods that do not merely consider production but also the ecological, technical, socio-economic and cultural spheres of the agro-ecosystem.

Major aims of this science are:

i. to increase both functionality and productivity of a business and an ecosystem;

ii. to deal with biodiversity conservation and nutrient recycling;

iii. to optimize local resources usage and economic viability of a farm.

Following these objectives, some practices - such as crop rotation and use of local varieties - essential to the microbiological and mineral balance of the soil can be recovered together with techniques well-known since past times but never applied on a large scale, e.g. the on-farm production of natural preparations and fertilizers [11]. Even planting local varieties of fruit trees and hedges, a good practice swept away by the advent of industrial agriculture, can help to increase biodiversity within a farm and promote a more balanced agro-ecosystem. A subsoil rich in organic and mineral elements and a greatly diversified topsoil make vegetation more durable and more resistant against diseases and insects.

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Present industrialized agriculture, as Altieri underlines, is no longer sustainable. In fact, conceiving a constant supply of water, some cheap power and climate exploitation with no abrupt changes is absolutely utopian nowadays.

For instance, the old system based on mechanization, e.g. forage drying, cannot work any longer because of the continuous rise in price of fossil fuels. Not to say about the global use of herbicides that counts about 2.6 million tons per year and costs 25 billion euros. Being excessive and uncontrolled, they negatively affects wildlife and pollinators, the quality of water and fishing activity, and pose a serious risk of poisoning humans and animals. In addition, arthropods and weeds show high adaptation to these substances, while abuse of monocultures indirectly select pests and makes them more resistant [10]. Table 6 illustrates the fundamentals of agro-ecology.

Enhancing biomass recycling, with a view to optimizing organic matter decomposition and nutrient cycling over time.
Strengthening the " immune system" of agricultural systems by enhancing functional biodiversity, natural enemies, antagonists, etc.
Providing the most favorable soil conditions for plant growth, particularly by managing organic matter and by improving soil biological activity.
Minimizing losses of energy, water, nutrients and genetic resources by enhancing conservation and regeneration of soil and water resources and agro-biodiversity.
Diversifying species and genetic resources.
Improving beneficial biological interaction and synergies among the components of agro-biodiversity, thereby promoting key ecological processes and services.

Table 6. Principles of Agro-ecology by Altieri (2012) [11].

As seen in Table 7, in a recent study Koohafkan et al. [12] formulated some questions indicating what can be defined as an agro-ecological farm.

1. Do they reduce poverty?
2. Are they based on rights and social equity?
3. Do they reduce social exclusion, particularly for women, minorities and indigenous people?
4. Do they protect access and rights to land, water and other natural resources?
5. Do they favor redistribution of productive resources?
6. Do they substantially increase food production and contribute to household food security and improved nutrition?
7. Do they enhance families water access and availability?
8. Do they regenerate and conserve soil and increase soil fertility?
9. Do they reduce soil loss / degradation and enhance soil regeneration and conservation?
10. Do practices maintain or enhance organic matter and the biological life and biodiversity of the soil?

11. Do they prevent pest and disease outbreaks?
12. Do they conserve and encourage agro-biodiversity?
13. Do they reduce greenhouse gas emissions?
14. Do they increase income opportunities and employment?
15. Do they reduce variation in agricultural production under climatic stress conditions?
16. Do they enhance farm diversification and resilience?
17. Do they reduce investment costs and farmer dependence on external inputs?
18. Do they increase the degree and effectiveness of farmer organizations?
19. Do they increase human capital formation?
20. Do they contribute to local / regional food sovereignty?

Table 7. Questions to define an agro-ecological farm. (Koohafkan, 2011) [12]

4. Cabannina breeding in Aveto Valley

4.1. Territory

Aveto Valley represents the natural settlement area of Cabannina cows. When a war correspondent, Ernest Hemingway wrote in his diary in 1945: “I have just passed across the most beautiful valley in the world”. In fact, it is one among the narrowest valleys of the northern side of the eastern Ligurian Apennine, where both the climate and the lands have unique characteristics thanks to the favorable altitude, which ranges from about 350 to 1800 meters, and the proximity to the sea. Rain is much more abundant than in the rest of the region, with about 2500 mm per year as peak value, especially in autumn and spring. In winter, snowfalls are stable, usually abundant, and temperatures can drop below 10 °C. In summer, the valley climate is humid and can become cooler at high altitude.

Figure 1. Aveto valley panorama, from Scabbiamara area.

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The river Aveto flows through the valley floor; it springs in Prato Lungo, an area just below the village of Acquapendente near Mount Caucaso. Slowly winding, it creates different landscapes where pastures alternate to woods, meadows and rocky canyons. Then it passes through the province of Genoa, to reach the province of Piacenza. In Confiente place (a term that means "confluence") it gets into the river Trebbia, a tributary of the Po river.

In this context, the Cabannina, a great grazer even in extreme conditions, perfectly adapted to the territory and evolved. In fact, it can climb to the ridges and resist cold temperatures as well as snow without any problem like other northern breeds, such as the Highlands of Scotland. Thanks to these features this breed could spread to other areas of Liguria where the animals can graze free on green pastures surrounded by woods all summer long and where many businesses breed only one race.

4.2. A short historical account

Modern cattle breeds derive from two domestication events of the ancestral "Bos Primigenius" occurred in South West Asia and in Asia, which gave rise to the "Bos Taurus" and "Bos Indicus" respectively [13-15]. Hence, it is believed that these animals may result from a particular population of Uri that lived in the Southern Alps area [16]. Their dairy characteristics and adaptation to the territory still show their ties with the Bos Primigenius.

In the past the breeder families of Aveto Valley gathered in consortia; alternately, every producer received milk by the other members and made cheese. Every family was given a wooden stick where some notches were carved to mean milk given to community, a form of barter locally named 'cangiu', i.e. 'exchange'. The animals were let loose in the pastures; for most of the year they were fed only with fresh forage and homemade feed supplements such as boiled potato skins, cooking water and bran [17]. When necessary, transhumance to Tigullio coast occurred. At the beginning of the twentieth century there were about 40,000 specimens that in the postwar period underwent a dramatic reduction in their number as countryside was abandoned, people migrated towards cities and intensive farms arose.

In February 1963, Cabannina cows risked their extinction on account of Law No. 126, "Regulation of Bovine Reproduction", - now repealed- requiring their forced slaughter and replacement with apparently more productive breeds. When this law became effective the Aveto breeders, strongly determined to preserve Cabannina, started their resistance against the Provincial Inspectorate of Agriculture. Attempts were made to combine different races, such as Bruno-Alpine, to the native heads, but with little results. In this regard, Maimone studied these two races years later in 1981 [18]. Combining the data collected by Orefice in 1978 [19], it was certified that Cabannina reproductive efficiency was better than Brown one and its economic life was greater thanks to the early age of its first birth and the reduced inter-calving period (84 days versus 140). Brown also appeared to have an inferior capacity to pasture, especially in villages where Cabannina was farmed, and could not stand the scarce food resources in the area showing phenomena of piroplasmosis which Cabannina breed could better fight against. In fact, originally Aveto Valley was a large swamp and over time native breed may have developed a natural resistance to the protozoa responsible for the disease.

Since 1974, the studies by Usai (1974), Orefice (1978) and Maimone (1981) [18-20] contributed to the promotion of Cabannina breed. In 1978 Orefice proved that in the town of Rezzoaglio there were about 500-550 heads of this typical breed. In 1981 when a census was made, out of 759 heads left only 158 were phenotipically Cabannina. Thus, an exemption to law No. 126 was decided in order to use Cabannina reproductors, and in 1985 the "Anagraphic Register of indigenous cattle populations and ethnic groups of limited diffusion " was established.

Despite various interventions Cabannina heads have steadily declined. From a survey conducted in 2010 by the Provincial Association of Breeders, only 254 heads are left on the Ligurian territory, mostly concentrated in Aveto valley. In the same year Cabannina breed has got one of the 193 Slow Food Presidia on the Italian territory. The network of Slow Food Presidia was established in 1998; it protects and preserves traditional products made according to old techniques, often present in remote areas of our country, and makes them distinguishable by a logo.

Figure 2. U'Cabanin trademark of Cabannina raw milk production.

In our case it is not the product to be preserved, but the breed as a part of it, similarly to other endangered species such as the Black Pig Nebrodi of Sicily and the Black Cock of Val di Vara in Liguria (www.slowfood.it). In 2007 a cheese made only from Cabannina raw milk began to be produced under the trademark "U Cabanin". Compared to the 1981 census the number of Cabannina heads has slightly increased, but the situation remains critical, being this breed still at high risk of extinction.

4.3. Cabannina cow

Breed standards required to register animals were defined in a specific Registry for Breeds under Limited Distribution. A recent dissertation from the Faculty of Veterinary Medicine in Parma [21], collected biometric data on Cabannina cows; such data prove how their average measurements have changed according to observations performed from mid'70s. In fact,

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compared against race specifications, height at withers, set from 1.18 to 1.20 m for females, and from 1.25 to 1.30 m for males, appears to be about 10 cm higher, as well as other parameters related to both sexes. Circumference of front shank, as well as head and rump bis-articular width remained unchanged.

Figure 3. Pasturing Cabannina cows in Scabbiamara area.

Natural breed evolution and feed were considered to be factors influencing the named changes, but the investigation even considered the influence from crossings with Brown Alpine cows after Law 126 came into force when they began to replace the crossings. Moreover, while in the past the cows were fed exclusively on forage and leftover food, such as potato peels, the recent introduction of feed concentrates, even not properly balanced ones, may have boosted the growth of Cabannina breed. Actually, its small size should be considered as a positive feature to keep because it makes these cows excellent grazers even under extreme conditions, and low feed consumers, which means a substantial economic advantage for breeders [21].

4.3.1. Rusticity

Cabannina breed shows a very low incidence of diseases, virtually no hoof problems, or mastitis. No dystocical parturitions and on average calving-conception occurs after 80 days [21].

4.3.2. Physiological reproductive features

Under normal conditions and in the absence of any drug treatment, the involution of the uterus, is completed within 4-6 weeks post-partum. A recent work about uterine involution [22], shows that the reproductive physiology of Cabannina is characterized by a rapid recovery of ovarian activity. In fact, the onset of first estrus can be observed 20 days after birth and the fertilizing opportunity occurs in the following cycle, at about day 40. Features that allow farmers to achieve the advantage of a calf per year, i.e. maximum productivity, maximum reproductive efficiency and excellent mammary functionality.

4.3.3. Physiological productive features

Milk from Cabannina cows was carefully examined at the laboratory of Veterinary Physiology - University of Milan – [22-23] as to the dimensional characteristics of its fat globules, which make it a product of great value. In fact, it was proven the presence of small fat globules resulting in a wide specific surface area (SSA); thus, intestinal lipases can easily attack them and milk results to be highly digestible [24]. Moreover, as fat globules interact with casein curd, their size also influence the processes of lipolysis, bacterial colonization and cheese ripening [25]. High levels of unsaturated fatty acids and desaturase indices were highlighted, with important antimicrobial and biological effects and influences on human health as described in recent literature [26-30].

During cheese making processes, titrable acidity is one among basic parameters to control as it indicates good quality and good preservation of the product. Its normal values range between 3.20 and 3.80 °SH/50 ml. in fresh milk [21]. In 1987, Zanetti et al. [31]found that a low titrable acidity represents a condition technologically limiting optimal cheese making. In fact, hypoacidic milk, takes longer to turn into cheese when rennet coagulation is used, thus affecting dairy processing negatively. Finally, even though both remained within normal range values, milk from Cabannina showed average values of SH ° definitely higher than milk from Friesian breed, which can positively influence yield in cheese making processes [32].

5. Petramartina farm

5.1. Farm structure and management

Petramartina farm is located at Scabbiamara in Aveto Valley at an altitude of 1000 m above sea level. (GPS coordinates: 44 ° 31'60 "N - 09 ° 22'60" E). It was established in 2009; since then, the owners and two workers have looked after the animals and manufactured dairy products. The farm represents a modern rural reality carefully merged with traditions. In fact, its products are sold directly to consumers or to small local retailers (EEC Reg. 852/04) according to the principles of short food chain. The business is articulated into several buildings: an animal shed, a service area, a dairy product manufacturing room, a seasoning room. An area devoted to direct sale and a classroom to provide educational services for students and adult parties have already been designed and will be realized to complete the farm.

5.2. Cattleshed, animals and their reproduction

The old cattleshed is under the house of the herdsman/milker as it used to be in the past when they derived heat from nearby animals. It is characterized by stalls for the cows, used only to milk them and shelter them in winter, when they rest on chipboard.

As shown in Figure 5, the cattleshed is built according to an old concept: with no separating bars or "educators", as they are not essential where human-animal relation is still strong. Manure removal is not mechanized; the pit where it is kept to be spread over crops the farm

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can use is just close to the stable. In spring, the animals can graze freely. They are usually let loose day and night and lead to the cattleshed only for milking. During the night cows in lactation are kept in the pastures closer to the shed, whereas animals in dry and heifers can graze in the fields farther away.

Figure 4. Petramartina Farm Logo.

Figure 5. The cattleshed.

Milking is performed at 6.00 am and 6.00 pm by a cart machine; it starts with tail binding and then pre-dipping follows, i.e. a neutral liquid detergent (foam on) characterized by a sanitizing and softening action is applied. Such a procedure makes it easier to milk cows. To finish, post-dipping is carried out using a highly viscose solution of benzyl alcohol to pro-

tect nipples; in fact, it forms a barrier against bacteria, thus reducing mastitis and related problems for Cabannina cows at lowest level possible.

NUMBER	COW NAME	AURICULAR BRAND	STATUS
1	BIONDA	IT010990001714	Production
2	MARTINA	IT010990009965	Production
3	NORA	IT010990009966	Production
4	NEBBIA	IT018990022884	Production
5	BELLA	IT010990013162	Production
6	PETRA	IT010990013163	Production
7	ESMERALDA	IT010990012274	Production
8	PICOLA	IT018990042730	Production
9	LILLY	IT018990032152	Production
1	RAYA	IT010990013806	NORA
2	LENA	IT010990013807	NEBBIA
3	SUSY	IT010990015551	ESMERALDA
4	MIRA	IT010990013805	PICOLA

Table 8. Animals in the stable in August 2011

As shown in Table 8, productive animals are currently limited to 9 but their number is constantly increasing when compared with previous years. Figure 6 proves that trend related to number of Cabannine cows assigned to milk production is becoming larger and is equal to 2.5 subjects/year, which can prompt towards implementing their relative abundance in the area, sustaining their reintegration within typical Ligurian breeding and, last but not least, ensuring biodiversity in cattle. Year 2011 was a greatly positive as 4 female cows directed to production were born.

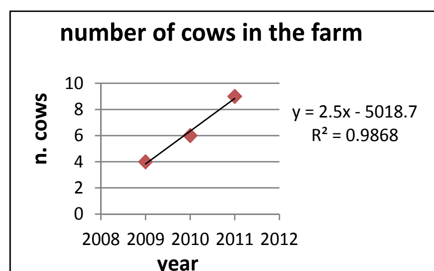

Figure 6. Cabannina cows increase from 2009 to 2011. Trend for forthcoming years.

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Management of reproduction represents one among breeders' tasks: to increase genetic variability artificial insemination is performed using Cabannino bull paillettes preserved by APA (Association of Provincial Breeders) since 20 years ago, which is essential to recover a cattle breed. Bulls are chosen carefully to keep blood lines as much as possible divided. This is important primarily because deleterious inbreeding must be avoided and, secondly, because genetic and phenotypic data about bull semen have not been collected so far.

NAME	INSEMINATION DATA	BULL	DELIVERY DEADLINE	DELIVERY
PICOLA	20-05-2010	PIPPO CAB	26-02-2011	06-03-2011
NORA	19-07-2010	PIP	19-04-2011	06-05-2011
NEBBIA	01-09-2010	BIMBOTTO	01-06-2011	08-06-2011
ESMERALDA	26-09-2010	LEO	26-06-2011	06-07-2011
BIONDA	05-11-2010	MANDRIN 35992	05-08-2011	23-08-2011
MARTINA	29-12-2010	FARFALLO	29-09-2011	-
LILLY	07-05-2011	LEO	-	-

Table 9. Insemination data and birth calendar.

Table 9 details dates about each insemination performed and deliveries occurred. As to inseminations, only one is necessary per animal, which can decrease reproduction management costs. As to calving-conception interval, data from the farm under investigation showed compatible with the breed trend, reaching about 80 days, against current 145 days related to Italian Friesian cows [21].

5.3. Dairy, seasoning room and dairy products

At Petramartina farm, dairy consists of two distinct working units: one devoted to cheese cooling where there is a milk cooling tank, the other for its processing with a boiler, a table for curd processing and a press.

Nowadays the farm, one among Slow Food Presidia, manufactures several dairy products:

- U Cabanin: matured cheese (60 days at least) from raw milk
- Nina:, a fresh 'caciotta' from raw milk
- Velleja: a fresh ricotta (sort of cottage cheese)
- Yocab: whole white yoghurt or with honey from the farm apiary
- Mamma d'oro: a creamy cheese to spread, particularly suitable for children
- Primo sale of Cabannina a refreshing light cheese
- Squacquero of Cabannina a creamy cheese

Figure 7. Petramartina dairy.

Since when named among Slow Food Presidia, Petramartina farm has been able to produce many different dairy products from just one ingredient, milk, which meets with the recent consumers' demand for products with a characteristic taste on which they can indulge. Physiologically, Cabannina breed cannot compete with milk production from Frisian. Being its quantity of milk per lactation equal to 26-30 kg, Cabannina raw milk sale would not be economically relevant. Nonetheless, the percentages of fat (about 3.7%), proteins (about 3.3%) and lactose (5.3%) make it particularly suitable for producing very good cheese, as recently confirmed by a recent study [11]. Briefly, Cabannina breeding is now possible thanks to its highly differentiated and tasty dairy products.

5.4. Pasture management and diet

The Ligurian hinterland is characterized by small sloping plains among its woods. Cabannina cows have very strong and resistant hoofs and a low live weight (about 4.5 quintals), and can move on this territory without great difficulties. For their correct management, a breeder should divide pasture into different areas where the animals can alternatively rotate to be provided with a constant supply of organic material, to avoid excessive exploitation of vegetation and to ensure a good cleaning of the whole undergrowth. Being highly adaptive, animals of this breed graze looser most part of the year, but from February to March and from October to November, depending on the weather.

The typical diet of Cabannina cows consists of pasture forage. During winter, when kept inside in stalls, the farmer must provide their whole daily ration, based only on hay originated from areas in Aveto Valley. Lactating cows are given an integration all year long at milking time, i.e. a feed supplement specifically made for this breed derived from raw materials typical of pastures in these areas (table 10).

Such a feed has an average protein content; it is to be administered daily with fodder as much as 2% of each animal body (30% maximum according to U Cabanin production protocols).

So far no studies on Aveto Valley forage and related nutritional value have been conducted, which makes it impossible to consider how the Cabannina diet is balanced. Nonetheless, such forage is pivotal to the exploitation of this breed whose production results to be constant even though it only feed on fodder from the pasture they graze.

Raw materials	Analytical components T.Q.:
wheat meal	protein 15,5%
(small/young) broad beans	Oils and fat 3%
wheat bran	crude cellulose 7%
maize	ash 7%
barley	
sugar cane treacle	
calcium carbonate	
Bicalcium phosphate	
Magnesium carbonate	
Sodium bicarbonate	
farina glutinata di mais	
vitamine e provitamine (vit.A, vit.D3, vit.E, vit.B1)	
oligoelementi (Mn,Zn,Fe,Cu,I,Cb,Se)	

Table 10. Raw materials from which Cabannina special feed is derived.

6. Conclusions

Petramartina farm can be defined as a modern agro-ecology reality according to the studies performed, as highlighted through direct comparison with the fundamentals of agro-ecology by Altieri (2012) [11] (table 11):

Pertramartina Farm	Yes	No
Enhancing biomass recycling, with a view to optimizing organic matter decomposition and nutrient cycling over time.	X	
Strengthening the " immune system" of agricultural systems by enhancing functional biodiversity, natural enemies, antagonists, etc.	X	
Providing the most favorable soil conditions for plant growth, particularly by managing organic matter and by improving soil biological activity.	X	
Minimizing losses of energy, water, nutrients and genetic resources by enhancing conservation and regeneration of soil and water resources and agro-biodiversity.	X	
Diversifying species and genetic resources.	X	
Improving beneficial biological interaction and synergies among the components of agro-biodiversity, thereby promoting key ecological processes and services.	X	

Table 11. Principles of Agro-Ecology by Altieri (2012) [11]

The farm totally follows the above mentioned agro-ecological principles.

Similarly, we can compare it with the questions proposed by Koohafkan (2011) [12] (table 12):

Petramartina Farm	Yes	No
1. Do they reduce poverty?	X	
2. Are they based on rights and social equity?	X	
3. Do they reduce social exclusion, particularly for women, minorities and indigenous people?	X	
4. Do they protect access and right to land, water and other natural resources?	X	
5. Do they favor redistribution of productive resources?	X	
6. Do they substantially increase food production and contribute to household food security and improved nutrition?	X	
7. Do they enhance families water access and availability?		X
8. Do they regenerate and conserve soil and increase soil fertility?	X	
9. Do they reduce soil loss/ degradation and enhance soil regeneration and conservation?	X	
10. Do practices maintain or enhance organic matter and the biological life and biodiversity of the soil?	X	
11. Do they prevent pest and disease outbreaks?	X	
12. Do they conserve and encourage agro-biodiversity?	X	
13. Do they reduce greenhouse gas emissions?	X	
14. Do they increase income opportunities and employment?	X	
15. Do they reduce variation in agricultural production under climatic stress conditions?		
16. Do they enhance farm diversification and resilience?	X	
17. Do they reduce investment costs and farmer dependence on external inputs?	X	
18. Do they increase the degree and effectiveness of farmer organizations?	X	
19. Do they increase human capital formation?	X	
20. Do they contribute to local/ regional food sovereignty?	X	

Table 12. Questions to define an agro-ecological farm (Koohafkan, 2011) [12]

The merits of Petramartina farm are obvious, with particular reference to its strong relation with territory, animals and humans. The main economic returns of this business undoubtedly are due to:

1. a low use of water for field irrigation and for animal management in the stables;
2. a reduced consumption of fossil fuel during different stages of feed preparation and preservation.

Some critical points can be identified:

3. in the current lack of direct production of forage; in fact, for the winter period it has to be purchased from other local businesses;

4. hay integration through the use of a feed supplement does not fully meet the agro-ecological principles, but the issue is absolutely irrelevant when compared to its irrational use in industrial breeding.

Noticeably, Petramartina farm is soon going to implement its properties by acquiring new pastures in the aim of improving the territorial hydrogeological structure. Moreover, farming is also designed to become an essential element in promoting environmental education at any school level for the east Ligurian territory through adequate didactic structures. A further step towards preservation and spreading of Cabannina breed and its derived products, as well as the creation of new jobs in the farming and marketing areas.

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Chapter 27

The SFIN Innovation System – Reflections on Food Cluster Management

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Additional information is available at the end of the chapter

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1. Introduction

The Skane Food Innovation Network works to help the food industry innovate, to make the future happen in a way that includes better food, increased competitiveness for the food companies within the region and a stronger culinary profile of the region. This chapter aims at presenting and discussing how a Triple Helix cluster initiative can be managed towards innovation. Skane Food Innovation Network (SFIN) is a triple helix cluster initiative located in the South of Sweden. SFIN represents a new type of innovation system. Government aids to individual corporations are no longer allowed due to EU legislation, which has forced Swedish public innovation funding to alter its form and organization. Today such funding is directed to regional industry clusters instead of individual companies. This has prompted innovation of the Swedish innovation system. SFIN is considered at the front line of this development through its highly developed and consistent triple helix model of innovation (OECD, 2012).

The chapter reflects upon how the innovation system works, both in practice and in theory. In practice, it builds on widespread institutional and industry legitimacy, twelve different networks organized around various nodes in the value system, the interplay between systemic innovation meetings and the formation of pilot projects, and not least, the direct involvement of entrepreneurs, customers and the shared value created in the joint action of the two groups. With a systematic approach - detect, develop and diffuse – to innovation, SFIN has moved towards a new innovation system.

The chapter builds on the idea of "bridging regimes" (Jönsson et al, 2011) and argues that innovation could be considered a collective cognitive process. It involves a case, showing the importance of recruiting intermediators that can bridge the different regimes that are imprinted in the actors and make them involved in realizing innovative solutions. Innovation

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inside an existing organization is particularly described and analyzed (Govindarajan and Trimble, 2010; Kotter, 1985). By innovation itself is meant any process or product that sells and creates new value in a market (Edquist, 2002). In theory, the innovation system may be described as a collective cognitive process. The chapter connects theoretical underpinnings from strategy theory and market theory with models of change, institutionalization and culture. Strategy is discussed as a consistent and long-term set of activities (Porter, 1996) and a clear and understandable modus operandi (Miles and Snow, 1984; Drucker, 1994). The system creates a "liquid environment" and increases the "adjacent possible" (Johnson, 2010), where ideas are detected and further developed in a market, defined as any self-referencing group with certain needs in common (Moore, 1991). Such a market view is consistent with the idea that any innovation established through replication (Christensen et al., 2006) in order to survive. Once the change is made (Kotter, 1996)– the developed new value creation is accepted, widespread and taken for granted, the new solution is institutionalized (Veblen, 1904; Hodgson, 1988; Scott, 1995) and part of a specific culture on one or more levels in society (Schein, 2010). The whole process of innovation is about bridging regimes: it could, in essence, be seen as managing stakeholder perceptions through the three stages of detection, development and diffusion, on the three levels of individual, organization and society.

1.1. Shaping the shaping of the future food industry

How do you shape the future? As a matter of fact, you don't have to do anything. It will shape itself around you, with or without your involvement or approval. But if you have an idea of what you want to see in the future, there is a challenge ahead. The Skane Food Innovation Network works to help the food industry innovate, to make the future happen in a way that includes better food, increased competitiveness for the food companies within the region and a stronger culinary profile of the region. This chapter aims at presenting and discussing how a Triple Helix cluster initiative can be managed towards innovation.

This chapter is about regional economic development and innovation is a key concept. It is used in the meaning of any process or product that sells and creates new value in a market (Edquist, 2002). The text revolves around the development of Skane Food Innovation Network (SFIN). In 2003 - winning the competition Vinnväxt - SFIN was boosted by national funding the coming 10 years. The objective was to systematically facilitate innovation in the since long established food cluster in the south of Sweden, also known as Skane. Reading the official program description today, almost ten years later, it is strikingly void of normative guidelines of how to go about creating the innovation system. The purpose of the program, called *Regional growth through dynamic innovation systems*, is

"to promote sustainable growth in the regions based on international competitive ability, by successively developing or further developing the functioning, dynamics and effectiveness of innovation systems in functional regions at an international level. A prerequisite for the programme is the active participation of players from the business community, research organisations, politics and public administration."

The concept of Triple Helix is mentioned in the original directions. The collaboration between business firms, universities and public sector organisation, innovation will result.

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This view of the innovation process could be interpreted in two opposite ways. On one hand, you could expect innovation as a result of the ”adjacent possible” in a ”liquid environment” (Johnson, 2010), where the mere coexistence of different individuals gives the future new combinations of ideas, DNA, artefacts or other things. This is what Edquist and Hommen (1999) call the ”systems oriented” view on innovation processes. On the other hand, there is a strong contender in the ”linear” perspective on innovation. Innovation is unidirectional process, where research and development efforts is initiated by public authorities or private firms, which is expected to produce new technology, which in turn provides new solutions to market needs.

1.2. Purpose and approach

The purpose of this chapter is to describe and interpret the development and the dynamics of the way SFIN facilitate innovation.

By innovation is meant any process or product that sells and creates new value in a market (Edquist, 2002). In theory, the model may be understood in terms of strategy as a consistent and long-term set of activities (Porter, 1996) and the need for a clear and understandable modus operandi (Miles and Snow, 1984; Drucker, 1994). The system creates a “liquid environment” and increases the “adjacent possible” (Johnson, 2010). A market is any self-referencing group with certain needs in common (Moore, 1991), in which any innovation has to be established through replication (Christensen, 2006) in order to survive.

A major impediment for innovation tends to be the idea that things should be done the way they always have been done. Innovations therefore often occur in settings where actors with different backgrounds join up with a mutual interest to solve the same problem. However, things can also go terribly wrong if the different backgrounds clash instead of fertilizing each other. Sidney Winter and Richard Nelson (1982) have discussed the importance of studying regimes in order to understand why or why not innovations tend to happen. Winter defines regimes in a sector as a specific set of not only regulative institutions and norms but regimes also regulate codified formal as well as tacit informal habits and routines related to common collective and individual practices and beliefs. These practices and beliefs shape and coordinate actions between various groups, individuals, and organizations in the sector. An important role for organisations working with facilitating innovation may be to work for “bridging regimes”, as argued in Jönsson et al, (2011).

We are both active in social sciences, business administration and ethnology. We use our set of models of concepts as ”temporary walking sticks [to] aid sense making as we go along” (Ghoshal, 2005). Ghoshal questions the pretense of knowledge, resulting from the idea that social sciences should be formed, tested and verified in the same way as natural science and causal theories are. Thus, our eclectic approach is a start trying to understand and verbalise the shaping of the shaping of the future. The approach is to use an eclectic frame of reference, where different models and concepts in the field of business administration are put together in order to show how concepts of strategy, markets, institutions and culture have human understanding and acceptance in common.

Our effort could be labelled action research (Lewin, 1946, Rapaport, 1970, Susman and Evered, 1978). Action research is defined by Rapaport (ibid.:499): ”*Action research aims to contribute both to the practical concerns of people in an immediate problematic situation and to the goals of social science by joint collaboration within a mutually acceptable ethical framework.*”

The empirical account of the chapter is based on our direct experiences of working inside SFIN since 2004. We both started off leading different projects for a few years. Since 2008 we have been part of the management team of SFIN, each heading a separate large operation. In 2010, the Skåne Regional Council, one of the major stakeholders of SFIN, decided to allocate the initiative Taste of Skane inside SFIN. The assignment is to develop Skane in terms of food experiences - tourism, restaurants, food education, and much more. In 2011, the same organisation gave SFIN the assignment of innovating the hospital meals, starting with the small regional hospital in Trelleborg where a role model is to be presented in early 2014.

So, from an eclectic frame of reference and first hand empirical experiences, this story will serve two general purposes - making sense of cluster management in SFIN and advocating the use of individual and common understanding of the subject of change. Individual come collective understanding, as we aim to show, is at the core of innovation.

2. Skane Food Innovation Network (SFIN)

2.1. The development phases of SFIN

During most part of the 20th century, the Swedish food market has been protected from international competition. The idea of national self-subsistence used to guide Swedish food policy. International competitiveness was not a major issue, since surplus production was limited. During the last decades, though, the Swedish food industry has been rapidly changing from a sheltered national industry into an industry exposed to strong international competition (Lagnevik 2006). In 2003, VINNOVA, the state agency for innovation and systems-oriented research, launched a regionally oriented program for research, technology and deployment/demonstration (RTD). The aim was to promote upgrading and renewal of local innovation and R&D capabilities and skill building in certain important growth areas with strong regional profiles. One initiative within the Vinnväxt (Winn-Growth) program was granted to the Skane Food Innovation Network, a triple Helix network formed in Skane, Sweden’s “bread basket” with approximately half of the nation’s food production and R&D.

After winning the Vinnväxt competition in 2003, this first period of the ”cluster initiative” was research oriented. This ”research phase” saw a large share of the funding funnelled into research and PhD student projects. It was characterised by a linear view of the innovation process. As a triple helix organisation, the reactions from the business part of the stakeholders gradually expressed doubts about how the process should gain their companies. The inherent tension between long-term research and short-term profitability pressures of larger companies needed to be resolved.

