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Edited by Ana Novo de Barros and Irene Gouvinhas

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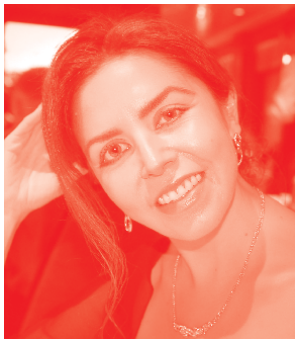

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Meet the editors

Ana Novo de Barros is an assistant professor with habilitation in the Chemistry Department, University of Trás-os-Montes e Alto Douro, Portugal where she is also the director of the Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB). She is the author of more than eighty-five international peer-reviewed papers and holder of eight national and international patents. She has supervised eight Ph.D. theses and forty master's theses. Her main research area is the valorization of by-products of different industries, giving an added-value for the circular economy and industrial symbiosis. She has been working in the evaluation of antimicrobial activity against gastrointestinal and diabetic foot wound bacteria of hospital patients, in the concept of One Health.

Irene Gouvinhas is a full-time integrated researcher at the University of Trás-os-Montes e Alto Douro, Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), Portugal, where she is collaborating on several R&D projects. She works in the areas of agrarian sciences and natural sciences with emphasis on the valorization of the major agrifood industrial by-products and waste for the recovery of compounds for several industrial applications. In her relatively short research career, she has already participated in several research projects funded by both national and European funds. She is the author of twenty-five JCR-indexed papers in specialized journals and two book chapters.

Contents

Preface

The progressive depletion of agro-diversity due to the emergence of new cultivars has led to a reduction of local varieties selected for millennia in traditional farming systems, which are now seriously threatened. The old varieties (landraces), now cultivated in traditional farming systems, are an important part of many production systems and are much appreciated for their taste and nutritional characteristics. A wide range of preharvest factors can modulate the appearance and nutritional value of the harvested products. These include biological factors (pathological, entomological, animal); physiological factors (physiological disorders, nutritional imbalances, maturity); environmental–cultural factors (climate, soils, water relations, light intensity); mechanical damage; and genetic variation. Creating and/or maintaining production conditions that minimize undesirable product appearance is essential. In recent years, consumers' perception of the roles played by the foods they ingest on their health status has evolved considerably. Foods are no longer seen only as a source of nutrients but are now viewed as a means to prevent disease and improve physical and mental well-being. This has changed consumer demands in the field of food production. Fruits, vegetables, and mushrooms are valuable sources of nutrients and contain many phytochemicals with biological activity. In addition, the use of genetic resources in the development of hypoallergenic and antioxidant products is an attractive solution. The identification/detection of antioxidants and antioxidant genes in different plant species is an interesting topic due to their importance for human health. A food traceability system is fundamentally based on product identification, data to trace, product routing, and traceability tools to ensure that consumers receive products that are safe and do not pose a threat to health. Another step in food valorization is the development of new products and new technologies for food processing.

This book contains fifteen chapters organized in three sections: "By-Products Valorization," "New Technologies," and "Innovation in the Food Sector."

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Section 1

By-Products Valorization

Chapter 1

Winery By-Products as Source of Bioactive Compounds for Pharmaceutical and Cosmetic Industries

Irene Gouvinhas and Ana Barros

Abstract

It is well established in the scientific community that agro-food wastes represent economic advantages and contribute to circular economy. For instance, wine industries of Região Demarcada do Douro involve the production of large quantities of by-products, such as stem, pomace, trimmed vine shoots, or wine lees, presenting a remarkable valuable composition in phytochemicals with putative health-promoting qualities. Nevertheless, the bioactive compounds obtained from these natural sources depends on the extraction process employed. In order to reduce production costs and optimize processes, new technologies—such as ultrasound-assisted extraction (UAE)—have been employed to decrease energy consumption and increase the product or process safety/control and quality. This work aims to characterize the phenolic compounds extracted from winery by-products (WBPs), namely grape stems, grape pomace, and wine lees of two grape (*Vitis vinifera* L.) varieties (Sousão and Tinta Barroca) from the same geographical site, as well as the antioxidant capacity. Wine lees and grape stems presented the highest concentration of phenolic compounds and the highest antioxidant capacity for Tinta Barroca variety, while grape pomace presented the highest values of these parameters for Sousão variety, demonstrating the high influence of the variety studied. Furthermore, wine lees revealed to be the winery by-product with the lowest antioxidant capacity and content of phenolics. These by-products revealed to be a rich source of phenolic compounds with high antioxidant capacities reveling to be of interest for pharmaceutical and cosmetic industries.

Keywords: Winery by-products, Ultrasound-assisted extraction, Bioactive compounds, Antioxidant capacity, Valorization

1. Introduction

The main strategies for the valorization of food wastes are related to their biotechnological transformation into chemicals or even the recovery of important substances, such as polyphenols that typically appear in the winery by-products (WBPs). Currently, the implemented alternatives for reducing the environmental impact of agronomic residues involves the development of new feeds and their use as soils amendments. Actually, these are the primary alternatives considered. Given their low added-value there is a need to search for new valorization alternatives.

Based on these premises, the content of bioactive phytochemicals of agro-food materials in general has allowed envisaging their use as donors of these kind of molecules to obtain materials that could contribute to enhance medical/nursing treatments.

Given the relevance of the winemaking companies, particularly at Douro region, and the high amount of underexploited wastes produced, the development of innovative applications for these materials urges [1]. On these materials ongoing research (also relevant studies developed by the research group) has revealed the valuable quantitative profile of bioactive compounds in WBPs, namely a variety of (poly) phenols and stilbenes that could be responsible for remarkable biological activities, such as anti-inflammatory, antioxidant, and antibacterial, among others [2–5].

However, for envisaging new applications for these materials, it is important to be aware about the close dependency of the phytochemical composition and therefore the biological power on an array of factors, namely the geographical growing conditions [3], the cultivar studied [4] and, most important, the extraction methodology employed [6]. In fact, the extraction methodology no just condition the phytochemical compounds obtained from a given plant material, but also is associated to environmental constraints, as well as to economic and toxicological issues depending on the solvent used [7]. To overcome these limitations, special attention has been paid to the extraction methods for bioactive compounds [8]. So, the use of eco-friendly techniques blended with reusable and non-toxic solvents is gaining a wide acceptance, due to its contribution to minimizing costs, heath related risks, and environmental impacts. As a valuable alternative to the traditional extraction methods, UAE arises as exceptional option to extract (poly)phenols, revealing to be an environment-friendly technology that offers several advantages over the conventional and non-conventional ones, such as a lower cost, versatility, and easily scale-up [7]. This technique has been already employed in diverse plant matrices and the outcomes reported have revealed it as one of the best alternatives to extract phenolic compounds from winery wastes [7, 9].

Based on these compositional features, potential applications for these materials, and specifically for grape stems, have been described by the research team, such as the spirits production, leading to an industrial alternative to traditional distilled spirits [10]. Beyond this application, recently, a preliminary study developed by the team demonstrated the stem extracts capacity to inhibit the growth of foot wound ulcers multidrug resistance bacteria (*S. aureus* and *Enterobacter aerogenes*) through disc diffusion and minimum inhibitory concentration assays [5, 11]. Whereby, WBPs are valuable candidates as wound healing agents for instance. Additional studies of our group also revealed that the quantitative (poly)phenolic profile of grape stems remains almost constant during storage for months, leading to the possibility to access this by-product all year-round, due to the preservation of the phytochemical composition [2], and thus, the biological activity expected.

In this work, we intend to generate new knowledge on the potential ability of WBPs (wine lees, grape pomace, and grape stems) bioactive compounds to be further used in pharmaceutical and cosmetic industries, using a sustainable and green extraction way, namely ultrasound-assisted extraction (UAE), enhancing the regional and circular economy.

2. Material and methods

2.1 Chemicals

Folin–Ciocalteu's reagent, 3,4,5-trihydroxybenzoic acid (gallic acid), acetic acid, both extra pure (>99%), and sodium hydroxide were purchased from Panreac

(Panreac Química S.L.U., Barcelona, Spain). Sodium nitrate, aluminum chloride, and sodium carbonate, all extra pure (>99%), and methanol were acquired from Merck (Merck, Darmstadt, Germany). Sodium molybdate (99.5%) was purchased from Chem-Lab (Chem-Lab N.V., Zedelgem, Belgium). The compounds 2,2-diphenyl-1-picrylhidrazyl radical (DPPH$^{\bullet}$), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)diammonium salt (ABTS$^{\bullet+}$), and potassium phosphate were obtained from Sigma-Aldrich (Steinheim, Germany), as well as the standards compounds for the chromatographic separation. Additionally, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Fluka Chemika (Neu-Ulm, Switzerland).

Ultrapure water was obtained using a Millipore water purification system. Chromatography solvents were of HPLC grade according to the analysis performed.

2.2 Sampling

The present work was carried on WBPs, namely, grape stems, grape pomace and wine lees of two varieties of *Vitis vinifera* L. (Sousão and Tinta Barroca), which are traditionally cultivated in the *Região Demarcada do Douro*, in northern Portugal. Plant material came from a farm located in Cima Corgo sub-region (Upper Corgo) (Sanfins do Douro - GPS: 41.1656, −7.2912, Average Altitude.: 730 m, vineyard altitude from 690 to 730 m), as demonstrated in **Figure 1**, where geology is essentially characterized by schist formations with occasional outcrops of granite in Mediterranean-like climatic conditions [12]. No irrigation was applied in the field trial of this investigation. WBPs were collected in 2020 growing season, at the wine company which possesses this vineyard. Once collected, samples were lyophilized, grounded to a fine powder, and stored, protected from light, at room temperature until analysis.

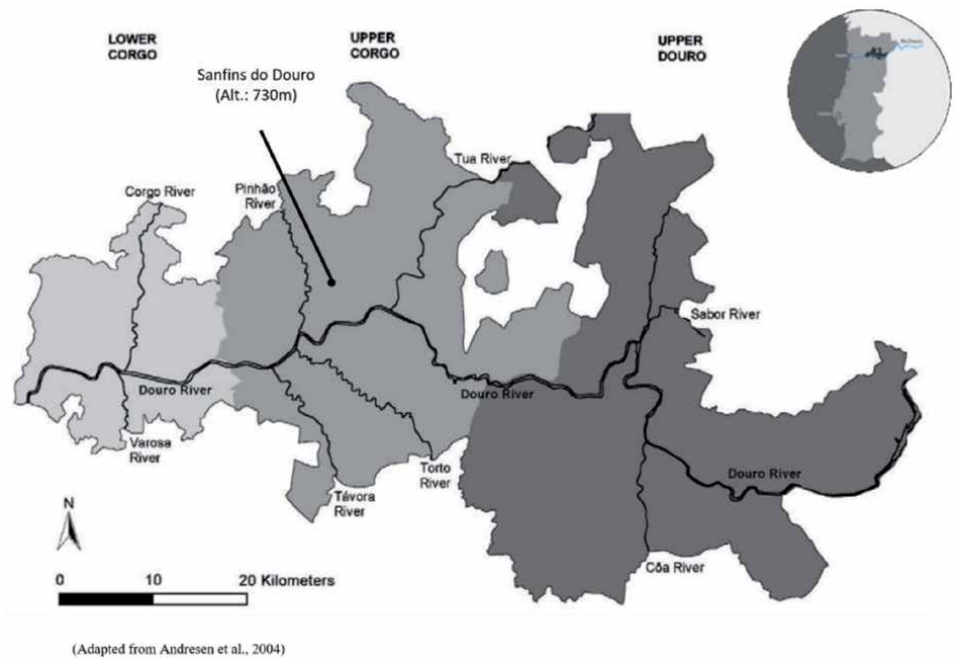

Figure 1.
Geographical origin of WBPs from the Região Demarcada do Douro *(Portugal).*

2.3 Ultrasound-assisted extraction (UAE) of bioactive compounds from olive seeds

For the phenolic compounds extraction, the protocol used was previously described by Lameirão et al. with some modifications [8]. The UAE was performed with an ultrasonic apparatus (VCX 500 Vibra-Cell™, Newtown, Connecticut, USA), using a 13 mm diameter tip with amplitude, temperature and time controller. The amplitude was employed at 50%. The powdered samples (2.5 g) were extracted with 50 mL of methanol:water (70:30, v/v) into the ultrasonic apparatus during 40 min and at 70°C. After ultrasonic extraction, the methanolic extracts were centrifuged (13,000 rpm, 4°C) for 15 min (Sigma Centrifuges 2–16 K, Germany) and filtered. Samples were stored at 4°C until analysis.

2.4 Phenolic content

The content in total phenols, flavonoids, and *ortho*-diphenols was determined according to spectrophotometric methodologies previously reported [13].

Briefly, the content of total phenolics in olive seed extracts was evaluated by the Folin–Ciocalteu spectrophotometric method, using gallic acid as standard, being the results expressed as mg of gallic acid per gram of dry weight (mg GA g^{-1} DW).

The content of *ortho*-diphenols in olive seeds was determined by adding Na_2MoO_4 (50 g L^{-1}) to the samples appropriately diluted, reading the absorbance at 375 nm. For the quantification, the gallic acid was used as standard. Results were expressed as mg GA g^{-1} DW.

For the assessment of flavonoid content, the aluminum complex method was performed, using catechin as standard. Results were expressed as mg of catechin per gram of dry weight (mg CAT g^{-1} DW).

All the assays were performed using 96-well micro plates (Nunc, Roskilde, Denmark) and an Infinite M200 microplate reader (Tecan, Grödig, Austria). For all analyses, three replicates (n = 3) of each sample were assessed.

2.5 Antioxidant capacity assays

The free radical scavenging capacity was determined by ABTS and DPPH spectrophotometric methods, according to the method described by [14]. FRAP methodology was also applied to measure ferric antioxidant power of WBPs extracts.

These assays were also performed using 96-well micro plates (Nunc, Roskilde, Denmark) and an Infinite M200 microplate reader (Tecan, Grödig, Austria), being the results expressed in mmol Trolox per gram of dried sample (mmol Trolox g^{-1} DW). All the analyses were made in triplicate (n = 3) for each sample [15].

2.6 Identification and quantification of phenolic compounds by RP–HPLC–DAD

The polyphenolic profile of WBPs extracts was assessed by Reverse Phase - High Performance Liquid Chromatography - Diode Array Detector (RP-HPLC-DAD), in an Agilent HPLC 1100 series equipped with a photodiode array detector and a mass detector in series (Agilent Technologies, Waldbronn, Germany), in accordance with the method previously described [13]. The equipment consisted of a photodiode array detector (model G1315B), an autosampler (model G1313A), a binary pump (model G1312A), and a degasser (model G1322A). The HPLC system was controlled by Xcalibur software (Agilent, version 08.03). A C18 column (250 x 4.6 mm, 5 µm particle size; ACE, Aberdeen, Scotland) was used, being the reverse phase HPLC

method based on a polar mobile phase with the mixture of solvent A: H_2O/HCOOH (99.9:0.1, v/v), and solvent B: CH_3CN/HCOOH (99.9:0.1, v/v). The following linear gradient scheme was used (t in min; %B): (0; 5%), (15; 15%), (30; 30%), (40; 50%), (45; 95%), (50; 95%) and (55; 5%). At this last time (55 min), return to 5% of B to stabilize and prepare the column for the next sample. The analysis was performed at 25°C, with a flow rate of 1.0 mL/min and a sample injection volume of 20 μL. All samples were injected in triplicate. For the quantification of the identified compounds, the respective standards were used at 280 nm. Concentrations were expressed in mg g^{-1} of dry weight (mg g^{-1} DW).

2.7 Statistical analysis

The results are presented as mean (n = 3) ± standard deviation (SD). The data obtained were subjected to variance analysis (ANOVA) and a multiple range test (Tukey's test) for a p value <0.05, using IBM SPSS statistics 21.0 software (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1 Phenolic content of wine lees, grape pomace and grape stems

In the present work, the determination of the phenolic composition and the antioxidant capacity of wine lees, grape stems, and grape pomace extracts of two grape (*Vitis vinvifera* L.) varieties (Sousão and Tinta Barroca) were performed. The phenolic content of these samples collected from Douro region (Northern Portugal) was presented in **Table 1**. As it can be observed, in general, grape stems were the WPBs with the highest content of total phenols (168.75 mg GA g^{-1} DW, on average), *ortho*-diphenols (166.39 mg GA g^{-1} DW, on average), and flavonoids (152.31 mg CAT g^{-1} DW, on average), followed by grape pomace and wine lees. Concerning this last winery by-product, it can be stated that the samples from Sousão variety showed the lowest content of these three studied parameters, being significantly different from the other variety and WBPs ($p < 0.05$). In fact, wine lees and grape stems presented the highest values of phenolic content in Tinta Barroca samples which has not been observed for the grape pomace extracts, which can be explained by the different phenolic compounds present in these WBPs.

Romero et al. obtained similar values in wine lees of total phenols (38–254 mg CAT g^{-1}) and flavonoids (16–146 mg CAT g^{-1}) content from the Tempranillo variety, with these ranges caused by the extraction solvent employed by these authors [16]. However, Pérez-Serradilha et al. [17] obtained higher values in this WBP of total phenols content (364 mg g^{-1}) than those obtained in this work after a microwave-assisted extraction optimized. Our research group have analyzed the phenolic content of grape stem extracts prepared with conventional extraction methods (hydro-methanolic solvents) [3, 4, 11, 18].

The values ranged between 32 and 123 mg GA g^{-1} DW for total phenols, between 35 and 116 mg GA g^{-1} DW for *ortho*-diphenols, and from 34 to 106 mg CAT g^{-1} DW for flavonoids, depending on cultivar, geographical localization, crop season, among other factors [4, 18, 19]. The values of the present work were slightly higher than the values obtained in those studies, maybe due to the new efficient extraction method performed in this work (UAE). Grape pomace has been also analyzed by other authors concerning its phenolic content, obtaining values around 40 mg GA g^{-1} DW for total phenols and around 14 mg CAT g^{-1} DW for flavonoids which are significantly lower than those obtained in this work.

	Phenolic content						Antioxidant capacity					
	Total phenols		*Ortho*-diphenols		Flavonoids		ABTS		DPPH		FRAP	
	Sousão	Tinta Barroca	Sousão	Tinta Barroca	Sousão	Tinta Barroca	Sousão	Tinta Barroca	Sousão	Tinta Barroca	Sousão	Tinta Barroca
Wine lees	[Z]15.44 ± 1.25[a]	125.39 ± 1.12[b]	118.91 ± 0.95[a]	136.03 ± 1.10[b]	18.50 ± 0.89[a]	128.34 ± 1.07[b]	1.71 ± 0.01[a]	3.28 ± 0.03[b]	1.24 ± 0.03[a]	1.58 ± 0.03[c]	1.54 ± 0.01[a]	1.96 ± 0.03[b]
Grape pomace	153.70 ± 0.53[d]	135.32 ± 2.76[c]	151.78 ± 1.89[c]	138.70 ± 1.42[b]	144.81 ± 1.75[c]	129.93 ± 0.93[b]	5.54 ± 0.09[d]	4.01 ± 0.03[c]	1.64 ± 0.03[d]	1.59 ± 0.01[c]	1.75 ± 0.01[d]	1.61 ± 0.02[b]
Grape stems	156.81 ± 1.29[e]	180.68 ± 2.77[f]	162.53 ± 1.01[d]	170.24 ± 1.88[e]	143.90 ± 2.13[c]	160.71 ± 1.44[d]	5.62 ± 0.02[d]	8.02 ± 0.02[e]	1.49 ± 0.07[c]	1.85 ± 0.02[c]	1.69 ± 0.01[c]	2.02 ± 0.01[e]
P-value	[Y]***		***		***		**		**		**	

[Z]*Data presented as Mean (n = 3) ± SD values for the same parameter evaluated followed by different superscript lowercase letters are significantly different at p < 0.001, according to Tukey's test.*
[Y]*Level of significance: N.s.: not significant (p > 0.05);*
**significant at p < 0.05;*
***significant at p < 0.01;*
****significant at p < 0.001.*

Table 1.
Total phenols (mg GA g^{-1} DW), ortho*-diphenols (mg GA g^{-1} DW), and flavonoids (mg CAT g^{-1} DW) content and antioxidant capacity (mmol Trolox g^{-1} DW) of WBPs from Sousão and Tinta Barroca varieties.*

In fact, these contents are particularly dependent of several factors, such as the agronomic conditions [3], cultivar [4] and extraction methods employed [6]. In this sense, in order to reduce production costs and optimize processes, new technologies—such as ultrasound-assisted extraction (UAE) or microwave-assisted extraction (MAE)—have been employed to decrease energy consumption and increase the product or process safety/control and quality. These techniques, already used at large scale, emerged as efficient, energy/time-saving and clean extraction methodologies, providing higher recoveries of bioactive compounds using low amounts of solvent, with particular advantageous for natural sources [8, 20].

3.2 Antioxidant capacity of wine lees, grape pomace and grape stems

The antioxidant capacity of the WBPs investigated in this work was performed by three methods (ABTS, DPPH, and FRAP), being the results presented in **Table 1**. As expected, due to the correlation of most phenolic compounds with antioxidant capacity, Tinta Barroca samples presented the highest values for all the methods concerning wine lees and grape pomace samples. In contrast, grape pomace samples from Sousão variety showed the highest antioxidant capacity for ABTS (5.54 ± 0.09 mmol Trolox g^{-1} DW), DPPH (1.64 ± 0.03 mmol Trolox g^{-1} DW), and FRAP (1.75 ± 0.01 mmol Trolox g^{-1} DW) methodologies, being significantly different from the Tinta Barroca samples ($p < 0.05$).

Romero et al. [16] also determined the antioxidant capacity of wine lees extracts from Tempranillo variety, obtaining values ranged between 0.46 and 2.197 mmol Trolox g^{-1}. The values obtained in the present work are in the range of those presented by these authors, concerning FRAP method. In literature, grape stem extracts have been also analyzed concerning this biological property, which present antioxidant capacities of 0.35–0.84 mmol Trolox g^{-1} DW, 0.15–0.76 mmol Trolox g^{-1} DW, and 0.33–1.03 mmol Trolox g^{-1} DW for ABTS, DPPH, and FRAP methodologies [4, 18, 19], which were lower than those presented in the present study essentially due to several factors, such as different extraction methods, extraction solvents or protocols, varieties, among others.

3.3 Phenolic profile of wine lees, grape pomace and grape stems

The phenolic profile of wine lees, grape pomace and grape stems was performed by RP-HPLC-DAD, being the results presented in **Table 2**. Fifteen compounds were identified, being grape pomace samples the ones with more phenolic compounds identified, including phenolic acids, flavanols, and anthocyanins. In this study, it was possible to observe the same behavior referred above, namely the significant highest content of the phenolic compounds identified in grape pomace extracts from Sousão variety ($p < 0.05$). Similar compounds were identified in wine lees, namely gallic acid, catechin, epicathechin, and malvidin-3-*O*-gluside, beside others which were found only in this by-product, namely protocatechuic acid (0.337 mg g^{-1} DW, on average), delphinidin-3-*O*-glucoside (0.190 mg g^{-1} DW, on average), and petunidin-3-*O*-glucoside (0.268 mg g^{-1} DW, on average). All these compounds were also determined in higher concentrations in Tinta Barroca samples which is in agreement with the previous results reported above. Grape stem extracts presented three phenolic compounds which were not identified in wine lees and grape pomace, namely isorhammetin-3-*O*-(6-*O*-feruloyl)-glucoside, caftaric acid, and ε-viniferin, which were also present in higher concentration in Tinta Barroca samples, being significantly different from Sousão samples ($p < 0.05$).