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The former CEO of SFIN retired in 2006, and a new one was recruited. From being an anonymous "industry body", SFIN now got a person with a strong background working with one of the most successful and innovative Swedish consumer products in a long time. Around 2007, "the entrepreneurial phase" began. Now there was an increased support of entrepreneurs, seen as innovation synapses. It could be regarded a reflex of large companies' urge for here-and-now innovation. SFIN now changed into a rhythm of making more frequent decisions. Each decision, though, concerned smaller amounts of money. In retrospect, the development could be described as the recognition of the systems view on innovation. The typical decision was about a business plan or project plan, and a subsequent need for financial support for implementing it. This kind of decision-making turned out to be very difficult, sometimes almost ad hoc, and the organisation then was struggling for criteria from which to evaluate the proposals. The result, and the problem, was that an inconsistency emerged, no other logic than stimulating individual entrepreneurs in order to create innovation, new firms and more jobs.

The orientations seen in this first half of the Vinnväxt program are direct reflections of what was evaluated and, hence, expected from SFIN by Vinnova. The new CEO, slightly flabbergasted, turned to the board for directions. None was given. Instead, the board and CEO together began articulating a strategic orientation for SFIN. The approach used was recognised from straightforward strategic planning and the traditional management of business. State a vision, formulate a mission and set goals and strategies in order to fulfil the mission. The process was clarifying the ideas and purposes of SFIN. A new organisation structure was created and still holds sway today.

2.2. The shape of the SFIN innovation system

The Skane Food Innovation Network is registered as a non-profit organisation. Its constituents are a number of partners, today about 40, and members, today more than 100. Partners pay an annual fee of about 4 000 euro, whereas members pay 300 euro. Partners qualify for board representation, members do not. The board mirrors the triple helix view - large and small companies, universities, and public authorities are all represented by the eleven board members. The Governor of Skane is chairman of the board.

The board has formulated a so called "VAMS", an acronym for Vision, Business Idea (Swedish: Affärsidé), Objectives (Swedish: Mål) and Strategies.

The vision states that SFIN is shaping the future food industry and meal experiences. The business idea is to offer the best network for cooperation of different competences, stage pilot projects and gain credibility from a concrete track record of innovation. From the high credibility, SFIN sustain and develop its network and receives funding from national and international authorities.

The objectives revolve around maintaining the networks in an inclusive manner, and hence increase the attractiveness and deepen and widen the competence base of the industry. Strategies are concerned with deep understanding of future needs and questions in the industry, creating a clear and attractive offer to participate, attracting people with vision and

drive, organising meetings between companies, entrepreneurs, universities and industry organisations, focusing commercialisation of innovations, taking the role as the network hub and constantly communicating our work and existence.

The operating core is organised in six different areas, each headed by one person. The areas cover as follows:

- Future Strategy: trend spotting, stakeholder relations, governance issues, finance and funding
- Career: student activities, trainee program, attraction of the food industry
- Entrepreneurship: coaching entrepreneurs in the industry and the adherent support systems
- Public Meals: increasing the status and competence of the public meal sector
- Packaging: develop innovative food packaging, small-scale, local producers etc
- Regional Food and Tourism: develop small-scale food producers, food tourism etc

The CEO and the area managers form a management team, also including the communications manager and one representative each from the two largest public stakeholders and funders, Region Skane and the County Council. The management team meet regularly three hours once a week.

From the different areas, various networks are formed. This model is an offspring from the initial CEO network, created by the new CEO in 2006. These networks are gatherings around specific topics, functions or roles in the system. Currently, 12 different networks meet more or less regularly. Apart from the CEO network, SFIN operates a communications network, a public meal network, a retailers' network, an HR network and seven more networks.

The logic is straightforward. In the networks, different ideas surface. Vital ideas, surviving the initial scrutiny of the network itself, are brought to the management team. The management team discuss the innovation potential of the idea. Today, there is a clear-cut evaluation criteria. The innovation has to be of a systemic character. There has to be a dedicated entrepreneur or coalition ready to test it in a pilot project. Finally, there has to be realistic plans of how the pilot project could reproduce itself, on commercial merits.

In summary, SFIN has over the past three years been compressed into six "business areas", a coherent network structure, a management control model and three distinct activities undertaken in order to create the future food industry and meal experiences: we *detect* a need or potential for innovation, we *develop* the solution in pilot projects, and we *diffuse* them to a wider market. This is the SFIN innovation system. This particular "modus operandi" has produced a wide variety of both short-term projects and long-term signature co-operations. The first one was an industry trainee program in open innovation. Another is the training program for elderly homes, "Meal Pleasure for Elderly". A third example is the retail concept "Locally Produced and Carefully Selected".

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On a deeper level, these different examples represent a maturation and consolidation of the early years of wayward - yet legitimate - experimentation. The strategic planning process undertaken in 2008 apparently missed out on the emotional aspect of change by avoiding making a mission statement. The organisation probably was not ready for it, but now the board and management team increasingly speak in terms of social responsibility, sustainability and social capital. The fourth example of this, and a strong confirmation of the credibility that SFIN has earned over time, is the most recent pilot project situated at the Trelleborg hospital. In the next section, the case of Meal Pleasure at the Trelleborg Hospital, will serve as a more detailed look into the dynamics of the SFIN innovation system.

3. The case of meal pleasure at the Trelleborg hospital

3.1. The background

In 2009, the public foodservice sector was slowly introduced into the SFIN operations. It was obvious that this was a forgotten part of the Swedish food industry. Half of the industry is foodservice, half is retail and consumer-oriented. Half of the foodservice sector is private restaurants and service sector offerings (gas stations, Seven Elevens and the likes), the other half is public sector meals. The major part is served in schools, hospitals and elderly care. Thus, this forgotten part constitutes one quarter of the entire demand in sales value, even more in terms of number of people being served. It is a rather consistent segment from a supplier's point of view, where public procurement law has streamlined the procurement of food and meal solutions. The guests, though, is a completely different thing. The elderly cannot be segmented by age, nor can school kids or patients in hospitals. This is a very demanding part of the food industry with diverse preferences and, on average, weak buying power.

The public foodservice sector is furthermore signified by a low average educational level, a predominantly female labour force, low pay, low rate of training and education on the job and the use of traditional cooking techniques. The most obvious example is the use of high tech ovens, which in fact are small ”computerised food factories”. Such an oven is expensive. Still it is often used merely as a traditional oven, a few hours a day, five days a week, due to lack of education and incentive.

One particular problem, unique to the public foodservice sector, is the obligation to follow the public procurement law. One important purpose of the law is to optimise the use of taxpayers money and avoid nepotism. This is done by organised procurement using the competitive forces in a marketplace. This works, unless there is a dysfunctional market. In the Swedish food industry, both the retail and the foodservice market are oligopolistic markets. In the foodservice case, there are two large dominating suppliers. These have been continuously intimidating municipalities and counties by frequent and systematic legal actions and court over-rulings of public procurement processes, a tactic aiming at coercing the public organisations to behave in a certain way (likely as to benefit large-scale suppliers).

The educational level of the staff and the status of the foodservice operations are related to the problem of public procurement. Increasing occupational status and educational level could make the public foodservice customers more demanding, in that way increasing the innovation pace in the Swedish foodservice industry. That is the hypothesis that motivated SFIN to start working with the public sector.

3.2. The rationales of the Trelleborg project

Region Skane, the county council, is responsible for healthcare, transportation, cultural and regional development of Skane. It has offered continuous support of SFIN since the start in 1994. Gradually, the confidence in SFIN has increased. In 2009, with 2012 looming, the need for a new procurement of hospital meals for about two thirds of the healthcare organisation was pressing the politicians. The same year, a hospital food process investigation was initiated. The result was a vision for making Skane a role model in food and meals in Northern Europe by 2025.

It was an ambitious vision and it was necessary to take action. A senior foodservice ”celebrity” working for SFIN saw it coming and used her leverage in the political sphere in Region Skane to suggest a pilot project to ”walk the talk”. Said and done, a pilot project in the small local hospital of Trelleborg; was decided upon. There was an existing kitchen ready to use. ”Just dust it off and get it running”, we were told.

Apart from starting up a dormant kitchen and start cooking tasty food, the short-term goal was, and still is, to innovate the hospital meal experience and to integrate the meal into the healthcare operation. In other words, we got an opportunity to give the meal a higher status in hospitals. The longer-term goal was set to disseminate the idea, to diffuse the model. This is done in SFIN by way of entrepreneurial incentive. This was decided in the early summer of 2011, just before the holidays. In August that year, the adventure started.

3.3. The SFIN networks paying off

SFIN got the assignment from the board of Region Skane, along with funding for two years. This funding covered SFIN expenses for the project, not for investments or employment of staff or other Region Skane-related costs. The line-up from SFIN was possible to achieve as a direct result of the network organisation. Various SFIN projects in the past had made it possible for a rather unique and unorthodox set of project members to form.

The common denominator of this group is a genuine interest in making a difference. Normally, there is an obvious attraction to public funding as ”easy money”, but these people all work long-term with SFIN on reasonable market-based terms.

Initially, the SFIN manager Knutsson worked as a project owner in SFIN alongside with two experienced and highly respected persons. Together, these three people formed a project core group and started to write a project plan. The project plan was accepted by the responsible politicians in late August 2011.

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The project organisation consisted of a political steering committee, a managing steering committee, a work group and a reference group. SFIN participated in all groups. The rest of the participants varied, but represented either the Region Skane corporate level, the Region Skane business development, the Trelleborg hospital or Regionservice (the facility management part of Region Skane coordinating real estate, food, transportation, laundry, and postal services).

At the time in Region Skane, each hospital manager was responsible for all service operations, including the food process, and the organisation of the healthcare. Regionservice had the role of an in-house service integrator, coordinating the service activities in the entire organization.

There were three distinct stakeholders. The hospital of Trelleborg, Regionservice and SFIN. In this context, SFIN was a fairly unknown organisation of (”self-entitled?”, some whispered) experts in the field, who had been given a powerful position in reshaping the hospital foodservice in Trelleborg and, more or less explicitly expected, the rest of the Region Skane hospitals. Adding to that, Regionservice had a rather bad reputation in the hospitals, not meeting expectations. Naturally, Regionservice representatives were suspicious and - perhaps - offended by the political decision to invite an innovation organisation from the outside ”that no-one ever has heard of”. Moreover, the project was running against the clock, as the contract with the external food supplier was to expire 31 August 2012. Regionservice, the coordinator and recipient of the SFIN innovations, had 15 months to go.

3.4. Colliding regimes – The first year's theme

The first meeting with a group from Regionservice and the Trelleborg hospital was held before the summer, before there was a project plan written. This was the first step in cooperating. The meeting was marked by confusion. There was no previous relations in the group to rely on. Knutsson, chairing the meeting, had nothing but a generally formulated political decision to hold on to. Focus was naturally put on the ”dusting off” the hospital kitchen.

SFIN, represented by an experienced, renowned and respected chef, made an initial sketch of the kitchen layout on the basis of available blueprints. The Regionservice real estate division appointed a project leader to organise the starting up the kitchen. This was a young and inexperienced person, as fresh as they come, who got this assignment as her first project. The tight time schedule and the goal to create something entirely new clashed. Soon it was discovered that the previous check of the building, about one year old, had been rough and, as it showed, insufficient. The original estimation was 11 MSEK. It rapidly grew to speculations of 70 MSEK. The needed capacity was 500 meals per day. Any sound investment calculus became impossible. Thereafter, Regionservice got silent and started its own investigation of the Klippan kitchen without the participation of SFIN.

There was obvious tension between Regionservice and SFIN. The director of support and service functions in Region Skane was contacted and introduced to the need to strengthen the Regionservice commitment to the collaborative nature of the project. A collaboration document was written and acknowledged. But the Regionservice investigation was already

on its way and the door was not opened to SFIN. At that time, it was also decided by the board of Region Skane that Regionservice should take on the direct responsibility for all support functions. That meant that the hospitals were relieved of the support functions, effective January 1, 2012. This stirred things up in Region Skane and the pilot project did not go unaffected. Regionservice got more powerful and the door to SFIN seemed firmly shut. In late January, the door came ajar, though. Suddenly, the real estate project leader forwarded a number of detailed questions from their kitchen architect and kindly asked that we respond the same day.

In the meantime, two people – one dietician and one gastrome – built a remarkable base of knowledge and learnings about the meals in the Trelleborg hospital. The explicit goal was to observe and analyse the potential and need for change in the meal organisation and the quality of the patient's meal experience. They worked in an exploratory fashion and became more and more independent of the project leader, working increasingly with the kitchen issue and the collaboration between Regionservice and SFIN. The first half of 2012 was a period where the project goals first got under real scrutiny. What does it mean to "innovate the hospital meal experience and to integrate the meal into the healthcare operation"? Frequently the phrase "establish a new norm for hospital food" was used in SFIN. That did not bring light to the more and more blurred question - "What do we have to do?" The pressure increased further from an increasing interest and high expectations of the project. And here we were, fumbling for structure and some guidance. And what about the food, where did *that* go in this huge project?

A hospital kitchen is an important part of the infrastructure. There is a certain minimum efficient scale, given the choice of building, production methods and technology. In the spring of 2012, there was hardly enough expected volume to guarantee an acceptable cost per meal. The projected daily volumes included 300-350 meals to the hospital and about 150 meals to a new psychiatric unit in Trelleborg (RPC), starting up in 2016. 500 meals per day rendered an unacceptable production cost and the entire credibility of the project was being questioned. In that situation, the municipality of Trelleborg caught the project's interest. In the municipality, there was a commonly known need to invest in new elderly care meal solutions. Contacts were made and a letter of intent was written in the spring. Left with a need to find out ways to share the meal organisation, this was a crucial step that in one blow could double the projected production volume in the kitchen. Also in the late spring of 2012, the political decision was made to invest in total 50,1 MSEK in the Trelleborg kitchen. And the project is delayed by one whole year. With the decision finally at hand, the building process got off to a new start. There was a new building meeting and in that meeting, there was a complete breakdown of communication between SFIN and Regionservice.

The demand and expectations from the hospital, RPC and seven different elderly homes in the town of Trelleborg turned out to be difficult to capture and align. The picture of the total demand was very blurred and the design of the kitchen and logistics turned out to be a bad compromise. A calm and focused foodservice manager in Regionservice now entered the project in order to strengthen the project in the eyes of Regionservice and, in some respect, mediate between SFIN and the real estate project group. In order to get hold of the produc-

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tion model, it became necessary to take a stand on the food issue - do we transport hot or cold food, or both? The decision was made by the experienced chef representing SFIN, in mutual understanding with the foodservice representative from Regionservice, to go with the cold alternative. The decision to work with chilled food was supported by the politicians, and opened up a possibility to solve the problem of supplying food to other hospitals in Skane. If the Trelleborg kitchen was expanded, it could serve so much more than merely the few patients and persons in Trelleborg. This, of course, had ramifications on the building project. But the political negotiations were kept secret. Knutsson was allowed to break the news of going for chilled food in a construction meeting. The impression was that SFIN had surrendered and resorted to the cook/chill method. In the meantime, a plan was outlined for a larger solution, which the politicians initially at first supported. However, one week later there was a 180° turn, and the old plan was only reconfirmed by the politicians. All of this wheeling and dealing went unmentioned to the real estate project group and it was no wonder that SFIN lost credibility in that group. That wasn't enough. The hospital management in Trelleborg had been completely forgotten in this intense and extremely pressured situation and was not happy.

3.5. Current state

As the last steering committee before the summer ended, no decision had been made to reorganise the project. As it happened, the project leader met with the service director/project owner the same morning. Referring the meeting, the project leader got an immediate positive response and the service director offered himself to chair the steering committee and to supervise a revising of the project plan and the project organisation.

So, as the summer is almost gone, the project is now divided into four distinct parts of the project (construction, food, meals, and continuous operations). In this scheme, SFIN has now got an explicit development and innovation responsibility for on one hand the kitchen design and a distinct food strategy, and on the other hand the meal experiences of patients, staff and visitors. The construction part of the project now resides directly under the service director. Articulated goals are now present in each respective part. All in all, order has been reinstated and roles and responsibilities are now much clearer.

In Trelleborg, base groups are formed within each clinic, and the hospital management is engaged in the formulation of the Trelleborg policy of patient meals. In that policy, the integration of hospital meals into the healthcare operation resides. The construction planning is underway, and a new, healthy menu is currently planned.

Of course, it remains to be seen, but by early 2014, SFIN and Regionservice will present a new way of working with food in the hospitals of Region Skane. The meal organisation is revamped, the status of the meals is increasing and the quality of the hospital food is dramatically improved by new production and distribution methods. Moreover, the cost of hospital meals is lower than before and the new kitchen turns the conception of high quality foodservice production upside down. From the rest of the country and from other parts of Europe, people start to benchmark and copy the new norm of hospital food, established by SFIN in tight cooperation with Region Skane. Fingers crossed.

3.6. Case study learnings

This case is a story where we want to show more details of the innovation process. We will dwell somewhat upon the difficulties we have experienced from working side by side, with an established organisation.

The story began with the importance of confronting the basic concept of value creation. Change stemmed from an understanding of (the potential for) value creation, in this case supported by external knowledge and experience, but also a strong political awareness and desire to improve the meals served at the Region Skane hospitals. The staff working in the areas in need of change did not, however, recognise the need to the same extent. This was an important learning in the project. Every organisation is shaped to produce a certain outcome. Ongoing operations create, over time, an inertia and inability to adapt to change. One of the participants in the project, a former foodservice manager, drily summed up the operation in one kitchen by saying ″this is like it was 25 years ago when I left it″.

This pointed to the need for implementing the wish for change to the staff. The success of an organisation is contextual, every organisation is part of a larger system. The supply chain of the hospital food operation relies on education, procurement, IT cooperation between different occupational groups and so on. To isolate and dissect a single part of a system in order to change only that particular part is doomed - the surrounding system will rein in the deviant.

Innovation is based, the case shows, on every individual's understanding, acceptance and estimation of the value of the ″new thing″. It was most difficult to convey to large groups of people that we shouldn't talk about just food, we had to take the entire meal into consideration. Hospital staff may have longed for ″finally getting better tasting food″ but the foodservice organisation had a clear view that ″cook/chill food is lousy!″. This was the real challenge of innovation, finding the motivation and mutual understanding among the kitchen staff and hospital staff at one hand, and the external innovation facilitating actors at the other. We needed to initiate and encourage change agents in a long-term process of changing attitudes and conceptions of how the meals in hospitals are valued and, hence, organised.

We tried to derive changing attitudes, values and conceptions to individuals influencing individuals. The case reveals the importance of tasting sessions, to organise ″base groups″ at every single clinic and to align top management support, middle management involvement and floor management dedication.

In essence, this is an argument for treating the innovation process as a cognitive process rather than the physical implementation of new work routines. The innovation in this case is the reconceptualisation of a hospital meal.

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4. Innovation in the light of strategy, markets and institutions

Looking at the Trelleborg case from a strategic point of view may give further insight on how actions can be planned in order to succeed in innovation facilitation. First of all, the way that SFIN runs its course follows the strategic planning paradigm. But, just to conceptualise a management system in terms of a list of words will not suffice. From theory we can deduce a certain frame of reference, guiding both sense-making and action-taking. The argument is the following:

- strategy is about understanding how you create value to a customer
- the flipside of a good strategy is inertia and a compromised ability to adapt to change
- success is contextual, every organisation is part of a larger system
- innovation is based on every individual's understanding, acceptance and estimation of the value of the new thing
- individuals are influenced by other individuals;
- innovation, defined as ″any process or product that sells and creates new value in a market″ (Edquist, 2002) is built on a collective cognitive process;
- The SFIN innovation system is built around a systems view on innovation and concerns the management of cognitive processes triggered by the meeting between people.

4.1. Innovation and strategy

Strategy has an everyday connotation as a plan. It is implicitly rational, built on analysis and take its form as an ex ante decision to make something special to happen. There is a wide literature on the concept of strategy, but a convergence could be discerned from a number of scholars in the past. In 1975, the Swedish scholar Richard Normann (1975) defined the way a company could dominate competitors, i.e. strategy, as an interplay between the organisation, its products and its markets. Strategy, Mintzberg (1978) has defined as ″a pattern in a stream of decisions″. A continuation of that definition is recognised in Miles and Snow (1984) and Porter (1996), who define strategy as a matter of activity fit. What counts is what you do, in total.

Miles and Snow (ibid.) present fit in terms of integration of an organisation's strategy, structure and management processes. Misfit breaks down an organisation, whereas tight fit is ″the underlying causal dynamic producing sustained, excellent performance and a strong corporate culture″ (1984:10). Early fit is the discovery and articulation of new patterns of strategy, structure and process and fragile fit signals vulnerability to change. Tight fit, thus, is the objective for adaptation. Tight fit is built upon

- discovery and establishment of a seemingly easy way to work together towards a chosen goal,
- simplicity and increased understanding by incumbents

- reduced need for elaborate coordinating mechanisms, creating slack resources to be re-allocated.
- the causal association between the way to work and achieved performance.

Miles and Snow combine the classic thoughts of Selznick (1957) with similar ideas of Normann (1975, 2001) and Porter (1980, 1985, 1996). The easy way to work, well understood by incumbents, could be regarded as a distinctive competence (Selznick, ibid.) and an interplay between the mental models of people, institutionalised organisational behaviour and the way a company runs its business. Normann (2001) indicates that a ″business landscape″, i.e. an industry structure and its prevailing business logic, could be a result of the ″mental maps″ of people. Drucker (1994) calls the same thing ″the theory of the business″. A later heir of these ideas is Jim Collins (Collins, 2001, Collins and Hansen, 2011), who in a massive empirical material derives an explanation of the success of certain companies. It is from disciplined people, thoughts and actions superior performance stems. In the healthcare organization of Region Skane, this is expressed in distinct occupational roles and a strong hierarchical organization. Even though people have to eat, and our wellbeing depends very much on what we eat, people in the medical profession seldom considers how the patient's eating affects its recovery. The "mental map" of healthcare needs to be redrawn.

4.2. Strategy, inertia and the difficulty of surprise

When, in SFIN, we speak about getting the unexpected to happen, there is one problem with innovation and strategy. Strategy is a matter of pattern, routines and familiarity. March (1991), among many others, is preoccupied with the choice a firm has to make between exploiting ″old certainties″ and exploring new possibilities: ″The essence of exploitation is the refinement and extension of existing competences, technologies, and paradigms. Its returns are positive, proximate, and predictable. The essence of exploration is experimentation with new alternatives. Its returns are uncertain, distant, and often negative. Thus, the distance in time and space between the locus of learning and the locus for the realization of returns is generally greater in the case of exploration than in the case of exploitation, as is the uncertainty." (1991:85)

This frames the challenge of SFIN and other innovation organisations: how do you help an organisation close the gap in time and space, i.e. between innovation experiments and profitable growth of business? The wider the gap, the less interested an organisation is to bet on something other than the proven way. Christensen (1997) distinguish between disruptive and sustaining innovation, where sustaining innovation is an incremental development of the existing products or methods. Disruptive innovation changes the game, for the customer or the organisation or both. Digging further down the theoretical underpinnings of innovation, Argyris and Schon (1978) introduced the idea of single and double loop learning to a wider public. Learning in a single loop is to learn to do better what you already do. Double-loop learning occurs when you question why you are doing what you do in the first place - you test the assumptions, values and policies underlying the particular action.

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So, the sustaining innovation is a matter of improvement of the existing solution to a problem. You run your thoughts through a single loop - how can we do this better? The degree of surprise by the unexpected is limited. Disruptive innovation, though, is by definition something other than the existing thing. Then, when you enter a ”going concern”, the accountant’s label on an organisation expected to live long, and you should get the unexpected to happen, you could expect resistance.

In more practical terms, Govindarajan and Trimble (2010) differentiate between the main organisation, what they call the ”performance engine”, and the innovation organisation, the ”dedicated team”. In order to succeed with exploration, call it innovation, the dedicated team has to work alongside with the people in the performance engine. A partnership has to be established between the exploring and exploiting parts of the organisation.

The main issue, Govindarajan and Trimble argue, is the work relationships among the participating members and decision-makers. Work relationships are analysed in terms of depth, power balance and operating rhythm. Deep relationships are defined as a continuous and tight relationship where two or more people work together with a specific problem. Power balance is a matter of influence and having the final say. It doesn’t rely on individual personal authority, but rather the primacy of certain organisational functions over other. Last, the operating rhythm concerns the pace and intervals in which the cooperating organisations work - if one part work in a monthly budgetary cycle and the other in a three-year development process, the operating rhythm will cause the work relation to wither.

What Govindarajan and Trimble teach us in SFIN is, at the very least, that as soon as we enter an existing organisation with an innovation mission, we must take good care of the work relationships we establish. Infrequent, casual meetings with imbalanced power distribution - one way or the other - and differing time horizons, we are by and large bound to fail to innovate. If we don’t get to understand each other, the existing view of reality will prevail. In order to redraw the mental maps of the hospital staff to include the hospital meals as an integrated part of the healthcare, we need to get our “partners” in the “performance engine” Regionservice and the Trelleborg hospital to take the patient meals in a double loop – why do we serve meals in the first place? We have to do this in close work relations, where we need to engage deeply in the realities of kitchen staff and healthcare staff, so that we can better understand how people think about and value the work they do. The change doesn’t start from our standpoint, it starts from theirs.

4.3. Innovation, markets and other institutions

The problem of innovating inside an existing organisation could be further discussed in terms of diversity and interdependence in the organisation (Kotter, 1985). Kotter claims that the larger the organisation, the harder it is to run it efficiently. If we look closer to his explanation, again work relations between people is the object of interest. His argument goes that a smaller organisation, with a narrow scope of technology, products and markets, creates less confusion and disputes than in larger organisation. The larger organisation depends on many people working together, so when interests divert among these interdependent individuals, the organisation gets stiff and bureaucratic. Kotter ponders: ”*it*

is not by chance that the economist's traditional model of a firm, where only 'rational' economic decision making occurred, and where power struggles and politics were nonexistent, was a small and technologically simple organization that operated in an environment without large customers, suppliers, unions, or governmental regulators, and that employed a relatively homogeneous labor force in a simple organization structure." (Kotter, 1985[1]). Thus, as soon as the organisation grows, the diversity and interdependence in existing relations have to be managed and led.

Max de Pree in his book "Leadership is an art" (1987) made a classic statement about leading an organisation - "the first responsibility of a leader is to define reality. The last is to say thank you. In between, the leader is a servant." In case of an organisation needing to change, the leader has to spell it out. Kotter (1996) has designed an eight-step model of change, claiming the need to explain, motivate and sustain the change. The now and here must be described, but also the then and there: where are we and where are we going. A small core of people, the "guiding coalition", has the initiative, but in order to succeed, more and more people have to be involved. One important motivator for others to engage is "short-term wins", necessary to underscore the meaning of and reward from participating. Kotter ends his list with the need to persevere and to gradually institutionalise the changes. New behaviour has to be established. If we choose to define "institution" as a pattern of routinized behaviour (Veblen, 1904, Hodgson, 1988, Scott, 1995, Cohen et al, 2004), the links between individual understanding, actions and innovation are again in focus. When changes are institutionalised, the diversity of action and interdependences are not considered.

Yet, the diffusion of certain behaviour is not automatic. Once the leader has pointed out the way, "defined the reality", people need to follow. Think about this definition of a market. A market is i) a set of actual or potential customers, ii) for a given set of products or services, iii) who have a common set of needs or wants, and iv) who reference each other when making a buying decision (Moore, 1991). There is a direct link between Kotter's (1996) eight-step change model and this view of a market. First, there has to be a need for the new thing. Then a group has to advocate the new thing and persuade others to buy it. Transferred to the concept of innovation, defined as "any process or product that sells and creates new value in a market" (Edquist, 2002), it is deeply dependent on a cognitive process where an idea roots itself and grow stronger as more and more people see it and value it. A successful innovation has gained a critical mass of buyers. A product offered by a company creates value to its buyer, otherwise there will be no repeat buying, nor any persuading elaborations to other potential buyers about the good qualities of the product.

The group that formulates the Trelleborg policy for patients' meals sets the stage for the base groups, which are now being formed and activated in the hospital. The base group activity is a conscious move to have the people reflect upon what they do and to discuss it with each other. In that way, under the supervision of the project members, there is a clear "guiding coalition" creating a sense of urgency. From this, the base group activities reduce

1 Here is an example of how innovations challenge existing norms. The Kotter book Power and Influence was read on an iPad, the new way of reading. In there, the pages are dynamically paginated. The traditional way to refer to a quote hence needs a paper copy - the old way is supported by norms and standards.

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the diversity of the staff's different conceptions of a hospital meal. This is the first ripple across the mental map of hospital food being redrawn.

5. Bridging regimes to boost innovation

The distance between the concepts of market, institution and culture could be seen as great. However, if we take the above definitions as our viewpoint and listen to Schein (2010) and his definition of group culture, the close relation between the three appears clearly and interestingly. The culture of a group Schein defines as ″*a pattern of shared basic assumptions learned by a group as it solved its problems of external adaptation and internal integration, which has worked well enough to be considered valid and, therefore, to be taught to new members as the correct way to perceive, think, and feel in relation to those problems*.″ (2010:36). In the western business community, there is an adage that ″culture eats strategy″. This means that no matter what strategy companies formulate, the implementation will be determined by the culture of the organisation. Culture is based on past experiences, what has been working. Strategy, defined as a plan, is about future competition and profits. Culture is based on inductive emotional consequence, strategy is based on deductive rational choice. The statement could be tested on other cultural levels than the organisation.

Culture, Schein (ibid.) suggests, could observed on a macro level (national, ethnic), organisational level (private, public, nonprofit, government), on a sub level (occupational) and on a micro level (units, teams, groups within a larger organisation). Thus, if we extend the argument of culture eating strategy, it becomes imperative to take existing cultures into account if enduring change is wanted. In that sense, there is wind in our sails now, as the Swedish healthcare establishment increasingly directs attention to deteriorating hospital food. However, the real challenge of re-conceptualizing the hospital meals is found at the occupational and work group levels. These cultures need to be confronted and overtly discussed if they are to be altered. And to the extent they are, the innovation project will succeed.

Following Levinthal (1991) and Scott (1995) regimes have three dimensions: i) cognitive rules, related to belief systems, ii) normative rules expressed in missions, goals, and identity, and iii) strategies and strategic orientations towards the surrounding external socio- technical and politico–economic environment.

The Trelleborg case shows the three dimensions in relation to the re-conceptualization of a hospital meal. There were plenty of cognitive rules about how a hospital meal should be, that turned out to be an impediment for innovation, sometimes in direct conflict to the normative and strategic dimensions. Furthermore, the regimes of the public staff, the political level and the innovation facilitating actor (SFIN) were difficult to bridge, as shown in the initial conflicts about the hospital kitchen.

The road to successful innovation work turned out to be the combination of people that could function as intermediaries between the different regimes in action. People working for SFIN had credibility among the hospital staff, while the experienced and renowned chef's

knowledge of food preparation could not be disputed. The SFIN project leader turned out to have unknown skills for intermediating between the outside organisation and the higher administrative and political level.

There is still work to be done before the new paradigm of hospital meals is successfully launched in Trelleborg, especially the implementation at ground level. The innovation is not a success until all levels are imprinted with the mission for change.

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Collaborative Innovation — A Focus on Food SMES

Maria Colurcio and Tiziana Russo-Spena

Additional information is available at the end of the chapter

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1. Introduction

1.1. Collaborative innovation: A set of agenda

During the last years, the topic of 'collaborative innovation' has become the dominant perspective in the innovation literature by the argument that innovations are effectively developed through the interplay between different parties from different organizations (Steinle and Schiele, 2002; Trott and Hartmann, 2009; Nootebom 2004, Chesbrough, 2006).

In the SME context, the development of well established networks for innovation has been understood as necessity more than a choice (Goss, 1991; Pratten, 1991, Rothwell and Dodgson, 1993,). A large body of literature indicates that participation in networks and engagement in partnerships are important for SME as these factors enable firm toto tackle new technological and market frontiers and to cope with the fast changing environment (Hanna and Walsh 2002; Van de Vrande et al. 2009).

Main advantages are the access to network competencies as well as the opportunities of engaging into supply chain innovation processes and of growing in collaborations with larger customers (Johnsen and Ford, 2006; 2007). At the same time, collaborative innovation creates challenges to SME resulting from the inability to nurture and maintain the necessary resources and capabilities for growing (Matthyssens, Vandenbempt, Berghman 2006), to build a competitive positioning (Colurcio and Russo Spena, 2009; Day and Nedugady, 1994) and to create value for the own company and for the network (Johnsen and Ford, 2006; Donada and Nogatchewsky, 2006; Ulaga, 2003).

Recently, the network perspective have triggered a fervent debate on the generation of knowledge and learning in inter-organizational and network collaborations (Dyer and Singh 1998; Nooteboom 1999, 2004, 2006; Lampela and Kärkäinen 2009, Hallikas et al. 2009, Lampela et al. 2008). Collaborative learning in innovation networks is said to stim-

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ulate the creation of new knowledge, processes, products and services as well as the motivation for networking itself (Araujo, 1998). Many contributions have demonstrated the role of learning in the context of innovation networks discussing it as particularly challenging but increasingly more important task for companies (Capaldo 2007, Lane and Lubatkin 1998; Inkpen and Tsang 2005, Dyer and Hatch 2006).

However, the integration of diverse knowledge sources and development of learning processes are carried out in relationships between a multiplicity of actors that may show different characteristics of asymmetry. The asymmetries become evident when the relationships involve actors with different positioning and power, as showed by Johnson and Ford (2007). Asymmetries in business relationships have been analyzed from their different perspectives and on their impact on innovation and network development (Mouzas & Ford, 2004 ; Johnsen and Ford 2001; 2006).

From other perspective a wide literature has identified enablers and barriers to network collaboration (Leonard, 1995; Szulanski, 1996; Knott, 2003). Among others Szulanski (1996) has recognized the main role of knowledge, motivation, trust and ambiguity while others researchers also considered the role of the context (the environment) (Nelson and Winter, 1982; Teece, 1986; Pihkala, Ylinenpaa and Vesalainen, 2002). Other studies have showed that the amount of social capital correlates significantly with the competitiveness of collaborative networks (Macke, Vallejos, Faccin, & Genari, 2010).

Despite these contributions we find that little attention has been paid to analyse the collaborative innovation in the context of learning and asymmetric relationships. We aimed at generating insights into attributes of relations and at identifying barriers and enablers to collaboration and learning in innovation networks from the perspective of SME.

We find the issue of collaborative innovation, asymmetry and learning very critical and under investigated to explain the competitiveness and the development of firms.

2. Asymmetric relationships

The study examining the way in which a firm innovates through inter-organizational and network collaborations (Capaldo 2007, Lane and Lubatkin 1998; Inkpen and Tsang 2005, Dyer and Hatch 2006; Nooteboom 2004) has only a more recent tradition.

Many researchers focus on partner characteristics, such as size, interdependence, cultural compatibility and relative influence (Holmlund and Kock, 1996; Pittaway and Morrisay, 2005). They show that these factors may leverage resources to drive innovation but that they can also inhibit innovation. Other authors (Pfeffer and Salancik, 1978; Håkansson and Snehota, 1995; Axelrod, 1964) - although stressing that networking is often the only way to realize innovation within a small size context - emphasize the organizational (resource) dependencies that emerge from networking. Similarly, other contributions that have focused on power unbalance (Hakansson; 1987; Håkansson and Gadde, 1992) find that this relational characteristic may become a barrier to the build up of a fruitful relationship and it may inhibit the

development of innovation capabilities for smaller partners. Power unbalance is a barrier since business relationships in such a situation are dominated very often by contracting more than by trust (Jeffrey and Reed, 2000; Blomqvist et al.2005); so they don't drive cooperation to innovation but simply consist of a contractual contribution of resources. Important studies are those by Ford and Rosson (1982) and Geser (1992) who have deepened insights about frequency, range and level of contacts between firms.

Although researchers focused on different characteristics which enable or inhibit the development of business relationships aimed at innovation, a common central theme emerged: the asymmetry of relationships. The concept of asymmetric relationships concerns different elements of the relationships such as size, power, resources, and competencies and strongly affects its innovation potential (Blomqvist, 2002; Whipple and Frankle, 2000; Bailey et al. 1998; Colurcio, 2009; Colurcio et al. 2012). On the topic of asymmetric relationships, a relevant contribution comes from Johnsen and Ford (2000; 2001, 2007) who have identified seven types of asymmetries in business relationships: mutuality, particularity, conflict, cooperation, interpersonal inconsistency, intensity, power and dependence.

All these characteristics express interdependency ties among partners involved in the relationship. These interdependencies unequally affect the partners of the relationships. Mutuality is conceived as an "attitudinal variable" since it concerns the willingness of an organization to advantage common goals in respect to its own goals; it requires a long-term perspective since a company may give up its own goals in order to advantage a counterpart. Over time this behaviour will create well-being for both (or all) the parts involved in the relationship. Particularity is the way to manage the relationship and concerns the interaction's level of standardization. It depends on the relational approach of the party and on its availability to customize the relationship and its contents. Conflicts are the essence of asymmetry since it conveys the inequality between the parties and conflicts are amplified by the level of the interdependence of the relationships. The interdependence strengthens the differences and in turn feeds relational pressure and conflicts. Co-operation concerns the willingness of the may concern specific goals or projects but it is mainly conceived as a way to work, as a perspective to manage the relationship and to extract value from it for all the involved parts. Interpersonal inconsistency relates to individual characteristics of subjects that interact in the relationship. Intensity stresses that the number of people who interact in a relationship affects the relationship and its value (cross-functional group; team working). Power and dependence hedges in different kinds of asymmetries and stresses difference in the partners' resource stock.

These characteristics may work both as facilitators and barriers to the development of innovation in a business relationship and affect the evolution of the relationship differently depending on value, culture and managerial system of the SME.

3. Learning process in collaborative innovation

Recently knowledge and learning processes have become the main topics in the agenda of many scholars studying collaborative innovation (Dyer and Singh, 1998; Gemunden et. al,

1999). Among them, the inter-organizational and collaborative network learning perspectives have emerged as distinctive approaches providing a starting point for the analysis of development of innovations in collaborative relationships. The table 1 summaries the similarities and distinctions among these perspectives on the base of three main dimensions of knowledge and learning process in interaction i.e. firm's knowledge base, attributes of knowledge and characteristics of relationships.

Dimensions	Inter-organizational perspective	Collaborative network perspective
Firm's knowledge base and capacity	• Absorptive capacity • Relational, interaction and collaboration capability, • Relative absorptive capacity	• Collaborative competency • Cooperative competency • Network competence • Coordinator or orchestrator capacity • Positioning and visioning
Knowledge and processes characteristic	• Tacit/explicit knowledge • Similarity of knowledge • Specialized knowledge • Similarity and shared routine • Resource and knowledge appropriateness	
Relation characteristics	• Strong/weak ties • Commitment/opportunism • Trust, shared value and culture • Similarity of processes • Shared inter-firm routine	• Multiple and connected relationships • Flexibility of network • Strong ties/loose ties • Managed/unmanaged network
Main references and studies	Dyer and Singh (1998); Lane and Lubatkin (1998); Adler, (2001); Johnson and Sohi (2003); Blomqvist and Levy (2004); Miles et al. (2000, 2004); Lampela and Kärkäinen (2006); Rasmussen (2007); (2002); Hurmelinna-Laukkanen et al (2007); Castaldo (2007);	Ford (1998); Holm et al (1999) Bloomqvist, (2006); Moller and Halinen (1999); Möller and Rajala (2007); Miles et al. (2000, 2004); Ritter et al. (2002); Möller and Rajala (2006); Dhanaraj and Parkhe (2006), Heikkinen and Tähtinen (2006)

Table 1. Learning in inter-organizational and collaborative perspective

The inter-organizational approach

Based on the foundation of organizational learning literature (Cohen and Lenvintal, 1990), the inter-organisational perspective (Johnson and Sohi 2003; Blomqvist and Levy 2004) stresses the firm's knowledge attributes of absorptive capacity (Levinthal, 1990; Koza and Lewin, 1998) as addressing the leveraging of firm's interaction (Lampela and Kärkäinen 2009, Hallikas et al 2009) in a dyadic relationship. Relational capability (Lorenzoni and Lipparini, 1999; Johnson and Sohi, 2003), collaboration capability (Blomqvist and Levy, 2004) and interaction capability (Johnson and Ford, 2006) are different terms used to refer to the ability of a firm to

recognize the value of external partner's knowledge and to build and maintain high-quality and productive-firm relationships. These relationships have been recognized as important for accelerating the firm's knowledge access with an effect on growth and innovativeness (Lampela and Kärkäinen, 2006). Moreover, the understanding on the interactive process of knowledge flow has been deepened by Dyer and Singh (1998) who emphasized the similarity of the knowledge base and the level of knowledge specialization in learning between partners. Similarly, Lane and Lubatkin (1998) introduced the concept of relative absorptive capacity to take a wide set of characteristics of the partner into account. In addition, Dhanaraj et al. (2004) have showed that the strength of a relationship between firms positively influenced the transfer of both tacit and explicit knowledge, leading to higher performance in learning. Kachra et al. (2008) found that the presence of stronger social relationships and social capital correlates significantly with the innovativeness of learning based collaboration (see also Knight, 2002). Furthermore, scholars studied the role of motivation (Szulanski, 1996, 2002) and appropriability (Hurmelinna-Laukkanen et al, 2007) and identified trust as one of the most effective enablers to inter-firm knowledge and resources transfer because high trust decreases situational uncertainty (Simonin, 1999) and opportunism (Lubatkin et al., 2001) and encourages higher commitment to the relationship (Capaldo, 2007). At the same time, some authors have evidenced also the "dark side" of strong inter-organizational relationships as obstacles for innovation mainly of radical type. The main reason is that a strong relationship locks firms into a narrow network, making them dependent on the inspiration by only a small number of external sources of creativity (Capaldo, 2007) and reduces the likeliness to explore new knowledge paths (Collinson and Wilson, 2006).

The collaborative network learning approach

The focus on structural and social dimension of relationships has been further debated by collaborative learning perspective focused on "learning by a group of organizations as a group" (Knight, 2002). According to Hallikas et al. (2009) the network learning literature represents a novel topic for learning research as the innovation phenomenon becomes increasingly occurring with multiple participating organizations. Emphasizing the aspect of multiple and organized relations (Håkansson and Snehota (2006) and open and interconnected business relationships (Ford et al 2003; Ford and Håkansson 2006), these studies have shed new light on the mix of diversity, variety and continuity of relationships and the way in which they are conductive to learning and innovation. Among them, Möller and Rajala (2007) argued that in innovation networks, knowledge exploration through weak ties, i.e. sources external to well established relationships, is needed, and flexibility of network is essential. They furthermore recognized the role of network orchestration defined by the nodal position held by an actor in a flexible network as crucial from a knowledge transfer point of view, especially *"because such an actor's task is to connect multiple actors in the net"*(Möller and Rajala, 2007; p 899).

The problem related to relational distance has also been discussed according to a cognitive perspective (Argyris and Schon, 1977). The lack of feedback for effective learning processes is seen as very likely when a relatively large number of agents interact with each other in various process steps. So it has been concluded that striving to learn more effectively in network means

to enable trust-based mutual communication and continuous feedback as well as that the coordination and co-operation link between the organizations must be strong and kept active (Blomqvist, 2004; Miles et al., 2000; 2004). Miles et al. (2000, 2005) pointed out that the ability to collaborate in network is a meta-capability for innovation. Similarly, Sivadas and Dwyer (2000) discussed cooperative competency as *"the ability of the partners to trust, communicate, and coordinate"* (ibid, p 40). Moller and Halinen (1999) and Ritter et al. (2002) have developed a concept of network competence to understand the capacity of firm to drive innovation success through the effectively management of actors in the network. Many others authors furthered the role of coordination or orchestrator (Dhanaraj and Parkhe, 2006; Heikkinen and Tähtinen, 2006) and discussed these aspects in term of capacity to 1) support absorptive competences among the network actors, 2) foster articulation and codification of tacit knowledge when it is reasonable and possible, and 3) develop long-term inter-firm relationships and network vision and identities for members (Hurmelinna-Laukkanen and Natti, 2007).

However the ongoing debate on the nature and structure of the network and its impact on learning is far from a final resolution and there are many contributions that support different perspectives. Recent research has found that an open unformed network comprising of numerous and loose ties has to be particularly well-adapted to facilitate new knowledge creation, whereas the more closely and coordinated structure has been found to better facilitate the diffusion, implementation and exploitation of existing knowledge (Hallikas et al., 2009).

4. Research aim

The above summarized literature studies the underlying principles of collaborative innovation at a rather abstract level or within the context of large companies (Chesbrough, 2003, 2006). Studies explicitly focusing on the SME network perspective look at advantages and opportunities for collaborations, whereas an in-depth debate about the asymmetric nature of relationships as well as the mechanisms that enable or hinder the development of effective collaboration and learning processes in SME innovation networks is yet missing. The main objective of our study is to contribute to bridge this gap.

More in detail the aim of this chapter is to provide a relationship approach to collaborative innovation in SMEs', specifically: 1) to investigate the dynamics of SMEs' relationships with partners different by size, power and resources and stock within a network 2) and exploring the barriers and facilitators to learning for Smes' innovation processes.

To address these efforts we chose to investigate in depth the food sector. We put two main points in the base of our business focus. *First*, food collaborative innovation has been analyzed so far mainly within the context of large, multinational firms (Fortuin & Omta 2008) stressing the role of this actors as transfer of formalized knowledge. *Second*, the topic of collaborative innovation of food sector SMS is particularly important as the food market is not very supportive to innovation. It is highly saturated (Sucher, 2007), consumers tend to be rather conservative concerning their food preferences (Rozin & Vollmecke, 1986), and the food industry is not extensively pushed by technical innova-

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tions (Moskowitz, 2008). Innovation is very much fraught with risk in the food sector; 60 – 80% of the new launched products fail (Grunert and Valli, 2001). To realize a successful food product innovation therefore a combined efforts of different network partners - like suppliers (often SMEs) and retailers – is needed for customizing the new product to the needs of the end-consumers (Gellynck, & Molnar, 2009).

Given the difficult market situation of food sector SME as well as the necessity to cooperate for being able to create and launch food innovations, there is a need for research that deepens understanding of how SMEs experience their relationships and configure modes of interaction with asymmetric partners. Also we want deepen the understanding of factors and barriers of food sector SME network learning, fostering integration and creation of new knowledge as antecedents and contribution to innovation and sustainable competitive advantage of all network.

4.1. Selection of industry and sample

For the study, we decided to investigate processing food SME that innovate in network collaborations. An aspect to study is how highly different partners like SME suppliers and large retailers can cooperate for innovation despite – or because of - large power asymmetries (Gellynck and Molnar, 2009; Colurcio et al. 2012). Beside product innovation, a network is also needed for the so-called "immaterial" organizational innovations like the adoption of quality standards and methods are of tremendous importance for food SMEs (Padel, 2001). Without network partners, these innovations would be out of their reach as they require inter alia scale economies in audit, control, certification activities.

Literature suggests that the opportunities for collaborative innovation depend on the market conditions (Chen and Chen, 2002).

Our sampling strategy followed three criteria: *First*, all selected companies had to be SME, i.e. have between 1 and 250 employees. *Second*, we based our sample on the "stylized model of agri-food vertical chain" originally defined by Raynaud et al. (2005:60) which is presented in table 2 below:

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
Supplier (e.g. fertiliser)	Farmer	1st Processor (e.g. mills)	2nd Processor (e.g. semi-finished good, end products)	Wholesaler	Retailer

Table 2. Stages of the agri-food chain

Within this model, we mainly focused on processors active in stages 3 and 4. We expected that these SMEs would have a high need to cooperate in innovation processes. *Third*, the accessibility of the company, i.e. the interest of the interviewee to participate in the study, has been crucial for the sample design (Merkens, 2007). The final sample consisted of interviewees from 167 Italian SME (148 small and 19 medium companies). All interviewees were indicated by the SME as a "person responsible for innovation".

4.2. Methods for data gathering and analysis

In our study, we were interested into the perspective of SME innovation managers on collaborative innovation. We particularly wanted to know what they perceive as barriers and enablers to inter-organizational collaboration and learning in innovation networks and gain detailed insights into attributes of network relations in innovation networks involving SME. We therefore decided to conduct semi-standardized interviews (Flick, 2009; p. 156). The basic assumption behind the interview format that combines open, theory-driven and confrontational questions is that the interviewees hold a subjective theory on the topic of the study. This subjective theory consists of explicit knowledge which the interviewee can easily articulate as a response to an open question but also implicit knowledge where he or she needs methodological support in the articulation through other types of questions (Groeben, 1990). Our interview guideline therefore started with open questions on what the interviewee understands as innovation, what the major challenges of his company are in that respect and what he sees as networks. Then, interviewees where asked to describe the most important attributes of network relations in order to identified trigger and opportunities for collaboration and learning in innovation. Here, we added theory-driven sub-questions for being able to fully understand the attributes of the network relationships: If the interviewee did not mention it him- or herself, we asked for the cooperation duration and evolution, particularity, intensity, dependence, and mutuality of the relationship. These questions have been developed according to the theoretical framework proposed by Johnsen and Ford (2000; 2001, 2007) who identified different types of asymmetries as relevant for cooperation in business relationships.

The interviewee was asked both for the benefits and learning points from the cooperation but also for difficulties, conflicts, and barriers to collaboration and knowledge sharing and development. In this case, the questions have been drawn to deepen the firm's capacity, knowledge and relationships attributes as theoretically discussed at the base of learning processes in interaction.

Like this, the interviewee was confronted with the opposite of the own subjective theory regardless whether he or she was in favour or not in favour of cooperation in innovation networks.

Interviews were held via telephone. They took approximately half an hour and were then recoded and transcribed ad verbatim.

Data analysis and interpretation took several steps. We first separated the interview transcripts of small (up to 50 employees) and medium (between 51 and 250 employees) companies because we were interested whether size matters. We then created bodies of text related to the questions, for example all answers related to the question on "From your perspective, what is Innovation.

We then analyzed the question-specific data sets with a bottom-up approach and used techniques suggested by Miles & Huberman (1994). Between two and four researchers coded the material independently, then came together, noted themes and clustered them considering the relations and linkages between each other.

Table 3 provides an outlook on the typologies we have recourse to in order to analyze above mentioned characteristics of the relationship within the innovation network.

Relationship Characteristic	**Definition**	**Consequences** **Customer unilaterally influence/ Customer and Supplier bilaterally influence**
Mutuality	Shared goals or common interests of firms.	– Formality/Informality in setting relationship goals – Use/purpose of writtren plans – Extent of interwining of goals and interest and experience
Particularity	Direction and uniqueness of interaction. Extent of standardisation of interaction.	– Standardization/adaptation of approach to interaction – Width of suppliers' customer portfolio – Building of confidence and emotional ties in interaction
Conflict	Differences between the parties over the direction of the relationship or over their respective contributions and benefits	– Conflict resolution efforts involving higher/lower - level actors – Development of approaches for coping with conflict
Cooperation	The extent to which companies work together to determne or implement a direction for the relationship Co-existence of co-operation and conflict	– Experience and inclusion of suppliers in co-operative projects – Development of personal expectations and contributions in interaction
Intensity	Level of contact and resource exchange between firms.	– Range, level and frequency of contact between customer and supplier – Extent of supplier involvement in contact and resource exchange
Power and dependance	Distinct types of power and dependance exist: Technical, Knowledge, Social, Logistic , Administrative.	– Strategic and operational aspects of relationship – Technical aspect of relationship and advisory roles – Decision-making process – Social/networking aspects of supplier's relationships – Development of information and knowledge – Develeopment of values built on history

Table 3. A typology for the analysis of size asymmetry

We analyzed data through a qualitative content analysis that is based on data from narratives and observations that requires understanding and co-operation between the researcher and the participants, such that texts based on interviews (Kondracki et al., 2002). Specifically, we analysed and categorized consequences of each characteristic of the relationship according a pattern of analysis based on abstraction (Graneheim, Lund-

man, 2003) since it emphasises descriptions and interpretations on a higher logical level including the creations of codes, categories and themes on varying levels. Then, we defined three different levels of intensity of the characteristic in the specific relationship: + = low level; ++ = medium level; +++ = high level.

5. Findings

The findings below describe how and in what types of networks SMEs cooperate for developing innovation and what the interviewees identified as barriers and enables to inter-organizational collaboration and learning in innovation network.

5.1. The collaborative innovation of SMES

The understanding of innovation is different for interviewees from small and medium companies within the two data settings. When asked what they perceive as innovation, interviewees from medium companies often mentioned the development of new technologies for the improvement of production processes and technologies. The small firms instead associated process and technological innovation with the incremental improvement of existing products and processes. Generally they talked about product innovation as the improvement of existing products and the extension of the product range. All firms not or least associated the development of completely new products (See Table 4).

	Small	Medium
Development of completely new products		+
Innovative appearances at the market	+	++
New production processes and technologies	++	+++
Incremental improvement of existing product/processes	++	++
New packaging solution	+	++

Table 4. What the firms understood as innovation

However, we observed a difference in the attributes of relationships depending on the size of companies. Table 5 provides a brief overview of these attributes. The general network of relationships comprises clients, suppliers, research centres, and other stakeholders. But looking specifically at innovation network it is understood by the large part of the interviewees

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as cooperation between processing companies and suppliers of raw material, equipment and services. Respect to the theoretical framework we adopted, in this table we didn't consider explicitly the attribute "conflict" since we investigated it through the variable "mutuality". However, although we didn't asked clearly about conflict, we will discuss it due to lateral information. The relationship with suppliers, especially with raw material and equipment suppliers looks very high cooperative to small companies are the suppliers were seen very often as source of innovation or as co-innovator partner. Suppliers act as development and implementation partners for new ideas; in some cases we even find common projects for developing innovations like new packaging solutions and new products. Suppliers also support small companies in designing and tailoring new production and logistic technology solutions for sustainable processes. Relationships to suppliers are characterized as long lasting, affordable and personal. In addition, small clients and experts or specialists are mentioned by small firm as important and strategic cooperative partners in some specific situation. In general collaborations judged to be important are those with specialized clients or firms that punctually provide services. Mentioned are market research companies or firms that have a high investment in R&D and don't want manufacturing or commercialize their new products or semi-finished goods by themselves. Interviewees from small companies reported least on universities and research centers and consumer collaboration. The cooperation with research centers is usually aimed at solving technical problems and has been described as project based with clear cooperation agreements.

Interviewees from medium companies defined network mainly as cooperation with suppliers of equipment and raw material and large distributors. According their view, a network is a strong cooperation within the supply chain, based on technology and market content, allowing to face competition and to guarantee the survival on the market. Suppliers are seen as important and they enable the medium companies to get access to update technology and complementary knowledge. The development of a win-win relationship is very important for the interviewees. Relationships with suppliers aret hus in most cases very stable (longer than ten years) and characterized by trust and reliability. Also relationships with large distributors have been recognized as very important to complement firm knowledge and demand a high management attention and regular contact. Particularly it is described as profiting when strategies are aligned, involve pear cooperation and partners can make reciprocal business deals. In that case, the company gains a profit because it can use the network of clients, take up new ideas, ask their clients to test the products with the end customer and in best case deliver products in exclusivity.

Only few companies named marketing agencies and consumers as important partners for the development of innovation.

Generally horizontal cooperation for innovation within the same branch is not so frequent within small and medium companies. Cooperation with competitors to reach common goals or to develop the whole branch for the profit is mentioned as generally scarce and without any effect when it comes to innovation or to a fruitful knowledge exchange.

<table>
<tr><th rowspan="2"></th><th colspan="2">Cooperation</th><th colspan="2">Particularity</th><th colspan="2">Intensity</th><th colspan="2">Power and Dependence</th><th colspan="2">Mutuality</th></tr>
<tr><th>Small</th><th>Medium</th><th>Small</th><th>Medium</th><th>Small</th><th>Medium</th><th>Small</th><th>Medium</th><th>Small</th><th>Medium</th></tr>
<tr><td>Small clients</td><td>+++</td><td>+</td><td colspan="2">+++</td><td>+++</td><td>++</td><td colspan="2">++</td><td colspan="2">+++</td></tr>
<tr><td>Large distributors</td><td>++</td><td>+++</td><td colspan="2">++</td><td>+</td><td>++</td><td colspan="2">+++</td><td colspan="2">++</td></tr>
<tr><td>Suppliers</td><td colspan="2">+++*</td><td colspan="2">++</td><td>+++</td><td>++</td><td>++</td><td>+</td><td colspan="2">+++</td></tr>
<tr><td>Universities/ research</td><td>+</td><td>++</td><td colspan="2">+++</td><td>++</td><td>+++</td><td colspan="2">+</td><td colspan="2">+++</td></tr>
<tr><td>Competitors</td><td>+</td><td>+</td><td colspan="2">+</td><td colspan="2">+</td><td colspan="2">+</td><td>+++</td><td>++</td></tr>
<tr><td>Federal Agencies</td><td>+</td><td>++</td><td>++</td><td>+</td><td>++</td><td>+</td><td>+</td><td>+++</td><td>+++</td><td>++</td></tr>
<tr><td>Specialists and experts</td><td>+++</td><td>++</td><td>++</td><td>+</td><td>++</td><td>+</td><td colspan="2">+</td><td colspan="2">+++</td></tr>
</table>

+ = low level

++ = medium level

+++ = high level

* very high

Table 5. Attributes of Smes Network

Regarding the particularity and evolution, interviewees reported that relationships develop and evolve over time built on increasing tied and personalised relationships. However, the particularity of the relationships of processing SMEs is described as high with small clients and research institute. With suppliers the particularity is a guarantee for the innovation since the benefit of innovation depends on the application and on the novelty of the equipment, especially for small companies. Processing SMEs however have innovation relationships with more than one supplier, so that from their side, the relationship with suppliers is less exclusive than the other way round. Relationships with large distributors are very often not particular; here, the mainly the bigger processing SMEs are usually just one innovation partners amongst many others for the distributers and often based on negotiated affair: technical know how is exchanged for marketing, knowledge on market trends for access to other markets, lower costs for product tests.

Asked about the intensity of the relationship, almost all interviewees mentioned the continuity and the frequency of exchange of information and knowledge with the main partners. Particularly, interviewees from small companies mentioned intensity mainly as team working and continuous knowledge and information sharing together with conjoint job training. Evidence shows the same dynamic in the intensity of relationship with the main partners also for medium companies. We however also find that the bigger the processing SME, the more

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intense cooperation with large distributors and the less intense cooperation with small clients, suppliers and Federal agencies are.

With respect to power dependence, only three few companies declared that the power is balanced and that there are no asymmetries in resources and power between the parts involved in the relationship. In tendency we find that the bigger the processing company, the more dependent are suppliers, and the less dependent is the company from large distributors. In addition, with the size of the company, its dependence from Federal agencies increases.

Mutuality of the relationships is generally mentioned at high level respect to all partners. Mainly small firms indicated as important to create a win-win situation that enables the (incremental) development of "new" products, services, production technologies and raw materials, and to solve common problems.

5.2. Benefits and difficulties in collaborative innovation

The reasons that companies provided on why cooperation for innovation is important include: 1) acquisition of know how on the market and trends, 2) the external view that helps to overcome blind spots, 3)the opportunity to enter new markets and to build up a market reputation, and 4) the possibility to join resources for radical new ideas.

Asked to medium firm for the benefits of cooperation in innovation networks, most interviewees mentioned the development of new products, new services, new production technologies and raw material. Very often, the interviewees mentioned that cooperation creates a win-win situation where they receive complementary know-how and information from each other, exchange concepts, solve together common problems without having to re-invent the wheel or get access to new markets. In some cases, interviewees stated that cooperation enables them to build up a completely new supply chain with producers of raw material in a developing country what makes the production and the products more sustainable innovation. Some medium firms additionally mentioned the opportunity to extend the firm's vision of network and to contribute to fostering the firm's influence on other relationships and partner's collaboration commitments. For the interviewees from small firms, especially the market effectiveness has been emphasized as important benefit during the interview, i.e. how the innovation allows them to improve the quality of their offering in a way that their products better fit the changing market requests.

Discussing on enablers and barriers of collaboration similar results emerged among small and medium firm. Cooperation, generally, has been reported ended due to missing reciprocity of efforts, changing interests or strategies of network partners or problems with the quality of the products. Small firms detailed this aspects in the missed promise of the partner or if he strives only for own benefit, the new product has now success on the market or can not be developed or the partners do not get along well. Rarely, cooperation ends because a contract ends and partners have new plans. Rarely, cooperation ends because a contract ends and partners has new plans. Small interviewees mentioned that cooperation with large distributers is difficult because of the small market power of their own company. For medium firms the challenges they faced are related to finding enough time for cooperation and clarifying

expectations. A barrier to cooperation is also the necessity to confidentiality of new ideas. Some interviewees underlined that the number of partners involved into an innovation project should not be too big because otherwise, the process is too slow.

Looking at the difficulties, more than half of the interviewees declared that collaboration is not always easy. More into detail, some interviewees mentioned the difficulty to share information in real time; others referred to procedural and routine problems arising from the interaction between organizations with different process rules. Technological and technical difficulties have been mentioned mainly with reference to supply chain and R&D relationships where a larger distance has been perceived with respect to knowledge and experience background of partners. One interviewee well elicited this issue indicating that it is difficult (though not impossible) to work with specialized suppliers because of their different views on time, technology and ways of working. Similarly, to work with universities or R&D agencies needs the parties to get first used to each other. Within the perception of the most of interviewees these aspects represents the major challenge of collaboration for innovation. It has been widely outlined by the interviewees that the firms really innovate if they are able to conduct and participate in exploratory activity outside of their organizational boundaries absorbing novel practices from external. In addition, a difficulty other interviewees referred to is the lack of a shared vision with the partners and missing clarity in communication and the definition of goals and expectations.

When asked to expand on the reasons of such difficulties, a majority of respondents referred to the presence of cultural, organizational and resource-related barriers. Particularly trust issues, distances (geographical, dimensional, technological and commercial) between firms and strategic competences exchange increases the risk perceived by the interviewees to integrate into and participate in innovation networks.

The lack of trust and benefits understanding have also mentioned as the main reason of the failure and interruption of long standing collaborations. Furthermore, the i) imbalanced power between cooperating parties, ii) insufficient resources and highest changes required, iii) quality problems or iiii) better alternatives have been found as reasons to break up business relationships. Finally, to avoid dependency from other firms is also mentioned; as well as the time and money investment to innovate within a network was seen as unprofitable in certain circumstances. In addition it is also remarkable that more than the half of firms who disclaims problem or conflicts in collaboration are mainly the smaller ones. Surprisingly, there is a strong tendency among these managers to regard relationships in some general way as a "good thing" and there is also a common belief that all partners work towards closer and mutual relations.

A general agreement among all interviews regarded the belief that the cooperation for innovation needs openness as well as transparency on interests and on the own contribution. The partners should operate at the same eye-level and respect each other, develop together something new while building upon existing knowledge and both benefit from the cooperation. Both should be ready to invest time and efforts into cooperation. Long lasting, personal relationships and trust has been said to be essential for cooperation. Transparency and openness, collegiality and a good gut feeling have been seen by all interviewees as essential for cooperation at the same eye level and to avoid conflicts.

Again, the trust issue has been mentioned as a key driver of networking orientation of SMEs with the distinctive feature that trust is perceived to be built gradually through the continuity of cooperation among partners. A deep understanding of shared risks as well as of mutual benefits of all network partners have been identified as trigger to the openness of firms and to team working allowing to focus on common goals amongst all network members. Personal and face to face daily contacts - often within existing long standing relationships –have been seen as the preferred way to collaborate and to solve conflicts.

5.3. Barriers and enabler to inter-organisational learning

Discussing on barrier and enabler to inter-organisational learning the main finding which is noticeable is that about all firms (there is only two exception,one for each market) declared that innovation partnerships allow them to increase their competence and knowledge assets. First of all the complementary nature of knowledge and competence predominates innovation relationships. Acquisition and upgrading of technical and technological competences have been seen as the main results of learning collaboration by about the half of investigated firms. Interviewees from the other half of firms declared that their company mainly benefits from the development of market capability and subsequent increases of the market perception of firm offerings' value.