Several compounds identified in this work have been also identified by other authors in these WBPs, namely gallic acid, catechin, delphinidin-3-*O*-glucoside,

	Wine lees		Grape pomace		Grape stems		*P*-value
	Sousão	**Tinta Barroca**	**Sousão**	**Tinta Barroca**	**Sousão**	**Tinta Barroca**	
1. Gallic acid	[Z]0.422 ± 0.001[a]	0.503 ± 0.003[b]	0.750 ± 0.002[d]	0.641 ± 0.002[c]	nd	nd	[Y]***
2. Isorhammetin-3-*O*-(6-*O*-feruloyl)-glucoside	nd	nd	nd	nd	0.203 ± 0.001[a]	0.338 ± 0.001[b]	***
3. Caftaric acid	nd	nd	nd	nd	0.171 ± 0.003[a]	0.225 ± 0.008[b]	***
4. Protocatechuic acid	0.216 ± 0.001[a]	0.458 ± 0.001[b]	nd	nd	nd	nd	**
5. *p*-coumaric acid	nd	nd	0.434 ± 0.005[b]	0.125 ± 0.002[a]	nd	nd	***
6. Delphinidin-3-*O*-glucoside	0.123 ± 0.002[a]	0.258 ± 0.001[b]	nd	nd	nd	nd	**
7. Catechin	0.559 ± 0.010[a]	0.798 ± 0.018[b]	1.501 ± 0.025[d]	0.993 ± 0.031[c]	nd	nd	***
8. Epicatechin	0.498 ± 0.001[b]	0.660 ± 0.005[d]	0.601 ± 0.007[c]	0.412 ± 0.003[a]	nd	nd	***
9. Petunidin-3-*O*-glucoside	0.215 ± 0.005[a]	0.321 ± 0.007[b]	nd	nd	nd	nd	**
10. Malvidin-3-*O*-glucoside	0.875 ± 0.023[a]	0.968 ± 0.012[c]	1.002 ± 0.024[d]	0.934 ± 0.017[b]	nd	nd	***
11. Quercetin-3-*O*-rutinoside	nd	nd	0,450 ± 0,002[c]	0.214 ± 0.012[a]	0.369 ± 0,003[b]	0.605 ± 0.010[d]	**
12. Quercetin-3-*O*-glucoside	nd	nd	0.458 ± 0,011[c]	0.385 ± 0.062[a]	0.374 ± 0,013[a]	0.423 ± 0.019[b]	**
13. Kaempferol-3-*O*-rutinoside	nd	nd	0.207 ± 0.001[d]	0.110 ± 0.001[c]	0.071 ± 0,001[a]	0.102 ± 0.003[b]	**
14. Kaempferol-3-*O*-glucoside	nd	nd	0.305 ± 0.003[c]	0.401 ± 0.001[d]	0.211 ± 0.001[a]	0.286 ± 0.006[b]	***
15. ε-viniferin	nd	nd	nd	nd	0.087 ± 0.002[c]	0.109 ± 0.004[d]	**

[Z]*Data presented as Mean (n = 3) ± SD values in the same row followed by different superscript lowercase letters are significantly different at p < 0.001, according to Tukey's test.*
[Y]*Level of significance: N.s.: not significant (p > 0.05);*
**significant at p < 0.05;*
***significant at p < 0.01;*
****significant at p < 0.001. nd: not detected.*

Table 2.
Content of individual phenolics (mg g^{-1} dw) of WBPs from Sousão and Tinta Barroca varieties. Statistical treatment notes: Data were subjected to analysis of variance (ANOVA) and multiple range test (Tukey's test) with a significance of p < 0.05.

epicatechin, *p*-coumaric acid, petunidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, among others [16, 17, 21, 22]. However, it is well known that the extraction method, the cultivars, the geographical conditions, the growing season, the plant diseases, among others, affect the chemical composition of WBPs, namely the secondary metabolites which are highly present in these matrices.

4. Conclusions

Nowadays, it is well established in the scientific community that wine has an important role in the prevention of some cardiovascular diseases, resulting from their content in bioactive phytochemicals with antioxidant capacity. Many of these compounds are derived from the solid parts of the grape cluster (stem, pomace, trimmed vine shoots, and wine lees). However, during the winemaking process, a complete extraction of these compounds to the juice/wine does not occur, and they may remain at high concentrations in certain wastes, such as in the stems. Indeed, the wine industry involves the production of large quantities of by-products, characterized by a valuable composition in phytochemicals with putative health-promoting qualities. Additionally, in light of the biological properties revealed recently, the search for natural bioactive compounds has paid attention on these materials as promising alternatives.

In this work, it was possible to observe the high content of phenolic compounds and the high antioxidant capacities demonstrated by several winery by-products, namely wine lees, grape pomace, and grape stems which were subjected to an ultrasound assisted extraction, obtaining higher values than those obtained by conventional extraction methods employed by the research group.

In this sense, the phenolics present in winery by-products may have an added-value to be used as an alternative to synthetic substances employed in distinct industries, giving rise to sustainable agro-industrial activities.

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Chapter 2

Food Waste and Agro By-Products: A Step towards Food Sustainability

Ajita Tiwari and Roshna Khawas

Abstract

Utilization of food/agricultural waste is having challenge and necessity in day to day life. It's an important aspect for all the industries (food) for the process of modification and recovery. The main aim is to minimize deterioration and maximize utilization of food which will lead to less problems in waste management and environment pollution. In some of the meat packaging and food processing industries, waste utilization treatment has been implemented for successful and substantial processing. In need of growing demands of high nutritive and cheap price foods, requirements are getting high simultaneously with increasing world population. So, there is urgent need of nutrient recovery from wasted utilization and sources of food/feed will help to reduce the shortage of world food supplies to the coming generation.

Keywords: Innovation, agro by products, food waste utilization

1. Introduction

The organic waste generated during different unit operation from various sources including commercial and domestic kitchens, food processing plants, restaurants and cafeterias. According to FAO [1], in the food supply chain approximately 1.3 billion tonnes of vegetables, meat, fruits bakery, dairy and other food products are lost [2]. In Asian countries nearly every year amount of food waste (FW) could rise from 278 to 416 million tonnes from 2005 to 2025.

Kitchen and yard waste are the primary source of municipal solid waste (MSW). This waste can be further utilized for converting into useful products/energy generation at very low-cost rather than dumping and landfilling [3].

The process may attribute towards environmental and economic factors, such as capacity of municipal landfill; price associated with transportation of waste materials and landfilling; rules and regulation adoption for environment protection; less utilization of commercial fertilizers; more recycling of household waste and quality improvement of compost products [4]. Composting FW reduces the waste volume, kills pathogens, decreases weed germination in agricultural fields, and destroys malodorous compounds [5]. In concept of agriculture (organic), organic-grade agricultural waste compost gaining popularity due of its advantage on physical, biological, and chemical soil properties of soil [6]. Various food industries produced a large number of by-products or wastes which cause a serious disposable problem with the environment. Approximately 1.3 billion tonnes of food per year [7] for human consumption is lost or wasted globally. As the food production is resource-intensive, food wastes and losses are indirectly accompanied by impacts

of environment, such as erosion of soil, deforestation, air and water pollution, as well as greenhouse gas emissions that occur in the processes of food production, storage, transportation, and waste management [8]. Domestic households generate the largest food-waste faction in the food supply chain [9]. As the food waste amount occurring high on the household level, in the food supply chain at the final stages, prevention must be taken at utmost importance to help prevent further climate change for food waste [10]. Recognition of appropriate management of waste has been implemented as essential prerequisite for sustainable development [11]. Historically, in the context of urban, removing potentially harmful substances or materials away from human settlements was the main focus of public waste management [12]. Increase in waste generation due to environmental, social and financial implications of unsustainable use of raw materials in the short and long term [13] waste management began to shift from a mere pollution prevention and control exercise, towards a more holistic approach.

This chapter presents the reasons for consumer food waste in a systematic, causes, replicable, systematic and transparent way. In this chapter it is reviewed and analyzed the observation collected from different studies which is carried out on the factors promoting or impending on consumer food waste. Food waste is generated when the unprocessed food is converted to suitable form for the human consumption, during the period of conversion of food it may lost, contaminated, discharged and degraded leading to the production of food waste. Nowadays waste management and its control are a great challenge from collection point to disposal unit and identifying of sustainable approach to solve the problem of waste management caused by the agricultural and industrial sectors, food supply chains and as well as retailers and final consumers. Some useful products for industrial purposes like biofuels or biopolymers are produced from the food waste. Fixation of carbon by composting and nutrients recovery can also be achieved and the final left out waste should be used as minimum desirable options for incineration and landfilling. The chapter reviews to provide an overview on food waste definitions, generation and reduction strategies, and conversion technologies emerging.

2. Food waste and agro by-products

Food wastes are usually organic residues produced by the processing of raw materials into food. Waste is characterized as a product that do not add value to a product whereas by-product is a secondary product obtained as a result of manufacturing of the main product, often with a market value. Many by-products require further processing before sale [14].

So, the wastes could be considered valuable by-product if appropriate technical means are available to generate value which exceeds the cost of reprocessing. Residues in this case cannot be considered as wastes but becomes a product of higher value. Utilization of food processing residues offer potential of converting these by-products to beneficial uses [15].

3. Agro by-products

Agro by-products or agro residues are mainly obtained from agricultural production, harvesting, and processing in farm areas and from agricultural processing industries such as oilseed extraction, brewery, malt production, cereal grain milling, fruit and vegetable processing. These by-products hold tremendous potential

source of protein supply for animal feed and can also be converted to biofuels, bioenergy and other products in a way that produces economic value.

3.1 Types of agro by-products

The agro by-products derived from various crops play a significant role in animal nourishment. These by-products are of various types and can be classified into different groups, such as by-products from fruit and vegetable processing industry, crop waste and residue, by-products from sugar, starch and confectionary industry, by-products from distilleries and breweries, by-products from grain and legume milling industry, and oil industry. The handling and technologies used for processing of by-products are generally based on their type [16].

3.2 Value addition of agro by-products

Adding value to agricultural by-product makes it more desirable and enhances their economic value. Crop residue or agro by-products usually represent relatively high amounts of cellulosic material that could be returned to the soil for its future enrichment in carbon and nutrients or could be made available for further conversion to biofuels, bioenergy and other products. Such agricultural by-products can play an important role in triggering the transition of sustainable energy. There are many economic benefits that can be obtained through value-addition to agricultural by-products. These benefits include enhancing the resource use efficiency of agricultural production, increasing farm incomes and reducing the costs of production and thus increasing the profitability of farming, producing novel products, creating jobs, minimizing the disposal of the by-products into the environment to ensure improvement in environment quality [17].

3.3 Utilization of agro by-products

Earlier these agro residues were treated as waste by agriculturists and used to disposed into the surrounding environment causing pollution. However, they realized the significance of these by-products and the invulnerable costs of animal feed and fertilizers, and harmful impact to the environment and started to utilize it as animal feed. The use of crop residues is a good way of discarding materials that could otherwise be a potential health and environmental hazard. Agro by-products plays an important role in improving the nutritional status of various forms of rations and feeds of livestock as these by-products contain numerous amounts of macro and micro nutrients that are necessary for body growth and productivity [18]. There are several ways of utilizing agricultural by-products as feed for livestock, a source of fuel, and as inputs into agricultural production and rural industries [17]. The increased utilization of agricultural by-products can provide a sustainable basis for small and medium industries in a rural area and stimulate rural economic development. Harnessing crop residues as manure and bio-fertilizers, and as raw materials for producing energy and consumer products, can expand the profitability of agricultural enterprises, improve the quality of the environment and enhance energy security [19].

4. The global food supply chain: food losses and waste

The global food waste challenge concerns about over escalating emissions of Green House Gas and other impacts of environment associated with food waste [20],

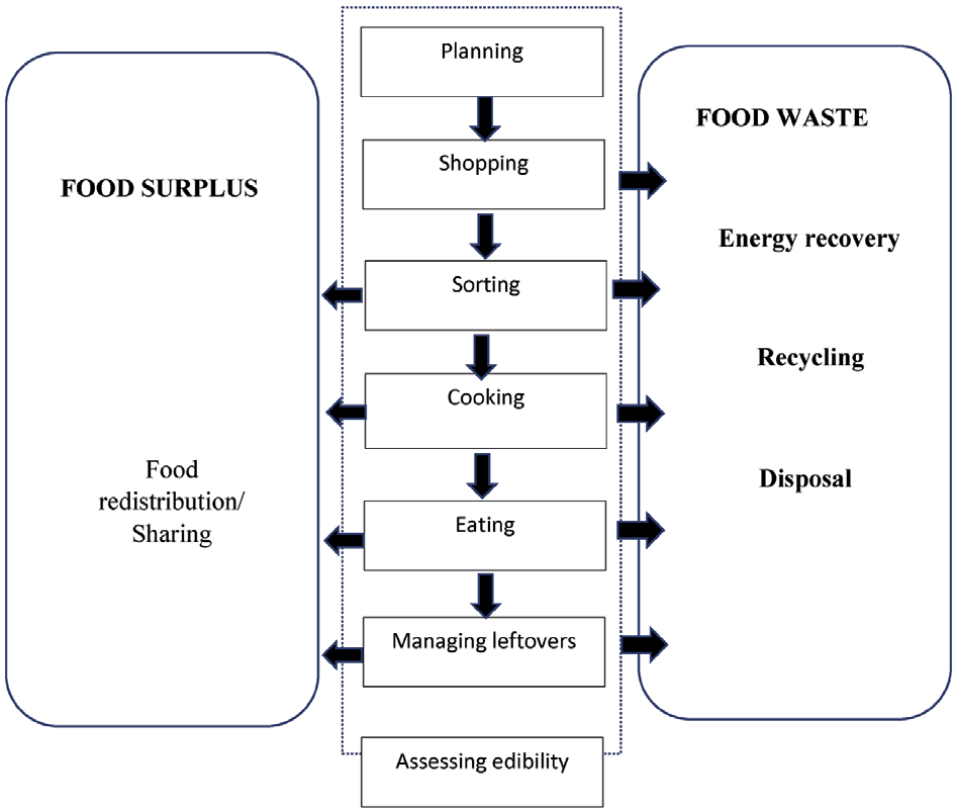

Figure 1.
Waste prevention and waste management.

as a priority waste stream, food waste is identified by growing number of policies (national and regional) [21–23]. In high range the food amount wasted in the global food supply chain (FSC) that could have otherwise been used to feed people food security is constantly pressing global issue and raises questions about it [24].

4.1 The waste hierarchy

The aim of the waste hierarchy is to recognize the problem and to generate the suitable environment friendly product from the waste. As shown in **Figure 1**, 'prevention' is the most favorable and disposal is the least favorable activity in the waste management 'pyramid'. It has been advised to consider the social and economic impacts as well as the environmental, the waste hierarchy, as a framework, primarily focuses on delivering the best environmental option by the European Waste Framework Directive (European Parliament Council, 2008).

5. Concepts in waste management and sustainability

An overview of these concepts is provided in the sections below:

5.1 Sustainable production and consumption

According to the United Nations Environmental Program [25] Sustainable Consumption and Production (SCP) defines "production and use of goods and

services that respond to basic needs and bring a better quality of life, while minimizing the use of natural resources, toxic materials and emissions of waste and pollutants over the life cycle, so as not to jeopardize the needs of future generations". In this context, the SCP is seen to approach strategy implementing for achieving sustainable development, economy encompassing, environment and society with the use of both social innovation and technologies.

Framework of SCP policies includes growth of economy without damaging environment, fulfilling basic human requirements, and avert the rebound effect. SCP illustrate the phenomenon of negative impacts from growing consumption outweigh and the benefits of efficient and improved technologies. It is an integrated approach, involves both the supply and demand (goods and services), by reducing the adverse situation of production and consumption [26]. On the sustainable production side, some examples are prevention from pollution, cleaner production, efficient for ecosystem, and productivity towards greenery [27]. SCP on the consumption side, connects product and the producer with the consumer, allowing more sustainable choices to be made. Some traditional examples are eco-labeling, management in supply chain, minimization in waste, recycling, sustainable procurement and resource efficiency measures [28].

5.2 Avoidable and unavoidable food waste

WRAP defines avoidable food waste as food no longer wanted, thrown away. Avoidable food is composed of material that was, edible, at some point prior to disposal, even though a proportion is not edible at the time of disposal due to deterioration.

Avoidable food waste includes foods or parts of food that are considered edible by the vast majority of people. Unavoidable food waste is described as waste arising from food that is not, and has not been, edible under normal circumstances. This includes parts of foods such as fruit skin, apple cores and meat bones.

5.3 Waste prevention and waste management

Sinclair Knight Merz Enviros (SKM Enviros) explains waste prevention are the activities that avoids generation of waste, for instance, food surplus reduction, whereas waste management as shown in **Figure 1**, includes the activities which deals with food waste once it has been generated, such as composting and anaerobic digestion.

6. Fermentation processes

Chemical transformation of product into value added products is the process known as Fermentation which is one of the oldest methods used for product transformation through microorganisms. Fermentation processes are mostly done in three types/methods such as solid state, sub merged and liquid fermentation. Selections of the fermentation process are product specific. To obtained bioactive compounds of industrial interest from various substrates such as wastes, solid state and sub-merged fermentation processes are used [29].

6.1 Solid state fermentation

Solid-state fermentation (SSF) is the fermentation procedure in which development of microorganisms takes place on solid substrates in the absence of open liquid [30]. It focuses to attain the maximum nutrient attention from the substrate

for fermentation by using the microbes such as fungi or bacteria. SSF further classified on the basis of seed culture used for fermentation is pure or mixed. In pure culture SSF, specific strains are used whereas, in with the mixed culture, different types of microorganisms are used for fermentation.

6.2 Sub merged/liquid fermentation

Submerged fermentation (SmF) is the type of fermentation in which the substrate is liquefied or put off in a water source. In industrial processes for high yield, low cost, and contamination SmF is mostly used. However, SmF has some disadvantages like physical space and energy or water requirements etc. [31]. Because of some advantageous SmF produced enzymes has been used over past of century as compared to SSF. Ease of process control and sterilization this fermentation process is easier to plan by researchers [32]. Pectinase, an enzyme production from fungi has been described by Favela-Tores et al. [33] using SmF. Pectinases are a gathering of related proteins engaged with the breakdown of pectin from an assortment of plants. Pectinases have various commercial as well as industrial importance.

6.3 Uses of fermentation for the production of bioactive/value added compounds

To elevate functional and nutritional values of the substrate to large extent SSF is a remarkable tool [34, 35]. For solid state fermentation several types of solid substrates generated from agro waste have been used which is contains of high nutritive value in terms of proteins, fibers, and minerals, respectively [36].

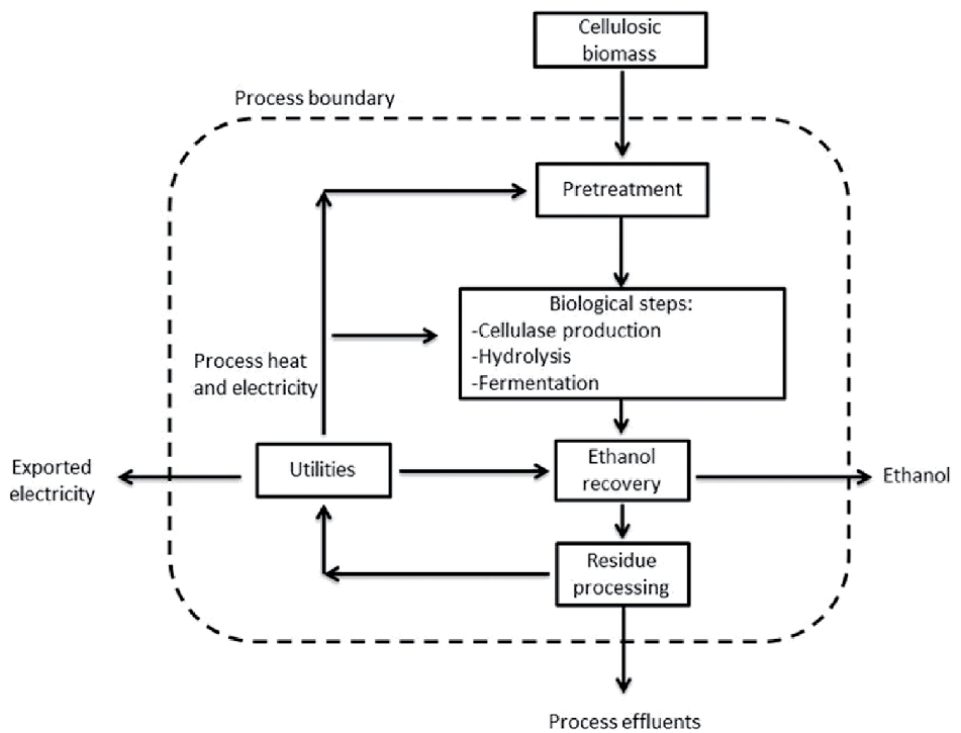

Figure 2.
Industrial waste and by-product streams via fermentation.

Figure 2 shows the outline of food industries waste through fermentation for production of various bioactive compounds. Real fact is that in human as well as animal diet these macro and micro molecules have tremendous value, therefore solid-state fermentation is an effective approach to improve their digestibility and bioavailability [37, 38]. Functional properties are the significant properties that define the pivotal phenomena of food, which are essentially used in food application [39, 40]. Also, the functional properties of food are always correlated with intrinsic components such as fats, proteins and starch, respectively.

From processing setups of the agro-industry especially the food industry produces a huge volume of wastes that are generally obtained [41]. The composition, quantity, and quality of wastes depend on the raw materials as well as the processing steps. Different type of wastes like date syrup, wheat straw, orange peel, and bran, rice straw and bran, banana, sugarcane bagasse and potato peel, soybean waste, apple pomace, oil press cakes, dairy waste, marine waste, brewery waste, food waste, etc. are produced by various types of food industries. Appropriate applications i.e., fermentations used for biotransformation of these wastes into valuable products having low cost and high nutritive value. Undeniably, use of wastes not only excludes the dumping problems but also resolves the pollution-related problems. Therefore, extra governing endorsements, as well as principal funds, are essential to bring these value-added products in the commercial market. The valorization of agro-industrial by-products to beneficial substances may not only provide future dimension to researchers but also decrease the existing environmental hazards.

7. Technologies for renewable energy generation from food/agricultural waste

Currently in many countries food waste are incinerated together with other combustible municipal wastes or landfilled for possible recovery of energy. However, due to these two approaches environment and economy of the countries are more stressed. Due to its composition of organic and nutrient-rich content, theoretically FW can be utilized as a useful resource for biofuel production through various processes of fermentation. It has attracted increasing interest in the production of biogas, hydrogen, ethanol and biodiesel as final products. Therefore, this section reviews all the food waste fermentation technologies for renewable energy generation.

7.1 Production of ethanol

The rapid global demand for the for the ethanol which has wide application in industries is increasing day by day. The main purpose of ethanol is to produce ethylene which is the main raw materials for the production of polyethylene and other plastics that is the reason for the high demand i.e. more than 140 million tonnes per year. Even the bioethanol has gained interest that is produced from cheap feedstocks [42, 43]. The source of bioethanol is the waste from starch and cellulose rich crops, e.g. sugar cane, rice and potato [44]. With the help fermentation in presence of *Saccharomyces cerevisiae* starch undergo breakdown resulted in the conversion of glucose by commercial enzymes and finally production of ethanol. In case of cellulose the breakdown due to hydrolysis is more difficult. If the FW contain large number of cellulose feedstocks than hydrolysis will become difficult, that is why, for the production of ethanol use of abundant and cheap wastes such as municipal, lignocellulosic and food waste has been explored as alternative substrates [45, 46].