To improve the ability to learn is also mentioned as core element of innovation relationships by about two thirds of respondents. The interviewees stated that collaborating in innovation networks allows them to i) better define what they want and what is expected by partners, ii) develop a clearer vision of their relationships, iii) gain better insights on external knowledge through understanding its meaning for the own organization. As main inter-organizational learning results medium companies valued the better understanding of causes and consequences of their actions that allows for the detection and correction of errors. Some interviewees stressed the unlearning issue as an open approach to innovation. Innovation in networks is mentioned to promote the company to proactively question its older routines, assertions and beliefs and fostering the need of dismissing or replacing outdated knowledge.

Among those interviewees who confirmed the learning results of innovation network relationships, only a minor part identified some barriers or obstacles to their learning processes. The latter have been mainly identified as cultural and power distances between partners or low commitment of partners too. These difficulties have been often seen in relation to the different business and value chain position of partners. In addition, some interviewees stated to be not aware of competences and activities of their partners and this obviously leads to an inadequate understanding and knowledge of the competences available in the network. A common language is required to reduce the distance between partners and to reinforce social and cognitive dimension.

At the opposite site, the complementarities of the partners' knowledge have been seen as the main stimulus to the learning processes. Cultural issues instead feed a contrasting debate: whereas some interviewees named cultural barriers as critical obstacles, others perceived them as a trigger allowing a different view on problem solving issues. When supported by personal and social ties, the different culture of partners has been seen as great opportunity for a more

effective knowledge integration allowing the firm to increase flexibility and therefore its ability to seize strategic opportunities.

6. Discussion and conclusion

The works aimed to advance the state of the art in research on network innovation in SMEs by developing a deep understanding of barriers and enablers to cooperation and learning. In particular, the work aimed at identifying networking attitudes, preferences and practices of food processing SMEs that are relevant to the innovation development in Italian food sector.

The main conclusions from our study are that food SMEs are orientated to collaborate with partners for innovation. Cooperation in innovation networks seems to be important to them – as long as it brings mutual benefits and partners cooperate at the same eye level. However, the innovation openness is focused on some privileged relationships with few partners often belonged to the current network of SMEs where long lasting relationship lleviates trust concerns. Like this, the results highlight the importance of trust in innovation relationships. The processes of developing collaborative innovation is little formalized and based on personal in nature and daily relationships.

We also find that size matters. We identified some differences between the partners of the collaborative relationship for innovation depending on small companies and medium companies in our sample. Small companies seems to work closer with small clients similar to their background of knowledge and routine. Medium companies are used to cooperate with universities and research institutions and like this gain access to very specific technological know-how. The stronger cooperation with the research world explains a wider opening towards the development of new knowledge and indicates that medium companies frame innovation in a long term vision. We didn't notice the same tendency for small food processing SME. This evidence highlights a critical point for Italian small companies, the difficulty to access some kinds of relationship which are not finalized to a specific project but to a wider cooperation of knowledge and development.

For all SMEs in our sample, suppliers are the most important partners with whom innovation cooperation is developed; while a strong innovation dependence characterizes the value chain relationships involving unbalanced partners. Mainly little 1st processing SME are usually more dependent on the bigger 2st processing for innovation whereas the latter are more dependent from large distributers. Like this, dependence of partners in network innovation along the agri-food supply chain always also includes effects of moving costs for innovation development down to the weakest partner. However, even in such a situation the involvement in the innovation network is of mutual benefit for both partners as the larger partner offers marketing and technological input for the improve of products. Surprisingly for small companies the matter of unbalanced power has not been mentioned as critical. The reason might be that they usually operate in a niche and grow together with small clients and suppliers in partnership.

At the basis of innovation collaborations the results highlight the research of resource and knowledge complementarities (Lampela and Kärkäinen 2009). The learn by doing approach

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of Smes to innovation (Holmlund, Kock, 1996) has been proved by our results and extended to networking and learning orientation of the Smes. Cognitive and relational benefits consist of knowledge developed through "on the job" interaction: by solving together common problems Smes improve their processes without having to re-invent the wheel and partners advance their knowledge about potentiality of Smes and needs of upgrading their involvement and interactions. In addition the results show the considerable benefits from the reference effect of the relationships (Ritter and Gemünden, 2004). For the more knowledgeable Smes the interaction for innovation allows the access in a wider network of connected relationships and to better position themselves in value networks.

In sum, the resource and power asymmetries seem to be perceived as a trigger for network and learning interaction. This suggests that the matter of asymmetries seem to be none the most critical to innovation and learning network relationships. A general view is that innovation partnerships tend to perceived to offer a lot also to the less powerful partners mainly in terms of learning and knowledge issue.

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Adding Value in Food Production

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Additional information is available at the end of the chapter

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1. Introduction

This chapter investigates the practice of value adding in food production, by taking a view from a food manufacturer that has found itself in recent years increasingly integrated into the food industry: small-scale farming Producers. Specifically, their construction and practice of value adding 'authenticity' to food products is revealed.

The objective of this chapter is twofold. One is to study the process of and provide a new perspective on value adding, with an emphasis towards 'authenticity' in food. This examination delivers new knowledge and provides future direction to small scale farming Producers and the wider food industry. The secondary objective is to provide a demonstration to the value of Applied Cultural Analysis in food development.

The practices of small-scale Producers will be explored through an ethnographic approach, combining participant observation and interview. This investigation intend to uncover how and what is value added by small scale Producers: in the values they infuse in their food commodities, of the value found in their way of life, and the value in their production practices. It is an examination of the small, unconscious acts revealed through ethnographic enquiry and the lens of cultural theory to reveal the practice of value adding 'authenticity' as a culturally constructed act. These findings will be demonstrated as important not only for Producers in understanding their practices, but also for the wider food industry concerned with value adding authenticity in their food products

1.1. Adding value – A problem definition

First of all we have to establish what *value* is. When following, in a business context, the end-user of a product or service appreciates its use, he or she values the consumption. In return for the value that the business firm produces, the customer pays for the value. The *economic value* is determined by the subtraction of cost (effort) from the perceived benefit of using the

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product in question (Peteraf and Barney, 2003). Perceived benefits, then could be anything – status, self-confidence, health, satiety, excitement or whatever the user gets from the product.

A simplistic view of adding value to food brings with it straightforward notions of 'adding' to raw ingredients and processing them in a way that increases economic return. A useful supporting definition of this starting view to 'value added' is provided by the United States Department of Commerce and Industry, which outlines any processing of food as a value added act in and of itself:

> Processed foods are "value-added" products, referring to the fact that a raw commodity or commodities are transformed into a processed product through use of materials, labour, and technology. Any product that requires some degree of processing is referred to as a processed product, regardless of whether the amount of processing is minor, such as for canned fruit, or more complex, such as for snack foods (2008; p. 3)

However, the processing of food is not as straightforward as gathering materials, labour and technology together equals automatically producing a value added product. In recent years, a increased emphasis on the addition of *culture* as a value is an evident phenomenon in the food market today that introduces an element of complexity and requires deeper investigation and further definition. Paul DuGay and Friedmann defines the addition of culture *as a value* that adds the "inscription of meanings and associations as they are produced and circulated in a conscious attempt to generate desire for them amongst end users" (DuGay & Friedmann, 2002, p. 7).

In close connection to the discussion of culture as value is the commercial value of *authenticity* in food products. Several large industry consultant groups refer to the importance of value adding, with statements such as:

> Key trends to impact the food and beverage market through 2012 and beyond… relate to purity, authenticity and sustainability, as consumers continue to look for products with added value. (Innova Market Insights, 2011)

> Added value still matters. Despite lingering economic uncertainty and mounting scrutiny of product health claims, consumers remain willing to spend a bit more on food that does, or stands for, 'something'. (Euromonitor International, 2010)

Value adding could be seen as the reinvention of product for economic gain. But what is the reinvention happening - what are the processes at play? Let's continue with the idea of processing as value adding in food. This processing of a raw commodity to product can also be described as a process of cooking. The french anthropologist Claude Levi-Strauss (1994), wrote in his classical article on the culinary triangle about the processes of cooking and described it as an "…act of mediation, where we transform raw materials into a cooked prod-

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uct… not only does cooking mark the transition from nature to culture, but through it and by means of it, the human state can be defined with all its attributes" (p. 164-5).

Focusing on the words of mediation, transformation and culture, cooking can be viewed as a process to *mediate* what and how is added in, or transited out; to *transform* into a final product is a practice to inscribe *cultural* meaning to the human state. It's the selection of these elements that positions the function of the cook (or food manufacturer) as a *mediator* at the conjunction of final product to the consumer and therefore places them in the position of cultural agent(s). Culture becomes an important part of the production in cooking - the combination of what cultural meaning and associations are selected and represented in this transformation *is* the challenge in 'cooking' and thus the 'value add'. Cultural processing and processes bind the selection of chosen elements that can be simplicity, rarity and tradition. Therefore to produce a 'value added' food product, an understanding of cultural processes is required – a complex task in which the food industry finds itself involved.

According to Mintel (2011), an astonishing 20,000 new "value added" products per month are launched in the global food industry. However, a demonstration of the complexity involved with cultural interpretation, and just how challenging creating a cultural product can be, is found in the exceptionally high failure rate, "exceeding 90 per cent for some categories, which suggests that firms have difficulty in developing products that appeal to enough people to warrant continued distribution" (Connor & Schiek, 1997).

Combined, this has created a food industry both focused on creating new added-value products while at the same time, working to minimise the potential risk of failure and subsequent economic loss. The sheer number of products also hints to the increasing complexity of adding value to products by the inscription of cultural values. These cultural values often take the form of ideologies, experiences, morals, mythology, magic and sometimes illusion infused within the product offer and branding, such as Coca Cola's inscription to transform a carbonated soft drink to the embodiment of the spirit and essence of America, summarised in their advertising plans:

> Coke is real, authentic, honest, passionate and refreshing. It taught us to sing in perfect harmony. It even introduced us to Santa Claus. Coke is the kind of cultural fabric that unites all of us in some way. It has always encouraged us to share who we are and what we believe in (Identity Advertising, 2007, p. 2).

The creation of 'value added' inscribed in food is thus an effort to elicit emotion from consumers and entice them to spend more money in return for feeling this emotion. Value adding is a basis for economic gain via emotion, and it is emotion that becomes critical in the evaluation of how successful a value add food product is. It is also one of the more difficult to interpret – one cannot simply walk up to a consumer and ask what their emotions regarding a certain product are – they would not be able to tell you. It leaves mediators (cooks) charged with the responsibility to decode cultural meaning and charge their products with it by their practices. It also leaves the assessment of their labours as ephemeral and complex. It is information that needs to be teased out, tantalisingly revealed and then hidden, uncov-

ered by other means - lending qualitative research methods such as ethnology and anthropology as critical in the understanding and evaluation of value added food.

For the food industry, the tension created between potential and seemingly boundless creative transformative opportunity available in developing 'new' value added products is balanced against the underlying understanding of risk in both the emotive assessment of the culture that is infused and also in economic terms has led to, as Belasco (1989, p. 210) states, "corporate conservatism", neatly outlining a response from the food industry to address both perceived risks: there has been increased focus to consumer-directed research, in some instances as broadly to elevate its importance into the heart of day to day business operations, while at the same time to reduce the costs in bringing a product to market. The emergence of qualitative research methods to uncover 'insight' in the food industry saw an increasing investment to understanding food consumption within a daily life context when developing value added products. This however, is a difficult task when we consider the unconsciousness of taste and how unaware we can be about the food we eat on a daily basis.

The environment in which we eat our meals also demonstrates the quiet absorption of food into the background of daily life. In a 2011 report, it was suggested that in an average person's day, 56% of the time spent eating (85 of 152 total minutes) is while engaged in another activity considered primary such as watching television, driving, preparing meals, or working (Hamrick, 2011, p. 5). We're not even paying full attention to over half the food that goes in our mouths, yet significant amounts of time and money are invested by large food manufacturers trying to get consumers to tell them why they eat what they do.

The complexity in dissecting everyday life within business constraints such as timelines / budgets in a climate of conservatism and cost reduction has conceivably led to an avoidance of radically innovative products.

The reduction of economic risk by bringing down costs in value adding is also demonstrated with the use of materials (such as synthetic / non-food additives, flavour enhancers, thickeners, colours, stabilizers), a change in business operations such as off shore manufacturing, combined actions of which have increased the length and complexity of the food chain and the distance – both geographical and mental - between the raw and the cooked. It is this distance created over the past 50 years that's had a hand in creating the demand for authentic products and places small-scale production in the position it finds itself today.

Given the ongoing involvement and importance of value adding authenticity in food by these large companies, it may seem surprising that small-scale food Producers were not run out of the industry long ago. Instead, these Producers find themselves in a strong and growing position within the food market for value added authenticity. For example, direct to consumer sales from small scale farming production have grown 104.7% from 1997 to 2007 (Martinez, 2010), and 60% of the Swedish population prefers to buy local produce (Ekelund, Fernqvist & Tjärnemo, 2007, p. 233).

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2. Understanding value adding

2.1. Food and cultural studies traditions

There is a long tradition in cultural and sociological food studies regarding the *consumption* or *meaning of food* and does provide a basis for understanding a consumer perspective, and how demand is created. Existing food studies credit food consumption for a vast array of cultural process from the reproduction of a stable society (Lupton, 1996; Goody, 1982), the decoder of the unconscious attitudes of a society (Levi Strauss, 1994), of nationality (Barthes, 1967), as an indicator of class (Mennell, Murcott & van Otterloo, 1992; Bourdieu, 1984), social significance (what is important in a given society) (Douglas, 1982), to the relation of food in feminist studies and body shape (Adams, 1990). Taken together, these studies point to food as central to culture, a suitably broad implication that requires narrowing.

In narrowing to the central theme of this chapter - value adding and authenticity - the central ideas that emerge from a consumer perspective is that consumption of small-scale, authentic food is enjoyed largely by the elite upper classes in westernised societies – the diets of the poor have remained relatively unchanged: they are excluded from this possibility (Bourdieu, 1994). However, Alan Warde (1997) argues that the emphasis is shifting from class formation towards the formation of new groups in society that share 'lifestyle' rather than social class, and the consumption of 'authentic' food is a representation of chosen lifestyle group. A study by Anthony Giddens (1991) takes a wider view, outlining that the agency of consumers is emphasised over both the social and economic structures in which they find themselves, and this is a crucial means of establishing an individual's identity. The emergence of consumption of small-scale authentic food is also evidently associated with the mitigation of food risk and potential for disease as explored by Ulrich Beck (1992) and Mary Douglas (1996, p. 123).

Further, in specific regard to the term of authenticity, a concrete definition is a little difficult to locate. It has been described as a product of shared systems of signification (Ashley et al, 2004, p. 7) and is a modern phenomenon (Appadurai, 1986). What constitutes as 'authentic' changes over time (Peterson, 1997), an example of which is exemplified with the shift in lard heavy foods recipes - such as Cassoulet - where goose fat may be substituted for cheaper and lighter alternative fat sources but still acceptably remains an authentic French dish.

In some cases, authenticity has been deciphered as an act of micro-resistance to dominant forms of cultural production (De Certeau, M; Giard, L & Mayol. P, 1998) - where one makes an appeal for authenticity in response to their perception of *in*authenticity in others. For example, you are what you *don't* eat. Josée Johnston & Shydon Baumann (2010, p. 70) provide a key view on authenticity with their claim that it "doesn't really exist" as it is socially constructed. The definitions of authenticity are difficult to define concretely and are deliberately vague in use here, as it is the cultural construction through practice that this thesis aims to reveal.

It's important to note that the term of 'consumer' can be applied to Producers in this chapter, also - after all, as we all are when it comes to food - the small-scale producers in this

study are consumers, too. There is argument that Producers are developing products they personally consume.

2.2. A new food economy

The increasing intertwinement of "culture" and "economy" is a focal point in both cultural studies and economics during recent years (duGay & Pryke (2002), Pine & Gilmore (1993), Lash & Urry (2002)). The framework of a "New Economy" based on cultural values with labels such as Experience Economy or Dream Society has been launched during the last decades. We will discuss some of the major writings in order to define their usefulness for understanding an emerging "New Food Economy" with cultural production of added value as a common denominator. Firstly, the definition of New Economy in use here is reference to the increasing intertwinement of symbolic cultural messages and economic processes, the desired outcome of which is economic gain. The use of symbolic cultural messages is a key element in the New Economy, as it involves the responsibility for consumers to develop skills in 'reading' cultural values and, as Lash & Urry (2002) and duGay & Pryke (2002) point out, brings in references to the New Economy as being an 'economy of signs'. DuGay & Pryke's argument also outlines that there are an increasing number of goods and services that can be regarded as 'cultural' goods. Their use of 'cultural' goods refers to the inscription of meanings and associations to elicit consumer desire for their purchase - exactly what our cooks are trying to achieve - but from this view it is unclear if food is part of the basket of 'cultural' goods in the New Economy. They further describe that:

> There is a growing aestheticization or 'fashioning' of seemingly banal products whereby they are marketed to consumers in terms of particular clusters of meaning, often linked to 'lifestyles' and this is taken as an indication of the importance of 'culture' to the production and circulation of a multitude of goods and services (as quoted in duGay & Prkye, 2002, p. 7)

The inclusion of 'banality' would seem to infer that food is indeed inclusive; as Jönsson (2005) outlines in his discussion of milk, he demonstrates an obvious connection in the New Economy due to rapid development pace and inclusion of cultural messages in added value products in the dairy counter. While the mention of rapid development would seem to indicate the there is something *new* happening with the 'fashioning' of meanings embedded in (banal) food products in the New Economy, Jönsson continues that this may not necessarily be the case. As he outlines, "the production of such "value-added products" can be seen as a part of a general conversion (to the New Economy), but it is suggested that it can just as well be that the economy is increasingly bent on trying to capture experiences that have always been conveyed via food" (2005). In other words, the meanings inscribed in value added food are an amplification of information that is already known, and communicated via experiences, bringing in an inclusion to the 'Experience Economy' as well. A concept discussed by Pine & Gilmore in 1993, they outline the construction of experience 'spaces' as key in the eliciting of emotion for economic gain. In this world, no one sells mere commodities … they sell 'lifestyles'. That the spaces Produc-

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ers develop to sell their products are in part to create emotion further embeds them in the experience economy.

It is here that I position small-scale Producers - within the New and Experience Economies, producing products inscribed with cultural values: but as will be shown, the inscriptions made utilise previously held cultural knowledge. The role of those who create cultural inscriptions is continued by Lash & Urry:

This process, they argue has been accompanied by the increased influence of what are often termed the 'cultural intermediary occupations' of advertising, designing and marketing; those practitioners who play a pivotal role in articulating production with consumption by attempting to associate goods and services with particular cultural meanings and to address those values to prospective buyers… these signifying practices in doing business is evident not only in the production, design and marketing of goods and services, but also the internal life of organisations as well (as quoted in duGay & Pryke, 2002, p. 7).

This further embeds the position of cooks as 'cultural intermediaries'. Another key supporting element of Lash & Urry's description is the increased importance accorded to signifying practices that create symbolic value in the production of goods and services, such as those of small-scale Producers under investigation here. It also calls into account for the wider food industry to understand that it's not only the communication messages that are interpreted here: it is the reading of the entire production process.

Accordingly, an investigation of understood cultural values and production processes over time provides foundational information in understanding the context of value adding in the New Economy. Searching the historical roots of the emergence of late modern value added food production, Warren Belasco stress the importance of the radical counter cuisine against mainstream food ways that began to emerge from 1966. Some of the more important elements were:

- A consumerist component offered survivalist advice and suggested what to avoid, especially processed 'plastic' food.
- New ways to make food more fun - e.g. through the delight of improvisation, craftsmanship, ethnic and regional cooking.
- The organic paradigm, which posited a radically decentralised infrastructure consisting of communal farms, cooperative groceries, and hip restaurants (Belasco, 1989, p. 4).

While the countercuisine initially was interpreted as a threat by the major food companies, the ideas soon became the main inspiration source for product development. The wide range of rural, authentic, hand made, old-fashioned, ethnic and light products launched the last decades owe a lot to the counterculture of the 1960s and supports the use of this theory as still applicable in the food industry today.

Further support that these elements are still in demand today is an appreciation that the decentralised infrastructure Belasco refers also includes Farmers Markets, Farm Shops and

specialist stores - all of which are preferred sales channels of Producers in this study. The emergence of the Farmers markets as an alternative food channel became evident during this cross over period between the second and third food regimes. The first Farmers Markets were reported in the US during the 1970's, with a slow expansion over the subsequent twenty to thirty years - however increasing at a faster rate in more recent years. The market in observation for this study has been in operation since 2000. As shown on the chart below, the number of operating Farmers Markets in the US grew by seventeen per cent to 7,175 from 2010 to 2011 alone.

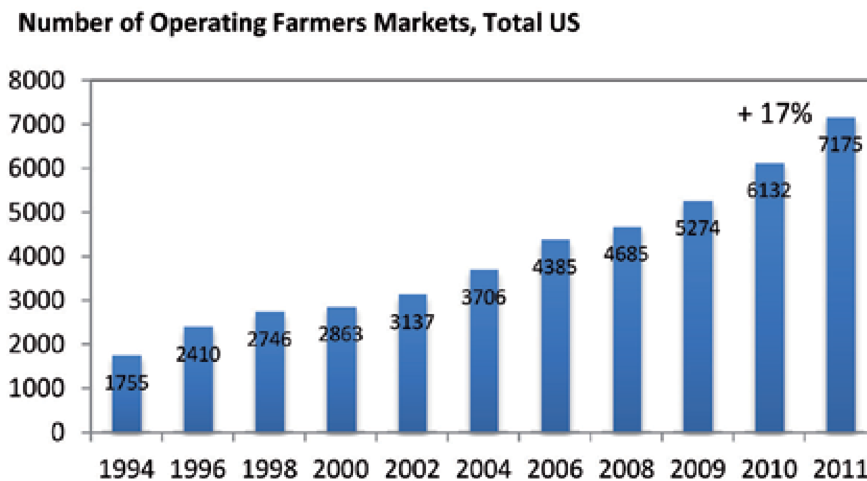

Chart 1. Number of Operating Farmers Markets, Total US Source: United States Department of Agriculture, 2011

Richard Wilk (2012), in his description of the contemporary food movement, also points to the growth of Farmers Markets, local food councils, regional food alliances, school food initiatives, community gardens and orchards, and popular interest in the quality of food over the past five years… and like Belasco, wonders if these will be exploited as a marketing opportunity or turned into an ideological foundation for change in the food landscape.

This counter also marked the emergence of the consumer as a voice of influence in the food industry, after the focus to internationalisation and mass industrialisation in the first and second regimes. A key point of Belasco's analysis is his argument that marketers who 'abused' the process turned this counter into mainstream belief. To what process does he refer? He describes the way in which marketers turned these beliefs into profitable products was by turning food messages to meet desired consumer lifestyle and identity values, and it was this focus to meeting consumer lifestyle needs that created their voice of influence. In order to communicate to identified values, marketers *repositioned* food added values. Belasco describes a deceptive 'picking and choosing' process where 'good' elements of products were chosen to anchor repositioning in lifestyle values, and the 'bad' elements were ignored or glossed over - for example, carbonated soft drink became 'low in fat' to embody consumer desire for a healthier lifestyle (Belasco, 1989, p.212).

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The success evident in relevant consumer re-positioning, demonstrated below with organic retail sales - even with potential drawbacks of its use, particularly when deception or excess 'glossing over' is involved - has lead to virtually an entire industry shift to how food products are communicated to consumers.

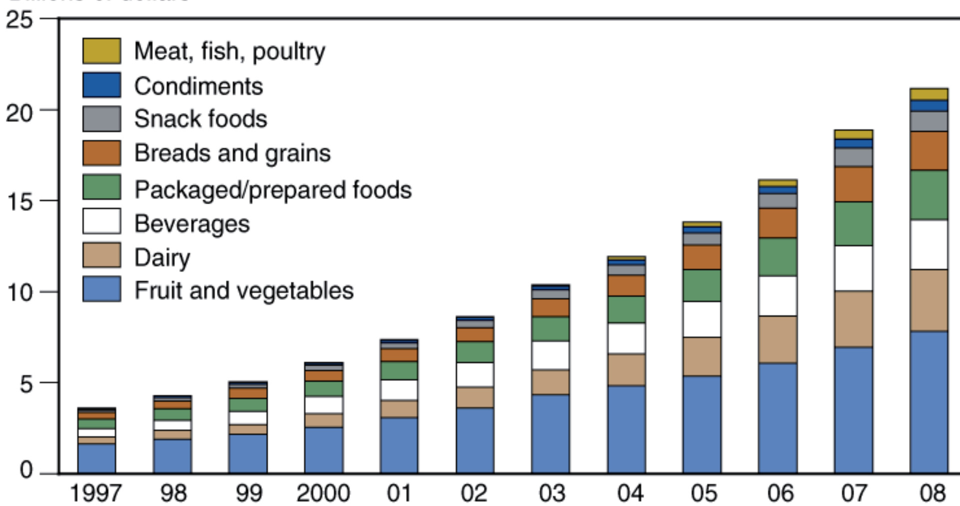

Chart 2. Retail Sales Growth of Organic Food Products. Source: Dimitri & Oberholtzer (2009, p. 13)

Products and their messages began to transform from functional communication style into symbolic lifestyle and experience messages - and it is this process of how consumers read these messages that underpins Belasco's argument in how the counter became mainstream, for without the culturally held knowledge to 'read' the message conveyed, for instance, using a picture of a tractor on a green field is a farm, is completely lost.

It is the process of developing symbolic representation and cultural construction of small scale Farmers through their production and selling methods that allows a simple understanding and competitive advantage in a confusing food landscape. This occurrence is made all the more interesting in a wider food industry of careful, researched product positioning and communication from larger scale manufacturers: to achieve this, the small scale Producers in this study are unconsciously just doing what they do.

I am a farmer's daughter, and my mother, she cooked jam and juice and all this that we also do, but she was never paid for it, but we are… and that is the difference between the 20th and 21st century! (Skåne Producer, November 2011).

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3. Method and materials

For this investigation a specific empirical study involving small-scale value-added producers in the Skåne region of Sweden was undertaken.[1] Taking an ethnographic research approach, observation and participant consumer observations were undertaken at a periodic Farmer's Market held in Malmo, Southern Sweden, over six Saturday mornings during 27 August 2011 to 15 October 2011. Ethnographic enquiry in this instance is a method to investigate value adding due to the focus on consumer / producer interactions and the evaluation of signs inscribed in products and material items used in display. It is also a relevant methodology to 'move the conversation forward' regarding value adding, as common industry methods such as quantitative surveys have apparently failed to provide underlying and unconscious motivations in their subjects of study. This particular market is advertised with an express requirement that all produce for sale there is made and sold by local Skåne small-scale farmers. As such it is a good market to begin investigation of producers and also analyse the material representation of space at the market, as each stall was under control of the individual producers themselves. Notes were taken directly after each of the six visits to the market (it is difficult to write while standing up in a crowd of people), in a nearby café, with a focus to the salient events from each visit to the market. Each visit on average lasted forty-five minutes to one hour.

Each visit to the market had specific item to review. In week one, which was also the first market day of the market season, the organiser of the market was interviewed, and introductory contacts with all twenty one stall-holders present on that day. The attention on this day was drawn to the ways in which the organiser the perceived competitive advantage of the Farmer's Market, the type of consumers she believes shops at the market, expectations of the coming weeks' market sales and also the best way to market Producers' product to consumers, (particularly for newcomers to the market, of which there were two). The second week the investigation of the market were directed to the specific intention to review the material representation of space – display items outside the products themselves and signage that producers commonly use.

The third week, focus was to body language and behaviour between stallholder and consumer while tasting and/or buying products. Week four was focused to obvious advertising promotional or 'special' deals available at the market. Week five required another review of material representation (a repeat of week two), and the final week was spent looking for details missed on the previous weeks.

1 The field work was undertaken by Rebecca Dare, for her Master's thesis in Applied Cultural Analysis at Lund University. This article is a revised version of the main parts of the thesis.

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Additional ethnographic observations were made during farm visits, and hour long depth interviews were also conducted with four producers during October and November 2011, in addition to an online questionnaire circulated to farmers in attendance at the market, of which fifteen responded. The online questionnaire, which participants were able to respond anonymously is a recognised departure from ethnographic methods, however was circulated primarily as a way of locating participants for deeper investigation.

However, the understanding of *why* certain questions were answered as they did in the questionnaire revealed again that qualitative methods uncovered much more about Producer value adding practices than straightforward quantitative line of questioning, and adds further support for the use of a cultural analysis approach in the articulation of meaning. All of the depth interviews were conducted with the use of an interview guide allowing to focus the majority of attention to the subtle emotions the became evident during discussions. What subjects created the most emotion? These subjects became focus for later analysis. In addition, web ethnography was also undertaken of Producers at market to understand their representations of self and product online, and was also taken into account during analysis and depth interview guide preparation as required.

Of the four depth interviews conducted, three were held on the site of farming production – which usually also housed home, office and Farm Shop where their produce was sold. This gave an opportunity to view participants in the scene of their day-to-day lives, which often involved family.

The main field work was conducted at the Farmer's market in Malmö in the South of Sweden. As other Farmer's markets throughout the world, it was built around a specific regional provenance (in this case Skane, the most southern part of Sweden, which produces around 50 % of the Swedish food production) the number of stalls selling produce that had been mediated and transformed in some way (such jams, smallgoods, rapeseed oils, breads, cheeses), and the stallholders were also the producers of the products that they sold.

Johnston & Baumann (2010, p. 70) have tried to decode the elements of Authenticity that consumers demand in high-value food. They claim that geographic specificity, simplicity, personal connection, tradition, or 'ethnic' connections are the manin elements of authenticity. They report that from a consumer perspective, a 'good' authentic product does not need to have all these elements, perhaps one or two… but a *really* authentic product from a consumer perspective has elements of all five. This market communicated most, if not all of these elements.

While watching Producers carefully setting up their stalls to display their product in a way they deemed the most appealing, and engaged in passionate descriptions about the product they sold with consumers, it became obvious that there was a very close connection they made with their product. Their products were tended with almost maternal care. Jars were carefully and individually placed, labels facing forward… not too cluttered, each jar having a right to stand on it's own. Esteemed flavours stood centrally, close to where the stallholder could easily access for offering tastings and making serving recommendations. It was explained that this set up and staging was taken very seriously, with the utmost 'attention that

must be paid to the small details in a display' – right down to the pattern on a table cloth used to conceal a rickety, aged wooden table. The care and thought placed into the display interestingly revealed a pattern of placing the stallholders themselves as co-stars and leads to the first Producer practice in adding value.

Observing the interaction between consumers and producers at the market, it became evident that exchanges were based not only on direct economic issues such a price. There was much more under discussion underway. Even though the elements of authenticitiy described by Johnston & Baumann were found at the market, the elements as such does not give full credit to the practices involved. And they certainly do not answer the question why certain producers and products seem much more authentic than others, although they share the same elements.

The study of the actual practices of value making made some new themes emerge. They are summarized under four headings; Co-creation, The farmer myth, Art and Magic.

3.1. Co-creation

The online questionnaire circulated to locate participants for this research included a question that asked: 'of your current distribution channels, which do you prefer? Please select one from below'. The majority of respondents (66.6%) indicated Farm Shop or Farmers Market.