7.2 Production of hydrogen

Hydrogen in the form of compressed gas gives high energy yield (142.35 kJ/g) which can also be produced from FW. The production of hydrogen is associate with the food waste containing higher amount of carbohydrate. The production rate of 0.9 to 8.35 mol H_2/mol hexose is generated from thee food waste according to recent studies [47]. The production of H_2 is influenced by many factors such as process configurations, pre-treatments and the composition of FW.

7.3 Production of methane

Methane is used as a fuel for ovens, homes, water heaters, automobiles, turbines, and other things. Because of its low cost, the production of methane via anaerobic processes is a good approach for management of waste, low production of residual waste and its utilization as a renewable energy source [48, 49]. In addition to biogas, a nutrient-rich digestate produced can also be used as soil conditioner or fertilizer. [50] investigated two-stage anaerobic digestion of fruit and vegetable wastes, in which 95.1% volatile solids (VS) conversion with a methane yield of 530 mL/g VS was achieved. [51] FW was converted to methane using a 5-L continuous digester fed with an organic loading rate (OLR) of 7.9 kg VS/m^3, resulting 70% VS conversion with a methane yield of 440 mL/g VS. [52] the methane production capacities of about 54 different fruit and vegetable wastes ranged from 180 to 732 mL/g VS depending on the origin of wastes.

7.4 Production of biodiesel

Biodiesel is synthesized through direct transesterification/acid catalyst using alkaline FW converted to fatty acids and biodiesel via various oleaginous microorganisms [53–56]. Many yeast strains produce microbial oil and then it can be used as the substitute of plant oils due to their similar fatty acid compositions. It also can be used as raw material for the production of biodiesel [57]. It has been found that the potential of FW hydrolyzate as culture medium and nutrient source in microalgae cultivation contributes for production of biodiesel [58].

In terms of prevention and concern towards economic and environment, management of FWs is utmost urgent and important to be implemented. The bioconversion of FW is economically viable for the conversion of biodiesel, ethanol, hydrogen, and methane. However, problems associated with FW in terms of transportation/collection should also be monitored. Nevertheless, the low or no cost of food waste along with the environmental benefits considering the waste disposal would balance the initial high capital costs of the biorefineries.

7.5 Production of bioactive compounds by fermentation of food waste

Bioactive and compounds are the two words which gives the term "Bioactive compounds". Scientifically, the meaning of this term is several molecules that have some biological activity.

These compounds are naturally present lesser content in plants and food stuffs, they are phytochemicals [59] and potentially able to growth in metabolism for the betterment of human health. Bioactive compounds are extremely heterogeneous class of compounds includes plant growth factors, alkaloids, mycotoxins, food-grade pigments, antibiotics, flavonoids and phenolic acids etc. with dissimilar chemical structures (hydrophilic or lipophilic), specific to ubiquitous distribution

in nature, significant amount present in foods and in human body, efficient against oxidative species and possess the potential biological action [60, 61].

Through various food industries a large number of by-products or wastes are produced worldwide due to which it leads to environmental degradation. So, nowadays many approaches and new techniques are introduced for the use of the wastes, because these by-products are an excellent source of various bioactive components and beneficiary for human health. The composition of these wastes mainly depends on the waste source/type. Approximately half of the waste produced from food processing factories is lignocellulosic in nature. The dissimilar types of waste produced by food industries can be fortified by various processes. One of the oldest approaches is fermentation and carried out in three types of processes, that are carried out such as solid state, submerged and liquid fermentation used for product transformation into value added products through microorganisms [34].

8. Future perspectives

Agricultural industries generate a huge amount of wastes and by-products during production, handling and processing of agricultural products. Disposal of these wastes has a serious financial and ecological concern due to its detrimental environmental effects [62]. Therefore, to discover alternative methods of recycling and reprocessing of these wastes is a significant target taken into consideration globally. These wastes and by-products represent huge potential which have not been fully exploited, causing a loss of economic opportunity. There is thus need to identify the reasons for underutilization of agricultural by-products so that they can be addressed through suitable strategies and policy interventions. Part of the reason for the underutilization of agricultural by-products is due to lack of awareness about their properties and potential economic benefits. Proper research and studies need to be carried out on assimilating different value-added product manufacturing process. Value addition of by-products generates economic value as it facilitates the process of economic diversification by opening up new agricultural market and providing alternatives to low-cost commodity production, by offering new perspectives for the management of resources and by providing economic opportunities and environmental benefits. Markets for agricultural by-products are essential for their commercialization, value addition and efficient utilization. The lack of markets for the by-products restricts the use of crop residue to produce biofuels. So, there is a need to establish markets and to keep operational expenses of its value addition low enough to encourage the production and utilization of value-added products. These by-products also represent potential solutions to the problems of animal nutrition. Technologies needs to be developed for better utilization considering factors, such as characteristics of individual wastes and the environment in which they are produced, reprocessed and utilizied, such technologies need to convey products that are safe not only for animal feed use, but also from the point of view of human feeding.

The proper utilization of agricultural wastes and by-products has the potential to support entire industries, increase income and valuable employment opportunities, develop rural areas and solve the problem of waste and environmental pollution.

9. Conclusion

The world Population is increasing rapidly with the decreasing trend of natural resources are at the same time. Raising concerns over the security of global food

due to the disparity between food wastage and food poverty, highlights the moral and social food waste dimensions. This chapter suggests that the first step towards a more sustainable resolution of the growing food waste issue is to adopt a sustainable production and consumption approach and tackle food surplus. The distinction between food surplus and food waste on one hand, and avoidable and unavoidable food waste on the other, are crucial in the process of identifying the most appropriate options for addressing the food waste challenge. This study proposes the food waste hierarchy as a framework to identify and prioritize the options for the minimization and management of food surplus and waste throughout the food supply chain. The proposed food waste hierarchy aims to challenge the current waste management approach to food waste, contribute to the debate about waste management and food security, and influence the current academic thinking and policies on waste and food to support more sustainable and holistic solutions.

Preventing food waste in agriculture and food processing requires improved infrastructure and technological solutions in harvesting, storage, transport and distribution, supported by large-scale investment and local policies. Waste management policies should be integrated and aligned with the wider policies on food, agriculture, food standards, food poverty alleviation and sustainable production and consumption.

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Chapter 3

Valorization Technologies of Marine By-Products

Amar Kaanane and Hind Mkadem

Abstract

Generally, in different countries, strategies to improve food security have focused on increasing food production, which contributes to climate pollution and increases stress on scarce natural resources such as water and land. Due to the increase of world population (estimated to be 9 milliards in 2050), to the limited biological resources and to the increase of environmental pollution, there is a need in innovation in food industry. This can be done by improving food quality through new technologies for valorization of food and food by-products. According to Food and Agriculture Organization (FAO), one third of world food production is lost or wasted along the food supply chain. In the sector of fisheries and aquaculture, 35% of the world's harvest is lost or wasted each year. Thus, the valorization of marine by-products should be an obligation to assure the world food security and to satisfy the growing demand for fishery products. The objectives of this study are: First to review the sources of by-products and their characteristics and second to describe and evaluate the different technologies that are or can be used to valorize marine by-products in production of marine oils and concentrated fatty acids.

Keywords: by-products, valorization, technologies, fish, marine oil, n-3 fatty acids

1. Introduction

According to the FAO, world fishery production has reached around 179 million tons in 2018, of which 156.4 million tons were intended for direct human consumption. This is equivalent to an annual supply estimated at 20.5 kg per inhabitant. While world capture fisheries production stagnates at around 96.4 million tons, aquaculture is experiencing continuous growth in the supply of fish for human consumption, contributing with 46% of the total supply [1].

Unfortunately, despite the expanding demand for fishery and aquaculture products and their importance to the food security of many populations, a large part of the catch is wasted [2–4]. From the catch to the finished product, unused secondary products are generated. Today, some of these by-products are used but a great amount is wasted [5]. In 2016, Jackson & Newton estimated that 11.7 million tons of by-products produced in processing plants around the world are not collected for the production of marine ingredients [6].

In the past, marine by-products were often discarded as waste, used directly as feed for aquaculture, livestock, pets or used in silage and fertilizers [2, 7]. However, in past two decades, other uses of marine by-products have appeared based on their important characteristics and their contents of high value molecules. In some

cases, compounds from by-products are identified higher in value than the starting material [8, 9]. Furthermore, with improved processing technologies, marine by-products can be now used differently and more efficiently.

Several reviews have been previously published discussing the possibilities of using marine by-products to produce high added value compounds [8, 10–15]. Different methods have allowed to produce useful molecules like proteins, gelatin, collagen, enzymes [7, 16, 17], biodiesel and biogas [18–24], natural pigments [25], minerals [26, 27], hydroxyapatite [28], chitin and chitosan [29, 30], creatine and taurine [31, 32].

The possible valorizations of marine by-products can be divided into three main categories: production of marine proteins (fishmeal, silage and hydrolysates), oils rich in polyunsaturated fatty acids (PUFAs) and preparation of high value compounds such as vitamins, enzymes, minerals, taurine and creatine, hydroxyapatite, biodiesel and biogas for human and animal nutrition, industrial or pharmaceutical uses. In this review, we mainly present the sources of marine by-products, their characteristics, and the possible technologies that can be used to produce marine oils and concentrated n-3 fatty acids.

2. Marine by-products and their characteristics

There is no one definition of marine by-products. In the past, marine by-products have been often considered as fish offal or waste [5, 7]. Actually, the term by-products designates all unused parts that can be recovered during production operations. They designate viscera, heads, trimmings, bones, cartilage, tails, skin, scales, blood, shells, carcasses, or damaged fish. Depending on the fishing period, reproductive elements such as eggs, milt or soft roe may be among these by-products [33].

In some works, the definition of by-products was reserved for feed. In others, the terms fish waste [34–37], waste streams [38], and rest raw material [5] have been used. In all cases, the biomass of by-products can be used to generate an added value unlike waste which has to be composted, burned or destroyed [5].

Generally, by-products can result from all aquatic food processing industries onshore or even during transformation on board. Marine by-products often constitute more than 50% of the body weight of processed fish [2, 4, 7, 39, 40]. However, this amount can reach up to 70% of the catch depending on catching species and area, postharvest conditions and industrial preparation processes [2, 7, 8, 11, 34, 41–44]. Processing operations like filleting, salting and smoking generate the most important amounts of by-products (50–75% of processed fish) [10], followed by the fish canning industry (30–65% of processed fish) and finally, the processing of crustaceans and mollusks [45]. It's estimated that the quantities of fish by-products generated by the processing industries will continue to increase due to the increasing demand for fishery products as source of valuable nutrients and a balanced diet for health [11, 45].

Knowledge of the properties of by-products allows their valorization into highly valuable products that could be higher in value than the fish fillets [8]. Analysis of the composition of by-products has revealed their richness in potentially valuable molecules such as proteins, essential fatty acids, oil, vitamins, minerals but also in bioactive compounds [4, 5, 34, 38, 46–48].

The by-products protein fraction is easily digestible and rich in essential amino acids. It can be used for production of peptides and amino acids, hydrolysates, gelatin and collagen, thermostable protein dispersions and protamine. While marine oils contain n-3 fatty acids [36, 37, 49–51], phospholipids, squalene, fat-soluble

vitamins, and cholesterols. Additionally, other valuable components can be extracted from marine by-products including nucleic acids, calcium, phosphorous, and hydroxyapatite [14, 28, 35] and other bioactive compounds such as astaxanthin [8], chitin and chitosan [25, 30], creatine and taurine [10, 15].

There are significant compositional differences between parts composing by-products [38]. In cases where the separation between the different parts of marine by-products is possible, the valorization will be optimal. For example, prioritizing the extraction of protein derivatives from the skin of the fish or oil from viscera and/or heads. Fatty fish by-products present an important raw material for the fish oil extraction industries especially during the high fat season. Aidos and co-authors studied the possibility of oil extraction and quality of oil from salted by-products of the maatjes herring (heads, frames, skin, viscera, etc.) by demonstrating that salt does not prevent the production of an oil of good quality [47]. A recent study showed that sardine cooking condensate and cooked by-products have a high potential for the recovery of oil with yields that can reach 32.9% during the fatty season [52].

The greatest valorization of these by-products depends on their handling according to the hygiene rules applied for food production [33]. Special care must be taken to maintain the temperature low during storage and transport to avoid alteration and to preserve their nutritional qualities as marine by-products are highly sensitive to degradation (oxidation, microbial spoilage and enzymatic reactions) [53].

3. Main valorization technologies of marine by-products

3.1 Production of marine oils

Marine oils are rich in PUFAs, especially, eicosapentaenoic acid (EPA, 20: 5 n-3) and docosahexaenoic acid (DHA, 22: 6 n-3) [52, 54–59]. These n-3 fatty acids have valuable benefits and medicinal properties. Numerous articles have described the benefits of n-3 fatty acids in regard to blood pressure, prevention and treatment of coronary artery disease [60, 61], atherosclerosis and thrombosis [62–64], hypertriglycemia [64, 65], schizophrenia and memory [66], stress and depression [67] and foetal development [57, 68–71]. However, the most widely discussed benefits relate to cardiovascular health [61, 65, 72–79] and the prevention and treatment of inflammatory diseases [57, 80–82].

These fatty acids are of marine origin, found mainly in fatty fish and seafood. They are obtained by consumption of algae, fungi and phytoplankton [83]. However, certain human groups, such as premature babies and ill people, are unable to synthesize them. Even in people not belonging to these groups, the amount of EPA and DHA synthesized by the body may not be enough because the biosynthesis of these two acids becomes slow with age as well as with bad habits such as smoking, alcohol intake and poor fitness habits [11, 84]. In this case, a diet based on marine lipids (fish and its derivatives) provides the needed intake of EPA and DHA [85, 86].

Marine oils are mainly composed of mixtures of fatty acids esterified with glycerol in triacylglycerides [11]. They are the main natural source of n-3 PUFAs particularly, EPA and DHA [37, 50, 51]. **Table 1** summarizes some variation intervals of EPA and DHA in certain oils extracted from fatty marine by-products. The variation depends on type of by-products used, the species, the catching season and the processing technology used for extraction and purification.

Species	By-products	EPA	DHA	Process	Ref
Sardinella maderensis	Liver	4.7	4.8	Solvent extraction	[87]
	skin	20.5	4.2		
Sardinella aurita	Liver	1.8	1.4		
	skin	10.4	2.5		
Cephalopholis taeniops	Liver	1.6	1.1		
	skin	3.1	6.9		
Cod (*Gadus morhua*)	Liver	8.6–11.4	11.8–16.2	Solvent extraction [88]	[89]
	Viscera	10.6–12.6	20.0–25.6		
	Trimming	14.2–16.5	30.4–33.8		
Saithe (*Pollachius virens*)	Liver	10.3–11.5	15.5–15.9		
	Viscera	7.3–13.6	9.5–23.2		
	Trimming	10.5–17.1	11.3–35.5		
Haddock (*Melanogrammus aeglefinus*)	Liver	13.1–14.8	15.2		
	Viscera	11.7–12.0	23.2–23.7		
	Trimming	14.6–16.6	33.3–35.7		
Tusk (*Brosme brosme*)	Liver	5.0	14.2		
	Viscera	7.0	25.5		
	Trimming	6.8	34.8		
Sardina pilchardus	cooked by-products	20.5–25.0	4.6–10.2	Batch hydraulic pressing	[52]
Tuna (*Thunnus obesus*)	Skins	4.2	23.6	CO_2 supercritical extraction	[90]
	Scales	4.8	23.5		
	Bones	5.1	21.6		
	Skins	3.6	21.8	Hexane Soxhlet extraction	
	Scales	4.5	21.5		
	Bones	4.7	20.0		
Sardina pilchardus	Heads, gut content, fins	14.20	18.59	Wet reduction method	[91]
Skipjack tuna	Precooked heads	0.1	25.5	Wet reduction method	[92]
	Non precooked heads	0.1	18.8		

Species	By-products	EPA	DHA	Process	Ref
Hake,	Offcuts	—	—	CO_2 supercritical extraction/Cold extraction/ Wet reduction/enzymatic extraction	[50]
Orange roughy,	Offcuts				
Salmon	Offcuts				
Jumbo squid	Livers				
Sardinella lemuru	Head Intestine liver	1.84 1.73 2.76	15.95 11.87 12.97	Solvent extraction	[37]
Sardina pilchardus	Heads	9.3	10.3	Enzymatic hydrolysis	[93]
Salmon	Frames without heads	9.3	11.3	Enzymatic hydrolysis	[94]
Black scabbardfish (*Aphanopus carbo*)	Heads, viscera, frames, skin, trimmings	2.7	6.2	Enzymatic hydrolysis	[95]
Sardine (Sardina pilchardus)	Heads, viscera, frames, trimmings)	—	—	Enzymatic hydrolysis	[96]
Salmon	Belly part	3.17	3.85	Pressing	[97]
	Belly part	4.45	3.62	CO_2 supercritical extraction	
	Trimmed muscle	3.53	3.46		
	Frame bone	4.27	3.60		
	Skin	3.87	3.26		
	Belly part	3.12	3.23	n-Hexane extraction	
	Trimmed muscle	3.22	3.98		
	Frame bone	3.85	4.32		
	Skin	2.79	3.09		
Indian mackerel (*Rastrelliger kanagurta*)	Skin	11.91–12.31	13.15–14.47	CO_2 supercritical extraction	[98]
		12.22	13.86	Soxhlet extraction	

Species	By-products	EPA	DHA	Process	Ref
Salmon	head, skin, viscera, backbone, frames, cuts off	2.46	2.97	Cold pressing	[99]
Salmon (*Salmo salar*)	Heads	7.7	11.9	Enzymatic hydrolysis	[100]
		8.4	12.1	Solvent extraction	
Rainbow trout (*Oncorhynchus mykiss*)	Roe	11.3	19.0	Enzymatic hydrolysis	[101]
		11.5	24.0	Solvent extraction [88]	
Rainbow Trout (Oncorhynchus mykiss)	Bones with leftover fish meat, skin, scales, fins	6.49–6.89	14.76–5.72	Isoelectric solubilization/precipitation	[102]
Nile perch (*Lates niloticus*)	Viscera	3.0	9.0	Enzymatic hydrolysis	[103]
Nile perch (Lates niloticus)	Heads	3.4	7.7	Enzymatic hydrolysis	[104]
Salmon (Salmo salar)	Heads	6.1	8.4		
Sardine	Heads	10.95	13.01	CO_2 supercritical extraction	[105]

Table 1.
EPA and DHA content (% of total fatty acid) in oil produced from marine by-products by different methods of extraction and purification.

Marine oils are produced from whole fish mainly small pelagic species, but also from by-products generated by the transformation industry. In 2018, it was estimated that between 25 and 35% of the total volume of fishmeal and fish oil produced came from by-products [2]. In production of marine oils, different techniques can be employed such as wet reduction process [52, 91, 92], solvent extraction [37, 87, 89, 106], supercritical fluid extraction [50, 90, 97, 98, 107, 108], urea complexation [108], cold pressing [99] and enzymatic treatment [9, 93–96, 101, 104, 109–112].

3.1.1 Wet reduction process

The traditional process for the production of marine oils is coupled with the production process of fishmeal. It is based on a heat treatment of the raw material which allows breaking the cell membrane to liberate the oil, pressing and separation [15, 106]. The principle of this process is based on the separation of the lipid phase from various fish compounds. The oil is separated by a decanter that separates insoluble compounds from the liquid phase, and a separator, separating oil and water. Another possibility is to use a tricanter, which separates solid, water phase and oil in one operation [8]. This process is mainly used in the treatment of fatty species containing high levels of fats such as anchovies, menhaden, sardines, Atlantic herring or their by-products [13].

On an industrial scale, the wet extraction technique is the most used method to obtain the oil and a substrate rich in proteins [106]. This process consists of 3 main stages:

Cooking, where the biomass is heated in a continuous screw cooker. Oil and water are separated from the solid protein. In order to recover an oil of high quality, the temperature and pressure must be adapted to the type and size of used biomass. Cooking can be done by direct injection of steam or by indirect steam heating in order to denature cell proteins and facilitate oil extraction [113, 114].

Pressing, a screw press squeezes the oil and water from the cooked biomass to separate the liquid phase containing the oil and the solid material. Press juice contains a considerable amount of suspended solids in the form of coagulated proteins, scales, edge fragments which have escaped the pressing. These particles are added to the fishmeal while the liquid phase undergoes centrifugation [115].

Centrifugation, which is now preferable compared to decantation, separates the oil from the aqueous phase. Draining and press water can be treated with live steam, allowing better separation of the oily phase on a horizontal centrifugal decanter [116]. In this step, water can be added also to wash the oil from any remaining impurities. At the end of this operation, the oil and stick water are obtained. The stickwater is evaporated to a concentrate before to add it to the pressing cake [36, 47, 117].

Quality and stability of the oil produced depend on process conditions. On one hand, the quality of oil depends on temperature, pumping speed and centrifugation. The marine oil is more stable if high pumping speed is used [118]. On the other hand, Chantachum and co-authors studied the influence of precooking on the separation and quality of tuna heads oil [92]. The results obtained have showed that cooking at 85°C for 30 min, followed by pressing at 140 tons/m^2 using a hydraulic press allows better release of the oily fractions which would be slowed down when cooked at a higher temperature due to the coagulation of proteins. The process conditions applied normally depend also on the type and quality of raw material used [117]. Comparison of quality of herring oils produced from three different types of by-products: heads, mixed by-products (heads, frames, skin, viscera, etc.), and headless byproducts showed that heads by-products and its oil presented the highest oxidation levels and the lowest R-tocopherol content. Heads contained the lowest PUFAs level and the highest amount of saturated fatty acids (SFAs) [55].

Oil recovery yield varies during the year depending on fat content of by-products. Extraction of oil from cooked by-products of *Sardina pilchardus* (skin, meat, bones and cooking condensate), using wet hydraulic pressing at 85°C for 30 min and centrifugation, gave an important oil yield varying between 6.0% and 32.9% depending on the fishing period [52]. Another study reported that the percentage of oil extracted from cooked by-products of *Sardinella gibbosa* was 8.96% [119].

Wet reduction process is more suitable to extract oil from fatty fish producing oil with improved quality and high level of n-3 PUFAs [50]. However, the main disadvantage of this technique is the use of high temperature during cooking that degrades oxidative quality of the oil and causes a loss of EPA and DHA contents due to hydrolysis and oxidation reactions [12, 111, 120].

In addition to the conventional extraction process called also wet reduction or hydraulic pressing, the extraction of marine oils could be obtained with several methods, such as enzymatic hydrolysis, physical fractionation, low-temperature solvent fractionation, supercritical fluid extraction and pH adjustement method to the isoelectric point (**Table 1**).

3.1.2 Enzymatic hydrolysis

The principle of enzymatic hydrolysis is based on the action of specific proteases at low temperature on protein tissue without use of solvents and high temperature [13, 50]. Firstly, the by-products are hydrolyzed by the use of commercial proteolytic enzymes and endogenous enzymes. These enzymes destroy the structure of cells walls. They broke down protein molecules to small peptides and amino acids which allows releasing the oil contained. After inactivation of the enzymes, the oil, hydrolysate and the insoluble residue are separated. The released oil can be centrifuged as previously described in the conventional process. For example, Batista and co-authors extracted oil from black scabbardfish (*Aphanopus carbo*) by-products using enzymatic hydrolysis with 1% Protamex [95]. The percentage of free oil released from the by-products has reached 36% of the total amount.