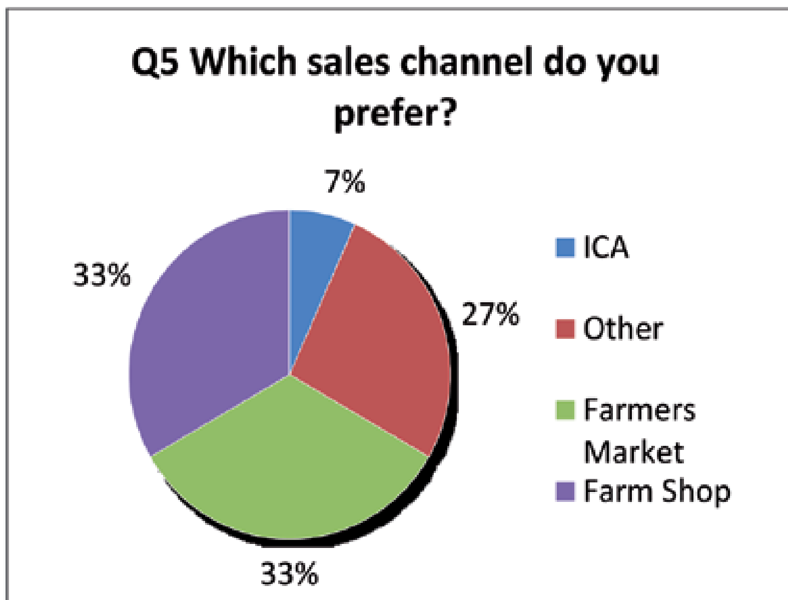

Chart 3. Preferred Sales Channel, Producers in Skåne, Sweden. Source: Online Questionnaire, August 2011. (Note: 'Other' is predominately 'Restaurants')

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This piece of information on its own is a little bit 'nice to know' as it fits with the preferred channels of the counter movement outlined previously. When asked about why Farmers Market or Farm Shop is preferred, most participants indicated it was personal selling and consumer contact that they like the most about these channels. There was a consensus that selling in this way was intensive work, but it was 'worth it' - there was significant return for labour. Which begs the question: What is it that they are getting in return? Is it purely economic gain?

Evident from the exchanges observed at the Farmer's market and discussed in later depth interviews indicated this return contained multiple components. A key component was one of education. Producers and consumers educate *each other* about the meaning infused in the food (consumers commonly rewarded the product for being *hand*-made), new ways of consumption and suggested occasions (try this with blue cheese, perfect for a summer party!) - important in meeting the consumer *and* Producer need in identity building and class positioning through cultural capital. As Producers spoke about their product, they educated consumers about varieties of produce included in the food (i.e.; The tomato used is Roma, best suited for this type of relish), and also, importantly, Producers gained ideas for new product development through suggestions from consumers. The drawing of new products into the discussion may at first seem a little offensive, but later discussions with Producers revealed that this type of communication is invaluable information for new product generation and development, and has been credited with sparking ideas for product and also material displays of space. Two examples of this involved a recent award-winning product with the unusual inclusion of a savoury, exotic spice to a sweet condiment – tapping into an emergent consumer trend for food exoticism. The second example regarded the co-creation of one producer's use of the ubiquitous 'chalkboard' sign:

When I started, I wrote by hand (the signage) and I do not know how many times I bowed and curtsied for customers and apologised, how it was awful and all of that. And then a lady comes and she says to me like this: 'But, it is proof it is handmade'. Huh! (*Laughs*)... Sigh! It was, of course! (Skåne Food Producer, November 2011).

It was also revealed during depth interviews that disagreements with consumers may occur (usually regarding their 'confusion', or as one Producer referred 'blasé and spoilt', when talking of one discussion over the use of plastic wrapping around a herb plant, the consumer thought this meant it was not grown at site and objected to its use), however Producer position was always made in reference to their relation as the solution to confusion rather than the cause of it. It was also revealed that Producers rarely discuss new product ideas with other producers, as 'the market in small in Sweden - you have to learn to stand on your own', indicating that there are limits to the farming community in Skåne. Exceptions to this were evident, however only when a philosophical connection and strong friendship had been formed over a number of years.

These exchanges with consumers are very much a two-way communications process, and, to reference to Prahalad & Ramasway, an exchange "between the firm (Producer) and the consumer (in) becoming the locus of value creation and value extraction" (2004, p. 6) and is

an invitational act of co-creation. The co-creation is the centre of value extraction and knowledge for Producers to build their producer offer and the way they sell their product, and as such is held at a high level of importance.

While Prahalad & Ramasway's (2004) investigation examples primarily involve larger corporations, such as car manufacturing giant General Motors – what stuck with the discourse with consumers was that small-scale Producers are routinely in a position that larger business pay large amounts of research money for… in direct contact with individual consumers who are eagerly sampling their products, freely offering feedback, sharing tips from other sources, with the added bonus that the consumer is speaking with a person that has a level of influence within the business structure to initiate change. Small-scale producers are very close to the consumer via their preferred distribution methods, and in a position where they can respond relatively quickly to changing consumer demand. However, as Prahalad & Ramasway articulate, this is achieved not by only just 'showing up' at the Farmer's Market or opening a Farm Shop. To do so, Producers must invite feedback. Do they? If so, how?

Exploring the practices that Producers engage to begin the co-creation process at market started to tease out more information. The approach to addressing this question firstly lay not by observing Producers that appeared to be engaging in a co-creation with consumers, but by observing Producers that were *not*. There were two stalls at the market during fieldwork, both of which consistently had uncrowded stalls while the remainder of the market teemed with activity (one sold shiitake mushrooms, the other corn).

There were two common denominators between these stalls: 1. Their product offer was not immediately apparent it was 'value added' and 2. Their stalls featured little to no elaboration in their stall displays – no or very limited signage, no extra items to add visual appeal, and symbolically represent a return to the farm / authenticity such as the previously mentioned flower arrangements or wooden crates. There was no elaboration in the space to communicate their values, which as discussed earlier, are important in the social construction of authenticity.

The absence of continuous consumer - producer dialogue at these stalls would seem to indicate that consumer feedback and discourse is in support of Prahalad & Ramasaway's theory in that it is *invitational*. This occurrence implies that there is prerequisites involved the staging of experience in order to co-create with consumers. The invitation extended by a Producer must materially demonstrate their authenticity by the use of symbolically loaded material objects, and further offer an experience such as tasting plates before consumers will engage in discourse. It would also seem to indicate that non-value added products are perhaps not necessarily seen as something in which to discuss in greater detail than necessary to complete a financial transaction - again bringing to mind Levi Strauss with his cooking as a mark of culture.

3.2. The farmer myth

The symbolic meaning communicated by the material display items in use is a key point here. As the symbolic loading in these items is culturally held, unconscious knowledge, further analysis is required. After all, the person that builds the displays first imagined it in their mind - perhaps they didn't realise how those images got there. The displays heavily

utilise cues for 'authenticity', and for discussion of this I'll elaborate on what I am calling the Farmer 'myth' utilising Barthes.

One of Barthes "lasting contributions… was the identification and interpretation of certain 'mythologies' that he drew from everyday life in France" (as quoted in Atkins & Bowler, 2001, p.6). Barthes utilises semiology as a tool to understand the basis for myth creation, and relates the expression of myths as inherent in food. As Barthes stated: "for who would claim in France that wine is only wine?" (1997, p.20) relating wine as a symbol *of* France and to the myth of what it means to be 'French'. Likewise, in the current food regime of counter-values to mass industrialisation, the myth of farming is utilised in small-scale food production to embody a 'return to the farm' in support of their authenticity.

The Producers are met with a challenge, though: this use of these 'return to farm' signs is not a unique in the food industry today, as Belasco previously outlined in discussion of the emergence of the food counter culture. Most of the items in use here to symbolise the return to farm can also be found represented on numerous food packaging, and in virtually every food distribution channel, including mass supermarkets and speciality stores. Items such as chalkboards, wooden crates, wicker baskets, striped awnings, are in common use in supermarkets and speciality stores around the world.

But what of our Producers in this, using the same material items, yet successfully inviting co-creation with consumers? With this proliferation of material displays in use in the market today, why are these displays not approached with cynicism, disbelief, or just plain dismissal? Here Baudrillard makes a contribution with the outline of two distinctive features of mythology to be drawn as relevant: "firstly the nostalgia for origins, and secondly the obsession with authenticity" (1996, p. 76). Baudrillard outlines that the obsession with authenticity is reflected with an obsession with certainty - specifically, certainty as to the origins, date, author and signature of a work, continuing:

> The fascination of handicraft derives form having passed through the hands of someone the markers of whose labour are still inscribed thereupon: we are fascinated by what has been created, and is therefore unique, because that moment cannot be reproduced (1996, p. 76).

In co-creation the feature of *certainty in myth* here is a distinct element in invitation: the certainty that in a confusing food landscape and the new economy of signs, where authenticity is hinted at almost every turn, that the Producers *are* farmers. There is certainty created in regards to the origins of the food, as the author and signature to the work, often accompanied by undeniable physical presence. What could be more certain than that? This invitational certainty is in direct contrast to Prahalad & Ramasway's theory where they propose that 'trust' (or certainty) is an outcome of co-creation (2004, p. 13). Here we propose it is a central element in use at the beginning of the co-creation process. It's the certainty that Producers can truthfully and safely take consumers from myth and into reality.

In the next section, the displays used at the Farmer's Market is contrasted to the spaces constructed at Farm Shops - with the consumer on the farm, the invitational space to co-create becomes something else entirely.

3.3. Art

When discussing five-year plans, most Producers indicated a desire to increase business through their on-site Farm Shops - all of the depth interviewees had a Farm Shop, and thirteen of the fifteen respondents to the online questionnaire did also. These shops are under their full control, and thus ideal to explore the more permanent environment (compared to the Farmers Market) in which they have constructed to sell their product. All the producers referred in depth interviews to a desire to elicit positive emotion from consumers from their Farm Shops, something they felt that large supermarkets could not do[2].

One Producer spoke about their delight with their shop layout creating happiness, astonishment and surprise emotional reactions from consumers:

In my farm shop I don't stock these types of everyday products (*sold at ICA, a large supermarket chain in Sweden*). No, what I have here has nothing to do with everyday commerce. There is no milk… I do not have those types of products, this is really speciality items… I really take it to myself, how I have been thinking when I planned the store. "What should it look like in the shop to make it inviting to the customer, inviting to enter and to have a look?" Often I say… (*Pauses: body language changes, pulling away a little bit - it's like a self reprimand for becoming a little too pleased with oneself, quickly decides on an acceptable rephrase, leans forward again*) …the customers usually stand in the door and say. "OOOOH what a lot of, AHHHH how nice!" Then they are of course happy, I think! (*Laughing*). Yes indeed! Most of them are perhaps a little astonished and surprised, because they don't expect anything more. (Skåne Food Producer, November 2011).

Arguably this is an effort to create a unique positioning and distance to the dominant food sales channel today (supermarkets), however the exclusion of what is perceived to be everyday products raises some important questions regarding the 'specialness' infused their products and reveals beliefs about the work they do. Why is an everyday food item such as milk not considered 'special', particularly when considering that a number of products for sale in this store could be considered as 'everyday' use items such as jams and mustards? Consideration of the space implies an effort to raise their product above the everyday and into a form of art, and in doing reveals Producer cultural capital and class aspirations.

To communicate their perception to the specialness, non-everyday, uniqueness of their products, Producers reveal a tacit, cultural understanding to their construction of space, and have turned their Farm Shops into spaces resembling Art Galleries. The Producer quoted earlier in this chapter created a large space, beautiful to look at, with a significant amount of

2 In the online questionnaire, Producers were asked to nominate the emotion they felt consumers experienced in different sales channels. 80% of participants believe customers feel 'negative' emotions such as neutral, bored or confused in Supermarkets.

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non-food items, and set up in a maze of stalls to encourage exploration and discovery. Another Producer housed several buildings, one of which featured a café with soaring cathedral ceilings, artwork on the walls, and with their produce barely visible in - again - a beautifully constructed space. The farm shop was located in the same building where the produce was 'cooked', separate to the main house that was a short distance away, however all cooking space was off limits and invisible to regular custom in the shop, as were all of the cooking spaces in the farm shops visited as part of this study.

In guided tours with Producers at their farm shops, there was always a respectable distance kept from the Product in store, to allow the viewer to take the entire scene in. The lighting scheme shined directly on produce, to illuminate its form. The display of goods was uncluttered, so as not to catch a wandering eye's attention from the main event. Discussions commenced about why the products on display were chosen with slightly hushed tones, even if we were the only people in the store. These actions struck as being very similar to behaviour that would be found in art galleries - here, a discussion was ongoing with an Artist. An artist creates. What are these spaces creating?

The construction of these spaces to resemble art galleries attracts the use of the term 'Experience Economy' as introduced in theoretical framework to elaborate and further embeds Producers as active participants in the Experience Economy. The art gallery spaces are carefully staged for consumption and construction - consumption of the producer, consumption of myth, consumption of art…and hopefully for our Producers, what follows is the consumption of authentic 'special' food products.

Tom O'Dell & Peter Billing (2010) outline a useful interpretation regarding spaces that are staged for consumption using a theoretical framework that combines the conceptual tools of Arjun Appadurai's (1996) 'scapes' and Henri Lefebvre's (1991) production of space. Herein they have developed an understanding that spaces where experiences are manufactured for consumption can be interpreted as "places in which the global and the local are entwined and where power relations played out, political interests are material interests are materialised, cultural identities are contested and dreams are redefined" (O'Dell & Billing, 2010, p.18). The building of producer's identity through practice is a key function of the space created. The environment feels very much staged for artistic expression and exploration… a space has been created that Pine & Gilmore (1993) could interpret as a carefully staged production to create experiences such as those available at Disney or the Ritz Carlton.

A main criticism of Pine & Gilmore's description of the 'Experience Economy' is that the spaces as provided as examples (such as Disneyland and the Ritz Carlton) are created with the assumption of a passive receptivity – consumers have no hand in creating the space and limited opportunity to personally shape it. However, as is known from the previous research finding, co-creation is a key element to Producer practices and the impact of consumer feedback on the construction of this space cannot be underestimated - regardless if the Producer is conscious of this or not. The inclusion of art indicates a desire for the accumulation of cultural capital in identity construction, not only for Producers, but for their consumers, also. The construction of this space seeks to confirm Producer position within upper 'cultural capital' class, and the investment of time, money and plan-

ning into the ongoing operation of their galleries demonstrates a long-term desire to retain this class positioning.

3.4. Magic

Magic, the art of influencing through supernatural means, could infer secret, hidden and disappeared acts in production practices without clarification of its use. Here, magic is described and applied using two references from the social sciences - Sir James George Frazer (Social Anthropology) and Marcel Mauss (Sociology).

In '*The Golden Bough*' first published in 1890, Frazer notes magic generally falls into two categories: the Law of Similarities and the Law of Contact or contagious magic. The former applies to the belief that like produces like - an example used was "the then current belief in among peasants in the Balkans that swallowing gold could relieve the symptoms of jaundice" (Barnard & Spencer, 1996, p. 341). The Law of Contact or contagious magic was causal idea of things that had once been in contact continued to influence on each other at a distance.

In describing themselves in their products, Producers emphasise 'them' as integral to the product offer. This introduces a belief that they hold continued influence in and over their product before, during and after consumer purchase, to continue the communication of their authenticity, thus asserting their influence at a distance via magical means. In discussions with Producers, the appeared to be little separation between Producers and their products … the way their products were referred was often in the same way that you would refer a person. The extension of themselves as represented in their products created a desire for their products to be treated as how they would personally like to be treated. For example, it encouraged a slight concern about selling in delicatessens where there is a perception consumers are particularly discerning and demanding, which means the chance of product rejection (and thus, themselves) is higher. Producers also held a strong resistance to sell in supermarkets where their product could become the 'little' with scant consumer attention (a bit like going to a crowded party and standing alone in the corner), or face the potential of having their product abused in store by careless staff.

The Contagious Magic in use by Producers is a practice to infuse a competitive advantage they believe to be what no one else has into their products: themselves. Producers are the magicians in their kitchen, infusing themselves in their products. Two quotes from research summarise this belief in infusion of self very well. One Producer mentioned that their soul must be in the product:

I have nothing to sell if there is no soul in my product… then I find it difficult to justify why the customer should buy my (product), then he can buy another, because what's unique in mine is that *I* cultivate it. It should be something that no one else has. (Skåne Food Producer, November 2011).

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While another in responding to a question asked in the online questionnaire: '*If your favourite product could talk, what would it say to consumers?*' The response neatly stating: 'I am special, unique and wonderful. And you?'

Additional theory to view producer practices using Magic is provided by Mauss in '*A General Theory of Magic*' (2001), which is that of a social phenomenon containing belief and rites.There must be a magician to produce an act of magic, and an audience to believe in this act. Rites are developed to enact and perform the magic. Magic is not a one-time occurrence; magical performances are repeated patterns of behaviour and when at it's most successful, refer to "those things that society as a whole considers magical" (Mauss, 2001, p. 22). As O'Dell (2010) describes, the inclusion of belief in this theory provides 'special problems' that will be revealed as particular relevant for this discussion:

> Magic is an institution only in the most weak sense, it is a kind of totality of actions and beliefs, poorly defined, poorly organised even as far as those who practice it… It's existence necessitated two different forms of belief that he (Mauss) called a 'will to believe' and 'actual belief' (p. 57)

The problem being, for acts of magic (or influence at distance) to be *not* seen as trickery, they require collective belief by *all* actors involved, with each actor having a role to play. The magician in this act is the Producers and it is here where belief does exist - but it is not without tension. Present and seemingly unwelcome is a level of uncertainty about their own skill and knowledge in their practice, that in conversation betrayed an oscillation between a 'will to believe' and 'actual belief'. This tension was managed by the development of magical rites, and as far as they could possibly manage, a self-imposed 'no-cheating' policy on these rites.

These rites specifically managed the symbolic values they use to describe their products as authentic - themselves in the product made it *hand*-made, *home*-made, *crafted*. The translation to practice is rather literal: to be *home* made, the production practice must be either in their home or in a nearby building as part of their faming complex, never in a removed and distant facility. To be *hand* made, their own hand must be involved in making the product, and they must perceive a level of craftsmanship involved in the transformational cooking process. One Producer described the importance of seeing and inspecting each individual raw main ingredient with his or her *own* eyes. Another Producer describes the importance of the cook (themselves) to be directly involved in the cooking, cooking in low batch volumes and never on a stove that could be considered 'industrial':

> I stir the pot myself. I never use a pot that does not sit on the stove. I never cook in volumes that are larger than 25 litres. No, no, no. Not ever. (Skåne Food Producer, November 2011).

…And also describes readying themselves psychologically as to not infuse the products with stress, which might negatively taint the flavour:

It is an experience, every day, every batch that is made; you do not always get the same batch or the next batch exactly the same with the same products. It depends on your peace of mind, on how much you put in your measuring cup. It has to do with… well, if you are under stress… all those things matter (to the final product). (Skåne Food Producer, November 2011).

However, these assertions were made at the same time limitations and exceptions were made obvious, and showed clearly the edges between a 'will to believe', 'actual belief' and deceit. It and also showed differences of opinion and poor definition where these edges actually lay between the Producers involved in this study - there was no common approach. Individuals had determined what fit with their magical act. For example, it's understandably difficult to grow all of the required raw ingredients for a product on the one farm, so while it is important that they farmed the 'star' ingredient in their products, it appears that consideration to all origins of the ingredients in use are not considered as heavily - one Producer mentioned popping into the local ICA store whenever an ingredient was needed, and had never checked the provenance of the product bought there for that purpose; while another mentioned checking provenance of all the ingredients they use. Another mentioned the recipe for one product was something they looked up on the Internet, hardly conjuring images of *crafted*, while a different Producer claimed that all recipes they used were the result of experimentation in their home kitchen. Another mentioned that they had employed Polish workers to pick their raw ingredients as a cost saving, and wondered if this were doing the 'right thing' in terms of authenticity. In this instance they looked but did not find an exception to the rules of hand and home made, as they were selecting the produce to cook from the yields themselves - but this Producer conveyed feelings of unease and insecurity about their decision. It appeared to be a compromise in their hand made claims.

As these edges are indistinct, blurry, and left to the Producers themselves to navigate, the inclusion of magic rites to infuse themselves in their products brings a host of anxieties in addition to an immense sense of pride. It has also turned their attention to and creates a high level of reliance on consumer approval. The consumer is seen as the ultimate judge of magical efforts, and also aligns with Mauss's outline of magic as an act that "society considers magical" (2001, p. 23) - everybody has to believe in the act of magic to be true. In this case, consumers are representations of society. When Producers are asked *why* they engaged in the business that they do, this is commonly the first aspect mentioned - it's a checking of consumer belief in their magic, expressed in a desire to please:

'(I make this) for the customer to enjoy!'

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'I do this to delight my clients'

'I can't sell anything unless the customer is satisfied directly'

The importance of bringing consumer satisfaction - and by extension, belief - through their produce underpins and adds meaning and reinforces *their* belief in their rites of magic. Feedback from consumers is actively gained from Producers when possible, and it is this information that is actively sought and helps guide further practices, as discussed 'Co-Creation and Certainty of Myth' finding.

The execution of these magical rites also brings small-scale food production to more closely resemble a creative processes (such as art or fashion) rather than a production process (such as Fordist production methods), and again brings food production to in closer alignment within the New Economy. In order to execute their magical rites, Producers must manage themselves, their lives and their work in a way that optimises their creativity and mood to enact their magical acts, which blurs the line between 'work' and 'play'. Here, Löfgren and Willim (2005) refer to the use of magic as central to emergent people and self-management methods in the New Economy, where the introduction of 'fun' has become a central concern.

This management of self is referred to as 'reflexivity' by Lash and Urry (2002) and described by McRobbie (2002) "as a form of self disciplining where subjects… are increasingly called upon to inspect themselves and their practices, in the absence of structures of support… reflexivity marks the space of self responsibility, self blame" (p. 522). Producers are highly cognizant in the requirement for individual responsibility in their rites, as I was informed 'you are held accountable for what you do'. Giddens' and Becks description of the concept as undertaking self monitoring activities' (McRobbie 2002, p. 522), is relevant in describing this accountability here and is further articulated by Lash and Urry:

Social agents are increasingly 'set free' from heteronomous control or monitoring of social structures in order to be self-monitoring or self-reflexive. This accelerating individualisation process is a process in which agency is set free from structure; a process which, further, it is structural change itself in modernisation that so to speak forces agency to take on powers that heretofore lay in social structures themselves. Hence for example structural change in the economy forces individuals to be freed from the structural rigidity of the Fordist labour process. That is, it is increasingly a pre-requisite… that the labour force becomes increasingly self-monitoring as well as develops an even greater reflexivity with respect to the rules and resources of the workplace" (2002, p. 5)

In this instance, the use of magic to add symbolic value of Authenticity in production methods has transferred Producer association away from the Fordist economic labour process, but embedded them more closely with the counter-structure that has developed in resist-

ance to it within the New Food Economy. Furthermore, their intense self-monitoring has also developed skills of greater reflexivity with respect to the rules of their workplace that extends across their day to day lives, and creates a unique set of challenges inherent in operating in this way, which is discussed in the Research Finding Summary. As McRobbie describes the fashion industry in London (2002), this may mean holding down additional jobs in order to make ends meet - one Producer interviewed shovels snow in winter.

4. Conclusions

4.1. Summary of findings

In this article, new knowledge regarding the value adding practices to inscribe authenticity in food products is provided. The perspective from small scale farming Producers was taken; where co-creation, myth, art and magic are key practices utilised to value add products with authenticity.

Value adding is a common practice in the food industry, and is increasingly undertaken by inscribing lifestyle and cultural capital messages in food such as health, knowledge and class position, often by claiming some sort of "authenticity". The theoretical understanding in thesis outlined that authenticity is a social construction, and taking the view from Producers in this study, to be characterised with references to handmade, homemade and crafted messages inscribed into their products. These messages are understood via deconstruction of cultural codes. An example of this was demonstrated by the cultural perception of 'farming' to constitute a myth market that the food industry has utilised heavily in marketing communication messages. However, it was the deceptive use of communication messages by large-scale food companies that created consumer uncertainty to the real origins and production processes of mass-produced food, and opened small-scale production an opportunity to provide myth certainty to consumers with their products.

To value add their concept of 'authenticity' to products, rites of magic, co-creation, delivery of certain myth, and development of art spaces are undertaken. These practices differentiate and have been developed in counter to the dominant food system in order to create competitive advantage. In doing, Producers reveal culturally constructed beliefs about their relationship to their product, understanding of symbolic meanings in representation of space and their desired identity via cultural capital. It also reveals ignorance to the underlying cultural structure and history of food that has lead to their position in the food industry today.

As pointed out in the application of theory from the New Economy, the entire production process is increasingly taken into account when reading food messages, and it is here that small-scale production finds competitive advantage in the marketplace. However, Producers' lack of knowledge about how and why they came to a position of prominence has created uncertainty about their conduct now and into the future. To combat this, Producers have created magical rites and a high level of self-reflexivity in regards to their practices of authenticity, and a heavy reliance on co-creation with their consumers to develop their prod-

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uct range and material representation of space. The design of this space creates a sense of certainty for consumers that they were engaged with farmers and one of its functions is to create an invitational space for discourse. However, in creating spaces and products that the consumer demanded, Producers merely replicate current marketplace dynamics and base their business information only on what information their consumers can tell them - paradoxically, they attempt to surprise consumers with information that the consumers themselves revealed to them. It is here that a key advantage of Cultural Analysis is shown. With a wide theoretical and historical view, the food industry is outlined and underlying motivations and desires that neither consumers nor producers can articulate is articulated.

This article found that, as Producers and cultural mediators, Producers' own search for identity and meaning is inscribed in their products. In order to invoke the Law of Contact or contagious magic and influence their products at distance, they transform and value add their products by becoming magicians and undertaking rites of magic in their kitchens. These rites, while infusing their products with the desired handmade, homemade and crafted values associated with authenticity has created limitations to production methods, and also highlights the thin line that can exist between magic and deception. As individuals possessing cultural capital in the form of knowledge, Producers acknowledge and inscribe this power and transform what cannot be reproduced into their products: themselves. In paying very close attention to consumers, Producers have tacitly understood that their products represent a desire for cultural capital and class position, and have in response turned their farm shops into unique, non-everyday, special spaces that resemble art galleries. In doing, they transform themselves from cooks to artists, bring food closer to the Experience Economy, and also reveal and entrench their own understanding of 'culture' as high art; as opposed to the everyday. These actions also force a re-consideration of what business these Producers are actually in: these practices point towards a closer alignment to the service and artistic industries than to food.

Regardless, the belief in art, myth and themselves as value added determinants of authenticity has created practices that restrict income flow, with some Producers living on the edge economically. It also potentially creates risk in their business model should their product offer fall out of consumer favour as mysteriously (to them) as it fell in.

4.2. Conclusions with applications and suggestions for future research

The knowledge of the value-adding practices co-creation, farmer's myth, art and magic that small-scale producers use have several applications for food producers.

Firstly, it calls for a view of where business involvement actually lies, and why. In this example, small-scale Producers have combined elements of food and artistic industries as an expression of cultural capital for themselves and their consumers. The 'blurring' of industry edges in the New Economy is in itself not necessarily a brand new phenomenon in the food industry as Jönsson (2012) has outlined in '*The Gastronomic Revolution*'. However, in this context it is evolving the question of *why* Producers are engaged in the business that they are, where more inspiration for application can be found. As Simon Sinek, cultural anthropologist and management and leadership author proposes in '*Start with Why*' (2009), inspired

leaders such as Steve Jobs, Martin Luther King, Orville Wright looked for their why in places based in altruism, common good and social betterment (which he suggests is another construction of authenticity), which in turn motivated and inspired others, and in doing turned their ideas into social movements. In other words, their why is the basis for construction of consumer desire. Sinek suggests that it is the articulation of why as the reason that consumers ultimately buy products or services. A further description in Sinek's book is in support of a key message in this article - that consumers will be unable to tell you what this *why* is. There may be some concerns regarding the why Producers in this study have employed, but it is currently a compelling and motivating proposition for them nonetheless.

A less individualistic, more collective and cooperative approach within the food industry, to educate and empower themselves and consumers, develop sustainable, kind and ethical business practices, and authentically communicate about how their food is *really* made could be a more compelling and sustainable why than what is currently employed. However, the thrust of this application remains for the wider food industry: using a cultural analytical approach, define what industries your business is involved and describe *why* it's important.

Additionally, it is the certainty that Producers can create through their value adding practices that constitutes part of their competitive advantage. The wider food industry, and mass production companies in particular, have heavily utilised and communicated the 'return to the farm' and 'farmer' myth. In so doing, they have shown just how powerful mythology is in our culture and how easily it can be communicated in marketing messages. Unfortunately, these messages in past use were often concealing large-scale production methods and laboratories that bear little resemblance to what is commonly perceived as a farm (or farmer). As this concealment was exposed over the years consumer distrust and cynicism crept in. What Producers are able to do in this climate is remain present in their farmhouse kitchens, and their Farm Shops, as consumers to come *to* them, and in doing, start to create a new view for consumers to build their perceptions regarding the myth of farming. The certainty that is created, and ability to begin changing the myth points to an opportunity not only for small-scale Producers to start using a heavier hand for the evolution of this myth, but for the wider food industry in developing certainty in myth-based marketing claims - by initially providing increasing transparency to business practices and building trust.

This knowledge also illustrates the magic in infusing authenticity - where a key element is the audience *belief* in the magical act. To develop rites without an audience means there is no magic; and conversely if you have an audience and there is no belief in these rites, again there is also no magic. There exists a thin line between magic and deceit. Magic is alike to authenticity in this regard: all actors involved have to believe it to be true. It is also a key element that provides competitive advantage, and Producers have selected a positioning that cannot be replicated: themselves. The authenticity in this study is predominately the Producers themselves; they have magically infused their sense of self as unique representations in homemade, hand-made and crafted production methods as part of their product offer. Which leads to ask the question to the wider food industry: What does authenticity mean to your business? Is it believable? Can it be replicated?

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A warning arrives in the form of the risk involved when an over-reliance on consumer feedback is evident in the production of practice. Some research methods, such as quantitative surveys, will merely reinforce current modes of behaviour and reveal only what lies at the surface, arguably providing a perfect template for continuation of business as it is today. This article illustrates that consumer feedback can be too much of a good thing if taken out of a wider cultural context. To build business with a large reliance on this practice is problematic - as mentioned in the Introduction, one cannot simply walk up to a consumer and ask them why they like a certain food product. They can't tell you. Co-creation discussions with consumers reveal more about the widespread consumer ignorance regarding the food industry rather than revealing how they could be surprised and astonished. There is nothing game changing about approaching a consumer in this way - it doesn't challenge the status quo, and will serve only to reinforce what already is. How can one be surprised if they can articulate what constitutes a surprise? A deeper and wider view provided through methods such as Cultural analysis theory and ethnology as a method will reveal culturally unconscious held beliefs, the structures that surround society that influence behaviour and uncover what the underlying motivations *really* are in value making and value adding.

Future research recommendations pay attention to assisting the development of knowledge of practices identified in this thesis, and in doing proposes a greater involvement and collaboration of actors across the food industry. The examination of value adding by small-scale food Producers in the New and Experience Economies is a relatively new area of study within the social sciences and warrants further attention.

Continuing the development of knowledge in regards to co-creation, a point of interest is made with reference to Douglas Holt (2004). Like Prahalad & Ramasaway, his approach is positioned at the crossroad of marketing, brand management and cultural studies, and in his publication '*How Brands Become Icons*' invokes the use of co-creation (what he terms as 'co-authoring') and mythology as a key to success in branding strategy. However he does not refer to the element of certainty or trust in myth. It appears to be a given fact that this commonly held cultural knowledge can and will be used by larger corporations in their branding and communication strategies, and begs the future study within these cross disciplines from a cultural analysis perspective as to the importance of certainty (or trust) as required before a marketing or communication messages is developed, particularly when mythology is in use.

The value shown in Cultural Analysis in this thesis is applicable not only to small-scale Producers in Skåne, Sweden, but other actors in the food chain as well. There is opportunity for mutual involvement and collaboration to the adding of values in food with a greater focus to education and involvement in the construction of consumer desire.