The enzymatic extraction is influenced by several operating factors, namely: nature of enzymes, temperature, pH, concentration of enzymes during hydrolysis, method and quality of grinding, and water content of the raw material [93, 110, 111, 120]. Optimal conditions of hydrolysis of sardine heads by Protamex were studied (temperature, hydrolysis time and enzyme-substrate ratio) [93]. Results have showed that optimum conditions were found to be similar to recover lipids and phospholipids (29 min, 31°C with 2.6 g.kg-1 enzyme). The same study has showed that hydrolysis could increase the extraction of lipids and phospholipids by 27% and 50%, respectively compared to classical extraction.

Šližyte and co-authors studied the enzymatic hydrolysis of mixtures of cod (*Gadus morhua*) by-products using Flavoenzyme and Neutrase [110]. The results demonstrated that the most important factor influencing the extraction yield is the added water regardless the type of enzyme. On the other hand, using an enzymatic process based on a proteolytic extraction of oil from crude tuna heads followed by a urea complexation (−5°C, 20 h) has allowed to obtain a mixture of DHA and EPA with a purity of 85.02% and a liquid recovery yield of 25.10% [121].

The enzymatic hydrolysis of salmon frames with Protamex, was able to separate the salmon frames into an aqueous fraction rich in soluble nitrogen (fish protein hydrolysate), an insoluble nitrogen fraction, an emulsion fraction, salmon oil and a bone fraction rich in protein and minerals. This process allows to separate oil from protein fraction that can be valorized to recover peptides, essential amino acids or other molecules [94].

Batista and co-authors studied the use of 0.5% of three commercial enzymes (Alcalase, Neutrase, and Protamex TM) and a water/fish ratio of 1:1 for production of protein hydrolysates and oil from raw and cooked sardine by-products from the canning industry [96]. Results have showed that the highest nitrogen solubilization and degree of hydrolysis were obtained with Alcalase and Protamex. The raw by-products were more easily hydrolyzed by these enzymes than the cooked sardine. The highest percentage of oil released was obtained from raw sardine, and Alcalase and Protamex were the most efficient. In another study, enzymatic extraction of oil from ground salmon heads at 55°C using different commercial enzymes (Alcalase, Neutrase, and Flavourzyme) showed that the highest oil recovery (17.4%) was obtained after 2 h by using Alcalase [111]. Same results were obtained later by other researchers [100, 101].

The enzymatic process can be chosen to extract the oil for many reasons. First, it is conducted under mild conditions protecting PUFAs from oxidation. Second, this technique does not use chemical solvents in addition to short time of hydrolysis [120, 122]. However, this process focuses more on the yield and production of protein hydrolysates than on the production of high quality oil. This disadvantage is due to the presence of lipase in the by-products which still active during the hydrolysis therefore it affects the oil quality [96]. Furthermore, this effect is more important if the hydrolysis is done in the presence of oxygen which increases significantly the free fatty acids in the oil, compared with the conventional method [8]. The main product from this process is the protein hydrolysates that are produced with higher yields compared with the traditional process [8].

3.1.3 Supercritical carbon dioxide

Extraction with supercritical carbon dioxide (SC-CO_2) is a promising technique, as CO_2 is nontoxic gas, nonflammable and clean solvent [123]. This technique can be carried out under mild operating conditions in an oxygen-free environment and at a moderate temperature, preventing degradation of PUFAs [12, 50, 90, 97, 98, 105]. CO_2 used is a green solvent. At or above critical temperature and pressure (31.1°C, 7.39 MPa), CO_2 is in a liquid state while at ambient temperature and pressure, CO_2 becomes a gas and evaporates [120].

This technique allows the extraction of lipids of low polarity, avoids the extraction of impurities and reduces the heavy metal content [12, 50, 90]. Rubio-Rodriguez and co-authors proposed to couple this technique with extraction-fractionation process to remove free fatty acids and improve fish oil quality, alternatively to physical and chemical refining [50]. Same authors compared different oil extraction methods from fish by-products, cold extraction or centrifuging, wet reduction, enzymatic extraction and supercritical fluid extraction. This study has showed that SC-CO_2 is an interesting method, operating conditions are suitable to prevent lipid oxidation and to reduce the amount of certain pollutants such as some arsenic products [50].

Another research has also compared 3 different oil extraction methods (supercritical carbon dioxide, n-hexane and traditional pressing) from Atlantic salmon by-products (belly part, trimmed muscle, frame bone and skin). The maximum oil yield was obtained by n-hexane extraction (total oil), followed by supercritical CO_2 extraction (highly selective technique extracting non-polar compounds) and the traditional pressing that has showed the lowest yield. Likewise, differences were noted in the oil quality parameters between the 3 studied techniques, the longer oxidative stability was obtained in the oil extracted by supercritical fluid CO_2 [97].

This technique was also compared to soxhlet extraction using hexane to produce oil from skins, bones and scales of bigeye tuna (*Thunnus obesus*). This study

has confirmed improved quality parameters of oil obtained by supercritical CO_2 technique extraction (low heavy metal content in the oil) [90].

Using the ground skin of Indian mackerel (*Rastrelliger kanagurta*), various techniques of supercritical CO_2 were studied by varying pressure (20-35 MPa) and temperature (45–75°C). This study has showed that oil yield increased with pressure and temperature and the highest yields were 24.7, 53.2, 52.8, and 52.3/100 g sample (dry basis) for the continuous, cosolvent, soaking, and pressure swing techniques, respectively, at 35 MPa and 75°C [98].

Supercritical fluid technology coupled with membrane, enzymatic or adsorption process have been shown to produce high-quality oil with best reduction of levels of contaminants compared to traditional refining of oils [124, 125].

Faced with all these advantages, the main drawback of this method is the high cost of the application on an industrial scale [12, 50, 124]. In this contest, the effect of supercritical CO_2 techniques on CO_2 consumption was studied. The results have showed that the total amount of CO_2 consumption decreases significantly with temperature and increases with pressure in all extraction modes using supercritical CO_2 method. A higher amount of CO_2 was needed for the continuous technique, compared to the techniques of cosolvent, soaking and pressure swing regardless of levels of pressure and temperature. Consequently, the best extraction technique of the oil with least amount of CO_2 consumption was achieved with pressure swing mode at 35 MPa and 75 C [98].

3.1.4 Solvent extraction

Solvent extraction methods are numerous. They have been studied by several researchers applied on marine by-products [37, 87, 89, 106]. Unfortunately, these techniques have many disadvantages. Large amount of hazardous solvent and important energy are required. Besides, marine oils are oxidized exhibiting a strong red-brown or brown color when extraction of oil is done at high temperature and longtime [90]. Generally, extraction by solvent is only carried out on a laboratory scale for analytical purposes [124]. Among the widely used techniques, Bligh and Dyer method is the most recommended for the total extraction of lipids from biological tissues [126]. Most of the published data on lipid content are related to this method [105, 127–129]. The effectiveness of the Bligh and Dyer method was evaluated compared to Soxhlet method. The results have showed that the Bligh and Dyer extraction method is more effective in extracting polar and non-polar lipids from fish compared to the Soxhlet technique [130].

3.1.5 Cold pressing extraction

Other innovative processes are coming to the market such as cold pressing extraction, a patented process originating from the olive oil production industry [13]. This technique allows protection of PUFAs content, producing high quality marine oil from different types of by-products [50, 99]. This process is well known to produce a lower-yield, but higher quality of oil [131].

Due to its high degree in PUFAs, marine oils are very sensitive to oxidation. The degree of oxidation increases in the presence of air (oxygen), light and heat during extraction and storage [8, 47]. This phenomenon mainly reduces the shelf life of marine oils [53]. All techniques using high-temperature or toxic solvents can induce degradation and loss of nutritional qualities of marine oil.

For this, looking for gentle extraction of marine lipids or using non-heat processes might generate more stable lipid fractions [8]. It is also necessary to protect the oil by stopping or slowing down the oxidation process during the production

and during storage. Adding antioxidants to the oil is one of the most used methods [132]. Using a modified atmosphere packaging [133], or encapsulation, which keeps marine oil away from oxygen and light [133, 134] can be also used. In addition to protecting the oil, the use of microencapsulation technology provides consumers with supplements n-3 fatty acids ready to consume.

3.2 Production of n-3 fatty acids concentrates

Another valorization of marine oils (produced from whole fish or from fish by-products) is their use in production of concentrated n-3 fatty acids in the form of free fatty acids, methyl and ethyl esters or acylglycerols [135]. Several processes can be used, the most important are urea complexation [108, 121, 136], molecular distillation [137], supercritical fluid extraction [98, 108], winterization [138, 139], fractionation by chromatography [120] and by enzymatic processes [111, 112, 140]. These techniques have been reviewed by many authors [13, 124, 135] and recently by [12]. The main challenge in the choice of concentration technique at industrial level is to reach higher yield and purity at lower cost [13, 124, 138]. **Table 2** outlines some methods to produce n-3 fatty acids concentrates with levels achieved of enrichment in EPA and DHA.

Concentration by winterization allows elimination of SFAs present in the oil, which crystallize at low temperatures [139]. Winterization is primarily designed for oils with a high content of SFAs. It allows elimination of stearic phase, by cooling the oil to 0–4°C. The degree of concentration of PUFAs by this process is evaluated as low as these interesting fatty acids could be lost in the stearic fraction [13]. However, this method produces n-3 PUFAs concentrate in natural form [138].

An alternative solvent winterization and enzymatic interesterification was studied to concentrate n-3 fatty acids in cod liver oil [138]. The optimization parameters considered were separation method, solvent, oil concentration, time and temperature of winterization. Likewise, enzymes used were examined for interesterification efficiency under different system air condition, time and temperature. Authors proposed the optimal conditions of the technique via winterization (0.1 g/mL oil/acetone, 24 h, −80°C, precooled Büchner filtration) and interesterification (Lipozyme TL IM, N_2 flow, 2.5 h, 40°C) improving n-3 fatty acid content to 43.20 mol%.

In another study, winterization was carried out on a bleached oil by a progressive cooling (30–5°C) in three phases. The effect of solvent type, solvent proportion, and agitation in the second cooling stage was studied. The results have demonstrated that using hexane has improved content of PUFA of 64.3% with 13% as a decrease percentage in level of SFAs compared to the fatty profile of bleached oil [139].

In addition to what is explained previously for CO_2 supercritical fluid extraction, this technique could be used to concentrate fatty acids. It's based on the use of CO_2 which, in the supercritical state, behaves like an extraction fluid and entrains fatty acids. In several passages, their concentrations therefore increase [13]. This method can achieve high concentration levels of n-3 PUFAs.

Supercritical carbon dioxide was used for simultaneous extraction and fractionation of fish oil from Tuna by-products [143]. The obtained oil was divided into six fractions based on molecular weight and the chain length of triglycerides in terms of fatty acid constituents. The results showed that the three first separated fractions were rich in SFAs followed by monounsaturated fatty acids (MUFAs), then PUFAs. While the three last fractions contained high levels of MUFAs and PUFAs.

Melgosa and co-authors studied the use of supercritical CO_2 as solvent in the lipase-catalyzed ethanolysis of fish oil. The effect of initial substrate ethanol/oil molar ratio (2–38), pressure (7.5–30 MPa), and temperature (323.15–353.15 K) on equilibrium conversion, reaction rate and oxidative status of the products were

Species	Type of oil	EPA	DHA	Process	Ref
Tuna (*Thunnus albacares*)	Tuna oil	EPA + DHA (85.02%) recovery 25.10%		Concentration by urea complexation	[121]
Rainbow sardine (*Dussumieria acuta*)	Oil from white muscle	15.39	17.45	Extraction [88]	[108]
	Concentrate oil	—	—	CO_2 Supercritical extraction	
		19.47	29.61	Urea complexation	
		17.74	25.51	Low-temperature crystallization with ethanol	
Pacific sardines (*Sardinops sagax*)	Crude oil from Skin-on fillets)	28.2	16.7	—	[140]
	Refined unhydrolyzed oil	26.86	13.62	Extraction and refining	
	Concentrate oil	33.74	29.94	Lipase-catalyzed hydrolysis	
Atlantic salmon (*Salmo salar*)	Crude Salmon by-products oil	3.71	9.02	—	[112]
	Salmon oil 40% hydrolysis	4.72	8.94	Lipolysis, filtration	
	Permeate	5.16	9.91		
	Esterified permeate	5.64	10.30	Enzymatic re-esterification	
Salmon	Oil from salmon heads	3.6	9.9	Enzymatic hydrolysis	[111]
	Permeate	5.6	11.6	Lipolysis	
	Permeate re-esterified	5.06	11.90	Re-esterification	
Arctic cod	Arctic cod liver oil	10.53	7.63	—	[138]
	Winterized oil	21.08	17.83	Alternate solvent winterization and enzymatic interesterification	
Tuna (*Thunnus thynnus*)	Tunafish oil	4.6	18.3	—	[141]
	Tunafish oil ethyl ester	5.3	23.7	Supercritical fluid chromatography	
Menhaden	Menhaden oil	13.5	12.6	—	[136]
	Menhaden oil after urea inclusion	29.4	41.8	Urea inclusion method	
Sardine (Sardinops sagax caeruleus)	Refined oil	14.51	12.55	—	[142]
	n-3 PUFAs concentrate	34.17	39.47	Chemical hydrolysis + urea complexation	
	n-3 PUFAs concentrate	46.26	40.32	Enzymatic hydrolysis + urea complexation	

Table 2.
Some methods of production of concentrated n-3 fatty acids in oil.

tested. The results have revealed the importance role of employing CO_2 in improving reaction kinetics by reduction of mass transfer limitations and prevention of n-3 PUFAs oxidation due to displacement of oxygen [144].

The results of supercritical fluid extraction applied on Rainbow sardine oil have showed that the highest decrease in SFAs and MUFAs were obtained at 50–60°C and 350 bars [108]. This technique was evaluated selective even in the fractionation of fish oil with lower content of EPA (4.6%) and DHA (6.7%) under conditions of lower pressure (100 and 200 bar) [145]. In another study, conditions (pressure, temperature and supercritical CO_2 flow rate) influencing concentration of fatty acids in fish oil by supercritical carbon dioxide were studied [146]. The results have demonstrated that fractionation by a supercritical fluid under optimal conditions: a pressure increase (at 5 kg/h flow rate) and flow rate increase (at 150 bar pressure), both determined a higher EPA + DHA concentration and decreased the EPA/DHA ratio. The same authors proposed to carry out a urea adduction during preparation to decrease the amount of SFAs in the starting oil before supercritical fractionation.

With regard to optimization conditions, numerous researches have worked on modeling, simulation, and optimization of the CO_2 supercritical fractionation of EPA and DHA esters in fish oil [147, 148].

Optimal conditions of application by using adjuvant material and modifying CO_2 volumetric density and temperature were also investigated in other studies to get the highest fractionation yield of EPA and DHA [149]. Likewise, Antunes-Corrêa and co-authors and Davarnejad and co-authors studied the optimal operating conditions (pressure and temperature) to fractionate fish oil. In the first study [150], the best results based on oil solubility were obtained using 7.8 MPa and 301.15 K. While in the second research [151], the maximal solubility of the fish oil (0.921 g of oil/100 g of CO_2) was obtained at optimum conditions of 40°C and 27.2 MPa. In both studies, EPA fractionation was recorded not possible and low, respectively.

The experience of CO_2 supercritical fluid chromatography was transferred to be used in laboratory in a pilot plant to produce EPA enriched mixtures. Fractionation was done on a silica adsorption column using CO_2 as supercritical solvent [152]. This allowed to achieve best purity of 93% in EPA ethyl ester fraction with a 24.6% yield. The study of the technical and economic feasibility to produce n-3 PUFAs ethyl ester concentrates from trans esterified fish oil using CO_2 supercritical fluid extraction has revealed that process cost is around 550 U.S $/kg DHA and EPA ethyl ester concentrate [141].

Other investigations have studied the use of enzymatic hydrolysis in production of n-3 PUFAs concentrates. This technique involves the use of specific enzymes (lipases), able to catalyze reactions such hydrolysis, ethanolysis or transesterification of triglycerides [124].

Concentration of Pacific sardines (*Sardinops sagax*) oil was carried out using lipase-catalyzed hydrolysis [140]. The results of this study have showed that hydrolysis with 250 U from *Candida rugosa* lipase has increased EPA concentration to a relatively constant level of 33.74% after 1.5 h. DHA levels were also significantly increased from 13.62% to 29.94% with 500 U after 9 h. This technique uses mild conditions (neutral pH and low temperatures), very important to preserve EPA and DHA from oxidation [140, 153].

Salmon oil produced from by-products of this species by controlled enzymatic procedure with Neutrase has followed a selective enzymatic hydrolysis under mild conditions, using Novozyme SP398 to enrich the n-3 PUFAs. The process used consist of a lipolysis, filtration in flat membrane device and enzymatic re-esterification with glycerol and Immobilized 1,3-specific lipase IM60 (Lipozym IM). This method induced a significant increase in the amount of PUFAs from 39.20 mol% of total fatty acids in the crude oil to 43.29 mol% in the re-esterified permeate [112].

The proteolytic extraction of oil from salmon heads using three different types of enzymes (Alcalase, Neutrase and flavourzyme) and the lipolysis of this oil to concentrate PUFAs were carried out. Lipolysis was done with Novozym SP398 to obtain a mixture of free fatty acids and glycerol (24 hours 45% hydrolysis). The mixture was then filtered. This process has allowed an increase of the PUFAs content from 41.6% in the crude oil to 46.5% in the permeate. Likewise, DHA and EPA percentages have increased from 9.9% to 11.6%, and from 3.6 to 5.6%, respectively [111]. The same authors used a re-esterification in the permeate with Lipozyme IM which permitted obtention of 5.06% and 11.90% in EPA and DHA contents, accordingly [111]. Moreover, other authors proposed combination of enzymatic or chemical hydrolysis with urea complexation to produce high concentrates of n-3 PUFAs. The enzymatic hydrolysis followed with urea complexation of refined sardine oil has increased the level of EPA and DHA from 14.51% to 46.26%, and from 12.55% to 40.32%, respectively [142].

Another technique, short path distillation was tested to purify Alaskan Walleye Pollock (*Gadus chalcogrammus*) and New Zealand Hoki (*Macruronus novaezelandiae*) liver oils [154]. Certainly, this process has reduced free fatty acids and lipid oxidation parameters, which is appreciated to produce purified oils. Consequently, the conduct of this operation at high temperatures may cause degradation of PUFAs or development of new undesirable compounds. The short path distillation was coupled to a previous enzymatic glycerolysis of sardine oil with glycerol [155]. This work showed that short path distillation is able to concentrate n-3 PUFAs in monoacylglycerols at suitable evaporator temperature (125°C) Same technique aided by a working fluid was evaluated efficient in removal of persistent organic pollutants in marine oils (PCDD/PCDF, dl-PCB and ndl-PCB) [156].

When comparing the effect of using urea complexation on the concentration yield compared with dry fractionation and low temperature solvent crystallization, results revealed that n-3 fatty acids were enriched in liquid fractions of all methods except by dry fractionation. The highest enrichment was achieved with the urea complexation method (83.00%) [157]. In the same context of valorization of marine by-products, application of urea crystallization on tuna oil recovered from liquid waste by-product from a tuna canning process allowed an increase in the concentration of n-3 PUFAs [158]. In another study conducted on concentration of fatty acids in sardine oil, the highest PUFA concentrations in low-temperature crystallization with ethanol were attained at −5°C, with EPA and DHA purities equivalent to 17.74 and 25.51%, respectively [108].

These authors also compared three different concentration techniques, supercritical fluid extraction (T = 40, 50, 60°C and 150, 250, 350 bar), Urea complexation (T = 1, −5, −10°C) and low-temperature crystallization with ethanol solvent (T = 10, 0, −5°C). The optimal conditions for each technique were determined. Nevertheless, the highest reduction of SFA and MUFA, the best increase in PUFA and the highest n-3 yield (47.53%), were obtained at −10°C in urea complexation method [108].

There are still several techniques used for the concentration of n-3 PUFAs, among which there is the use of polymeric membrane separation [159]. Optimal conditions of this method were found to be at the temperature of 36.19°C, pressure of 4.82 bar and stirring rate of 43.01 rpm with a desirability value of 0.99. With these conditions, a concentration of n-3 PUFAs of 34.98% was achieved.

Synthesized poly-vinylidene fluoride (PVDF) asymmetric membranes are also tested in concentration of n-3 PUFAs [160]. Conditions of preparation of PVDF membranes influences significantly results. In this work, PVDF membrane prepared at a coagulation bath temperature of 0°C resulted in the best n-3 PUFAs enrichment (40.4%) at 5 bar and 30°C.

4. Conclusion

Marine by-products (viscera, heads, trimmings, bones, cartilage, tails, skin, scales, blood, shells, carcasses, damaged fish, eggs, milt or soft roe), generated by marine transformation industries, constitute a good opportunity of valorization into highly valuable products. Their characterization determines the choice of the most suitable and efficient valorization method among all possibilities available, production of marine proteins (fishmeal, silage and hydrolysates), oils rich in polyunsaturated fatty acids (PUFAs) and preparation of high value compounds such as vitamins, enzymes, minerals, gelatin, collagen, chitin and chitosan, taurine and creatine, hydroxyapatite, natural pigments, biodiesel and biogas.

In this context, several studies have been carried out to explore possible technologies that can be used in the valorization of the marine by-products into marine oils and concentrated fatty acids. In addition to the conventional extraction process called also wet reduction process or hydraulic pressing, solvent extraction, supercritical fluid extraction, urea complexation, cold pressing or enzymatic hydrolysis processes could be used to transform these by-products into marine oils highly rich in PUFAs very demanded by food, nutraceutical and pharmaceutical industries.

For more advanced enhancement, the concentration of fatty acids in marine oils is also widely practiced. Several techniques can be used such as winterization, urea complexation, short path distillation, supercritical fluid extraction, low temperature solvent crystallization, fractionation by chromatography or by enzymatic processes. Combined methods were also tested like solvent winterization and enzymatic interesterification, urea adduction before a supercritical fractionation. Many studies have focused on comparison between these techniques to provide differences, advantages, disadvantages, or even optimal conditions of operating.

The main challenge in the choice of extraction and concentration techniques at industrial level is to reach higher yield, purity, quality, stability at lower cost and low unwanted environmental effects.

Conflict of interest

The authors declare that there is no conflict of interest.

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Chapter 4

Animal Waste and Agro-by-Products: Valuable Resources for Producing Fish at Low Costs in Sub-Saharan Countries

Renalda N. Munubi and Hieromin A. Lamtane

Abstract

Animal and crop production throughout the world generate high amounts of wastes or by-products annually that may possess added value compounds with high functionality. These wastes and by-products may cause negative environmental impacts and significant expenses if not well managed and or controlled. Much of these wastes and by-products is valuable and cheaper source of potentially functional compounds such as proteins, lipids, starch, micronutrients, bioactive compounds, and dietary fibbers. In aquaculture, feed is expensive, and the existing body of literature has shown that animal manure and its extracts can be successfully incorporated into fishpond to increase fish production at a low cost. In addition, crop residues such as rice bran, maize bran, and seed cakes are commonly used as pond inputs in small-scale aquaculture. Animal waste and crop residues are added in a fishpond that filter-feeding fish can use directly as feed, and these may form a major proportion of the detritus in the pond. These resources also stimulate the growth of phytoplankton that are rich in protein and are the basis of the food web that can support the growth of a range of herbivorous and omnivorous fish. Therefore, technically, wastes are used as direct feed, a source of minerals for autotrophic production and a source of organic matter for heterotrophic production. In this context, animal manure and crop residues have been used to provide great opportunities to improve food security. The purpose of this review is to project the potential of animal waste and agro-by-products as a sustainable alternative as aquaculture inputs to reduce poverty, malnutrition, and hunger in developing countries.

Keywords: animal waste, fish farming, crop residues, farming systems, valorization

1. Introduction

Aquaculture is one of the world's fastest growing food production sectors with great potential for food supply, poverty alleviation, and enhanced trade and economic benefits, as targeted by sustainable development goals SDGs. The contribution of aquaculture to global fish supply increased from 3.9 percent in 1970 to over 41.3 percent in 2011 amounting to 63.7 million metric tonnes valued over USD

119 billion [1]. Its average growth rate of 8.8 percent has outpaced capture fisheries (1.2%) and terrestrial farmed meat production (2.8%) [1]. Aquaculture accounts for around 50 percent of seafood supply globally [2]. This quantity is expected to increase substantially as population increases (**Figure 1**). Aquaculture has gained much importance globally due to a decline in wild stock from natural water bodies; thus, aquaculture plays a key role in augmenting dwindling catch capture fisheries. It is well known that among other challenges facing the aquaculture sector, availability and quality of feeds affect its growth particularly in sub-Saharan (see for example [4–10]). Despite this challenge, aquaculture has been considered as one of the economic activities that contribute to poverty reduction, food security, and nutrition in the sub-Saharan Africa [4, 11, 12] and Asian countries [1, 13–17].

In order to realize the contribution of aquaculture in the alleviation of poverty and improvement of food security, development agencies should broaden their focus beyond poor/subsistence producers to include small and medium enterprises adopting a value chain perspective [18]. Bangladesh, which is among developing countries, has proven that aquaculture intervention in resource poor and marginalized group marked an increase in income savings and frequency of fish consumption [19]. Although small-scale fish farmers play a big role in poverty reduction and food security, the intensification from extensive to semi-intensive is essential [20]. However, for the intensification to take place, there should be an increase in investment in technological innovation and transfer through (i) Nutrition, feeds and feeding management, and (ii) low-impact production systems. This paper discusses

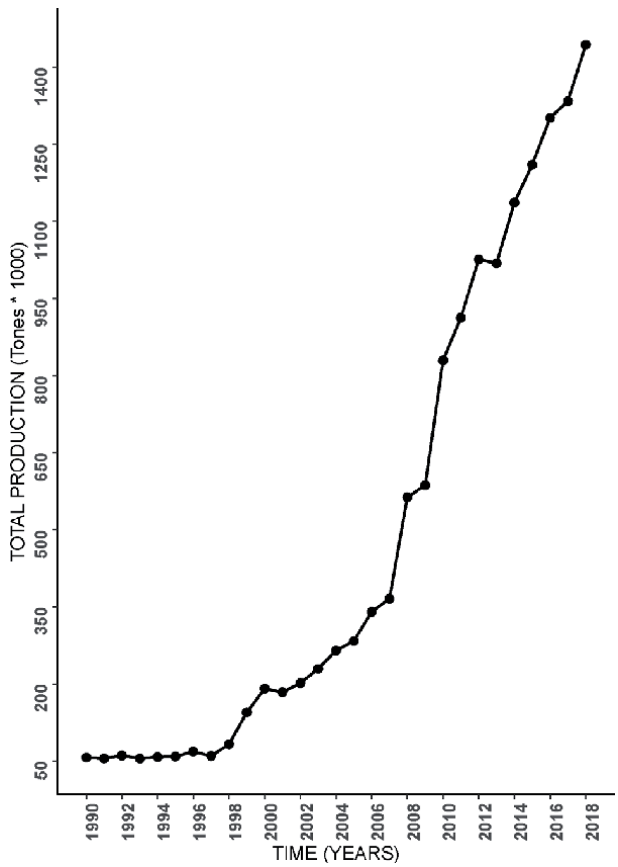

Figure 1.
Freshwater Aquaculture trend for African countries from 1990 to 2018 (data analyzed by this study see [3]).

the valorization of animal waste/by products and plants/crops-by-products to produce fish at low cost in order to increase nutrition and reduce food insecurity.

2. Aquaculture production in Africa

Africa's fisheries output is dominated by capture fisheries, but the contribution of aquaculture to the total amount of fish produced in the region has grown at a steady pace over the past decade (**Figure 1**). In these countries, fish is produced from capture fisheries and aquaculture. However, fish catches from wild sources have been declining, due to multiple anthropogenic pressures including climate change, overfishing, habitat destruction, invasion of non-native species, illegal, and unregulated fishing, and poor governance [21]. For example, consumption in the Eastern Africa region was projected to increase from 4.80 kg in 2013 to 5.49 kg by 2022 [22]. This implies that in order to meet the gap between fish production and the increasing demand for food fish, aquaculture production must double by 2050 to satisfy the Africa's fast-growing human population [23]. An appropriate way of keeping this sector growing constantly is the development of new researches aimed at determining the benefits of using different and cheap resources of feeds and determining how these strategies influence economic and productive parameters.

Country	Total production	Percent
Egypt	930,344	70.476
Nigeria	160,114	12.129
Ghana	70,628	5.350
Uganda	70,095	5.310
Zambia	17,500	1.326
Kenya	12,160	0.921
Tanzania	11,000	0.833
Zimbabwe	10,500	0.795
Malawi	5036	0.381
Rwanda	4526	0.343
Mali	3524	0.267
Congo	3185	0.241
Cã'te d'Ivoire	3000	0.227
Benin	2802	0.212
Lesotho	2500	0.189
Madagascar	2372	0.180
Algeria	2045	0.155
Angola	1752	0.133
South Africa	1503	0.114
Burundi	1455	0.110
Others	4045	0.306
Total	1,320,086.62	100

Table 1.
Freshwater aquaculture production (tones) in Africa by country in 2018. (data analyzed by this study see [3]).

In the aquaculture sector, Africa produced about 1400 tonnes of fish from freshwater aquaculture in 2018, but most of this came from Egypt, which contributed more than 70 percent of the total production (**Figure 1**, Data obtained in [3]). Major aquaculture producers in 2018 with more than 10,000 tonnes include Egypt, Nigeria, Ghana, Uganda, Zambia, Kenya, Tanzania, and Zimbabwe. Production has increased three times for the past ten tears from 563,000 in 2008 to 1,440,000 in 2018 (**Figure 1**, [3]). In general, African aquaculture production is overwhelmingly dominated by finfishes (99.3%), with only a small fraction of production from marine shrimps and mollusks [23]. Among the freshwater cultured finfishes, tilapia farming is the main product, which is also the most popular fish from a consumer perspective. Aquaculture production in Africa is also increasing as presented in **Table 1**.

3. Aquaculture production systems

In Africa, aquaculture systems are made up of extensive and semi-intensive systems. Small-scale earthen ponds (extensive systems) are characterized by low inputs and low yields. However, semi-intensive systems are characterized by human intervention where by fertilization is done to improve feed availability, hence, improved fish yield. In East Africa, semi intensive mainly used to produce *Oreochromis niloticus* and *Clarias gariepinus* in either monoculture or polyculture [24]. They consist mostly of earthen ponds, liner ponds and concrete ponds.

Other systems include cage particularly in areas with large water bodies including East Africa great lakes. Cage culture involves holding organisms under captivity within an enclosed space while maintaining free exchange of water. Cages use the existing water bodies, therefore, require comparatively low capital outlay and use simple technology, they can be used not only as a method for producing cheaply and high-quality protein but also for cleaning up eutrophicated waters through the culture and harvesting of caged planktivorous species. Although fish farming in cages in the existing water body is considered inexpensive relative to pond construction and its associated infrastructures [25], the feasibility and profitability of fish cage culture is influenced by the cost of input invested and revenue collected from output.

Although not common, re-circulating aquaculture system (RAS) has been used in some countries particularly South Africa. RAS refers to a fish farming technology that reuse wastewater from tanks/rearing premises [26]. Water reuse in RAS is supported by both inline and end pipe treatment using a series of mechanical filter for solid waste removal, bio-filter for dissolved nitrogenous waste removal and sludge pond to settle suspended solid [27]. RAS technology is termed as sustainable advanced production system that provides constant and independent production conditions and reduces water consumption compared with semi-intensive pond aquaculture, RAS technology provides high fish productivity with better effluent control of environmental conservation [28, 29]. Some of the sub Saharan countries have benefited from high temperature to which RAS performs efficiently [30]. The adoption of the system is low due to high cost of initial capital investment in tanks and high cost of electricity required in running the system and feeds. This has therefore called for sustainable aquaculture by integration of fish with livestock. Such integration involves the recycling of livestock wastes and processing by-products as manure and/or direct food for fish. Today, aquaculture in developing countries is mostly a small-scale activity and is usually not practiced as

a stand-alone economic activity, but rather as subsistence farming integrated with agricultural activities such as horticulture and rearing of livestock.

4. Organic manure and fish growth

The production volume and market share of aquaculture products are advancing extremely rapidly. However, feed is usually recognized as the single largest cost to producers, hence, the best way of reducing the cost of fish production is using organic manure and supplementary feed when available. Animal manure is widely used in developing countries in fish production in earthen ponds. The quality of manure as a fertilizer varies depending on the source of animal and the quality of feed fed to the animal [31, 32]. Research showed that pig, chicken and duck manures increase fish production more than cow and sheep manure. For example, in Asia, fish farming is probably the only branch of animal husbandry in which the use of manures is a traditional management tool. In Sub-Saharan Africa, ponds are fertilized using organic manures such as cow dung, sheep, poultry or rabbit manure [33]. The use of animal manure to fertilize ponds has been widely practiced in many countries in order to increase plankton so that there is more natural food for fish to eat, hence, high fish production. Manuring is therefore considered a cheap and preferred source of nutrient to increase fish production.

Pond fertilization with animal manure stimulates production of bacteria, phytoplankton, zooplankton, and benthic organisms [34]. The use of animal waste (livestock) has been studied under integration systems in Africa [35–37] and extensively in Asian countries [38, 39]. Benefits of integrated Agro-aquaculture systems have been reported in resource poor areas particularly in developing countries [38, 40, 41]. Studies conducted in sub-Saharan countries on the integrated aquaculture and agricultural systems are presented in **Table 2**.

Several studies showed that organic supplements contributed to fish yields by supplying P, N and C for algal growth and by stimulating detritus production and heterotrophic utilization. It is well known that high fish yields can be achieved through abundance of plankton in the cultural system [46]. Africa has vast resources of livestock and poultry, which play a vital role in pond fertilization. Livestock wastes including animal manure and poultry by-products are valuable resources in fish farming [47]. Livestock manure contains protein content of about 15 percent, energy (1250) kilocalories per kilogram, manure, and soluble vitamins [48].

Name of IAA	Country	Fish spp.	Livestock	Author(s)
Fish-cum-vegetable	Kenya	Various	—	[42]
Unknown	Malawi	Tilapia	—	[41]
Fish-cum-vegetable	Tanzania	Tilapia	—	[43]
Fish-cum-vegetable	Tanzania	Tilapia & Catfish	—	[36]
Fish-poultry-vegetable	Tanzania	Tilapia	Poultry	[44]
Fish-poultry-vegetable	Tanzania	Tilapia & catfish	Poultry	[45]
Fish-cum-poultry	Tanzania	Tilapia	Poultry	[37]
Fish-cum-rabbit	Rwanda	Tilapia	Rabbit	[35]

Table 2.
Studies on the integrated agro-aquaculture in sub-Saharan countries.

5. Fish feed availability and the concept of valorization

One of the solutions of fish feed availability is to entice animal feeds producing industries to consider also the production of fish feed [49]. However, the big issue here will be affordability; of these industrial feed products; most of our farmers belong to subsistence income bracket, hence, they might not afford these feed products. The use of floating pellets needs higher investment [50], which in most cases is lacking among smallholders; and unless the government intervenes in addressing the problems through either credit facilities or the provision of subsidies, the situation is not likely to get any better. It has been established that profitability in aquaculture is influenced by the cost of feed [51]. In Sub-Saharan countries, justification for industrial scale production of fish feed is not a priority despite the availability of raw materials [4]. Therefore, in order to feed fish, farm-made feeds can be made using locally available ingredients including animal by-products and plant residues.

In Tanzania, more than 80 percent of fish farmers relied on locally available feed ingredients as a major feed supplement for their cultured fish [43]. These local feed ingredients are categorized into four groups, (i) animal by-products, (ii) agricultural by-products, (iii) plant leaves and weed, and (iv) industrial by-products. It has been reported that the early growth phase of tilapia in 1991–2000 was significantly contributed by the use of alternative sources of protein including fishery by-products, terrestrial animal by-products, and a wide range of plant by-products [52]. In this chapter, discussion is cantered on the valorization of two broad categories of ingredients, plant and animal based ingredients.

5.1 Plant based ingredients and by-products

In addition to fertilization, feeding in ponds is done using supplementary feeds formulated on farm or purchased from cottage fish feed production industries. In some cases, cereal bran such as grains as energy source (**Figure 2**) and soybeans as

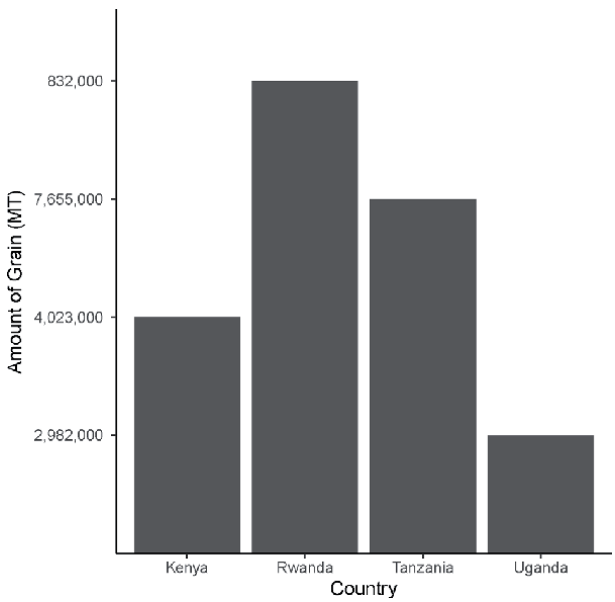

Figure 2.
Amount of grains required for fish feed compounding in East Africa. Source [53].

source of protein (**Figure 3**) are used in aquafeeds to increase pond productivity. The production from this system ranges from 1000 to 2500 kg/ha/year [33]. Most farmers prefer this system since it is less expensive in terms of feed inputs. Ten edible plant leaves were evaluated (see in [54]) as potential feed ingredients for aquatic animal, the results suggested that some of the plant leaves used contributed on growth performance, immune system, and disease resistance for the fish. Other important plant leaves which have been subjected to experiments to see whether they can be used as ingredients for fish feed formulation includes cassava leaves [55] and *Moringa* leaf [56]. In another study results showed that the integration of vegetables (*Brassica oleracea*) as pond inputs increased fish production and net yield than those reared under non-integrated systems [57]. In general, the amount of grain and soybean required in the four East African countries is given in **Figures 2** and **3**. Another experiment (see [58]), showed that when wheat bran, rice bran, and groundnut bran were used as agro-industrial by-products to examine their economic effectiveness in fish production, there were variability in growth rate and economic benefits, suggesting that variability of agro-by products reflects the growth rate of fish.

5.2 Animal based ingredients and by-products

According to the circular economy approach which focuses on the "reduce, reuse and recycle" of resources, waste from animal and food can be valorized leading to the production of proteins and other valuable compounds [59, 60]. For example, chicken, pig and cattle manures are substrates for production of housefly (*Musca domestica*) maggots which are in turn used as fish feed, or as supplement to fish meal in fish feed formulation [61]. Maggots are readily available and are accredited for having high nutritional value with an amino acid profile with biological value

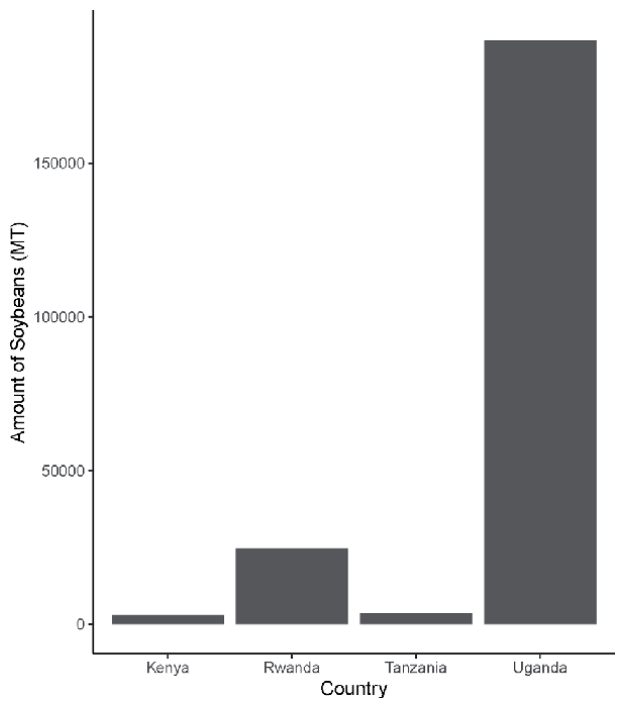

Figure 3.
Amount of soybean required for fish feed coumpound in four countries of East Africa. Source [53].

exceeding that of soybean and groundnut. Maggots can be harvested, processed into a meal that can be used to substitute or replace fish meal [61, 62]. Maggot grown on a mixture of cattle blood and wheat bran contained 92.7% dry matter, 47.6% crude protein, 25.3% fat, 7.5% crude fiber, 6.25% ash, and an amino acid profile comparable to fish meal [59] suggesting that animal wastes utilization can be used to produce insects which can be utilized as fish feed hence, reduce feed cost significantly, thus leading to a viable and sustainable aquaculture industry. The replacement of 25 percent fishmeal in catfish feed, culture with maggot gave high growth performance and profitability than fishmeal based diet [63, 64]. Several researches [65, 66] have been reporting on the use of red worms, black soldier fly, common housefly, and yellow mealworm as a source of protein to replace fish-meal. It is envisaged that the valorization of animal and animal by-products such as animal blood, offal of poultry, residues of traditional brewery waste, animal manure and fish wastes may contribute significantly on fish production hence, food nutrition and security.

6. Conclusion

It is clear that fish consumption in Sub-Saharan Africa is increasing. In order to maintain the present amount of fish consumption, considerable additional quantities of fish are required through aquaculture. In turn, aquaculture requires feed as a major input for increasing production. Since commercial fish feed production in most of the sub-Saharan countries is limited, considerable investments are required in local and low costs feed manufacturing. Raw materials of plant and animal origin are sufficiently available in the region albeit the possible competition from livestock and human consumption. Therefore, valorisation of animal and agro-products in the Sub-Saharan countries is imperative/inevitable for increasing fish production at low cost.

6.1 Recommendations

In order to increase food nutrition from aquaculture production through valorization of agro-by-products in the sub-Saharan countries, the following are recommended:

- Strengthen the use of Public Private partnering by putting more emphasis in services related to the collection of feed ingredients and preservation
- Public Private partnering must be embedded into an economic vision for aquaculture development
- Recognize small scale farmers as commercial ones and encourage small-scale farmers to work together by forming associations (work groups)
- Provide credit facilities for the private sector particularly for the small-scale holders
- Put emphasis on public private research partnerships and knowledge sharing on valorisation
- Provide capacity building and general education for small holders in order to improve their technological, managerial and commercial skills in handling agro by-products

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6.2 The way forward

With the ever-increasing human populations in sub-Saharan countries, the demand for food would increase and natural resources will become even scarcer. This situation will be more worsen with severe climate changes. These trends necessitate for a critical assessment of the situation to enable devise informed solutions in addressing issues pertaining to agro by-product processing and valorization.

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Section 2

New Technologies

Chapter 5

Innovation in Food Products Using Ozone Technology: Impact on Quality Assurance

Carlos Martín Enríquez-Castro, Manuel Pérez-Nafarrate and Jesús Enrique Gerardo Rodríguez

Abstract

Ozone application is a non-thermal technology used in food preservation, which is a powerful oxidant agent used in water and air treatment specially in disinfection processes for agriculture and food industry. The objective of this revision work is to publicize ozone applications in the growing, harvest, and postharvest handling of fruit and vegetables (F & V) across México. Ozonated water by foliar spraying and irrigation were proved to be effective in the control of pathogens, bacteria, and bugs. The use of Ozone was effective to heighten quality parameters of F & V, such as color, flavor, and soluble solids in mango, sugarcane, citric fruits, and nopal, increasing shelf life of fresh products up to 15 days after harvesting. Several protocols mentioned to fulfill the requirements of the producer were developed by TRIO3. The methodology proposed and the designed equipment by the company suggest a wider approach of this green technology in agriculture.

Keywords: ozone, climacteric fruits, ozonated water, foliar spraying, microbial growth control

1. Introduction

Ozone (O_3), also called trioxygen, is a gaseous substance whose molecule is formed by three oxygen atoms linked with an angular geometry. The ozone is formed by applying on the oxygen molecule enough energy to divide it forming various molecular structures [1]. This homonuclear molecule is the oxidant agent most powerful used for water and air treatment specially in disinfection processes for agriculture and food industry. Ozone is friendly with the environment and classified as Generally Recognized as Safe (GRAS) according to the Food and Drug Administration (FDA) [2].

Ozone is a universal disinfectant that reacts with the contaminant agents. It suppresses bad color and undesirable odor at the same time, destroying molds, bacteria, virus and algae [2, 3]. The deodorant action of ozone is due to the oxidation of chemical compounds such as ketones, hydrocarbons, acids, sulfides and nitrogenated derivatives [4]. Ozone oxidizes the cell wall, breaking its membrane and attacking to the DNA and RNA constituents. For this reason, the microorganisms are unable to develop immunity to the action of ozone as they do against other

chemical compounds [1, 4, 5]. The use of ozone as sanitizer is effective without compromising health of consumers [6]. Traditional sanitizers are cheaper than ozone, however, sometimes they are not appropriate when a new outbreak emerges or when the presence of new food pathogens is detected [7, 8].

Ozone as a non-thermal technology is used in food preservation, which helps to improve the organoleptic characteristics of food following good manufacture practices [9]. Ozone can be pumped into a postharvest cold room. In water applications, ozone is drawn into a low negative pressure water stream using a Venturi injection system (Mazzei Company, LLT). The excess of not mixed ozone must be captured and destroyed to avoid the corrosion and personal injury [2]. A useful method of destroying the remanent ozone is combining UV light and catalytic agents such as granulated activated carbon [10]. The aim of this review is to describe several memories in the application of ozone in agriculture. Adhered to this, every project mentioned was adjusted to the state regulations achieving the expectations of the producer.

2. Biological factors involved in the deterioration of fruits and vegetables (F & V)

Some of the variables affecting the postharvest conditions of raw materials are related with their metabolic processes. Respiration as a process in living cells moderates the release of energy through the breakdown of carbon chains and the formation of new compounds, necessary for the maintenance and generation of synthetic reactions after harvest [11]. A low respiration rate in fresh materials increase postharvest life of perishable fruits. Ethylene concentration acts as a maturation hormone due to its biological activity in ripening climacteric fruits. Therefore, ethylene concentration can be monitored using precise instrumental methods [12]. Fundo et al. (2018) monitored the quality parameters of Cantaloupe melon juice using 30 min O_3 and 60 min O_3 treatments. They observed a diminution in color, vitamin C, total carotenoids, and antioxidant activity; but an increase in total phenolics registered in ozonated juices.