The inclusion of experience and art to food also indicates a need to more fully understand these complementary culture-production industries in this context. To facilitate this discussion, it is further recommended that Producers push past their currently defined limits of their community and involve with industry bodies such as the Skåne Food Innovation Network in their provision of opportunities for collaboration through networks and connections. Education forums with a Cultural Analysis perspective regarding value adding will

help assist those in the manufacturing and production of consumer goods in the food industry understand their position and role as cultural mediators.

In closing, the role of small-scale food Producers is one that can be promoted with their collaboration, and in doing, can help build understanding that they do have a unique position and practices within the food industry. This will provide small-scale Producers with the confidence to further evolve their practices for greater economic return and business stability in the longer term as consumer demands in the food industry continue to evolve.

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Section 6

Products Management of Food Industry

Chapter 30

Water and Wastewater Management and Biomass to Energy Conversion in a Meat Processing Plant in Brazil – A Case Study

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Additional information is available at the end of the chapter

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1. Introduction

A commitment to sustainability and an understanding of the concepts of "cleaner production" are current requirements for achieving environmentally-friendly industrial practices. Such concepts promote the minimization of fresh water consumption, a reduction in wastewater production and the recycling of wastes. Hence, in a world where water scarcity and climate change are a reality, actions to protect fresh water resources and enhance renewable energy capacity are mandatory for any type and size of industry. With reference to solid wastes, social and environmental responsibility goes beyond the obligations determined by law and relies on substantial technical research to establish a strict environmental management policy.

Meat processing plants worldwide use approximately 62 Mm^3 per year of water. Only a small amount of this quantity becomes a component of the final product. The remaining part becomes wastewater with high biological and chemical oxygen demands, high fat content and high concentrations of dry residue, sedimentary and total suspended matter as well as nitrogen and chloride compounds (Sroka et al., 2004). Of the components usually found in these effluents, blood can be considered as the most problematic due to its capacity to inhibit floc formation during physicochemical wastewater treatment and its high biochemical (BOD_5, biochemical oxygen demand during decomposition over a 5-day period) and chemi-

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cal oxygen demand (COD). In fact, even with correct handling during meat processing, this activity generates 2.0 and 0.5 liters of blood as effluent for each bovine animal and pig, respectively (Tritt & Schuchardt, 1992). The treatment of both the solid wastes and the wastewater from the meat processing industry represents one of the greatest concerns associated with the agro-industrial sector globally, mainly due to the restrictions that international trade regulations have imposed over their use and the related environmental issues.

In order to meet this challenge, one of the largest meat processing companies in Brazilian initiated a series of investments in scientific research to improve its environmental performance. Biomass as an energy source, air pollution control, and water and wastewater management were the main issues addressed in research projects carried out from 2003 to 2010.

The Brazilian agro-industrial sector consumes large amounts of fresh water and produces large amounts of residues and by-products, which can potentially be used as energy sources. The Brazilian legislation itself admits the need for water management in industrial plants to implement cleaner production techniques, which include the conscious uses of water. There are several legal documents that promote the recognition of water as public property and a finite resource with economic value. These legal norms and legislation are gathered in a single official document called "Set of legal regulations: water resources" (Brazil, 2011) and promote: (1) the rationalization of water use and its conservation, reconditioning and sustainable management; (2) investment in pollution control, reuse, protection and conservation as well as the use of clean technologies to protect water resources; (3) the practice of water reuse to reduce discharges of pollutants into receiving waters, conserving water resources for public supply and other uses which demand high quality water; (4) the practice of water reuse to reduce the costs associated with pollution, contributing to the protection of the environment and public health; and (5) the creation of guidelines to regulate and encourage the practice of direct reuse of non-potable water. Official Brazilian reports highlight that the costs of water treatment have been raised by the contamination of water resources and water shortages (aspects of quality and quantity) in certain regions of the country. Consequently, they emphasize that high quality water should not be used in activities that tolerate water of lower quality (Brazil, 2011).

Regarding the solid waste materials generated in agro-industries, these are commonly generated during the processing of crops, but are also produced by all sectors of the food industry including everything from meat production to confectionery, such as peelings and scraps from fruit and vegetables, food that does not meet quality control standards, pulp and fiber from sugar and starch extraction, sludge from physicochemical and biological wastewater treatment and filter sludge. The co-digestion of energy crops and a variety of residual biomasses may be a good integrated solution for energy recovery from such waste materials, particularly with wastes that are unsuitable for direct disposal on land, as proposed by Schievano et al. (2009). These authors evaluated the suitability and the costs associated with many substitutes for energy crops in biogas production such as: swine manure, municipal solid waste, olive oil sludge, glycerine from biodiesel production and other agro-industrial by-products and residues. They concluded that farms could implant biogas plants to treat

their own biomass generated and other urban and agro-industrial organic wastes, providing power for the neighborhood and improvements in the agrarian economy.

The use of the biosolids originating from the physicochemical treatment of meat processing wastewater can reduce the costs associated with its disposal (which has been prohibited in many locations by strict regulations) as it can directly and significantly reduce the mass and volume of such wastes, allowing energy recovery and generally lower toxic gas emissions when compared to fossil fuels. As long as emissions are below the specified legislative limits, changing energy policies lend support to the use of this type of biomass as a fuel source, as part of a move towards achieving low carbon economies.

The EIA Annual Energy Outlook 2011 reported that the global marketed energy consumption is expected to rise by nearly 50 percent from 2009 through 2035 (US EIA, 2011). Unless the world energy matrix is altered, fossil fuels will account for 90% of this increase.

The requirement to reduce carbon dioxide emissions has sparked interest in the use of many types of biomass as alternative energy sources. Since biomass is produced by the photosynthetic reduction of carbon dioxide, its utilization as biofuel can essentially be carbon neutral with respect to the build-up of atmospheric greenhouse gases, increasing both the demand for the characterization of alternative fuels and encouraging the proliferation of scientific papers concerned with this subject (Demirbas, 2004, 2005; de Sena et al., 2008, 2009; Floriani et al., 2010; Obernberger et al., 2006; Virmond et al., 2010, 2011 2012a, 2012b; Werther et al., 2000).

Brazil is currently implementing advanced programs aimed at the use of biomass energy, and several experimental and commercial projects are being implemented, such as those presented by Lora and Andrade (2009), to provide important information in order to overcome the technical and commercial barriers which inhibit the extensive implementation of bioenergy. The solid wastes produced by the meat industry have been applied mostly to the production of animal feed, which include the slaughter wastes and the wastewater treatment solids as main ingredients (Johns, 1995; Tritt and Schuchardt, 1992). However, diseases such as BSE (Bovine Spongiform Encephalopathy) have led to restrictions over the use of these wastes for feed production.

The first actions taken by the case study meat processing company, between the years 2003 and 2004, as shown by de Sena et al. (2008), were related to the in-depth investigation of the physicochemical treatment carried out at the wastewater plant with regard to its solids removal, mainly to achieve an increase in the chlorine-free biomass obtainment with a view to its utilization as a biomass fuel for steam generation. The data obtained indicate that the raw wastewater has a high organic load comprised basically of blood and organic materials that cause the red color, the greater part of the turbidity, the high concentration of total solids, oils and greases, the BOD_5 and the COD. The combustion of these wastes, especially the sludge from the wastewater treatment plants, might be a nobler utilization for economic reasons, however, many parameters related to the combustion must be monitored due to the formation of pollutants such as polychlorinated dibenzodioxins (PCDD), polychlorinated dibenzofurans (PCDF), volatile organic compounds (VOCs), NO_x, and SO_2. The authors

showed that the physicochemical treatment carried out at the meat processing wastewater plant provides around 20% of sludge, an organic solid residue, using the chlorine-free coagulant ferric sulfate (instead of aluminum or ferric chloride). In order to avoid discharge and subsequent environmental problems, the authors performed a preliminary combustion test with a mixture of biosolids and sawdust in a mass ratio of 4:1. The results suggested that the use of the biosolids as an alternative energy source would offer a favorable solution, reducing disposal and processing costs, as well as avoiding environmental and health problems for staff and the community close to these processing plants, thus establishing a cheaper and cleaner energy source for the meat industry segment (de Sena et al., 2008).

Another point related to sustainability in the meat processing industry is the associated farm residues, like pig and chicken manure. A reasonable solution for these wastes is their anaerobic digestion to produce biogas and/or fertilizers. Many farms in Brazil are implementing biodigestors in order to obtain biogas to produce electrical or thermal energy. Boersma et al. (1981), for example, studied the energy recovery from biogas produced from pig waste and verified an economy of 86%, showing a very good potential for this kind of solution. Also, carbon credits could be sold when the biodigestion process together with the energy recovery are applied to the farms.

Biogas is composed basically of methane, carbon dioxide, hydrogen sulfide, and other components in lower concentration. The gas production and the proportion of each compound are dependent on the biodigestor parameters and the chemical composition of the substrates (Lucas Jr., 1994).

A typical composition of a biogas is 55-65% of CH_4, 35-45% of CO_2 and a low concentration of H_2S. The presence of H_2S can cause corrosion problems when using the biogas as a fuel and also, when it is emitted to the atmosphere, its greenhouse potential is 21 times higher than that of CO_2. A high concentration of methane is desirable, as its presence increases the calorific value of the gas, making it more attractive for energy production.

In this context, this chapter was designed to highlight complementary research projects that have been carried out between 2005 and 2010 to implement actions to reduce the fresh water consumption, promoting water recycling and reuse, and to further investigate the application of biomass residues as energy sources and gaseous emissions in combustion processes.

2. Case study – Meat processing plant

The industrial plant which formed the basis of this case study is located in the west of Santa Catarina State (southern Brazil), where water pollution and overexploitation, the uneven distribution of rainfall over the seasons and long periods of drought, especially in summer, have become a significant problem. The activities of the meat processing plant of this case study include the slaughtering and processing of poultry and swine, while the poultry hatchery plant includes all activities involved in poultry growth: breeding, hatching, rearing, food production and waste handling.

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The meat processing plant has its own drinking water treatment plant (DWTP) and wastewater treatment plant (WWTP). The major water resource of this unit is from a river called Rio do Peixe. Its DWTP produces around 8,600 m^3 d^{-1} of drinking water, and the WWTP treats around 7,900 m^3 d^{-1} of wastewater. As described by de Sena et al. (2008), after the flotation process with a continuous capacity of 350 m^3 h^{-1}, the treated effluent undergoes a biological treatment, while the biosolids are transported by pumps to a three-phase centrifugal system, which separates oil, water and solid parts (biomass). Afterwards, the biomass is dried in an industrial rotating granulator drier with an operating capacity of 400 kg h^{-1} (model Bruthus, Albrecht, Brazil), where the moisture content was reduced from approximately from 80 wt% to 10-20 wt% in order to make the burning process feasible.

Figure 1 shows the wastewater treatment process of the case study meat processing plant.

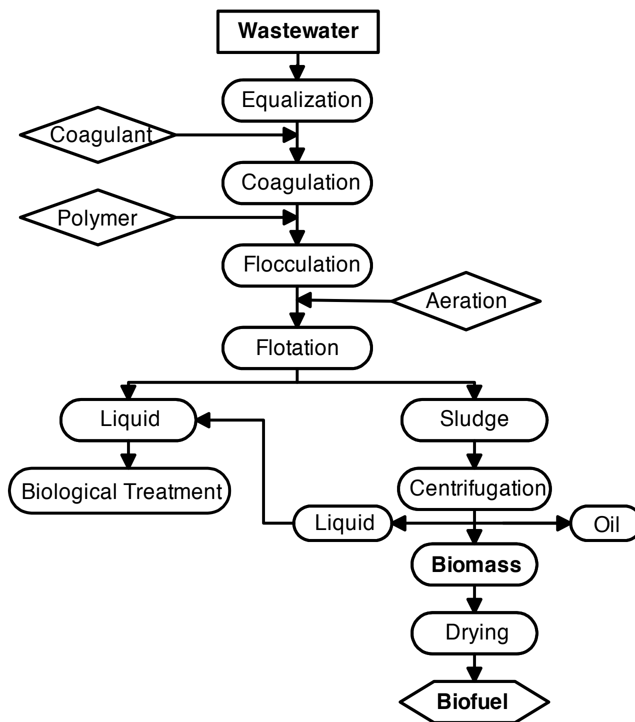

Figure 1. Processes involved in the wastewater treatment plant and obtainment of biofuel (de Sena et al., 2008)

The wastewater treatment plants of meat processing units in Brazil usually undergo the same type of treatment process, where a flotation system is the most commonly used solid-liquid separation step, due to the natural characteristics of these effluents, which possess high oil and grease contents. To increase the flotation performance the use of coagulants and coagulation aids are mandatory. Dissolved air flotation (DAF) has become an attractive separation process because of its well-known higher efficiency in terms of organic matter abatement, although the increase in costs associated with the production of micro-bubbles and

system maintenance must be considered. Flotation processes are preferred in relation to sedimentation considering their faster solids separation, the lower moisture content of the sludge produced and the smaller area requirements. Coagulants themselves are very efficient for floc formation during the coagulation process, but the use of coagulation aids (*e.g.*, anionic polyacrylamide polymers) after the rapid mixing of the coagulant-wastewater to disperse the coagulant, have been shown to increase of floc size and to provide higher floc stability and high solid separation rates. Since the coagulation and flocculation (addition of coagulation aids during gentle dispersion) are successive steps applied to neutralize the suspended particles and achieve strong flocs, the addition of these reagents must be carefully and precisely controlled to enhance solid-liquid separation. If some variables related to the process efficiency are not properly controlled, such as pH, reagent concentrations, mixing speed and contact time, the whole process will be unsuccessful. During the increase in floc size air bubbles of different diameters are incorporated into the flocs and this is responsible for the flotation phenomenon. Flotation efficiencies may vary from 60 to 95% of organic matter removal, according to the technology applied.

When the flotation of the solids is complete, the froth on the surface is separated from the water and skimmed off. It is collected in chambers and is pumped to a three-phase centrifuge where another polymer, a cationic polyacrylamide, is added to improve the oil-water-sludge separation. The water undergoes biological treatment and the oil is collected and sold as a raw material for the soap and detergent manufacturing industries. The remaining solid fraction is the sludge which was formerly used as an ingredient for animal food and feeds, especially the pet segment. However, due to the above-mentioned restrictions regarding its use in feeds, there are currently two other available options for the correct discharge of this so-called waste: combustion/incineration or land disposal. The combustion of the sludge for steam generation was the option chosen in this case study due to both economic and environmental aspects, since the use of an existing waste as part of the fuel content will decrease the fuel costs for internal energy supply, and the amount of sludge added to the fuel used (wood chips) could be properly controlled with regard to the gaseous emissions. On the other hand, land disposal might bring extra costs associated with transportation and long-term storage. All of the results obtained, as well as their pros and cons, are discussed in detail in the following sections.

The importance of the Brazilian poultry industry can be verified by its strong presence in the rural regions, mainly in the southern and south-eastern states. In many cities, poultry production is the main economic activity. The poultry hatchery unit of this case study, as in the case of the meat processing unit, also has its own WWTP. The wastewater originated from the processes of this unit is characterized by a high organic content, with the presence of nutrients such as nitrogen and phosphorus, as well as persistent organic compounds such as the residues of sanitizing products (*e.g.* pesticides) and veterinary drugs (Genena, 2009). The treatment system for the poultry hatchery wastewater comprises a screening stage (primary treatment), followed by equalization and finally biological treatment (secondary treatment: stabilization ponds). The treated wastewater is then discharged into a river (surface water).

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3. Water and wastewater management

The water and wastewater management (W2M) proposed for the pilot plant aimed to minimize the water consumption and evaluate the possibilities for water and wastewater reuse in the food industry. The W2M, described in a previous publication (Luiz et al., 2012a), proposed strategies for water management in slaughterhouses considering the restrictions imposed by Brazilian legislation and hygiene concerns particular to the food industry. The objective was to present alternatives for the minimization of water consumption and wastewater production.

The proposed W2M is a practical model of industrial water management, which consists of seven stages:

1. Collection and analysis of documents;
2. Measurement of water consumption and wastewater production (water balance);
3. Verification of the points of greatest water consumption;
4. Minimization of water consumption with emphasis on the points of greatest water consumption;
5. Evaluation of the potential for water reuse and recycling without reconditioning;
6. Evaluation of the potential for water reuse and recycling after reconditioning; and
7. Maintenance of water management.

The points identified as being associated with major water consumption were: (1) pre-cooling of giblets, (2) washing of poultry carcass before pre-chilling, (3) transportation of giblets, poultry necks and feet, and (4) washing of swine carcass after buckling. The potential for reducing the fresh water consumption in-line with the current Brazilian legislation in these four process steps was approximately 806 m^3 d^{-1} (Luiz et al., 2012a).

After the minimization of water use, the most important action is the evaluation of direct recycling and reuse of wastewater without reconditioning or treatment (direct reuse). The direct reuse could be "in processes without direct contact with food products, that is, in non-potable uses (*e.g.*, as cooling water, for flushing toilets or as irrigation around the plant), thus saving fresh potable water" (Luiz et al., 2012a). Hence, according to the water balance carried out, "the wastewater with the possibility for direct or indirect recycling or reuse was evaluated physically, chemically and microbiologically to verify if and where it could be recycled and reused" (Luiz et al., 2012a). The four types of wastewaters which offered the possibility of reuse originated from: (1) the defrosting of refrigerating and freezing chambers, (2) the purging of condensers, (3) the cooling of smoke fumigator chimneys, and (4) the sealing and cooling of vacuum pumps. These residues had similar water quality parameters; hence they could be mixed before reuse, totaling approximately 1,383 m^3 d^{-1} of wastewater. Depending on the final use, this mixed wastewater could be reused without major treatment or following simple filtration; thus, this approach can be considered as direct wastewater reuse.

The theoretical reduction in water consumption, after applying the principles of water minimization and wastewater reuse, was 25.6%, representing a financial saving of around $434,000 per year (Table 1). However, new regulations need to be elaborated together with national environmental, sanitary and water supply agencies, processing industries and research institutions aiming at the legalization and promotion of water reuse in the food industry.

Condition	Water flow (m^3 day^{-1})	Water saving (%)	Annual Costs[1] ($)
Production in 2007	8616.0	-	1,539,353
Theoretical production after water minimization	7810.0	9.4	1,366,000
Theoretical production after wastewater reuse	7216.8	16.0	1,256,996
Theoretical production after water minimization and wastewater reuse	6410.0	25.4	1,104,731

[1]Considering costs in 2007: $0.10 and $0.42 per m^3 to treat water (DWTP) and wastewater (WWTP), respectively, in the case study meat processing plant. Data reproduced from Luiz et al., 2012a.

Table 1. Water and financial savings

3.1. Tertiary and advanced treatment for indirect wastewater reuse

Additionally, tertiary treatments are a good alternative to produce high quality indirect reuse water, reducing the percent of fresh water consumption. Tertiary treatments can be applied to recondition secondary effluents (*i.e.*, after secondary activated-sludge treatment), further increasing the possibilities for indirect wastewater reuse inside or outside the building. For example, an industrial wastewater treatment plant can produce high quality tertiary wastewater to be used as reuse water in its processes that do not involve contact with the food product, without the risk of adverse effects in terms of the product quality and human health. Alternatively, it can provide this high quality reuse water for another industrial activity, which does not require fresh potable water for all of its processes.

To improve the quality of the wastewater to be reused, it is necessary to disinfect it and to decrease or eliminate the concentration of biologically persistent organic compounds. The inefficient removal of these organic compounds from the wastewater before reuse or discharge into natural watercourse is promoting their accumulation in fresh water bodies and causing environmental and human health problems and, especially, harming the aquatic animals (Esplugas et al, 2007; Liu et al., 2009; Luiz et al., 2009, 2010, 2011; Oller et al., 2011).

Biologically resistant pollutants or persistent organic pollutants (POPs) are compounds which are not eliminated through the metabolic activity of living organisms (mainly bacteria and fungi) in natural waters and soils (Oller et al., 2011). Thus, conventional primary (removal of suspended compounds) and secondary (such as activated sludge) wastewater

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treatments are inefficient in removing these pollutants (Luiz et al., 2009). These compounds are present in municipal wastewaters primarily as pharmaceuticals and personal care products (PPCPs) (Esplugas et al., 2007), and also in industrial wastewaters which contain a large number of synthetic and toxic compounds, mainly polar and non-polar hazardous compounds, pharmaceuticals, phenols, pesticides, endocrine disruptor compounds (EDCs), and non-biodegradable and toxic chlorinated solvents (Esplugas et al., 2007; Liu et al., 2009; Luiz et al., 2009, 2010, 2011, 2012a; Petrović et al., 2003).

Due to the large variety of recalcitrant organic contaminants, the tertiary treatment applied to produce water for reuse must be exceptionally efficient. The advanced oxidation processes (AOPs) are an excellent alternative. During AOPs, highly reactive oxidizing radicals are formed, mainly hydroxyl radicals (•OH) (Koning et al., 2008). These radicals are non-selective, promoting the oxidation of all organic and inorganic contaminants and, in the presence of a sufficient amount of oxidant and optimized reaction conditions, complete mineralization can be reached, the final products being CO_2, H_2O and inorganic anions. Thus, AOPs are applied to totally or partially remove recalcitrant organic compounds, increasing the biodegradability of wastewater (Rizzo, 2011).

3.1.1. Proposed tertiary treatments of slaughterhouse secondary wastewater

In previous studies carried out by our group we evaluated the different options of tertiary treatments to produce high quality reuse water to be used in processes without contact with food products, that is, non-potable uses (*e.g*. as cooling water, boiler feed water, toilet flushing water or for irrigation around the plant) (Cornel et al., 2011; Luiz et al., 2009, 2011, 2012a). However, since Brazilian legislation only allows the use of fresh potable water in the food industry, our research, using real wastewater and aiming to obtain reclaimed water with drinking water quality which adhered to Brazilian legislation, was carried out in bench-scale and pilot-scale.

The tertiary treatments evaluated for the slaughterhouse secondary wastewater included: UV, H_2O_2, O_3, and AOPs (H_2O_2/UV, O_3/UV; O_3/H_2O_2/UV; TiO_2/UV; and H_2O_2/TiO_2/UV). Additionally, for the best combinations, the kinetics of the photo-induced degradation of color, UV_{254}, total organic carbon (TOC) and/or total coliforms were evaluated (Luiz et al., 2009, 2010, 2011, 2012a,b).

Two main problems were encountered during this research. The first issue was the variation in the quality of the target slaughterhouse wastewater over time, which affected the treatment efficiency and the determination of the best treatment (Luiz et al., 2011). The second issue was the high concentration of nitrate and nitrite: 45.9(±17.7) mg NO_3^--N L^{-1} and 3.74-3.77 mg NO_2^--N L^{-1}, respectively (Luiz et al., 2012). The Brazilian drinking water standard (Brazilian Ministry of Health Administrative Ruling 518/2004) allows 10 mg NO_3^--N L^{-1} and 1 mg NO_2^--N L^{-1}, respectively.

In order to remove the recalcitrant organic compounds and the nitrate/nitrite, to reduce the color and turbidity, and to disinfect the secondary wastewater in a single treatment, micro-

filtration followed by an AOP employing $H_2O_2/TiO_2/UV$ was identified as the best combination evaluated (Figure 2).

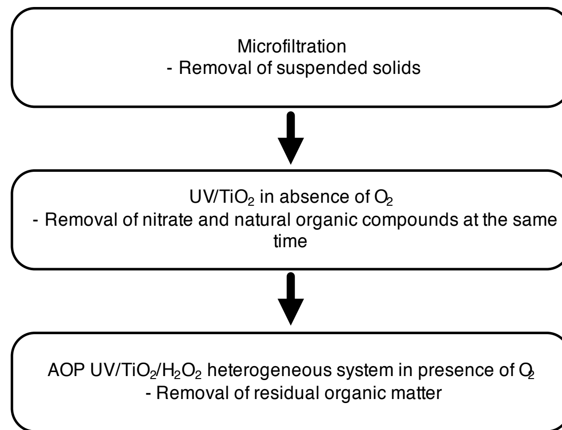

Figure 2. Process proposed for the treatment of secondary wastewaters with high concentration of nitrate/nitrite and recalcitrant organic compounds.

The photocatalytic removal of nitrate/nitrite is more effective in the absence of dissolved oxygen, because if O_2 is present this oxidant agent will be a better final electron acceptor than nitrate or nitrite. The catalyst is activated by the absorption of high energy photons, promoting the excitation of the electrons from the valence band (VB) to the conduction band (CB), and consequently an electron (e^-) and a positive hole (h^+) are formed in the CB and VB, respectively (Luiz et al., 2012b). The electron reduces the oxidizing agent adsorbed on the catalyst, and the hole oxidizes the organic compound or H_2O. In the latter case, the oxidation of H_2O produces •OH radicals, which will also oxidize organic matter (Ahmed et al., 2010).

Therefore, during the photocatalytic removal of nitrate/nitrite by UV/TiO_2, nitrate and nitrite ions will be the final electron acceptor and they will be reduced to N_2 gas. The natural organic compounds or residual, biologically persistent, organic pollutants (in the case of industrial wastewaters) will be the hole scavengers (electron donors, reducing agent). In cases where the natural concentration of organic compounds in the aquatic medium is not sufficient to promote the reduction of nitrate/nitrite to below the desired concentration, a carbon source should be added. Formic acid is a good alternative since its residue can be completely decomposed into the harmless compounds CO_2 and H_2O (Luiz et al., 2012b; Rengaraj and Li, 2007; Sá et al., 2009; Wehbe et al., 2009; Zhang et al, 2005). Finally, the heterogeneous AOP system $UV/TiO_2/H_2O_2$ was applied in the presence of O_2 to remove residual organic matter and achieve the required standard of drinking water quality (Luiz et al., 2012b).

The proposed treatment was also successful in removing recalcitrant organic compounds present in the secondary treated slaughterhouse wastewater, which include antibiotics, pharmaceuticals and personal care products which are commonly found in industrial, but predominantly in sanitary and domestic, wastewater (Luiz et al., 2009). One such compound

found was the macrolide antibiotic erythromycin A and its removal and degradation products resulting from direct ozone attack and hydroxyl radical attack (AOPs O_3/UV, O_3/H_2O_2 and UV/H_2O_2) were evaluated. However, the research indicated that the degradation of organic micropollutants, such as erythromycin, in the AOP may be faster than under ozone treatment, because the hydroxyl radical attack (AOP treatments) is not selective and is usually diffusion-controlled. On the other hand, the direct attack of ozone is selective and is typically targeted toward functional groups with a lone valence electron pair where the electrophilic addition of ozone occurs (unsaturated compounds with carbon-carbon double or triple bonds - π bonds, aromatic rings, amines and sulfides) (Luiz et al., 2010, 2012a).

3.1.2. Poultry hatchery wastewater treatment

Industrial wastewater is generally comprised of various effluent streams generated at different points in a particular process. Its physicochemical characteristics can present considerable variation over time due to, for instance, changes in operating procedures and cleaning activities. Therefore, the complexity and variation of its composition are typical attributes of industrial wastewater (Genena, 2009).

The poultry hatchery wastewater of this case study was collected and passed through the stages of screening and equalization. The wastewater variability was investigated over a period of 48 h and its quality was evaluated by chemical oxygen demand (COD) analysis, which is an overall pollution indicator and represents the amount of organic matter present in the sample. The COD values ranged from 218±2 to 997±5 mg O_2 L^{-1}, which confirms the high variability in the nature of the poultry hatchery wastewater (Genena, 2009).

Wastewater in the poultry hatcheries originates mainly from the washing of equipment and utensils. Therefore, a series of diverse compounds may be present, such as veterinary drugs administered to the animals through feed and excreted by them in urine, and sanitizer agents and pesticides used in the cleaning and disinfection of the work environment (Genena, 2009). These compounds, which are persistent compounds, are very harmful to the environment, presenting high toxicity and bioaccumulation (Almeida et al., 2004). They are complex and often difficult to degrade in the biological treatment systems commonly present in industrial wastewater treatment plants.

The food industry is constantly seeking ways to improve the quality of its wastewater through changes in treatment systems. The growing concern regarding emerging and persistent compounds has resulted in researchers focusing their attention on alternative methods of wastewater treatment to minimize or avoid the discharge of these pollutants into water resources, since the biological treatment processes typically used by the food industry are not able to destroy these types of compounds (Genena et al., 2011). The application of oxidative elimination methods, *e.g.*, direct oxidation with ozone or hydrogen peroxide and AOP have been highlighted as strong alternatives for the treatment of wastewater containing compounds which do not degrade easily (de Sena et al., 2009; Genena, 2009, Genena et al., 2011; Luiz et al., 2009, 2010; Tambosi et al., 2009).

The proposal for the use of physicochemical processes for the treatment of the poultry hatchery wastewater of this case study was based on the value of 4.6 for the COD/BOD_5 ratio (low biodegradability) and the presence of persistent compounds. Therefore, the application of different AOPs (H_2O_2/Fe^{2+} – Fenton, $H_2O_2/Fe^{2+}/UV$ – photo-Fenton and H_2O_2/UV) for the poultry hatchery wastewater treatment was investigated. The wastewater treatment process by photo-Fenton reaction was found to be the most appropriate, resulting in better organic matter removal efficiency (approximately 91.9% of COD and 66.3% of TOC). Additionally, the COD/BOD_5 ratio obtained for the treated wastewater indicates that all physicochemical treatments applied improved the biodegradability, *i.e.*, there was an increase in the amount of material susceptible to degradation by biological processes, reaching a value of 1.5 in the photo-Fenton process. Thus, the biological process can be considered as a post-treatment stage, which would reduce the total costs of the wastewater treatment process (Genena, 2009).

An important consideration in the degradation processes is the potential for the generation of toxic intermediates or compounds which are even more toxic than their parent molecule, and thus it is necessary to monitor the process using toxicity assays (Bila et al., 2005). The *Daphnia magna* acute toxicity evaluation showed that all treatments promoted a significant reduction in the wastewater toxicity effects, and a 94% reduction was reached in photo-Fenton process (Genena, 2009).

Photo-Fenton and Fenton processes result in the formation of a sludge, which is usually deposited in landfills. Thus, better alternatives are being proposed and among them is sludge combustion for power generation. However, in this case study the amount of sludge obtained was insufficient for the determination of its calorific power (Genena, 2009).

The poultry hatchery wastewater was submitted to analysis by liquid chromatography coupled to mass spectrometry (LC/MS) with the objective of investigating the presence of persistent compounds. The presence of imazalil (pesticide) was confirmed among the investigated compounds. Imazalil is an organochloride compound used as a fungicide in the industry for sanitization (Genena, 2009, Genena et al., 2011). Organochlorine pesticides are typical persistent organic pollutants and are the subject of worldwide concern due to their persistence, bioaccumulation and potential negative impacts on humans and animals (Guan et al., 2009; Zhang et al., 2007). The biological treatment of wastewater containing micropollutants, like pesticides, is often very complicated or even impossible, because many pesticides are highly toxic to wastewater biocoenosis (Genena et al., 2011).

The treatment of ultrapure water to remove imazalil has been investigated applying the photo-Fenton (AOP) and ozonation processes. *Tert*-butanol (t-BuOH) was used in the ozonation process as an •OH scavenger to ensure that the study was focused only on the direct attack of imazalil by molecular ozone. For both processes the detection and identification of by-products were carried out, applying sophisticated analytical techniques such as LC/MS and LC/MS^n (liquid chromatography coupled to mass or multiple tandem mass spectrometry). The toxicity induced by these by-products was also investigated. For each process of oxidative treatment, four degradation products not yet known were detected and their structures were elucidated. The toxicity analysis (*Daphnia magna* assays) revealed a decrease

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in toxicity over time for both treatments, indicating that the by-products were not more toxic than their parent molecules (Genena, 2009, Genena et al., 2011).

4. Biomass-to-energy actions

The biosolids originating from the wastewater treatment system of the meat processing plant, sawdust and their mixture in a mass ratio of 1:9 (w/w) were characterized as fuels. The correlations between the fuel properties, the operating parameters for the combustion and the gaseous emissions were then investigated in order to evaluate the feasibility of applying this organic residue as a substitute fuel for thermal energy generation.