One of the most relevant stages of growth and development in F & V is probably the ripening or maturity. During ripeness, the enzymatic changes promote softening, hydrolytic conversion of storage material-starch to free sugars, and pigmentation changes, especially in climacteric fruits [13]. Flavor changes are considered an important topic in the metabolic pathway of F & V depending on sugars, acids and volatile compounds changes during ripening. As the fruit ripens, there is an increase in the synthesis of their volatile compounds [14].

2.1 Physical damage and pathological deterioration

In order to assure a good maturity index of raw materials, some factors such as water loss, changes in size, and changes in shape or surface must be considered, particularly when a marketable size is reached. Therefore, raw materials that presents physical damage must be separated to prevent postharvest losses. Measurement of texture, either manually or instrumentally can help to detect the maturity index of the fruit. Paciulli et al. [15] measured texture to frozen vegetables such as asparagus stems, zucchini and green beans. They observed a softening effect on the vegetable structure probably due to the blanching treatment affecting the inner cell wall. Depolymerization and/or solubilization reactions of the tissue occurred as a consequence of thermal treatment. In addition, it is advisable to avoid the improper handling or biodeterioration by microorganisms, insects, rodents or birds [16].

3. Applications of ozone

The harvesting of fresh F & V, its management during the freezing, packaging and processing involves the use of water. The presence of water on the agricultural products can increase the probability of contamination of plant pathogens and microorganisms of major concern in food safety. Inappropriate application of procedures related with prevention of contamination and disinfection of water can have severe consequences. Special attention on the microorganisms present in the water recirculation system must be taken because of a rapid reproduction and adaptability in adverse circumstances [16]. Ozone used for vegetable washing and disinfection either gas or dissolved in water has been effective in reducing several microorganisms such as *Escherichia coli*, Penicillium Italicum, and *P. digitatum* [17, 18]. In this way, damage and deterioration of the fruit can be prevented, and postharvest life increased [19]. Ozone is used to eliminate pests and insects without harming the food quality and the environment. Applied at proper doses, ozone diminishes the proliferation of bugs, and is an excellent choice to replace chemical products.

Feston et al. [20] used ozone and observed a reduction of bed bug (*Cimex lectularius* L.) in different life stages. They concluded that LC_{50} and LC_{90} values were higher in nymph and adults than the observed in eggs. The damage caused by insects it is not only restricted to what the vector physically damages and ingests, but also what is defecated over the plant. All these factors promote fungal development like Fusarium spp. and Aspergillus spp., and in most of the cases, they are responsible of promoting the development of mycotoxins [21].

3.1 Ozonated water by foliar spraying

Ozone applied in a foliar way allows the addition of nutrients in the plant and the elimination of pests and diseases. The effectiveness of this chemical agent in reducing microbial load of foods includes the presence of fungi, molds, spores, viruses, bacteria, eggs, and nymphs of insects as shown in **Figure 1**. Ozone has no unfavorable effects on the organoleptic characteristics of foods including textural and nutritional quality. Besides, ozone acts directly in the metabolism of the microorganism suppressing its development [19, 22]. During foliar spraying, plant diseases can be reduced due to the effect of nutrients and ozone added. Another benefit observed of using foliar spraying is that the dose of fertilizers applied in cultivars and the operating costs can be decreased. According to Ali [23] an appropriate foliar spraying improved the quality parameters of papaya using 2.5 ppm and 3.5 ppm ozone. Weight loss, firmness, ripening and soluble solids concentration were maintained and preserved for a longer storage period.

3.2 Ozonated water in the irrigation system

There are different ways to carry out an irrigation system: by gravity, sprinkling, dripping, micro sprinkling or sub-irrigation, and capillary diffusion as shown in **Figure 2**. The ozone irrigation system in cultivars allows the incorporation of nutrients and oxygen, the strengthening of roots, thickening of the tree trunk, and the increase of soluble solids and sugars. Ozonated water is effective to eliminate virus and bacteria and control in nopal-vegetable such as Fusarium spp. and Aspergillus spp. [24]. The nematodes present in their root are destroyed due to the sanitizing effect of ozonated water. The mature fruit (tuna or prickly pear fruit) can be sprayed with ozonated water to guarantee the innocuity and integrity of fruit [25].

Figure 1.
Ozonated water by foliar spraying in mango cultivars.

Figure 2.
Ozonated water in the irrigation system.

Ozonated water irrigation provides oxygen to the root and the stream that flows in its path reaching the root of the plant. The inner membrane of viruses, bacteria, fungi, algae, spores, and other microorganisms are destroyed using ozone dissolved

Commodity	Ozone treatment	Effects on quality	Reference
Broccoli	0.04 μL L^{-1}, 7 d, 10 °C	Delay of metabolic process & oxidative reactions	[2]
Cucumber	0.04 μL L^{-1}, 17 d, 3 °C	Enhancement of appearance. Higher values of Firmness (N) than the observed in control simples.	[2]
Mushroom	0.04 μL L^{-1}, 14 d, 4 °C	Blotch in cap surface was diminished.	[2]
Apple and pears	1.5 μL L^{-1}, 100 d, 20 °C	Total soluble solids and firmness were sustained.	[6]
Orange juice	Ozone variable dose	Control of decay, abatement of ethylene, removing of pesticides and residues.	[7]
Fresh cut lettuce	1 mg L^{-1}, 10 d, 4 °C	Storage life increased, decline of respiration rate & enzymatic browning.	[8]
Cantaloupe melon juice	7 ± 2.4 gL^{-1} for 30 & 60 min	Increase of polyphenols and vitamins.	[5]
Mango	10 μL L^{-1}, 3 d, 25 °C	Delay of ripening, Improvement of quality characteristics.	[7]
Papaya	2.5–3.5 ppm	Weight loss reduction, higher firmness values.	[8]
Soil and water deposits	Ozone variable dose	Bactericidal action on responsible for food delay (*P.aeruginosa*)	[6]

Table 1.
Uses of ozone in several fresh produces and its benefits.

in water, and promotes strength and productivity in the plant preventing diseases. This technique is widely applied in fruit trees, vineyards and nopal-vegetable among others [26]. **Table 1** shows uses of ozone in several materials and its benefits.

Several studies have been conducted to identify the vector disease in the plant as follows: leaf yellow curl virus in tomato [27], whitefly (*Bemisia tabici*) affects cotton [28], tropical fruits [29], and potato [30]. Ozonated water irrigation used in a regular way effectively eliminates plant disorders leaving no smell or trace [19]. Several researchers emphasize the effectiveness of ozone [30–34]. Contigiani et al. [35] applied ozonated water effectively in strawberries to reduce the risk of pathogen proliferation. They found significative differences ($P > 0.05$) in the fungal control (*B. cinedea*) using ozonated water for 5 min (2.73 mgL-1). Additionally, quality attributes were preserved. Ozone seems to be a very promising technology to reduce post-harvest losses.

4. Innovation project: processing of mangoes (*Mangifera indica*) using ozone technology

4.1 Introduction

Mango (Ataulfo variety) cultivated in the tropical areas of south of Mexico has a supreme quality but achieves a fast state of maturity and decomposes easily due to a high microbiological load. Local growers apply higher doses of fertilizers and pesticides several times per year to avoid the infestation of mango tree, specifically whitefly and their larvae. Effective post-harvest techniques to avoid major economic losses must be employed.

4.2 Methodology

The research team of Pérez-Nafarrate [36] designed processes using ozone gas and ozone water for disinfection and packaging of mango in an industrial plant in Guerrero, Mexico. The accomplishment of concise food safety regulations was according to studies realized by Tran et al. [37]. In order to preserve the physico-chemical characteristics of mango fruit, the next premises were followed:

Ozone gas used in a regular basis (one dose every third day) should eliminate gradually the presence of pests.

Gas monitoring was performed using an ozone analyzer (Model IN-2000, L2-LC, in USA Incorporated).

Ozone concentrations (1.5, 2.5, 3.5, 5 ppm) applied (96 h, 30 ± 3 °C) in the storage container should reduce the ethylene content generated as part of the fruit ripening process.

4.3 Results and discussion

The most effective ozone dose (3.5 ppm) preserved the organoleptic characteristics of the fruit and delayed the ripening for 15 days. According to producers, optimal shelf life of mango is 10 days [37]. Soluble solids, color, flavor and empiric firmness were improved (data not published) and a sensory evaluation of the fruit was developed by the operators. More and Rao [38, 39] used a combination of UV-C irradiation (210–280 nm), guar gum (2.5%), and ozone water (400 mg-h-1) to preserve organoleptic characteristics of mangoes at 32 ± 3 °C after 19 days.

Compliance with good manufacturing practices diminish the fruit deterioration, and the economic profit increased. Harvesting of climacteric fruits, including mangoes, papaya, and banana; requires storage temperature and relative humidity similar to that observed in subtropical environments. According to Pérez-Nafarrate [36], key factors to conduct an ozonated water experiment consist on reliability and accuracy of the measurement devices, type and growth stage of the microorganism, ozone concentration and time of contact with the product.

5. Innovation project: use of ozone in the harvesting and processing of nopal

5.1 Introduction

The nopal vegetable (*Opuntia ficus-indica*) grows in semi-arid zones around the world, it has a high dietary fiber content, and is usually consumed Mexico [40, 41]. Physical and morphological characteristics of nopal allow a quick adaptation to low precipitation and excessive heat (**Figure 3**). The nopal consist of two main parts: the edible part (84–90% water), and prickly pear (45–67% of reducing sugars) also called "tuna" [42]. Relative humidity is a crucial variable in nopal for development of pests and diseases. The most commonly microorganisms present in nopal are: cactus weevil *Cactophagus spinulae* (*Gyllenhal*), [43]; boll weevil (*Cylindrocopturus biradiatus Champs*), [40]; white worm (*Lanifera cyclades Druce*), zebra worn (*Olycella nephelepsa Dyar*), [44]; and cochineal (*Dactylopius coccus costa*), [45]. The most important include the bacterial stain (*Bacterium sp*.), rotting of the epidermis, anthracnose, prickly pear gold [46], the thickening of cladodes and the presence of bold among others [47]. The objective was to give added value to nopal-vegetable (raw and processed) using ozonated foliar spraying and ozonated water irrigation.

Figure 3.
Manual harvesting of nopal in México.

5.2 Methodology

Due to the nature of nopal, good manufacture practices in soil and water are required to control outbreaks of diseases. The experimental plantation was located in Matehuala, San Luis Potosi; México. The producers gave light watering to nopal seedlings every 8 or 15 days during the dry months as shown in **Figure 4**, and the irrigation program was monitored periodically to establish the ozone generator requirements. Salinity, hardness, pH and sedimented solids were registered for 15 days before installing the irrigation system. The ozone solubility control included the measurement of pressure and temperature, concentration of carbonates, nitrates and metals [6]. Once the nopal "pencas" (thick and fleshy central nerve that has leaves) were cut, they were placed in recyclable plastic boxes preserving the thorns to avoid the oxidation reactions [48].

5.3 Results and discussion

Ozonated water favored the organoleptic integrity of the nopal, reducing oxidation reactions and retaining green color in sliced raw nopal. Other goals achieved were the reduction in microbial load and the increase in shelf life (data not shown). The final product was packaged and sealed in plastic bags with 500 g and 1000 g. An investment proposal to install a rural cooperative company with capacity of processing 500 kg per day was presented to the cooperative.

Technical specifications and cost of a nopal sider, an immersion washing machine, a centrifugal dryer, and a vibrator dryer with cold were included in the investment project for $1,500,000.00 pesos.

Figure 4.
Sustainable management of the nopal irrigation system.

Good manufacturing practices are essential to accomplish nopal processing with ozone. Water quality and soil requirements monitoring were key factors to improve the postharvest life of nopal opuntia. An innovative option for rural population centers was presented to detonate the local economy. Almost 50% of inhabitants in Mexico are distributed in rural areas, therefore ozone technology should be affordable to most of the population.

6. Innovation project: use of ozone to counteract *Huanglongbing* in citrus trees

6.1 Introduction

The *Huanglongbing* (HLB) disease or "yellow dragon", affects the worldwide production of citrus (lemon, tangerine, orange). *Diaphorina citri* (Kuwayama), the insect vector of HLB, is the main responsible as shown in **Figure 5** [49, 50]. HLB is mainly distributed in Asia (Malaysia, Thailand and Vietnam) and Africa. Farmers invest up to 50 insecticide doses per year to control the vector and avoid the spread of the disease [51]. Symptoms of this plant disease include damage in tree and fruit, sparce yellow foliage, and stunting among others as shown in **Figure 6** [52], shortening of the productive life of the plant, and detriment of final quality product [53].

Figure 5.
HLB in citrus tree. Source: www.gob.mx/agricultura.

Figure 6.
HLB symptoms in Mexican lemon tree. Source: www.gob.mx/agricultura.

Since 2010, HLB has been spread to the American continent especially those areas with frequent rainfall and template temperatures. HLB in Mexico is a serious threat to the 526 thousand hectares of citrus distributed in twenty-three Federal Entities. This in turn represents a production risk of 6.7 million tons per year with a value of more than 400 million dollars. Around 67,000 producers engaged to citric industry generated approximately 70,000 direct jobs and 250,000 indirect jobs, all of them endangered by HLB [54]. Specific research in Mexico to counteract this pandemic is urgently recommended.

6.2 Methodology

In 2013, a collaboration protocol was signed between TRIO3 Food Technologies, the National Institute of Forestry, Agricultural and Livestock Research (INIFAP, for its acronym in spanish) and Los Limones Orchard, located in Tecoman; Colima (18°46′38.04′′ N, 103°48′56.74′′ W). Pérez-Nafarrate [55] evaluated the effect of ozone to eradicate the HLB disease in the productive region previously mentioned. Two main objectives were proposed: first, the early detection of infected trees in the growing season; and second to evaluate the damage spread by the vector in those trees with an advanced stage of the disease, and register its total loss [56]. Fifteen hectares planted with real lemon were micro-sprinkler irrigated with ozonated water, and nutrients were added to roots of trees every third day during six months. The sprinkler irrigation process was evaluated at the end of the experiment.

6.3 Results and discussion

Ozone applied in Los Limones Orchard considerably reduced the population of larvae and adult vectors increasing the citrus production (data not shown). After ozonated water process concluded in lemon trees, other benefits were observed by farmers and technical staff including the improvement of the foliage coloration, and strengthening of trunk and root trees, which are very promising. Showler [57] used an organic mixture composed of corn meal, humic acid, molasses and fish oil to suppress greasy spot in infected trees. An increase in soluble solids content and a weight loss reduction in treated trees, as a result of the healing treatment during three years, provided similar results as ozone did. According to Perez-Nafarrate [55], the insecticide and plaguicide action of ozonated water proved to be faster than traditional methods.

HLB, like many other plant diseases, be diminished effectively with ozonated water. Gas concentration used, soil quality and the effectiveness of the irrigation system were the critical parameters to observe in the experiment. This clean and affordable technology is efficient to control HLB, but more research is still needed to eradicate this bacterium from Mexico and its surroundings.

7. Innovation project: use of ozone to control diseases of sugarcane

7.1 Introduction

Sugarcane (*Saccharum spp hybrids*) is a tall perennial grass traditionally grown in tropical zones and used for obtaining sugar concentrates, molasses and other derivatives [58, 59]. Good manufacturing practices and biological control of diseases allow the correct development of this plant. It is common to find a considerable number of microorganisms in sugarcane such as bacteria, fungi and viruses, all these entities cause several diseases in the cultivar [60] as shown below:

Most common organisms found in sugarcane include: red rot (*Colletotrichum falcatum*) [61]; wilt (*Fusarium sacchari*) [62]; grassy shoot (*Exitanius indicus*) [63]; leaf scald (*Xanthomonas albilineans*) [64]; smut (*Sporisorium scitamineum*) [65, 66]; brown rust (*Puccinia melanocephala*) and orange rust (*Puccinia kuchnii*) [67].

The diseases of major economic importance in sugarcane are described as follows:

Mosaic disease. Is produced by Sugarcane mosaic virus and attacks young plants making areas appear on leaves pale green and yellowish within a normal green. The reeds become stunted causing production declines [68].

Eyespot. This name is derived from the powdery black mass of spores associated with this disease. The affected plants show a reddish elliptical lesion surrounded by a yellowish structure that varies in size. The spores of the fungus Ustilago scitaminea are transmitted by wind, rain, irrigation water, seeds or animals [69].

Rust. Produced by the fungus Puccinia melanocephala manifested by many elongated spots on the leaves, show no growth and the stem become thin. The yellowish spots look at the whole sheet [70].

Leaf scald. The causal organism Xanthomonas albineans is a bacterial disease first observed in Cuba in 1979. X. albineans causes sudden death of entire stems and seedlings affecting significantly sugarcane yields [64]. Leaf scald is also called in Latin America "gomosis", a plant disease characterized by an abundant production of gum. Gomosis caused by Xanthomona axonopodis involves widespread dwarfism in plant [70].

Ratoon stunting disease. RSD caused by Leifsonia xyli spp. was observed in Cuba for the first time in 1953. Several stunted stems very thin are present within the plant and short internodes. Damaged stems increase as the number of cuts in the field is higher [71].

The ozonated water irrigation provides a greater contribution of oxygen to the root. Ozonated water is free of viruses, bacteria, fungi, algae, spores and any other microorganism. Growth is achieved faster than usual with more liveliness and strength as well as more productivity. This irrigation method is beneficial for plants, and is currently used in fruit trees, vineyards and crops in general. Ozonated water achieves the prevention of plant diseases such as leaf scald and rust reducing the microbiological load. Chemical products such as pesticides and fungicides are reduced improving the shelf life of fresh products [60].

7.2 Methodology

On July 4 2008 was signed a collaborative project between the National Institute of Agrarian Innovation (INIA, for its acronym in spanish) and the National Institute of Research of Sugarcane in Havana-Pinar del Río. Red ferritic soil was used to grow up a 9-month agamic seed from 5 varieties identified as follows: C323–68, C86–56, C86–456, C88–380, and C90–530. The experimental design (divided plots 5 x 6 x 2) included 180 buds from each variety with two replicates, 5 varieties and 6 treatments. Finally, sugarcane certified quality seeds (P. Vista Florida cv. 115–2014) previously treated with ozonated water were introduced among the producers as shown in **Figure 7**.

The exposure time in ozonated water was 10, 20, and 30 min respectively. The treatments included a hot water-chemical treatment, a biological one, and a sample control [52]. The measurements were carried out one and six months after sowing. The main objective was to evaluate the effect of ozonated water on sugarcane seed growing. The viability and growth of agamic seeds of sugarcane used was also evaluated. The ozone equipment used is shown in **Figure 8**.

Figure 7.
Certified sugarcane seed P. Vista Florida. Cv. 115–2014.

Figure 8.
Equipment used for the ozonation of water.

7.2.1 Treatment with ozonated water

An irrigation and sprinkler system with ozonated water to promote the growth of sugarcane is shown in **Figures 9** and **10**. The fungicide was reduced progressively to control and eliminate fungus and pests as the amount of ozonated water increased. Previously, 90 buds were submerged in ozonated water during 24 h. Thirty buds per variety were immersed in ozonated water (1 ppm) for 10, 20, and 30 min respectively.

7.2.2 Witness without treatment

Thirty buds per variety were soaked on water for 24 h without applying any treatment.

Figure 9.
Ozonated water irrigation system as applied on sugarcane cultivation.

Figure 10.
Ozonized water by foliar spraying.

7.2.3 Hydrothermal + chemical treatment

Thirty buds per variety were submerged 24 h in water, then immersed in hot water at 51 °C for one hour. Immediately they were treated with propiconazole (Tilt EC 250) at 0.4% (w/w) for 15 min. This time was considered enough to eliminate leaf scald and red rot diseases.

7.2.4 Biological treatment

This treatment started on July 4 2008 with the immersion in water of 30 sugarcane buds per variety for 24 h, and then submerged for 30 min in a water solution of Nemacid (EDOCA, Amealco, Querétaro) at 2% (w/w). This organic insecticide is used in the control and inhibition of nemathodes. Quivican experimental station was used to plant the sprouted seeds treated with Nemacid. After 30 days, the sprouting count was carried out and analyzed in the laboratory. All the experiments were made by duplicate.

7.3 Results and discussion

All the treatments showed significant differences ($p < 0.0001$). Best results obtained on seeds immersed in ozonated water for different time intervals are shown in **Figure 11**. The sprouted seeds treated with 10 min-ozonated water immersion reached the highest germination level: 10 seeds in total. The sample control seeds and seeds treated with 30 min-ozonated water reached an acceptable germination rate: 9 and 8 respectively. Nemacid and hot water samples presented the lowest germination rate: 7 in total. Using ozonated water did not affect the viability of the seed. The experimental ozonated water immersion system proposed to producers of sugarcane reduced drastically eyespot and leaf scald. This treatment was beneficial for agamic seed viability showing a different response in every treatment, especially that with 10-min immersion time.

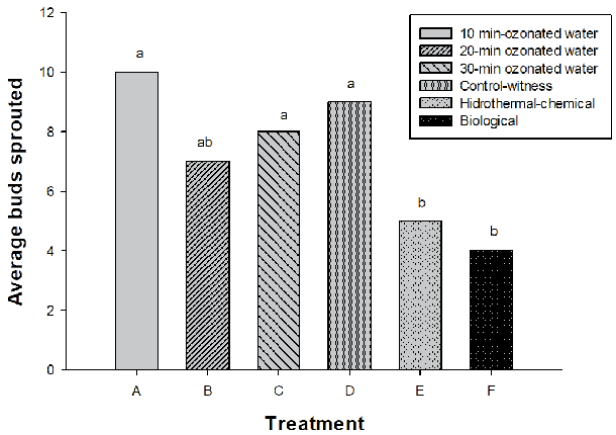

Figure 11.
Influence of ozonated water in sprouting of sugarcane agamic seed.

8. Conclusion

Ozone has demonstrated be useful for management of postharvest of climacteric fruits such as mangoes and papayas. This ecotechnology is cost effective and is very promising to use in developing countries of Latin América. Using ozone watering in Nopal opuntia crops, proved to be very effective to control diseases and increase plant health. An adequate ozone irrigation system prevents growth of bacteria and bugs in citrus trees, and other fruits, and is an alternative to reduce HLB infestation. Sugarcane and other important crops growing in tropical areas increase yield using ozonated water, and an early diagnose of diseases reduce losses and increase the economic benefit in productive areas. TRIO3 acknowledges all the producers and scientific investigators involved in the protocols developed during these last 10 years.

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Conflict of interest

The authors declare no conflict of interest.