4.1. Biomass properties

The fuel properties often form the basis for the selection of the most appropriate technology for the biomass-to-energy conversion process. Depending on these properties, a biomass fuel may not be suitable for specific conversion options, partially for technical and sometimes for environmental reasons. The characteristics of the biomass are influenced by its origin and also by the entire processing system preceding any conversion step. Biomass presents a wide variation in its physical and chemical properties. Many publications have investigated the effects of the biomass properties on thermal conversion processes (Demirbas, 2004; Jenkins et al., 1998; Obernberger et al., 2006; van Paasen et al., 2006; Werther et al., 2000; Werther, 2007). The use of biomass as a fuel in combustion processes is frequently desirable in the agro-industry sector because the residues, such as wastewater sludge, usually present high calorific value. However, burning biomass containing different mineral matter compositions may create various problems which can affect the boiler operation or make the firing of the biomass in conventional combustion systems unprofitable.

Wood and wood-based materials are extensively used as fuel for thermal energy generation particularly in the Brazilian food industry, which requires large amounts of steam.

In order to evaluate the potential for the utilization of the biosolids originating from the case study plant for co-combustion with sawdust, a sample of the biosolids obtained from the physicochemical treatment (LFP) was chemically and physically characterized, and its composition was compared to that of sawdust (SD), taken as a reference fuel. Additionally, a sample of a mixture of LFP and SD in a mass ratio of 1:9 w/w (LFPSD1:9) was also characterized and the results compared to SD and LFP properties.

The methodology applied for the biomass characterization and the results obtained for LFP, SD and LFPSD1:9 were reported by Floriani et al. (2010) and Virmond et al. (2008, 2011), and are summarized in Table 2.

Carbon (C), hydrogen (H) and oxygen (O) are the main components of solid biofuels. Carbon and hydrogen contribute positively to the HHV (higher heating value). The content of hydrogen also influences the LHV (lower heating value) due to the formation of water. The

content of greases was also measured in the LFP composition (34.39 wt%, raw) and it contributes considerably to the high energy content of the LFP (LHV of 25.77 MJ kg^{-1}, daf). The presence of chlorine in the biomass (0.18 wt%) occurs due to the utilization of chlorine-based products for hygiene purposes at the plant and is incorporated into the wastewater as well as into the remaining biosolids (LFP). The nitrogen content of the fuel mixture LFPSD1:9 (1.36 wt%), even though much lower than the concentration found in LFP, can still cause problems in terms of NO_x emission during its combustion. The variability of components present in the biomass is mainly due to the chemical compounds used as ingredients during meat processing operations, especially salts and additives. The sulfur content in LFP is mainly due to the conversion of sulfur-containing proteins, but some may remain from the precipitation agent used in the wastewater treatment (ferric sulfate).

	Units	SD[1]	LFP[1]	LFPSD1:9[2]
Proximate analysis[3]				
Ash	(wt%, db)	0.43	12.30	1.75
Moisture	(wt%, raw)	19.97	15.00	50.23
Volatiles	(wt%, daf)	79.78	85.29	83.08
Fixed carbon	(wt%, daf)	20.22	9.58	17.01
Ultimate analysis[3]				
Carbon	(wt%, daf)	55.30	58.04	51.06
Hydrogen	(wt%, daf)	7.14	9.01	6.64
Nitrogen	(wt%, daf)	0.21	9.24	1.36
Sulfur	(wt%, daf)	< 0.01	0.34	0.03*
Oxygen	(wt%, daf)	37.34	22.68	40.94
Chlorine	(wt%, daf)	< 0.01	0.18	< 0.01
Fluorine	(wt%, daf)	< 0.20	< 0.20	n.d.
Phosphorus	(wt%, daf)	0.01	1.03	n.d.
Lower Heating Value[3]				
LHV	(MJ kg^{-1}, daf)	16.62	25.77	20.31
LHV	(MJ kg^{-1}, raw)	16.55	22.60	19.76

[1]Data reproduced from Virmond et al. (2011); [2]Data reproduced from Floriani et al. (2010); [3]Maximum experimental uncertainties equal to 0.30%; db is on a Dry Basis; daf is on a Dry and Ash Free basis; *Value previously presented by Floriani et al. (2010) corrected; n.d. is Not Determined; LHV is Lower Heating Value

Table 2. Biomass properties

In previous publications (Floriani et al., 2010; Virmond et al., 2008, 2011), the authors have addressed the effects of the LFP ash composition on the fouling and slagging tendency in the combustion systems, showing that the occurrence of this problem can be reduced when burning a mixture of the biosolids with wood residues such as SD compared to LFP alone.

As shown in Table 3, the ash melting temperatures of LFP are much lower than the values estimated for LFPSD1:9 through mass balance analysis considering a homogeneous mixture, and its utilization is recommended in low blending proportions.

	Units	SD[1]	LFP[1]	LFPSD1:9[2]
Ash composition[3]				
Fe_2O_3	(wt%, db)	4.44	32.40	9.34
CaO	(wt%, db)	31.27	17.40	22.77
MgO	(wt%, db)	11.64	1.30	4.59
Na_2O	(wt%, db)	1.67	1.70	1.14
K_2O	(wt%, db)	10.44	1.70	8.77
SiO_2	(wt%, db)	15.69	4.90	17.84
Al_2O_3	(wt%, db)	12.30	1.70	8.22
TiO_2	(wt%, db)	3.94	0.00	3.07
P_2O_5	(wt%, db)	2.74	36.30	8.50
MnO	(wt%, db)	2.06	n.d.	n.d.
SO_4	(wt%, db)	3.02	n.d.	n.d.
Ash melting temperatures				
Deformation temperature	(°C)	>1150*	750	1335
Softening temperature	(°C)	>1170*	990	1359
Hemispherical temperature	(°C)	>1190*	1010	1361
Fluid temperature	(°C)	>1230*	1040	1364

[1]Data reproduced from Virmond et al. (2011); [2]Data reproduced from Floriani et al. (2010); [3]Maximum experimental uncertainties equal to 0.30%; db is on a dry basis; n.d. is not determined; [4]Data reproduced from Llorente & García (2005) for eucalyptus sample

Table 3. Biomass ash properties

It was observed that the main element found in the sludge ash was phosphorus, followed by iron. This is considered a problem because P forms compounds with lower melting temperature, which may have influenced the results presented in Table 3. As expected, the mixture

of biomasses maintained a relatively high ash melting temperature, which is a desirable aspect when considering the combustion of solid fuels. Additionally, the design of the equipment and the definition of the operating conditions are extremely important to control, or even avoid, the occurrence of such problems.

Concentration (mg kg^{-1}, db)				
	Limit[1]	**BS_{Flot}**	**BS_{Cent}**	**BS_{Biol}**
Trace metal				
Hg	5	< 0.50	< 0.50	< 0.50
Cd	5	< 0.50	< 0.50	0.64
Cr	800	6.7	28.4	26.7
Cu*	800	16.2	29.8	182.1
Ni	200	1.9	9.9	22.0
Pb	500	1.3	3.4	6.1
Zn*	2000	88.2	183.8	1090.3
As	75	0.57	< 0.50	< 0.50
Mo*	75	0.50	1.7	4.4
Co*	5	< 0.50	< 0.50	4.1
Micronutrient				
K	-	427	599	6903
Fe	-	9360	25600	20900
Al	-	1750	498	3420
P	-	6350	15900	28400
Secondary nutrients				
Ca	-	1520	5080	18600
Mg	-	148	259	7185
S	-	3140	6630	9810

db is Dry Basis; [1]Upper limit of pollution for the disposal of sewage sludge in the environment (EU, 2000); * Trace elements, biologically essential in small quantities. Reproduced from de Sena et al. (2009)

Table 4. Results for the determination of trace metals and nutrients in the sludge samples

Besides the determination of the biomass properties for biofuel applications, the organic and inorganic contents of the sludge generated from the WWTP were characterized, since it is necessary to assure that sludge containing high pollutant loads is not applied as fertilizer, in order to avoid contamination of agricultural soil and cultivated plants, *i.e.*, to avoid the

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transfer of contaminants into the food chain. Inorganic and organic pollutants not removed during physicochemical wastewater treatment processes are either bio-chemically degraded or adsorbed by the sludge. The characterization of the sludge was reported by de Sena et al. (2009), where the trace metal, PAH, PCB and PCDD/PCDF concentrations in the WWTP sludge were determined. Trace metals might end up in the effluent from the meat processing plant through sources like equipment, sanitizers and cleaning agents, as well as equipment and pumps used in the wastewater treatment plant itself. Also, some metals such as arsenic, copper and zinc are occasionally added to animal feed as mineral food supplements and/or as growth promoters (US EPA, 2004). The group of PAHs, generated undesirably mostly during manifold incomplete incineration processes, includes numerous compounds with three or more condensed aromatic rings. PCBs synthesized for specific applications as non-inflammable insulators, hydrolic liquids and plasticizers are pollutants which today are ubiquitously found in the environment, although they were phased out from production worldwide at the end of the 1970s. PCDDs/PCDFs are not intentionally produced by humans but they are released into the atmosphere as sub-products of incineration and combustion processes, both domestic and industrial, when carbon, hydrogen, chlorine and oxygen together with copper as a catalyst are present. The incineration of municipal or clinical wastes, iron ore sinter plants and non-ferrous metal industries (Quass et al., 2000) as well as the chemical synthesis of chlorophenols and electrolysis of sodium chloride are sources of PCDDs/PCDFs. These compounds accumulate in sludge due to their extreme lipophilicity, and it is very difficult to assess the various sources of these compounds and their pathways into the environment and into the food chain (Klöpffer, 1996). Tables 4, 5 and 6 show the results of the biomass characterization, including 3 (three) types of sludge (BS) collected at different points in the WWTP. BS_{Flot} refers to the sludge remaining after the flotation process, BS_{Cent} to the sludge remaining after the three-phase centrifugation, and BS_{Biol} to the sludge collected from the activated sludge bioreactor (not primarily intended for combustion purposes).

Compounds	**Concentration (µg kg^{-1}, db)**				
	TEF[1]	**Limit[2]**	**BS_{Flot}**	**BS_{Cent}**	**BS_{Biol}**
Polycyclic Aromatic Hydrocarbons (PAHs)					
Naphthalene (Nap)	-		110.0	< 40.0	< 30.0
Acenaphthylene (Acy)	-		84.0	< 30.0	< 30.0
Acenaphtene (Ace)	-		< 7.0	< 7.0	< 6.0
Fluorene (Flu)	0.001		20.0	< 7.0	< 6.0
Phenanthrene (Phe)	0.001		84.0	< 8.0	< 7.0
Anthracene (Ant)	-		< 2.0	8.0	8.0

Compounds	Concentration (μg kg⁻¹, db)				
	TEF[1]	Limit[2]	BS_{Flot}	BS_{Cent}	BS_{Biol}
Fluoranthene (Fla)	0.001		320.0	510.0	< 10.0
Pyrene (Pyr)	0.001		97.0	72.0	11.0
Chrysene (Cry)[3]	0.01		92.0	< 1.0	5.0
∑ PAH_{LMW}	-		816.0	616.0	113.0
Benzo[a]anthracene (BaA)[3]	0.1		24.0	< 1.0	4.0
Benzo[b]fluoranthene (BbF)[3]	0.1		38.0	25.0	< 3.0
Benzo[k]fluoranthene (BkF)[3]	0.1		11.0	< 1.0	< 1.0
Benzo[a]pyrene (BaP)[3,4]	1.0		2.0	< 1.0	5.0
Dibenzo[a,h]anthracene (DbA)[3,4]	5.0		< 4.0	< 4.0	< 4.0
Benzo[g,h,i]perylene (BgP)	0.1		12.0	< 6.0	< 6.0
Indeno[1,2,3-cd]pyrene (InD)[3]	0.1		< 10.0	< 10.0	< 10.0
∑ PAH_{HMW}	-		101.0	48.0	33.0
∑ PAH		≤ 6000	917.0	664.0	146.0
∑ TEF_{PAH}	-		11.0	3.0	6.0
Polychlorinated Biphenyls (PCB)					
PCB non-ortho					
PCB 77, 81, 126*, 169	< 0.1		< LOQ	< LOQ	< LOQ
PCB mono-ortho					
PCB 105, 114, 118, 123, 156, 167, 189	< 0.005		< LOQ	< LOQ	< LOQ
∑ PCB	-	≤ 800	< LOQ	< LOQ	< LOQ

db is Dry Basis; [1]TEF for dioxin and dioxin-like compounds (Nisbet et al., 1992; van den Berg et al., 2006); [2]Limit for solid disposal onto soil (EU, 2001); [3]Carcinogenic isomers; [4]Isomers of PAH with TEF comparable to the most toxic polychlorinated dibenzo-dioxins and -furans; *Isomer of PCB with highest TEF; LOQ is Limit of Quantification. Reproduced from de Sena et al. (2009)

Table 5. Results for the determination of PAHs and PCBs in the sludge samples

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	Concentration (ng kg^{-1}, db)			
	TEF[1]	**BS_{Flot}**	**BS_{Cent}**	**BS_{Biol}**
Polychlorinated dibenzo-p-dioxins (PCDD)				
2,3,7,8 – TCDD[2]	1.0	0.1	0.4	0.4
1,2,3,7,8 - PeCDD	1.0	0.6	1.3	2.2
1,2,3,4,7,8 - HxCDD	0.1	0.3	1.5	1.7
1,2,3,7,8,9 - HxCDD	0.1	0.2	1.9	3.3
1,2,3,6,7,8 - HxCDD	0.1	0.7	1.3	1.8
1,2,3,4,6,7,8 - HpCDD	0.01	0.8	4.2	4.6
OCDD	0.0003	2.1	21.1	23.1
∑ PCDD	-	4.8	31.7	37.1
Polychlorinated dibenzofurans (PCDF)				
2,3,7,8 – TCDF	0.1	0.2	0.3	1.3
2,3,4,7,8 – PeCDF	0.3	0.5	2.1	2.1
1,2,3,7,8 – PeCDF	0.03	0.7	1.5	3.8
1,2,3,4,7,8 – HxCDF	0.1	0.6	1.2	2.0
1,2,3,6,7,8 – HxCDF	0.1	0.6	1.6	2.3
1,2,3,7,8,9 – HxCDF	0.1	1.7	2.9	3.1
2,3,4,6,7,8 - HxCDF	0.1	0.7	1.9	3.8
1,2,3,4,6,7,8 - HpCDF	0.01	2.6	4.2	5.3
1,2,3,4,7,8,9 – HpCDF	0.01	1.4	3.1	3.6
OCDF	0.0003	2.1	4.4	10.2
∑ PCDF	-	11.1	23.2	37.3
PCDD:PCDF Ratio	-	0.43	1.37	0.98
∑ TEF PCDD/PCDF	100*	1.4	3.8	5.4

db is Dry Basis; [1]TEF for dioxins and dioxin-like compounds (van den Berg et al., 2006; WHO, 2005); [2]Isomer with highest acute toxicity; *TEF (ng kg^{-1}) limit for solid disposal onto soil (EU, 2001). Reproduced from de Sena et al. (2009)

Table 6. TEF and concentrations of PCDDs/PCDFs in the sludge samples

A study by de Sena et al. (2009) verified low pollution loads for the sludge (BS) originating from the WWTP, with respect to the most relevant inorganic and organic priority pollutants as monitored by the US EPA, at the case study meat processing plant located in the south of Brazil. Although other pollutants such as veterinary drugs, pesticides and surfactants were not investigated in this first analytical approach, they are of high concern. However, this study was a preliminary report for future monitoring of other food processing segments located in different regions of Brazil.

4.2. Biomass combustion

Co-combustion of agro-industrial residues in thermal power plants is not necessarily a low cost alternative for the thermal treatment of wastes. There is the possibility of interaction between the components and the main fuels in such a way that either the operating behavior of the conversion system is improved or the emissions are reduced (Werther, 2007). The emission of pollutants generated during combustion is strongly related to the biomass properties. Pollutant formation mechanisms and many other parameters related to the combustion process must be monitored due to the formation of highly problematic compounds such as NO_x, SO_2, benzene, toluene, ethyl-benzene, and (o-,m-,p-)xylenes (BTEX), polycyclic aromatic hydrocarbons (PAH), polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDF) (Chagger et al., 1998; Kumar et al., 2002; Mckay, 2002; Stanmore, 2004; Watanabe et al., 2004), and have to be controlled in order to comply with the stringent limits set by recent environmental legislation. Chlorine-associated, high-temperature corrosion and the potential corrosion problems associated with burning biomass fuels have been previously discussed (Nielsen et al., 2000). Fuel nitrogen can be a problem in terms of NO_x emissions. The conversion of nitrogen in systems fired by solid fuels (mainly coal, but also biomass) has been reviewed in detail, as well as the combustion characteristics of different biomass fuels, the potential applications of renewable energy sources as the prime energy sources in various countries, and the problems associated with biomass combustion in boiler systems (Demirbas, 2004; Werther, 2000).

The pollutant emissions due to incomplete biomass combustion can be effectively controlled by an optimized combustion process, *i.e.*, enhanced mixing, sufficient residence time at high temperatures (>850 °C), and low total excess air (Demirbas, 2005), as well as the appropriate choice of the combustion device.

Given the higher energy value of the biosolids (LHV equal to 22.60 MJ kg^{-1}) compared to sawdust (LHV equal to 16.55 MJ kg^{-1}), as shown in Table 2, the substitution of 10 wt% of sawdust with this residue can increase the thermal energy production by approximately 4% compared to the combustion of sawdust alone, leading to a sawdust saving of 1950 ton per year, besides the economic benefits related to reduced landfill disposal. However, the gaseous emissions have to be monitored so as not to infringe current legislations.

The evaluation of the feasibility of the utilization of the biosolids originating from the WWTP studied was performed through combustion testing of the biosolids as the sole fuel in a pilot-scale cyclone combustor (model Drako, Albrecht, Brazil) with a burning capacity of 100 kg h^{-1}, as described by Virmond et al. (2011), and in a furnace equipped with a recip-

rocating-grate coupled to a boiler at the meat processing plant with the capacity to process 12000 kg h^{-1}, as reported by Virmond (2007).

Grate firing is one of the main technologies that are currently used for biomass combustion aiming at heat and power production. Grate-fired boilers can fire a wide range of fuels with varying moisture content and show great potential in biomass combustion (Goërner, 2003). The plant-scale furnace was operated at a fuel feed rate of 2604 kg h^{-1} (moisture content of approximately 50 wt%) at 900 °C, with 59% excess air and without gas recirculation. The combustion test lasted for approximately 2 h after the system had reached steady state.

In the pilot-scale plant the combustion of SD was carried out at a fuel feed rate of 32 kg h^{-1} (moisture content of 9.16 wt%), with gas recirculation of 20% at an average temperature of 642 °C in a cyclone combustor (Drako, Albrecht, Brazil), which has been described by Virmond et al. (2011). The average temperature at the front stage of the combustor was 1052 °C and at the outlet it was 1024 °C, and an air-to-fuel (A/F) ratio of 9.08 (theoretical A/F ratio of 7.23 based on the fuel composition) was used. For the LFP combustion test, the conditions applied to the cyclone combustor were: fuel feed rate of 43 kg h^{-1} (moisture content of 11.44 wt%), with gas recirculation of 20% at an average temperature of 800 °C. The average temperature at the front stage of the combustor was 868 °C, at the outlet of the combustor it was 1080 °C, and the A/F ratio was 11.82 (theoretical A/F ratio of 7.76 based on the fuel composition). CO, O_2, SO_2, C_xH_y (measured as CH_4), NO, and NO_2 emissions were measured using a Greenline MK2 (Eurotron) analyzer and BTEX emission measurements were based on the adsorption and desorption of gases which were analyzed by gas chromatography. Emissions of BTEX were expressed as Total Organic Carbon (TOC). The detailed methodology for emissions sampling and analysis during the combustion tests, as well as the complete set of results, have been previously reported by Floriani et al. (2010) and Virmond et al. (2007, 2008) for LFPSD1:9 combustion, and by Virmond et al. (2011) for SD and LFP. Thus, only the main points are highlighted herein. The gaseous emissions observed in the combustion tests and the respective regulation limits were corrected to the reference oxygen content (O_{2ref}) of 7% and are given in Table 7.

Since no data on the combustion of meat processing or slaughterhouse wastes had been previously published, no comparison was possible. However, the results for the contaminants reported were compared with the emission limits established by national and international environmental agencies, such as the Brazilian guidelines issued by The National Council of the Environment (CONAMA, 2002) for gaseous emissions in the thermal treatment of wastes; the American guidelines issued by the US Environmental Protection Agency (US EPA, 2002) for emissions from commercial and industrial solid waste incineration units; and the German Guidelines 17.BlmSchV (17.BlmSchV, 2003) for emissions from biomass combustion and from biomass co-combustion. Concerning the combustion tests performed in the pilot-scale cyclone combustor with SD and LFP as fuels, the emissions of CO, CO_2, C_xH_y and TOC were well controlled and their concentrations remained below the regulation limits considered for both biomasses, except CO in the SD combustion test in relation to the German guidelines, which refer to waste incineration and are stricter than the other regulations.

	CO (mg Nm^{-3})	CO_2 (%)	C_xH_y (mg Nm^{-3})	NO_x[1] (mg Nm^{-3})	SO_2 (mg Nm^{-3})	TOC (mg Nm^{-3})
SD biomass[2]	93.58 ±21.97	10.32 ±0.03	0.00 ±0.00	241.58 ±26.31	0.00 ±0.00	1.27 ±0.57
LFP biomass[2]	63.33 ±10.30	10.33 ±0.05	0.00 ±0.00	1727.43 ±229.93	363.54 ±90.80	1.23 ±0.12
LFPSD1:9 biomass[3]	734.83 ±12.39	10.39 ±0.02	554.44 ±7.91	497.94 ±19.04	128.69 ±4.31	1.72 ±0.83
CONAMA [4]	*124.88*	*n.a.*	*n.a.*	*560.00*	*280.00*	*n.a.*
US EPA [5]	*196.16*	*n.a.*	*n.a.*	*796.02*	*57.09*	*n.a.*
17.BImSchV (24 h; <50 MW) [6]	*140.00*	*n.a.*	*n.a.*	*373.33*	*186.67*	*9.33*
17.BImSchV (24 h)[7]	*70.00*	*n.a.*	*n.a.*	*280.00*	*70.00*	*14.00*

[1]NO_x expressed in terms of NO_2; TOC is Total Organic Carbon; [2]Data from Virmond et al. (2011); [3]Data from Floriani et al. (2010) and Virmond et al. (2007, 2008); [4]CONAMA 316/02, thermal treatment of wastes (CONAMA, 2002); n.a. is Not Applicable; [5]US EPA, solid waste incineration (US EPA, 2000); [6]17.BImSchV 24 h, <50 MW, co-combustion of wastes (17.BImSchV, 2003); [7]17.BImSchV 24 h, direct combustion of wastes (17.BImSchV, 2003)

Table 7. Gaseous emissions from the combustion tests at O_{2ref}=7%

The effect of the biomass composition on gaseous emissions was clearly observed, especially considering the N and S fuel contents in LFP, which led to concentrations of these pollutants being higher than the established limits.

The use of the biosolids originating from the meat processing plant investigated in this study as a fuel in the pilot cyclone combustor was shown to be feasible; however, further research is required concerning the control of SO_2 and NO_x emissions to avoid exceeding the very strict emission limits as well as the occurrence of fouling and slagging.

In relation to the combustion test performed with the mixture of SD and LFP (LFPSD1:9), the high levels of C_xH_y and CO emitted indicate incomplete combustion. This can be attributed to the high moisture content of the biomass (50.23 wt%, as shown in Table 2), the lower combustion temperature (approximately 900 °C) compared to the other two tests performed in the pilot-scale combustor (approximately 1000 °C) and the absence of gas recirculation. Additionally, the control of the operating conditions of the large-scale plant is more difficult to achieve, due to the restricted testing time or minimal variation from the normal operation with the SD. Firing a biomass with low moisture content and flue gas recirculation could provide better oxidation conditions. Due to the lower nitrogen concentration found in LFPS1:9 compared to LFP, as well as to the low operating combustion temperature, NO_x emissions remained below the limits established by CONAMA and US EPA.

The co-combustion of LFP and SD with lower-N fuel content reduced the NO_x in the gaseous emissions compared to the burning of LFP alone. In fact, this option is the most feasible in Brazil considering the relatively high NO_x emissions related to both the fuel nitrogen and

to the fact that wood and wood-based materials are extensively used as fuel for thermal energy generation in the Brazilian food industry. Chlorine, PAHs and PCBs are among the elements or compounds that must be studied in greater depth as they are precursors to the formation of dioxins and partly of furans.

Industrial solid wastes must be disposed of safely, and co-firing them with SD has been shown in other studies by the authors, which are currently underway, to be profitable using the same pilot-scale cyclone combustor and different biomasses. The advantages are both a reduction in the consumption of primary fuels and the recovering of energy from wastes inside the plant, which would normally be disposed of in landfills, potentially causing environmental problems.

4.3. Biogas production and combustion

During this study, only preliminary tests were performed, such as the determination of the chemical composition of the biogas and the exhaust gases when the biogas was burned. Measurements were performed at several partner pig farms close to the case study plant that rear pigs from 45 to 110 days of life. At these farms there were relatively small horizontal-type biodigestors (approximately 450 m^3) in which the input consisted only in pig wastes without previous treatment. The hydraulic retention time was approximately 30 days and the entrance flow 8 m^3 d^{-1}.

Table 8 shows the average chemical composition and calorific values of the biogas. To analyze the composition of biogas, a biogas analysis kit was used (Alfakit, Brazil) which is based on colorimetric methods.

	Average chemical composition	Reference values[1]
CH_4 (v/v%)	45-65	57
CO_2 (v/v%)	35-55	34
H_2S (v/v%)	>1020	-
CH_4/CO_2	0.82-1.86	1.7
HHV (kJ Nm^{-3})	17996-25995	-
LHV(kJ Nm^{-3})	16200-23400	-

[1]Data reproduced from Silva et al. (2005); HHV is Higher Heating Value; LHV is Lower Heating Value

Table 8. Average chemical composition of biogas

At the time this study was completed the farms were burning only biogas to avoid harmful emissions and to provide a better disposal/reuse options for the waste. At all farms there were simple flares to burn the gas, and thus the gaseous emissions were evaluated. CO, SO_2, NO, NO_2, O_2, and C_xH_y were measured using a Greenline MK2 (Eurotron) analyzer and the sampling point was located at the top of the chimney. Results are not presented in this docu-

ment because each burner presented different burning efficiencies and they were still in the adjustment phase. However, by the end of the project, all gaseous emissions were below the limits imposed by Brazilian Legislation. The SO_2 requires greater caution due to the presence of H_2S and therefore a pre-treatment has to be considered before the process can be considered adequate. H_2S can easily react with iron oxides and hydroxides, requiring the presence of water, and this can thus be considered a good method to remove the H_2S from biogas (Zichari, 2003).

5. Conclusions

The water balance analysis carried out considering all processing steps at the case study plant indicated that the minimization of the fresh water consumption at the four major consumption points could account for some 806 m^3 d^{-1}. Concerning wastewater reuse, the four streams identified as having a real possibility for reuse totaled approximately 1383 m^3 d^{-1}. These need simple reconditioning treatment before application to processes without direct contact with food products, that is, in non-potable uses (*e.g.* cooling water, toilet flushing water or irrigation around the plant), thus saving fresh potable water. The theoretical fresh water reduction after water minimization and wastewater reuse was 25.4% with a financial saving of around $434,622.00 per year.

Additionally, to reduce even further the percentage of fresh water consumption, indirect wastewater reuse could be carried out after reconditioning by applying tertiary treatments, such as advanced oxidation processes (AOPs), to treat the secondary effluent (after secondary activated sludge treatment). The tertiary-treated water effluent could then be used in other processes without contact with food products. The tertiary treatment model proposed was a heterogeneous AOP system ($UV/TiO_2/H_2O_2$) which can be applied to urban, rural or industrial effluents where the factors inhibiting their reuse as water of potable quality are the presence of suspended solids (even at low concentration), dissolved organic matter, recalcitrant micro-pollutants (trace compounds) and high concentrations of nitrate and nitrite. However, laboratory tests should be carried out with real wastewater to evaluate the efficiency of each process step.

For the treatment of the effluent from the case study poultry hatchery, a chemical or physicochemical process would be the best option due to the low biodegradability of the effluent (COD/BOD_5 = 4.6) and the presence of persistent compounds, which are not removed by biological processes. All treatments evaluated, particularly the photo-Fenton reaction, resulted in an increased biodegradability of the effluent, in other words, an increase in the portion of the material susceptible to degradation by biological processes. Thus, a biological process should be added as a final step in the effluent treatment, as a post-treatment mainly to remove the previously-formed more biodegradable compounds (with lower molar mass) and the nutrients that are not eliminated by the physicochemical process, *e.g.*, nitrate. Also, a comparison of the pros and cons (especially costs and efficiency) of the two treatments (a) photo-Fenton + simple biological treatment (such as stabilization ponds) and (b) UV/

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TiO_2/H_2O_2 could be carried out in terms of their effectiveness in the treatment of slaughterhouse secondary effluent. Hence, the most important consideration to be evaluated is which treatment can ensure that the standards and limits set by legislation are achieved, thus avoiding undesirable impact on the environment (such as the discharge of persistent organic compounds into rivers) and providing economic benefits.

Regarding the solid wastes, the substitution of 10 wt% of the sawdust with the biosolids originating from the physicochemical wastewater treatment can increase the thermal energy production by approximately 4% compared to the combustion of sawdust alone, leading to an economy of 1950 tons per year of sawdust besides providing savings in relation to landfill disposal. Additionally, co-combustion is the most feasible option for energy recovery from this waste in Brazil, making it possible to control the burning process, to avoid the occurrence the fouling and slagging and to meet the emission limits established in the relevant legislation. Considering that wood and wood-based materials are extensively used as fuels for thermal energy generation in the Brazilian food industry, the mixture of such a small mass fraction of this solid waste with sawdust should not require significant changes to the current operating conditions.

Industrial solid wastes must be disposed of safely, and co-firing them with sawdust was shown to be profitable using a pilot-scale cyclone combustor in studies currently underway in our research group. The biogas produced from pig wastes has great potential to become another important bioenergy option for the Brazilian agroindustrial sector. Additionally, anaerobic digestion has other environmental benefits besides the production of a renewable energy carrier, which include the possibility of nutrient recycling and reduction of waste volumes. Nevertheless, studies are needed to investigate the effects of variations in the input to a biodigestor, how the waste composition influences the overall stability of the process and the product quality, and options for the biogas application.

The comprehensive technical-scientific analyses of the actions concerning water, wastewater and solid waste management carried out in the case study meat processing plant indicated that environmentally, financially and socially sustainable practices can be successfully implemented in any type and size of food processing plant.

Nomenclature

AOPs – Advanced Oxidation Processes

BSE – Bovine Spongiform Encephalopathy

BTEX – Benzene, Toluene, Ethyl-benzene and (o-,m-,p-)xylenes

BOD_5 – Biochemical oxygen demand (5 days)

COD – Chemical oxygen demand

DWTP – Drinking Water Treatment Plant

HHV – Higher Heating Value

LFP – Biosolids originating from the physicochemical treatment of the meat processing industry wastewater

LFPSD 1:9 – Mixture of LFP and SD in a mass ratio of 1:9

LHV – Lower Heating Value

PAH – Polycyclic Aromatic Hydrocarbons

PCB – Polychlorinated Biphenyls

PCDD/PCDF – Polychlorinated dibenzo-p-dioxins and Polychlorinated dibenzofurans

SD – Wood Sawdust

TEF – Toxicity Equivalent Factor

TOC – Total Organic Carbon

W2M – Water and Wastewater Management

WWTP – Wastewater Treatment Plant

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Seaweeds for Food and Industrial Applications

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Additional information is available at the end of the chapter

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1. Introduction

Marine macroalgae, or the term seaweeds, are plant-like organisms that generally live attached to rock or other hard substrata in coastal areas. The classification into divisions is based on various properties such as pigmentation, chemical nature of photosynthetic storage product, the organization of photosynthetic membranes, and other morphological features. Traditionally, they belong to four different groups, empirically distinguished since the mid-nineteenth century on the basis of color: **blue-green algae** (phylum: Cyanophyta, up to 1500 species), **red algae** (phylum: Rhodophyta, about 6000 species), **brown algae** (phylum: Ochrophyta, classes: Phaeophyceae, about 1750 species), and **green algae** (phylum: Chlorophyta, classes: Bryopsidophyceae, Chlorophyceae, Dasycladophyceae, Prasinophyceae, and Ulvophyceae, about 1200 species). However, each of these groups has microscopics, if not unicellular, represantatives. All seaweeds at some stage in their life cycles are unicellular, as spores or zygotes, and may be temporarily planktonic. The blue-green algae are widesperead on temperate rocky and sandy shores and have occasionally been acknowledged in seaweed floras. Seaweeds are found growing throughouth the world oceans and seas none is found to be poisonous (Bold and Wyne, 1985; Guiry, 2009; Lobban and Harrison, 2000). Why seaweed is important? Most people don't realize how important marine macroalgae are, both ecologically and commercially. In fact, seaweeds are crucial primary producer in oceanic aquatic food webs. They are rich both in minerals and essential trace elements, and raw materials for the pharmaceutical and cosmetics industry (Chapman, 1970). Seaweed is a very versatile product widely used for food in direct human consumption. Its classified taxonomically as algae and they represent a food group that is not normally ingested in unprocessed form to any great extent in Western societies. Humankind is no strangers to the use of algae as a food source. Even if seaweeds have been used as a human food since ancient times, particularly in the region bounded by China, the

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Korean Peninsula and Japan. But the commercial exploitation of this resource is only a few decades old, after World War II, when the focus was set on a possible insufficient protein supply due to the rapid increase of the world population. Today those countries are the largest consumers of marine algae as food. However, as nationals from these countries have migrated to other parts of the Earth, the demand for seaweedfor food has followed them, for example, in some parts of the North and South America. Nowadays, seaweeds are major coastal resources which are valuable to human consumption and environment in many countries. Edible seaweeds were widely consumed, especially in Asian countries (e.g., Japan, China, Korea, Taiwan, Singapore, Thailand, Brunei, Cambodia, and Vietnam, but also in South Africa, Indonesia, Malaysia, Belize, Peru, Chile, the Canadian Maritimes, Scandinavia, South West England, Ireland, Wales, California, Philippines, and Scotland) as fresh, dried, or ingredients in prepared foods. Their photosynthetic mechanism is similar to that of land-based plants. They are generally more efficient in converting solar energy into biomass, mainly because of their simple cellular structure and being submerged in an aqueous environment with access to water, CO_2, and other nutrients. Same time, macroalgae are considered as the food supplement for 21st century, because they contain proteins, lipids, polysaccharides, minerals, vitamins, and enzymes. In common, seaweeds are rich in vitamins A, E, C, and Niacin with similar content in red algae (Rhodophyta), brown algae (Ochrophyta) and green algae (Chlorophyta). The concentration of vitamins B_{12}, B_1, panthothetic acid, folic, and folinic acids are generally higher in greens and reds than in browns. The brown algae possess organic iodine in greater amounts. Marine algae are similar to oats in protein and carbohydrate values. The green and red algae appear higher in crude protein far tested about 2 to 4 %. All algae contain high content of cabohydrates (sugar and starches) in polysaccharide biochemical structure which is a natural nontoxic colloidal substance that has been used as mucilaginous material referred to as gel. The nutrients composition of seaweed vary and is affected by species, geographic area, season and temperature of water. These sea-vegetables are of nutritional interest as they are low calorie food, but rich in vitamins, minerals and dietary fibres. Seaweeds, which have traditionally been used by the Western food industry for their polysaccharide extractives 'alginate, carrageenan and agar' also contain compounds with potential nutritional benefits. Seaweeds have recently been approved in France for human consumption (as vegetables and condiments), thus opening new opportunities for the food industry. These seaweed ingredients must meet industrial and technical specifications and consumer safety regulations. It is also an ingredient for the global food and cosmetics industries and is used as fertilizer and as an animal feed additive. Total annual value of production is estimated at almost US$ 6 billion of which food products for human consumption represent US $ 5 billion. Total annual use by the global seaweed industry is about 8 million tonnes of wet seaweed. Seaweed can be collected from the wild but is now increasingly cultivated. It falls into three broad groups based on pigmentation; brown, red and green seaweed. Use of seaweed as food has strong roots in Asian countries such as China, Japan and the Republic of Korea, but demand for seaweed as food has now also spread to North America, South America and Europe. China is by far the largest seaweed producer followed by the Republic of Korea and Japan but seaweeds are today produced in all continents. Red and brown

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seaweeds are also used to produce hydrocolloids; alginate, agar and carrageenan, which are used as thickening and gelling agents. Today, approximately 1 million tonnes of wet seaweed are harvested and extracted to produce about 55 000 tonnes of hydrocolloids, valued at almost US $ 600 million (McHug, 2003).

2. Historical background on the use of seaweeds

The use of seaweed as food has been traced back to the fourth century in Japan and the sixth century in China. In 1750's, an English physician successfully used ash from kelp (Phaeophyceae) which is rich in iodine to treat goiter. Kelp was also used to treath obesity in 19 th. century, and agar was used as a laxative. Seaweeds were used as a source of iodine. And their crude extracts were used for clarification in brewing. Another hydrocolloid, carrageen, found initially in the red seaweed *Chondrus crispus* was known in Ireland since 1810. Alginic acid, a hydrocolloid found in all brown seaweeds, was discovery first by Charles Stanford in the 1880s. Development of a large scale alginate industry began California and in Scotland in the late 1920s and early 1930s, respectively. *Laminaria japonica* was cultivated in China from the 1950s. The hydrocolloids have found increasing industrial and food applications in those years. Today China, Japan and the Republic of Korea are the largest consumers of seaweed as food. However, as nationals from these countries have migrated to other parts of the world, the demand for seaweed for food has followed them, as, for example, in some parts of the United States of America and South America. Increasing demand over the last fifty years outstripped the ability to supply requirements from natural (wild) stocks. Research into the life cycles of these seaweeds has led to the development of cultivation industries that now produce more than 90 percent of the market's demand. In Ireland, Iceland and Nova Scotia (Canada), a different type of seaweed has traditionally been eaten, and this market is being developed. Some government and commercial organizations in France have been promoting seaweeds for restaurant and domestic use, with some success. An informal market exists among coastal dwellers in some developing countries where there has been a tradition of using fresh seaweeds as vegetables and in salads. Various red and brown seaweeds are used to produce three hydrocolloids: agar, alginate and carrageenan. A hydrocolloid is a non-crystalline substance with very large molecules and which dissolves in water to give a thickened (viscous) solution. Alginate, agar and carrageenan are water-soluble carbohydrates that are used to thicken (increase the viscosity of) aqueous solutions, to form gels (jellies) of varying degrees of firmness, to form water-soluble films, and to stabilize some products, such as ice cream (they inhibit the formation of large ice crystals so that the ice cream can retain a smooth texture). Seaweeds as a source of these hydrocolloids dates back to 1658, when the gelling properties of agar, extracted with hot water from a red seaweed, were first discovered in Japan. Extracts of Irish Moss, another red seaweed, contain carrageenan and were popular as thickening agents in the nineteenth century. It was not until the 1930s that extracts of brown seaweeds, containing alginate, were produced commercially and sold as thickening and gelling agents. Industrial uses of seaweed extracts expanded rapidly after the Second World

War, but were sometimes limited by the availability of raw materials. In the 1950's, it was found that *Gracilaria* spp. treated with alkali produced higher strengthgels. After several years, *Gracilaria* can be cultivated successfully on a commercial scale, it is now used more widely. Once again, research into life cycles has led to the development of cultivation industries that now supply a high proportion of the raw material for some hydrocolloids. Today, approximately 1 million tonnes of wet seaweed are harvested and extracted to produce the above three hydrocolloids. Total hydrocolloid production is about 55 000 tonnes, with a value of US $ 585 million. Alginate production (US $ 213 million) is by extraction from brown seaweeds, all of which are harvested from the wild; cultivation of brown seaweeds is too expensive to provide raw material for industrial uses. Agar production (US $ 132 million) is principally from two types of red seaweed, one of which has been cultivated since the 1960-70s, but on a much larger scale since 1990, and this has allowed the expansion of the agar industry. Carrageenan production (US $ 240 million) was originally dependent on wild seaweeds, especially Irish Moss, a small seaweed growing in cold waters, with a limited resource base. However, since the early 1970s the industry has expanded rapidly because of the availability of other carrageenan-containing seaweeds that have been successfully cultivated in warm-water countries with low labour costs. Today, most of the seaweed used for carrageenan production comes from cultivation, although there is still some demand for Irish Moss and some other wild species from South America. Seaweed meal, used an additive to animal feed, has been produced in Norway, where its production was pioneered in the 1960s. It is made from brown seaweeds that are collected, dried and milled. Drying is usually by oil-fired furnaces, so costs are affected by crude oil prices. Approximately 50 000 tonnes of wet seaweed are harvested annually to yield 10 000 tonnes of seaweed meal, which is sold for US $ 5 million. Fertilizer uses of seaweed date back at least to the nineteenth century. Early usage was by coastal dwellers, who collected storm-cast seaweed, usually large brown seaweeds, and dug it into local soils. The growth area in seaweed fertilizers is in the production of liquid seaweed extracts. These can be produced in concentrated form for dilution by the user. Several can be applied directly onto plants or they can watered in, around the root areas. There have been several scientific studies that prove these products can be effective. In 1991, it was estimated that about 10 000 tonnes of wet seaweed were used to make 1 000 tonnes of seaweed extracts with a value of US $ 5 million. However, the market has probably doubled in the last decade because of the wider recognition of the usefulness of the products and the increasing popularity of organic farming, where they are especially effective in the growing of vegetables and some fruits. Cosmetic products, such as creams and lotions, sometimes show on their labels that the contents include "marine extract", "extract of alga", "seaweed extract" or similar. Usually this means that one of the hydrocolloids extracted from seaweed has been added. Alginate or carrageenan could improve the skin moisture retention properties of the product. Pastes of seaweed, made by cold grinding or freeze crushing, are used in thalassotherapy, where they are applied to the person's body and then warmed under infrared radiation. This treatment, in conjunction with seawater hydrotherapy, is said to provide relief for rheumatism and osteoporosis (De Roeck-Holtzhauer, 1991).

3. Sources of seaweed

A seaweed may belong to one of several groups of multicellular algae: the red algae, brown algae and green algae,. As these three groups are not thought to have a common multicellular ancestor, the seaweeds are a polyphyletic group. In addition, some tuft-forming blue green algae (Cyanobacteria) are sometimes considered as seaweeds — "seaweed" is a colloquial term and lacks a formal definition. Two specific environmental requirements dominate seaweed ecology. These are the presence of seawater (or at least brackish water) and the presence of light sufficient to drive photosynthesis. Another common requirement is a firm attachment point. As a result, seaweeds most commonly inhabit the littoral zone and within that zone more frequently on rocky shores than on sand or shingle. Seaweeds occupy a wide range of ecological niches. The highest elevation is only wetted by the tops of sea spray, the lowest is several meters deep. In some areas, littoral seaweeds can extend several miles out to sea. The limiting factor in such cases is sunlight availability. The deepest living seaweeds are some species of red algae. A number of species such as *Sargassum* have adapted to a fully planktonic niche and are free-floating, depending on gas-filled sacs to maintain an acceptable depth. Others have adapted to live in tidal rock pools. In this habitat seaweeds must withstand rapidly changing temperature and salinity and even occasional drying

3.1. Red seaweeds

Red seaweeds have had a more diverse evolution than the green and the brown. Many species cannot stand desiccation and dominate the inter-tidal rock pools. Others tolerate desiccation, such as the purple laver which can often be seen stretched out like a dry black film over mussle beds on rocky beaches. Red seaweeds such as *Polysiphonia lanosa* are epiphytes, these are plants that grow on other plants for physical support. In this case the epiphyte benefits from the host's buoyancy lifting it closer to the sunlight. The red colour of the seaweeds is due to the larger amount of red phycoblin pigments overriding the green pigment chlorophyll. The main biomass of red algae worldwide is provided by the Corallinaceae and Gigartinaceae. The red algae *Gelidium*, *Gracilaria*, *Pterocladis* and other many red algae are used in the manufacture of the important agar, used widely as a growth medium for microorganism and other biotechnological and food applications. Another important red seaweed alga is *Eucheuma* used in the production of Carrageenan, an important product used in cosmetics, food processing and industrial uses, as well as a food source. Some of the most significant caargeenan species include *Betaphycus gelatinae*, *Eucheuma denticulatum*, and several species of the genus *Kappaphycus* including *K. alvarezii* (Lobban and Harrison, 1994).

3.2. Brown seaweeds

Laminaria sp. ′kombu′, Undaria sp. ′wakame′, Hizikia fusiforme ′hiziki′ is edible and an important resource Asia countries especiaqlly China and Japan. They are consumed raw, boiled or dried material with sweetened green beans, jelly, crushed ice, and coconut milk in Southern Vietnam (Tsutsui et. al., 2005). Laminaria sp. was in plentiful supply in Japan, mainly from the northern island of Hokkaido, where several naturally growing species were available.

Undaria sp. has been harvested from natural resources for many years in the China, Japan and Korean region. Another algae Cladosiphon okamuranus 'mozuku' as salad in Okinawa-Japan (Thoma, 1997; Zhang et.al., 2007; Zhu et.al., 2009). Sargassum sp. is known as horsetail and it is eaten as soup or dressed with soybean sauce, or after being processed in Korea (Madlener, 1997) and in Hawaii (Novaczek, 2001). In the Pasific region, Rosenvingea sp. or slippery cushion, Turbinaria or spiny leaf are eaten as soup or omelet Colpomenia sp. or paperly sea bubble as chop soup, stew or salad. Hydroclatharus sp. or sea colonder, Dictyota sp. or brown, Padina or sea fan ribbon weeds as a food dressing, soup or stew (Novaczek, 2001).

3.3. Green seaweeds

Green seaweeds are found on both sandy and rocky beaches. Many can tolerate low salinity and will colonise areas where rivers meet the sea. The green colour of the seaweed is due to the green pigment chlorophyll required for the photosynthesis of light. Using only chlorophyll means that green seaweeds require good levels of light and therefore will not thrive in shadowed areas or too any depth. It does give them an advantage, the ability to live higher up shore without competition from the red or brown seaweeds. The green saeaweeds *Ulva* sp., *Enteromorpha* sp., *Monostroma* sp., *Caulerpa* sp., *Codium* sp., are commonly known as source of food. In Asia countries especially Japan, dried fronds of edible *Monostroma* sp. and *Enteromorpha* sp are being known as 'aonori-green laver-ele ele-lulua-lumi boso'.These algae are eaten by humans as edible raw, dried, or cooked. They used in preperation of 'nori-jam' soup (Lobban and Harrison, 1994; Novaczek, 2001).

4. Nutritonal composition of edible seaweeds

Proximate composition (moisture, ash, protein and oil content), total dietary fibre content and physicochemical properties of three brown and two red edible Spanish seaweeds, namely: *Himanthalia elongata* (sea spaghetti), *Bifurcaria bifurcata, Laminaria saccharina* (sweet kombu), *Mastocarpus stellatus* and *Gigartina pistillata* were studied. Ashes (24.9–36.4%) were high in all samples. Protein content ranged from 10.9 to 25.7%, being much higher for *Laminaria* (25.7%) followed by the red seaweeds (15.5–21.3%). Minor components were lipids (0.3–0.9%) in all samples except for *Bifurcaria* (5.6%).In conclusion, these seaweeds can be estimated as a good source of food fibre, protein and minerals for human consumption (Gómez-Ordóñez et al., 2010). Mineral content was determined in several brown (*Fucus vesiculosus, Laminaria digitata, Undaria pinnatifida*) and red (*Chondrus crispus, Porphyra tenera*)edible marine sea vegetables. Seaweeds contained high proportions of ash (21.1–39.3%) and sulphate (1.3–5.9%). In brown algae, ash content (30.1–39.3%) was higher than in red algae (20.6–21.1%). Edible brown and redseaweeds could be used as a food supplement to help meet the recommended daily intake of some essential minerals and trace elements (Rupérez, 2002). Sea spaghetti (*Himanthalia elongata*), Wakame (*Undaria pinnatifida*), and Nori (*Porphyra umbilicalis*), on fatty acid composition, amino acid profile, protein score, mineral content and antioxidant capacity in low-salt meat emulsion model systems. The addition of seaweeds caused an increase in ω-3 polyunsaturated fatty acids (PUFA) and a decrease in the ω-6/ ω-3 PUFA ratio. In general, addition

of seaweeds to products increased the concentrations of K, Ca, Mg and Mn. The presence of Nori caused an increase in levels of serine, glycine, alanine, valine, tyrosine, phenylalanine and arginine, whereas Wakame and Sea Spaghetti produced no significant changes in amino acid profiles in the model systems. López-López *et.al*., 2009). The nutritional compositions of 34 edible seaweed products of the *Laminaria* sp.,*Undaria pinnatifida*, *Hizikia fusiforme* and *Porphyra* sp. varieties were analyzed.The marine macroalgae varieties tested demonstrated low lipid contents with 2.3 ± 1.6 g/100 g semi-dry sample weight(s.w.) and proved to be a rich source of dietary fibre (46.2 ± 8.0 g/100 g s.w). The pure protein content of seaweed products varied widely (26.6 ± 6.3 g/100 g s.w. in red algae varieties and 12.9 ± 6.2 g/100 g s.w. in brown algae varieties). All essential amino acids were detected in the seaweed species tested and red algae species featured uniquely high concentrations of taurine when compared to brown algae varieties (Dawczynski *et al*., 2007). The total lipid, protein, ash and individual fatty acid contents of edible seaweeds that had been canned (*Saccorhiza polyschides* and *Himanthalia elongata*) or dried (*H. elongata*, *Laminaria ochroleuca*, *Undaria pinnatifida*, *Palmaria* sp. and *Porphyra* sp.) Total lipid content ranged from 0.70±0.09 to 1.80±0.14 g/(100 g dry weight). The four most abundant fatty acids were C16:0, C18:1ω9, C20:46 and C20:5ω3. Unsaturated fatty acids predominated in all the Brown seaweeds studied, and saturated fatty acids in the red seaweeds, but both groups are balanced sources of ω3 and ω6 acids. Ash content ranged from 19.07±0.61 to 34.00±0.11 g/(100 g dry weight), and protein content from 5.46±0.16 to 24.11±1.03 g/(100 g dry weight) (Sanchez-Machado, *et. al*.,2004).

5. Edible seaweed in foods

Red macro-algae (*Gracilaria* spp.) are used as a fresh food in Hawaii. Species commonly marketed include *G. coronopifolia*, *G. parvispora*, *G. salicornia* and *G. tikvahiae*, however, these seaweeds have a short postharvest life of about 4 days (Paul and Chen, 2008). Seaweeds are a rich source of phytochemicals having anti-oxidant and antimicrobial properties. Presence of fibres and minerals helps in improving the mineral content reduce the salt content. The adding of seaweeds or their extracts to food products will help in reducing the utilization of chemical preservatives (Gupta and Abu-Ghannam, 2011). Edible sea‐weeds contain various bioactive compounds with potential health benefits and their use as functional ingredients opens up new prospects for food processing, meat product formula‐tions included. Seaweeds basically contain high proportions of polysaccharides along with various other potentially beneficial compounds such as good-quality protein and essen‐tial fatty acids, particularly long-chain n-3 polyunsaturated fatty acids (PUFAs). Alginates are the most abundant ionicpolysaccharides present in brown seaweeds (Fernández-Martín *et al.,* 2009). Some seaweed polysaccharides are used by food industry as texture modifiers because of their high viscosity and gelling properties. In Asia seaweeds have been used for centuries in salads, soups and as low calorie dietetic foods. The diatery fibre which constitutes 25-75 % of the dry weight of marine algae and represents their major component, is primarily souble fibre.(Jiménez-Escrig and Sánchez-Muniz, 2000). In particular, miyeok (*Undaria pinnatifida*) is often served in soup,salad, and sidedishes.

Gamma irradiation at 10 kGy is sufficient to sterilizefreeze-dried miyeokguk without significant deterioration in the sensory quality,and thus,the freeze-dried and irradiated miyeokguk at 10 kGy fulfills themicrobiological requirements as spacefood (Song *et al.*, 2012). The sausages were produced with two types of carrageenan (i- and j-) in four levels (0%, 1%, 2% and 3%). Carrageenan had a better effect on such characteristics as pH, weight loss and lipid oxidation of the sausages, as well as, on sensory attributes. The carrageenan level of 3% negatively affected the firmness of the sausages.Carrageenan added at levels up to 2% had a positive effect on the physicochemical and microbiological characteristics of the lowfat fermented sausages. (Koutsopoulos *et al.*, 2008). Cultivated *Ulva rigida* was utilized by using marination technology. Fresh and boiled (at 100˚C for 2 min.) *Ulva rigida* were marinated with two different formulations by using 2 % lemon salt and 2 % vinegar. The marination of *Ulva rigida* were made at room temperature for 20 days. Marinated fresh and boiled ulva rigida by using lemon salt and vinegar can be an alternative for human foods (Kılınç *et al.*, 2011). Breads were made by using *Lemna minor* (Tekogul *et al.*, 2011) and *Ulva rigida* (Turan *et al.*, 2011). The shelf-life of breads by using *Ulva rigida* were determined as unacceptable on day 5 at room temperature whereas on day 10 at 4˚C. When compared with control groups, the shelf-life of breads containing *Ulva rigida* were determined longer shelf-life. Breads prepared with *Ulva rigida* extended the shelf-life of breads for 2 days in two different storage period. *Lemna minor* extended the shelf-life of breads. The shelf-life of breads with *Lemna minor* were extended the acceptable limit on day 8 at room temperature whereas on day 12 at 4 ˚C. But control group extended this acceptable limit on day 3 at room temperature, on day 8 at 4 ˚C (Tekogul *et al.*, 2011).

5.1. Fermented seaweed

Brown edible seaweeds as a sole source of nutrition for the growth of lactic acid bacteria. Growth kinetics of lactic acid bacteria (LAB; *Lactobacillus plantarum*) was studied using three species of edible Irish brown seaweeds *Himanthalia elongata*, *Laminaria digitata* and *Laminaria saccharina*. The results of this study present an indication of the potential of fermentation of seaweeds using LAB with a possibility towards the development of a range of functional foods (Gupta *et al.*, 2011). Low molecular weight polysaccharides from seaweed as prebiotics. *Gelidium* seaweed showed significant increase in bifidobacterial populations. Agar and alginate bearing seaweeds indicate prebiotic potential (Ramnani *et al*., 2012). Brown macroalgae contain high concentration of mannitol and laminarian. *Clostrium acetobutylicum* ferments these seaweed extract substrates to butanol. Seaweed fermentation exhibited triauxic growth: glucose-mannitol- laminarin.Butanol yields in seaweed and pure glucose fermentations were comparable (Huesemann *et al*., 2012).

5.2. Seaweeds used as fertilizer and biogas production

Seaweed are used as a fertilizer which is suitable for use in organic agriculture (López-Mosquera *et al.,* 2011). Energy-rich methane can be harnessed from seaweed deposits by anaerobic digestion. However, the high heavy metal content in the seaweed and its digestates limits their use as fertilisers. The efficient utilisation of seaweed for biogas

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production, and the partial heavy metals mobilisation to enable the metal removal for improved fertiliser quality (Nkemka and Murto, 2012). The red alga *Chondracanthus squarrulosus* was cultured under semi-controlled conditions to valuate growth (biomass production) with agricultural fertilizers (ammonium nitrate, ammonium sulphate and urea) vs. analytical grade inorganic salts; sodium nitrate (analytical grade) and seawater were used as controls (Pacheco-Ruiz *et al*., 2004).

6. Conclusion and outlook

Seaweeds are being studied on the use of many industrial applications such as food, cosmetics, chemistry, paint, medicine, etc., at nowadays. In Western countries has traditionally concentrated on the extraction of compounds used by pharmaceutical, cosmetics, and food industries (Wijesinghe and Jeon, 2012a). Biologically active compounds of seaweeds (phlorotannins, carotenoids, alginic acid, fucoidan, peptides) have been demonstrated to play a significant role in prevention of certain degenerative diseases such as cancer, inflammation, arthritis, diabetes and hypertension. Therefore, seaweed derived active components, whose immense biochemical diversity looks like to become a rich source of novel chemical entities for the use as functional ingredients in many industrial applications such as functional foods, pharmaceuticals and cosmeceuticals (Wijesinghe and Jeon, 2012b). Commercially available varieties of marine macroalgae are commonly used to as ′seaweeds′ Conventional, macroalgae can be classified as brown algae (Phaeophyta), red algae (Rhodophyta), and green algae (Chlorophyta), depending on their nutrient and chemical composition. Red and brown algae are mainly used as human food sources. The protein content of seaweed species varies greatly and demonstrates a dependence on such factors as season and environmental growth conditions. For example, the protein content of brown algae species, e.g., *Laminaria japonica*, *Hizikia fusiforme* or *Undaria pinnatifida*, is relatively low with 7–16 g/100 g dry weight (d.w.) (Jurković *et al.*, 1995; Kolb *et al.*, 1999; Rupérez and Saura-Calixto, 2001). On the other hand, red algae, e.g., *Palmaria palmata* (Dulse) and *Porphyra tenera* contain 21–47 g protein/100 g d.w. (Fleurence, 1999; Rupérez and Saura-Calixto, 2001).The protein in algae contains all essential amino acids (EAA) and all EAA are available throughout the year although seasonal variations in their concentrations are known to occur (Galland-Irmouli *et al.*, 1999). For example, the proportion of EAA is 45–49% in *Hizikia* sp. and *Eisenia bicyclis* (Arame). In both these brown algae varieties, Ecological factory is the first limiting EAA, followed by Lys (Kolb *et al*., 1999). The EAA contents of some species (e.g., *Porphyra* sp.) can be compared with those of soy and egg protein (Fleurence, 1999; Galland-Irmouli *et al*., 1999). In addition to, high concentrations of Arg, Asp and Glu peptides are found in many macroalgae species (Fleurence, 1999). The fat content of marine macroalgae accounts for 1–6 g/100 g d.w. (Fleurence *et al*., 1994; Jurković *et al*., 1995; Herbreteau *et al*., 1997). In some brown algae varieties, such as *Hizikia* sp. and *Eisenia bicyclis* (Arame), only 0.7–0.9 g/100 g d.w. of fat content were found (Kolb *et al.*, 1999). Brown seaweeds are rich in fucose rich sulfated polysaccharides fucoidans (Wijesinghe and Jeon, 2012a). Polysaccharides produced by marine seaweeds form the basis of an economically important and expanding global industry. Key products are agars, agaroses, algins, and carrageenans.

These are used on ingredients in food, pharmaceuticals and diverse consumer products and industrial processes (Renn, 1997). Red algae (e.g., *Porphyra* sp.) have high concentrations of eicosapentaenoic acid (C20:5, ω -3, EPA),with 48.0–51.0% of total fatty acid methyl esters (FAME), and marginal concentrations of arachidonic acid (C20:4, ω-6, ARA), with 2.1–10.9% of total FAME and, linoleic acid (C18:2, ω -6, LA), with 1.3–2.5% of total FAME (Fleurence *et al*., 1994; Takagi *et al*., 1985). In contrast, brown algae (e.g., *Laminaria* sp., *Undaria* sp., *Hizikia* sp.) have high concentrations of oleic acid (C18:1, ω -9, OA) with 4.1–20.9% of total FAME, LA with 4.0–7.3% of total FAME as well as α-linolenic acid (C18:3, ω -3, ALA) with 3.6–13.8% of total FAME but low concentrations of EPA with 5.9–13.6% of total FAME (Fleurence *et al.*, 1994; Takagi *et al.*, 1985). Interestingly, in *Porphyra* sp., *Laminaria* sp., and *Undaria* sp., the concentrations of docosahexaenoic acid (C22:6, ω -3, DHA) and docosapentaenoic acid (C22:5, ω -3, DPA) were below the detection limit (less than 0.1% of total FAME (Fleurence *et al*., 1994; Takagi *et al.*, 1985).The types and abundance of carbohydrates vary strongly between algae species. Typical carbohydrates in red algae varieties consist of floridean starch (α-1.4-binding glucan), cellulose, xylan, and mannan. The water-soluble fibre fraction is formed by sulfur-containing galactans, e.g., agar and carrageen (Jiménez-Escrig and Sánchez-Muniz, 2000; Van den Hoek *et al*., 1993). The typical carbohydrates in brown algae varieties consist of fucoidan, laminaran (β-1.3-glucan), cellulose, alginates, and mannitol. Brown algae fibres are mainly cellulose and insoluble alginates. Alginates are Ca, Mg, or Na salts of alginic acid (1.4-linked polymer of β-*d*-mannuronic acid and α-*l*-guluronic acid). The amorphous, slimy fraction of brown algae fibres consists mainly of water-soluble alginates and/or fucoidan. Main reserve polysaccharides of Phaeophyta are laminaran (β-1.3-glucan) and mannitol (Kolb *et al.*, 1999; Dawczynski *et al*., 2007). The typical algae carbohydrates are not digestible by the human gastrointestinal tract and, therefore, they are dietary fibres. The content of total dietary fibre ranges from 33–50 g/100 g d.w. (Jiménez-Escrig and Cambrodon, 1999, Lahaye, 1991). Accordingly, the fibre content of seaweed varieties is higher than those found in most fruits and vegetables. The human consumption of algal fibre has been proven to be health-promoting and it benefits are well documented in the scientific literature. The consumption of this dietary fibre has been related to the following health promoting effects: **1)** its consumption promotes the growth and protection of the beneficial intestinal flora (Fujii *et al*., 1992, Goni *et al*., 2001), **2)** its consumption, in combination with high glycemic load foods, reduces the overall glycemic response, macroalgae fibre acts as a hypoglycaemic (Goni, Valdivieso, & Garcia-Alonso, 2000), **3)** its consumption greatly increases stool volume (Jiménez-Escrig & Sánchez-Muniz, 2000) and **4)** its consumption reduces the risk of colon cancer (Guidel-Urbano & Goni, 2002). In addition, seaweeds varieties are rich sources of vitamin C, vitamin B-complex, e.g., folic acid and B_{12}, and vitamin A precursors, such as β-carotene (McDermid and Stuercke, 2003, Takenaka *et al.*, 2001; Watanabe *et al*., 2002). Because the seaweed species are rich in beneficial nutrients, in countries such as China, Japan, and Korea, they have been commonly utilised in human alimentation (since ancient times) (Lahaye, 1991). For example, Japanese people consume more than 1.6 kg algae (d.w.) per year *per capita* (Fleurence, 1999). In addition to their importance as traditional the Asian foods, macroalgae species are utilised industrially as a source of hydrocolloids, such as agar, carrageen, and alginate (Jiménez-Escrig & Sánchez-Muniz, 2000). Over the past few decades, the consumption of seaweed products has increased

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in European countries. Currently, approximately 15–20 edible algae strains are being commonly marketed for consumption in Europe. These seaweed varieties differ greatly in their quality, colour, consistency, and nutrient content. Nowadays, for this reason the studies of algology evaluate and compares the nutrient and chemical contents of many commercially available seaweed products which were locally purchased in European food stores and speciality shops (Herbreteau *et al.,* 1997; Dawczynski *et al*., 2007).

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Due to the increase in world population (more than seven billion inhabitants) the global food industry has the largest number of demanding and knowledgeable consumers. This population requires food products that fulfill the high quality standards established by the food industry organizations. Food shortages threaten human health, and also the disastrous extreme climatic events make food shortages even worse. This collection of articles is a timely contribution to issues relating to the food industry. The objective of this book is to provide knowledge appropriate for students, university researchers, and in general, for anyone wishing to obtain knowledge of food processing and to improve the food product quality.

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