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Chapter 6

Advanced Optical Technologies in Food Quality and Waste Management

John Chauvin, Ray Duran, Stanley Ng, Thomas Burke, Kenneth Barton, Nicholas MacKinnon, Kouhyar Tavakolian, Alireza Akhbardeh and Fartash Vasefi

Abstract

Food waste is a global problem caused in large part by premature food spoilage. Seafood is especially prone to food waste because it spoils easily. Of the annual 4.7 billion pounds of seafood destined for U.S. markets between 2009 and 2013, 40 to 47 percent ended up as waste. This problem is due in large part to a lack of available technologies to enable rapid, accurate, and reliable valorization of food products from boat or farm to table. Fortunately, recent advancements in spectral sensing technologies and spectroscopic analyses show promise for addressing this problem. Not only could these advancements help to solve hunger issues in impoverished regions of the globe, but they could also benefit the average consumer by enabling intelligent pricing of food products based on projected shelf life. Additional technologies that enforce trust and compliance (e.g., blockchain) could further serve to prevent food fraud by maintaining records of spoilage conditions and other quality validation at all points along the food supply chain and provide improved transparency as regards contract performance and attribution of liability. In this chapter we discuss technologies that have enabled the development of hand-held spectroscopic devices for detecting food spoilage. We also discuss some of the analytical methods used to classify and quantify spoilage based on spectral measurements.

Keywords: Spoilage, valorization, spectroscopy, hyperspectral imaging, artificial intelligence

1. Introduction

Food waste is a significant problem in both developed and developing economies [1]. The global seafood industry faces unprecedented challenges as demand increases, consumer preferences change, and expectations of quality increase. Consumers are demanding more transparency and a commitment to sustainability while access to at-capacity or overfished fishery resources is strained and food production and retail profit margins are thin. Global food supply chains can be a cause of improper food storage, which leads to by-product waste. Whether through a lack of quality control or a hold in the distribution process, food spoilage can occur even before the product reaches markets. Approximately 35% of fish are lost to waste

globally with between 30% and 35% loss in most regions of the world [2]. Seafood's perishability is largely to blame.

A major goal to improve the overall valorization of food and reduce agro-food waste or diversion to by-products is earlier and rapid detection of spoilage. Like medical evaluations for disease, early detection can lead to quicker response from manufacturers or consumers to increase the shelf life of food products. Microbiologists and food scientists have developed a variety of methods to detect surface microbials and pathogenic microorganisms including culturing and colony-counting methods, polymerase chain reaction (PCR)-based amplification for DNA analysis, immunoassay analysis, chromatography, and mass spectrometry [3, 4]. Unfortunately, these techniques have limited versatility and restrictive methodologies that are not practical with on-site and on-demand food quality and safety control [3–5]. However, spectroscopic technologies have shown great promise for enabling early detection of spoilage to help minimize food waste.

Another problem affecting consumers and contributing to global food waste is the lack of transparent pricing for food products as a function of shelf life. Alongside government and industry regulation, intelligent dynamic pricing based on projected shelf life at retail and other upstream points along the supply chain can encourage efforts to reduce waste. This requires new tools for tracking food products at all points along the supply chain. These tools must be easy to incorporate, objective, verifiable, and provide data on quality, provenance, and freshness.

A pioneer in the development of food quality and traceability technologies, SafetySpect is developing a new handheld quality, adulteration, and traceability (QAT) scanner to address many of these issues. Utilizing hyperspectral multi-mode technology to provide species identification and direct measurements of freshness/spoilage in a handheld device can address challenges of waste and mislabeling. In seafood and meat processing, distribution, and storage supply chains in developed and developing economies, this is likely to meaningfully decrease food waste and increase sustainable access to safe, healthy, and nutritious foods. It will also decrease costs and increase profit within supply chains by providing better attribution of liability and verification of supply contract performance. This transparency will provide incentives to upstream supply chain participants to improve operational methods that can result in the degradation of product or unnecessary, accelerated spoilage.

1.1 Current trends for examining fish quality

The main approach to improving valorization and by-product management is early detection of spoilage. A common method for detecting spoilage in fish is the Torry Freshness Score [6]. This systematic scoring method was developed in the UK to provide an objective assessment of fish quality. It uses the human senses to examine specific parts of the fish. For example, an evaluator will observe gill odors, skin tension, opaqueness of the eyes, and overall smell of the fish and provide a freshness rating between 0 (lowest) to 10 (highest). However, this manual approach to evaluating fish samples is time consuming and may be more susceptible to evaluator bias or human error. This motivates the development of technologies that enable rapid evaluation of fish quality with minimal human interpretation.

Spectroscopic approaches offer a robust, non-destructive means of detecting and evaluating the extent of food quality issues. In recent decades, advancements in micro-electro-mechanical systems (MEMS) and micro-electro-opto-mechanical systems (MEOMS) have enabled the development of miniaturized spectroscopic devices that can be used for analysis at all points along the food supply chain, from farm fields to distribution centers to retail markets. Hyperspectral imaging (HSI)

combines spectroscopy and imaging to enable evaluation of an object's spectroscopic composition at a high spatial resolution, thus providing a more comprehensive evaluation tool for any given sample [7–9]. As the scale and complexity of food supply networks continues to grow, there is an ever increasing need for low-cost, portable, analytical devices to combat the corresponding growth in vulnerability of food products to adulteration, contamination, and fraud [10]. In the next section, we discuss a variety of technologies that have enabled the recent development of portable and handheld spectroscopic devices that can and have been used for evaluating the quality of food products.

2. Spectroscopy and hyperspectral imaging technologies

2.1 Infrared spectroscopy

One of the most common approaches used for quality control of food products involves the analysis of vibrational spectra via infrared spectroscopy. The spectral peaks and valleys formed by the fundamental vibrational modes (and their harmonics) of key structures within organic molecules can be used to detect the presence of abnormalities or measure the abundances of specific chemical components. The near infrared (NIR) and mid-infrared (MIR) spectral regions are of high interest in food analysis applications.

2.1.1 Infrared detectors

The rapid proliferation of visible digital camera technology over the past few decades is due in large part to the use of inexpensive silicon-based detectors which can sense wavelengths in the visible region and in the infrared region up to about 1050 nm. For longer wavelengths, however, detectors composed of different materials are required. Indium gallium arsenide (InGaAs) detectors have become the dominant technology for detectors on the market, surpassing germanium (Ge), lead sulfide (PbS), and lead selenide (PbSe) detectors [11]. Unfortunately, these detectors are generally more costly than silicon-based detectors. Furthermore, for wavelengths beyond 1700 nm, the noise in these detectors becomes so high that cooling is required to keep it to a manageable level [12]. To minimize the cost of these more expensive detectors, developers of handheld infrared spectrometers have sought to simplify detector designs by reducing the number of elements required.

2.1.1.1 Near-infrared spectroscopy (NIRS)

NIRS covers the approximate wavelength spectrum of 780 to 2500 nm. Within this range lie signals from the vibration of organic chemical bonds such as oxygen-hydrogen (O-H), carbon-hydrogen (C-H), nitrogen-hydrogen (N-H), and sulfur-hydrogen (S-H), as well as their overtones [13]. Instrument cost and robustness is generally better for NIR than for MIR [14]. However, NIR spectral peaks tend to be weak and broad with significant overlapping of absorption peaks because of a combination of vibrational spectra from multiple chemical bonds, making straightforward interpretation difficult, if not impossible [15]. Spectral preprocessing techniques (e.g., smoothing, detrending, and taking derivatives) and multivariate statistical methods (e.g., nonlinear partial least squares, Fisher determinant analysis, and artificial neural networks) are invoked to extract the information hidden in the spectra. Despite these disadvantages, the advantage in terms of lower cost, increased safety for the environment and operators, and superior chemical

specificity and applicability to a broad range of sample types has made NIR spectroscopy a popular approach for food analysis [11, 15]. These advantages have in turn encouraged the development of numerous portable NIR spectrometers based on a variety of designs.

2.1.1.2 Dispersive NIR spectrometers

In conventional dispersive spectrometer designs, broadband light is passed through the sample and into an entrance slit to create a narrow line of light which is imaged onto a detector. A dispersive grating is inserted in the path causing the image of the narrow line to be spread out into a spectrum where the light is separated into its various wavelengths. These are then focused onto an array detector. **Figure 1** shows a basic dispersive spectrometer based on the Czerny-Turner design which uses mirrors to minimize the overall size of the design.

One major disadvantage of the design shown in **Figure 1** is the need for an array InGaAs detector which can be expensive. Alternative designs requiring only a single-element detector have been developed to help mitigate this expense. For example, instead of collecting all wavelengths simultaneously, spectrometers based on the Fabry-Perot interferometer design shown in **Figure 2** collect the wavelengths sequentially. This design features one fixed and one moveable half-silvered mirror aligned along the same optical axis. As the light bounces between the mirrors, constructive and destructive interference determines the spectrum of light that passes through the other side of the moveable mirror and onto the detector. When the spacing between the fixed and moveable mirrors equals an integer number of half wavelengths, maximum constructive interference occurs leading to a peak in the output spectrum at that wavelength. As with the Michelson interferometer, the spectral range of interest is thus examined by translating the moveable mirror over a specific spatial extent. An example of a compact instrument based on the Fabry-Perot design is Spectral Engines' MEMS Fabry-Perot spectrometer, the NIRONE [18].

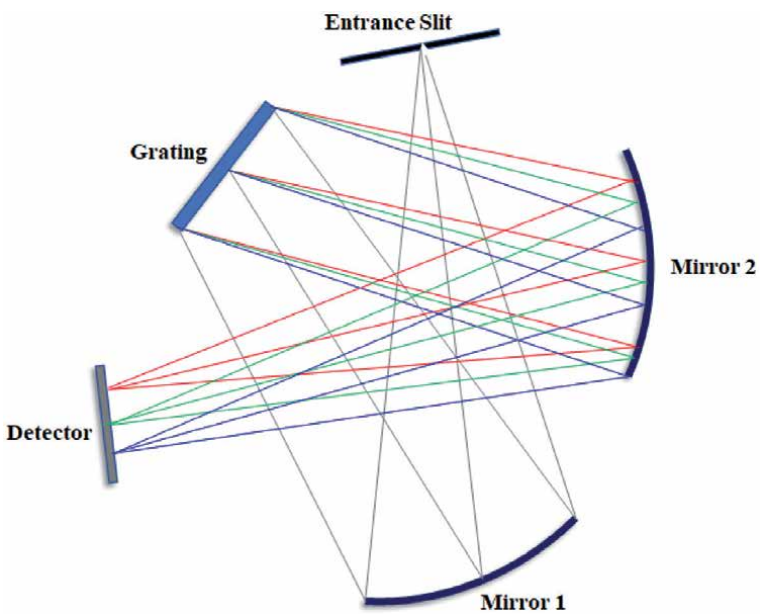

Figure 1.
Dispersive spectrometer based on the Czerny-Turner design [16].

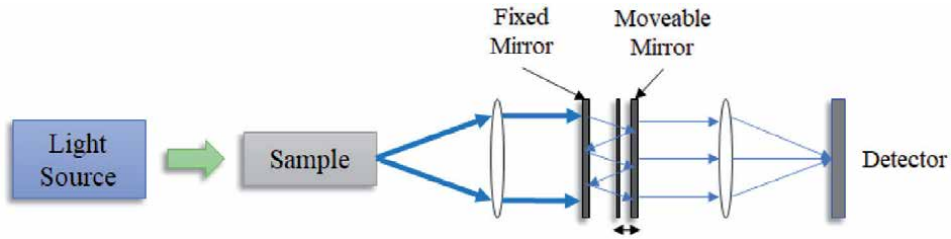

Figure 2.
Fabry-Perot spectrometer design [17].

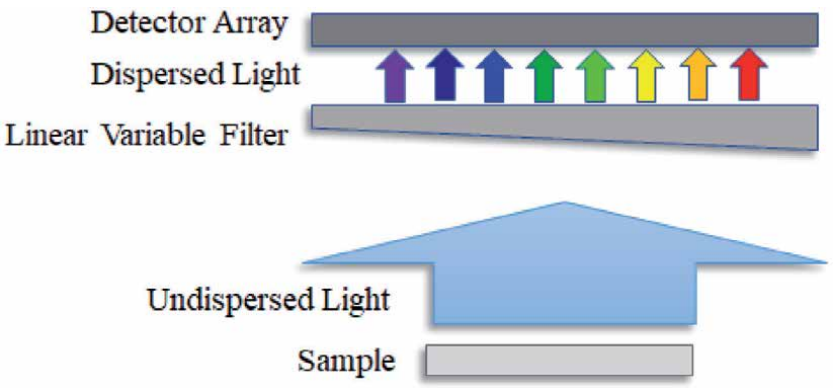

Figure 3.
Diagram showing the operating principle behind the LVF used in the MicroNIR OnSite [20].

2.1.1.3 Linear variable filter (LVF) NIR spectrometers

One problem common to all dispersive designs like the Czerny-Turner is that the light must be allowed to disperse over a given spatial extent such that the wavelengths can separate before reaching the detector. This causes limitations in designing for compactness [19]. One way to surmount this problem is with the use of LVFs which are generally formed from wedge-shaped optics and behave much like a Fabry-Perot interferometer but scan by lateral position along the filter instead of by movement of a mirror along the optical axis [12]. The LVF can be applied directly to a detector array, leading to a simple and compact mechanical design with no moving parts (see **Figure 3**). Viavi's MicroNIR OnSite features an LVF applied to a 128-pixel InGaAs array [21].

2.1.1.4 Hadamard spectrometers

The Hadamard spectrometer design has a couple of key advantages over the conventional dispersive design. First, it overcomes the slow scanning process of dispersive techniques where individual wavelengths must be collected one after another. This is often referred to as the multiplexing or Fellget advantage. Additionally, Hadamard spectrometers tend to be more sensitive and the sensors themselves have a higher optical throughput, resulting in what is termed the Jacquinot advantage [22]. **Figure 4** shows the basic layout for this type of spectrometer, the key component of which is the mask positioned just before the focusing lens. This mask blocks out a certain portion (usually ~50%) of the diffracted light at a time. The blocking elements are moved in discrete steps to form a binary matrix where the elements

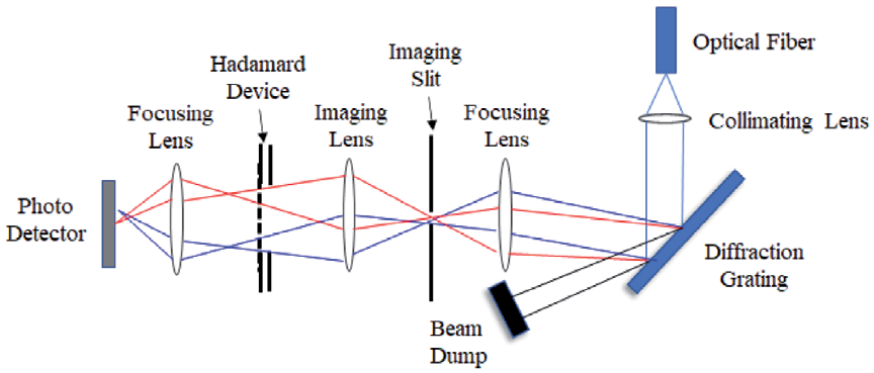

Figure 4.
Basic design layout for a Hadamard spectrometer [23].

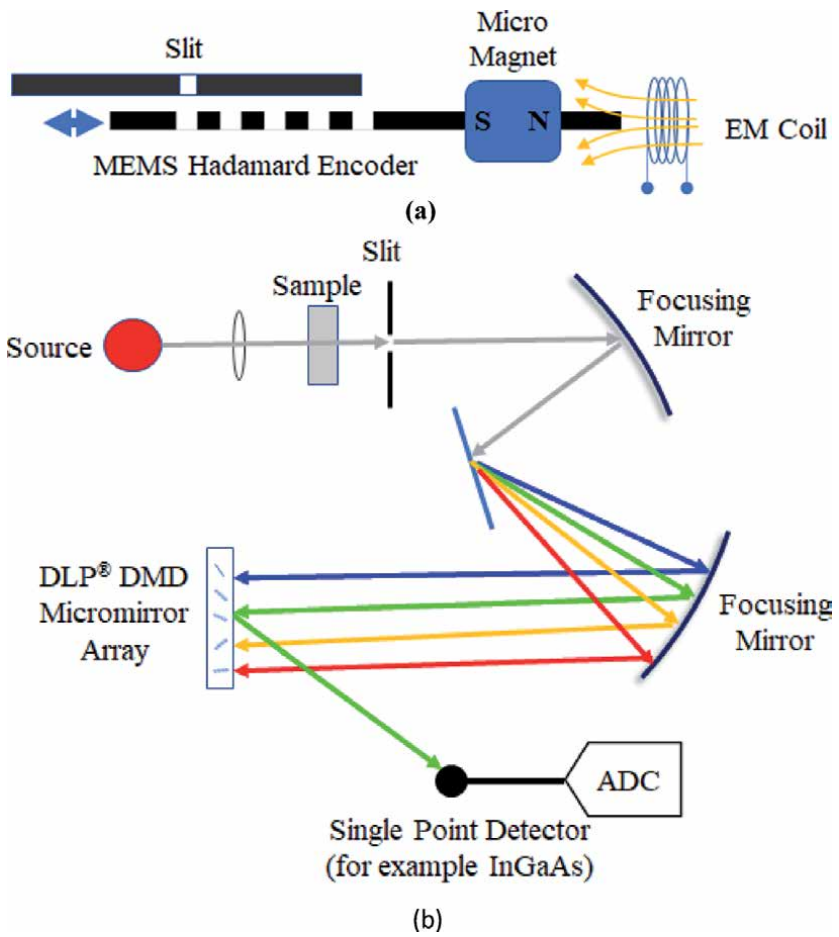

Figure 5.
Hadamard spectrometer designs and devices. (a) a coil and magnet [23] mask design; (b) layout of Texas Instruments' DLP® DMD-based NIRscan Nano optical engine [12].

of each successive row are shifted by one position to the left. The detector readings are recorded with each shift and pieced together to form a data vector. A Hadamard transformation using the binary matrix is applied to the data matrix to yield the measured spectrum [12].

The Hadamard mask itself can be implemented in a variety of ways. Early designs used a coil and magnet to move the mask linearly in front of a separate entrance slit (see **Figure 5(a)**). The microPHAZIR NIR spectrometer by Thermo Fisher Scientific uses a programmable MEMS diffraction grating as the Hadamard mask. Texas Instruments offers two NIR Hadamard spectrometer devices based on its Digital Light Projection (DLP®) digital micromirror device (DMD) technology, the DLP NIRscan and the DLP NIRscan Nano (see **Figure 5(b)**) [12]. The DMD contains an array of individually pivotable micromirrors which can be aligned and flipped in sequence to form the Hadamard mask [12].

2.1.1.5 Applications of handheld NIR spectrometers for food analysis

Advancement in MEMS technology and LVFs has led to the rapid miniaturization of NIR spectrometers, thus enabling the development of portable NIR spectrometers. Portable NIR spectrometers have been used to evaluate the quality of fruits and vegetables primarily during the pre-harvest stage while on the vine/tree. Such analyses focus on maturity parameters to enable determination of optimal harvest dates and include measurements of soluble solids content (SSC), titratable acidity, pH, weight, size, firmness, juice content, juice weight, pericarp thickness, and others [15].

NIR analyses of meat and fish are typically performed for shelf-life estimation and freshness evaluation. Examples include traceability analysis of pasture-fed lambs and stall-fed lambs, authenticity testing for pork and pork fat in veal sausages, moisture, protein, and fat analysis in Iberian pork muscles, fat characterization in Iberian ham, freshness evaluation in beef sirloin and beef eye of round, shelf life estimation of pork meat, and monitoring and control of the drying process in fermented sausages [13].

Portable NIR spectrometers have also been used to measure quality factors in milk and beverages. Components such as fat, protein, lactose, and moisture percentages have been measured to determine milk quality [15], and NIR spectral differences have been exploited to distinguish between organic and non-organic milk products [24]. Quality of rice wine, tea drinks, and beers have been evaluated via NIR measurement of alcohol, nitrogen, apparent extract, and non-sugar solids percentages, polyphenol and free amino acid concentrations, and bitterness and beer distinction factors [15].

2.2 Mid-infrared spectroscopy

The mid-infrared (MIR) spectrum covers a range of wavelengths from ~2500 nm to ~5000 nm and contains many of the fundamental absorption bands of organic components. Spectra in this range are very sensitive to chemical composition, leading to high specificity. Furthermore, organic functional groups produce well-delineated absorption bands in this region, a feature that can be exploited to individually separate different components present in a mixture by their unique fingerprints in the absorption spectrum [14]. Given the high cost of InGaAs detectors and the need for cooling to lower noise to a manageable level, MIR spectrometers generally feature single-element detector designs. Most exploit a technique based on the Fourier transform.

2.2.1 Fourier transform infrared spectrometer (FTIR)

One subset of FTIR spectrometers is based on the Michelson interferometer design that was used for Michelson and Morley's speed of light measurements.

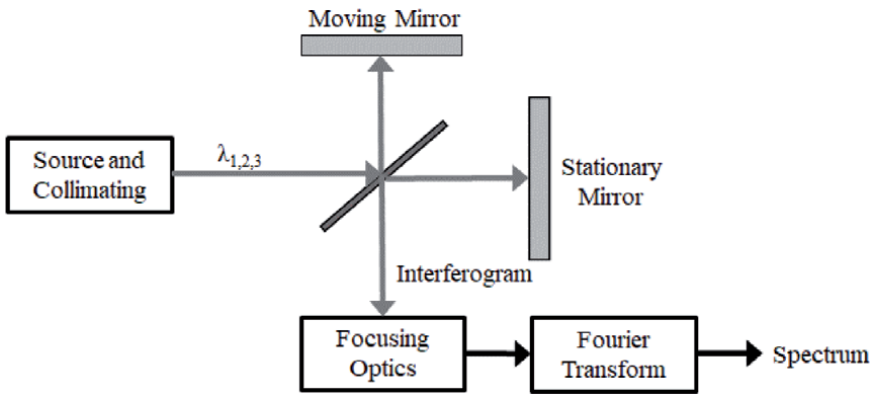

Figure 6.
Diagram of an FTIR spectrometer based on the Michelson interferometer [25].

This interferometer consists of two optical pathways oriented perpendicular to one another (see **Figure 6**). A collimated broadband light source enters from the left and strikes a half-silvered mirror (i.e., beam-splitter) oriented at 45°. Half of the beam then passes through this mirror and strikes a stationary mirror at the end of the pathway where it is reflected back toward the beam-splitter. The other half of the incident beam is directed toward a mirror that is allowed to move back and forth along this pathway. Upon reflection from this mirror and arrival at the beam-splitter, the two reflected beams produce an interference pattern which is focused on a single-element detector. The sample is typically inserted between the beam-splitter and the detector. After the moving mirror is swept through its full range of motion and the full interferogram recorded, these patterns are processed to produce the spectrum. Processing in this case is done with a Fourier transform which converts the sensor response as a function of spatial mirror position to a function of frequency. The Fourier transform accomplishes this by determining the optimal mixture of sine and cosine functions that can replicate the sensor response.

Like Hadamard spectrometry, FTIR spectrometry has several advantages over dispersive spectrometry such as that used in most NIR spectrometers. It enjoys both the multiplexing (Fellget) advantage and the throughput (Jacquinot) advantage. This latter characteristic serves to significantly reduce the noise in the sensor output. As this design includes only one moving component, the mirror in the path with the variable length, FTIR instruments have a mechanical design that it is highly robust to breakdown. Finally, many FTIR instruments include a HeNe laser that acts as an internal calibration standard, eliminating the need for calibration during operation (Connes advantage) [26]. SiWare Systems' NeoSpectra-Scanner is an FTIR NIR spectrometer with a MEMS-based Michelson interferometer design [27].

Another popular design for FTIR spectrometers is based on the property of attenuated total reflection (ATR). As shown in **Figure 7**, broadband infrared light is directed into a high refractive index crystal typically made of germanium, silicon, zinc sulfide, or diamond [28]. The ends of this crystal are cut such that the angle of incidence for the light will result in total internal reflection through the crystal. Although the light wave does not propagate outside of the crystal, an evanescent wave can still pass through the top of the crystal where the sample is placed. This evanescent wave interacts with the sample and absorbs portions of the infrared light, resulting in an attenuation of the light that reaches the detector. One of the primary advantages of this technique is that the light does not have to travel through the entire sample as it does for other designs, which often results in severe

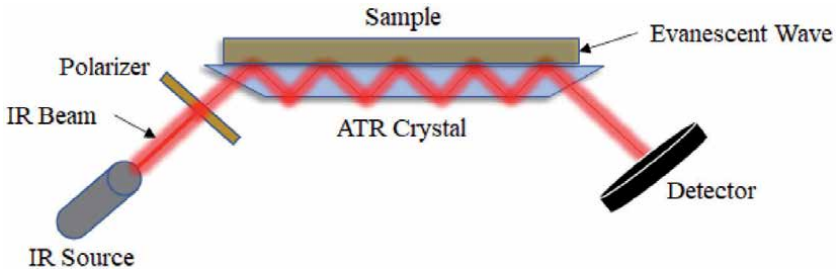

Figure 7.
Diagram illustrating the ATR concept [28].

attenuation and loss of signal. An example of a handheld FTIR spectrometer based on the ATR design is the Ocean MZ5, a miniature ATR-FTIR spectrometer produced by Ocean Optics [29].

2.2.2 Applications of handheld MIR spectrometers for food analysis

MIR spectrometers have long been used for food analysis, but most have been conducted in a laboratory setting. Examples include detection of food spoilage bacteria in meat and dairy produce, brand authentication of a range of Trappist beers, and adulteration of milk and of beef burgers [10]. More recently, portable MIR devices have been used for the simultaneous analysis of sugar and amino acid concentrations in raw potato tubers, the measurement of quality factors in tomato juices, and the measurement of fatty acid content of marine oil dietary supplements [30].

2.3 Raman spectroscopy

Raman spectroscopy is often seen as complimentary to infrared spectroscopy given the relative nature of the phenomena involved. While infrared spectroscopy measures the absorption of energy, Raman spectroscopy measures the exchange of energy with radiation provided by a monochromatic light source (usually a laser with a wavelength in the ultraviolet to NIR range). This exchange causes a shift in the source's wavelength. Molecules are infrared active only if the vibration induced by the source results in a change to the dipole moment, whereas the Raman shift is caused by changes in the molecules' polarization [10]. Thus, these two methods provide mutually exclusive information. Raman peaks tend to be much sharper than infrared peaks and data collection tends to be faster, but the Raman effect is inherently weaker. Furthermore, Raman spectrometers tend to be more expensive to manufacture than their infrared counterparts.

2.3.1 Raman spectrometers

Figure 8 shows an example design for a Raman spectrometer. Light from the laser is directed to the sample and the output is passed through a notch filter to separate out all but the Raman scattered light. A spectrograph grating then disperses this light into its constituent wavelengths and onto a detector. Metrohm's Mira M-1 is an example of a portable Raman spectrograph with a 785 nm laser [32]. Laser wavelengths for other Raman spectrometers can range from the ultraviolet (UV) to the NIR bands. Since spectral sensitivity and resolution increase with decreasing laser wavelength, UV lasers tend to be optimal for applications featuring bio-molecules [33].

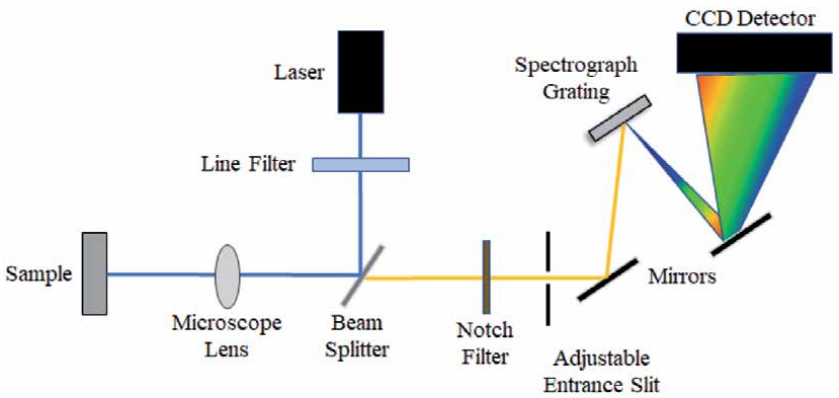

Figure 8.
Basic diagram of a Raman spectrometer [31].

2.3.2 Applications of handheld Raman spectrometers for food analysis

Recent applications of portable and handheld Raman spectrometers for food analysis include the detection of organophosphate and organothiophosphate pesticides on apple skins, the detection of fungicides and parasiticides on citrus fruits and bananas, authenticity and origin of vegetable and essential oils, detection of marker compounds for illegal alcoholic beverages, detection of adulteration in beef burgers, identification of rapid meat spoilage, and prediction of pork quality on a slaughterhouse line [10].

3. Artificial intelligence and machine learning techniques for spectral analysis

Once the spectroscopic data has been collected, sophisticated algorithms, and capable processors to host these algorithms, are needed to convert this data into useful information. The microchip revolution that started back in the 1960's has continued unabated [34] and silicon vendors continue to innovate with even mature technologies such as field programmable gate arrays (FPGAs) [35] with transistor counts that exceed one billion in number. This growth of computing power coupled with advances in spectroscopy have enabled modern machine learning algorithms to be implemented that can lead to significant positive changes to food safety, adulteration and fraud.

In this section, we discuss key machine learning algorithms that have been applied to spectroscopy in general and to food valorization applications specifically. We first examine methods used to extract from the data features that are non-redundant and information-rich and can be used for accurate classification and quantification of food spoilage and food quality.

3.1 Feature extraction

Most spectral datasets contain subsets of features that are highly redundant or subject to high amounts of noise. The inclusion of such features in a classification or regression algorithm generally leads to suboptimal performance. Feature extraction is the process by which redundant or noisy features are removed from the dataset, leaving a smaller set of features with a high amount of signal content. Here, we discuss popular methods for feature extraction that have been used for spectroscopic applications.

3.1.1 Principal component analysis (PCA)

PCA is a common method of feature extraction enabled through dimensionality reduction. PCA provides dimensionality reduction by representing the variance in the data within the smallest number of components possible. Each principal component is a linear combination of the original components and is calculated in an iterative fashion by identifying the weight vector that, when applied to the original data components, contains that largest amount of the remaining variance and is orthogonal to the previously calculated principal components. As a result, the majority of the variance (typically ~99% or more) is contained within the first few (typically 3–5) principal components, meaning the others can be safely ignored with negligible loss of information. These principal components are also eigenvectors of the data's covariance matrix and can be computed by eigendecomposition. The corresponding eigenvalues are proportional to the variance represented within each principal component and can be used to identify the principal components which are considered "significant." According to the Kaiser criterion [36], eigenvectors with eigenvalues less than 1 can be considered insignificant.

In additional to its dimensionality reduction benefit, PCA tends to yield principal components that provide good separability between data collected from different classes. It is this property that makes PCA such an effective tool for feature extraction. Principal components also provide qualitative clues to key underlying molecular constituent differences and relative abundances, since their spectral characteristics, often are the key features in the second, third and higher principal components.

PCA is widely used in food chemistry studies [37] and specifically for analysis of food spoilage. For example, in 2020 Saleem et al. [38] presented a new method for predicting microbial spoilage and detecting its location in bakery goods using HSI. HSI cameras monitored baked goods over a period of time as they were allowed to spoil. PCA was applied to difference images created by subtracting images collected at the beginning of the monitoring period, when the goods were fresh, and images collected at later times. The researchers then used PCA to separate pixels representing spoiled portions of the good from unspooled portions.

3.1.2 Sparse representation

Similar in concept to PCA, sparse representation methods are mathematical processes applied to data with the goal of transforming the data to a new representation containing as few non-zero elements as possible. This is achieved by conducting a trade-off between goodness-of-fit and sparsity. The transformation attempts to produce an accurate reproduction of the original data but is regulated with a cost penalty the increases with the number of non-zero components. Example sparse representation algorithms include basis pursuit, sparse dictionary learning, L1-regularization, and non-negative matrix factorization (NMF) [39].

With respect to HSI, sparsity can also be enforced through wavelength selection processes that identify a small number of information-rich wavelengths and discard all other wavelengths. Lei and Sun [40] developed a sparse coefficients wavelength selection and regression (SCWR) method for NIR spectral calibration to select the wavelengths that contributed most to the determination of the spectral response. They applied this method to a dataset if NIR spectra from potatoes with dehydration loss as the response variable. A model based on 23 selected wavelengths (from an original set of 200) predicted hydration loss with an accuracy that exceeded those yielded by common competing methods.

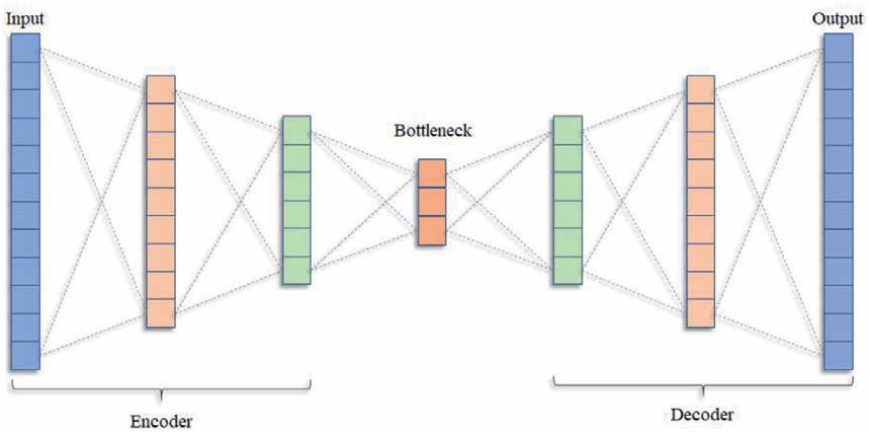

Figure 9.
Autoencoder neural network showing bottleneck which separates the encoder and decoder portions.

3.1.3 Autoencoders

Benefitting from recent advancements in both algorithms and processing technology, neural networks and their derivatives have experienced rapid development over the past decade. Another method for dimensionality reduction and feature extraction is based on a particular type of neural network called an autoencoder. An autoencoder network contains input (e,g., spectral measurement) and output layers of the same size but includes hidden layers in between with gradually decreasing numbers of nodes (see **Figure 9**). During training, the network weights are updated until the output is the same as the input within an acceptable tolerance. The layers from the input to the bottleneck center thus effectively encode a compressed version of the input signal. This set of layers is referred to as the "encoder" section. The compressed signal can then be uncompressed in the later layers (called the "decoder") to form a copy of the signal in the output layer. This process has the added benefit of removing noise in the input during encoding such that the decoded copy is more representative of the true response. In 2021, Vasafi et al. [41] made an initial application of an autoencoder in the field of food production process control by using it to detect anomalies such as changes in fat, temperature, added water, and cleaning solution during milk processing. Anomalies were found to result in significantly higher reconstruction error at the autoencoder output layer as compared with the control (i.e., "normal") data.

3.1.4 Partial least squares regression (PLSR)

PLSR is a well-known and often used means of conducting regression in the presence of noise. Regression provides a function that predicts a response from a data input (as opposed to classification which assigns the input to a class). While both PCA and PLSR are derived from experimental data, PCA is more qualitative by nature, often used in an exploratory manner, and is an unsupervised learning method. PLSR on the other hand is more quantitative and is a multi-dimensional evaluation that is linear. Both methods rely on computing a maximum covariance, PCA in the original data and PLSR in the data and response.

PLSR works best when the observed variables are highly correlated and noisy [42], which is a benefit in hyperspectral analysis where data at nearby wavelengths can be highly correlated. Also, PLSR assumes that the data set is linear and that that projection holds in a new subspace. However, if linearity does not hold up for the

model, there are several ways to deal with this problem that include polynomials, splines or small neural nets [43]. There is also an easy way to deal with non-linearity [44], the basic idea being to expand the data matrix with square, cubic, or cross product terms.

PLSR continues to be a popular tool for food analysis and quality control applications. Jiang et al. [45] invoked PLSR to model the relationship between NIR spectra from hyperspectral images of chicken to total *Pseudomonas* spp. and *Enterobacteriaceae* counts (PEC) to predict PEC rapidly. Cavaglia et al. [46] similarly applied PLSR to predict density and pH in ATR-MIR spectra from alcoholic fermentation samples.

3.1.5 Wavelets

Wavelets are mathematical functions that, like Fourier analysis, transform data into its constituent spectral components. However, unlike the standard Fourier transform, wavelet transforms can provide frequency information for specific locations in the temporal or spatial domain. Wavelets of different shapes (called mother wavelets) focus on different portions of the frequency spectrum and are typically used in combination to analyze the full spectral bandwidth of concern. Each mother wavelet can also be rescaled to form daughter wavelets to change the resolution in the temporal or spatial domain and thus examine higher or lower portions of the frequency spectrum in more detail. Some wavelet examples are shown in **Figure 10**.

Wavelet analysis has been used in spectroscopic applications as a means of extracting useful features from specific regions of the spectra. For example, Qi et al. [47] applied a single wavelet form at seven different scales to extract features from shortwave infrared (SWIR) hyperspectral reflectance images from peanuts. Using these features, they were able to distinguish moldy portions of peanuts from healthy portions. Wavelet analysis can also be applied in the image domain to extract 2D features. Ji et al. [48] applied wavelet transforms to hyperspectral visible-NIR images of potatoes (after first applying PCA) to decompose the original images into sub-band images at different scales to extract textural features that would enable the identification of bruising in the potatoes.

3.1.6 Splines

Splines are piecewise linear or polynomial functions that are combined to approximate a given set of data. Splines are often used as smoothing functions to approximate data curves while eliminating the "roughness" caused by noise. Like the other techniques discussed above, splines benefit the feature extraction process by focusing on the true signal within the data.

One application of splines common to chemometric analysis is the regression analysis method of multivariate adaptive regression splines (MARS) [49]. MARS

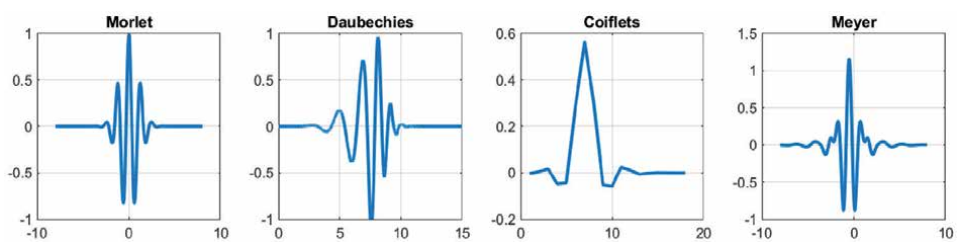

Figure 10.
Wavelet examples from different wavelet families.

models nonlinearities in data by fitting splines to specific regions of the input variable range. The regions are separated by "hinge" functions that have a value of zero for all locations except within the region of applicability. The transition points that link consecutive splines are called "knots." In a forward process knots or splines are added to yield a close fit to the data. In a backward process the least contributing terms are pruned to minimize overfitting. Garre et al. [50] compared a model developed using MARS to similar regression models developed to predict the amount of waste in food production and quantify model uncertainties. The MARS model achieved a precision comparable to that of more sophisticated machine learning models such as random forest methods developed to deal with the high variability in decision trees while maintaining low bias [51].

Closely related to spline regression is Savitzky–Golay filtering in which the data points are convolved with a set of filter weights, much like a weighted moving average. However, as the filter moves to each successive data point, a polynomial of degree p is fit to the data within the filter window, and the point in the center of the window is replaced by the polynomial value at that point [52]. One key benefit of Savitzky–Golay filtering is that it tends to preserve high frequency signal components while rejecting high frequency noise (often found in CCD or InGaAs arrays or photon-starved detection systems) whereas standard finite impulse response (FIR) filters tend to remove these signal components [53].

Savitzky–Golay filtering is a popular pre-processing technique that has been used extensively for food spectral analysis. Examples since 2020 include the use of Savitzky–Golay filtering in pre-processing NIR spectra to improve classification performance in the identification of allergens in powdered food materials [54], filter noise from FTIR spectra of instant freeze-dried coffee and MIR spectra of fruit puree samples [53], and NIR reflectance spectra of Indonesia rice flour-based food to enable accurate classification and level estimation of added sweeteners [55].

3.2 Classification

Automatic detection of food spoilage requires an algorithm that can successfully classify a food product (or part of a food product) as spoiled or healthy, either by detecting the presence of contaminants or by classifying physical changes to the product. A variety of sophisticated machine learning algorithms have been developed over the past few decades to provide accurate classification, and many of these have been used in spectroscopic and food quality applications. Here, we discuss two of the most popular classification algorithms, the support vector machine (SVM) and the artificial neural network, both of which take as input a set of features that are typically generated using the methods described in the previous section. We also discuss deep learning methods, which have advanced rapidly since 2012 and are being used in a wide variety of applications including food analysis. Unlike more conventional machine learning methods, deep learning methods include their own automatic feature extraction process [56].

3.2.1 Support vector machines (SVM)

An SVM is a supervised learning algorithm that seeks to find the separating hyperplane between data points of different classes that minimizes classification error. The position of the hyperplane is determined by the set of points (called "support vectors") that are closest to it. The basic concept of the SVM is intuitive when the hyperplane is linear and the classification is binary (see **Figure 11**).

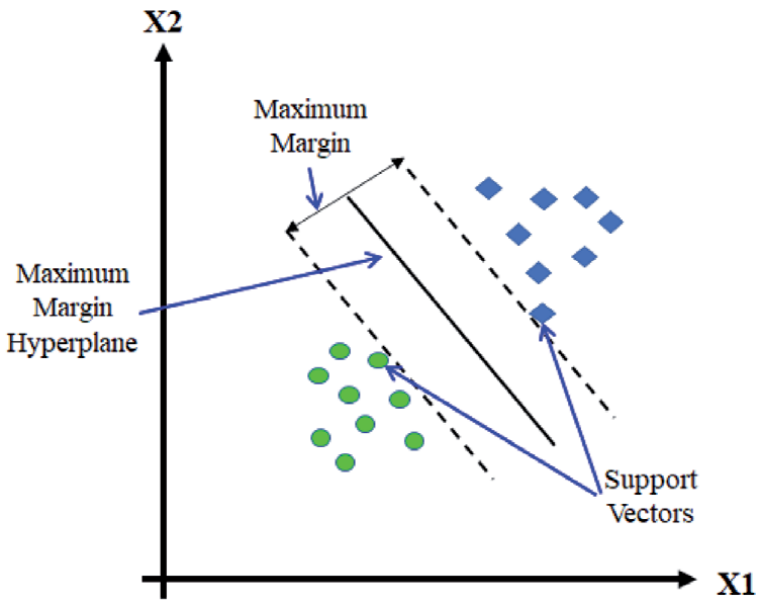

Figure 11.
Separating hyperplane determination for a Support Vector Machine. The hyperplane is positioned to maximize the margin between the support vectors.

However, SVMs can also be applied to data whose classes are not linearly separable by transforming the data from the original space into one in which they are linearly separable. This is often accomplished using the so-called "kernel trick" in which a kernel function compares vectors in the new space without performing the actual transformation, thus minimizing computation cost. Common kernels include linear, radial (i.e., Gaussian), and polynomial.

A reliable and robust machine learning classifier, the SVM has been used in many hyperspectral imaging food analysis applications. A few examples since 2020 include the detection of spoilage in visible-NIR imagery of baked goods [38], detection of bacterial foodborne pathogens in visible-NIR imagery [57], and detection of fish fillet substitution and mislabeling through accurate classification of fillet species from imagery collected from visible-NIR, fluorescence with UV excitation, SWIR, and Raman spectral bands [58].

3.2.2 Artificial neural networks

Artificial neural networks are another popular supervised classification method that has been surging in popularity with the advances in processing technology over the past few decades. Conventional artificial neural networks are based on the multilayer perceptron (MLP) architecture (see **Figure 12**) which was designed to resemble neurons in the brain. Such neurons accept some number of input values and remain dormant until the sum of inputs rises above a certain threshold value, at which point the neurons "fire." This nonlinear thresholding effect is enabled in artificial neural network nodes by nonlinear activation functions that determine each node's output value. Common activation functions include the sigmoid, hyperbolic tangent, and rectified linear unit functions.

Artificial neural networks are trained by initializing the network weights (usually with random values) and comparing the predicted results at the output layer to known target values. An error metric is calculated based on the difference between the prediction and target values, and the network weights are updated by

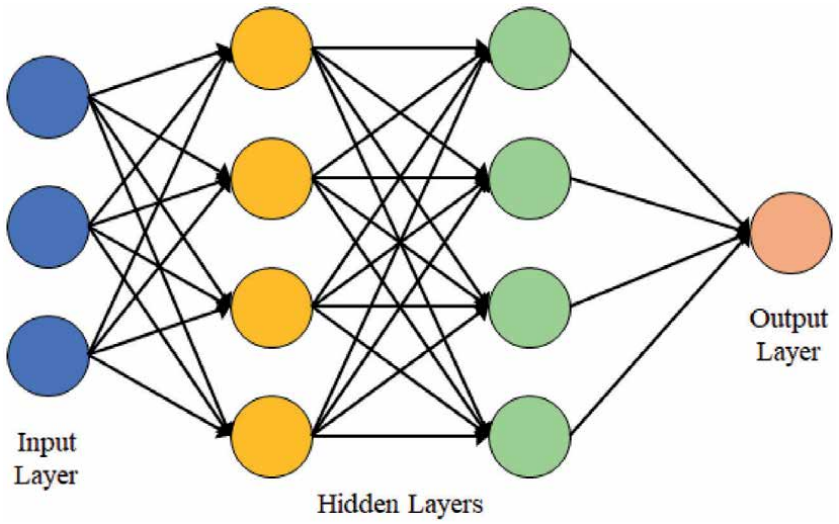

Figure 12.
A simple illustration of a multi-layer perceptron neural network architecture. Each circle represents a neural network node and each arrow represents the weight that connects a node in one layer to a node in the subsequent layer.

calculating partial derivatives of the error with respect to each weight, starting with the output layers and moving backward toward the input layer in a process known as backpropagation. This entire process is repeated until the error is brought below an acceptable tolerance or other stopping criteria are met.

Like SVMs, artificial neural networks have been widely used for spectral classification applications due to their ability to achieve high accuracy. In 2020, Balabanov et al. [59] developed a vison-based system with an artificial neural network to detect defects in apples passing on a conveyer belt by analyzing HSI in the visible and NIR spectral ranges. Although once popular, these MLP-based neural networks are rapidly being replaced by deep learning neural networks which not only offer superior performance, but also ease the data processing pipeline by eliminating the need for manual feature selection and extraction.

Prior to the advent of modern sophisticated processing technology, neural networks were limited in size due to their computational loads which grew with the number of layers and the number of nodes within each layer. As this processing technology advanced, more and more layers could be added to neural networks to improve their performance (although the theoretical reason for why this is the case is still poorly understood). Furthermore, as shown in **Figure 13**, layers could be added to perform different operations on the data, such as convolution and averaging (often called "pooling" in this context). The network then performs feature extraction by learning the weights in the convolutional layers which yield accurate classifications. In essence, the network learns which filters should be applied to the data to best extract the signal within. Pooling layers following the convolutional layers then apply averaging to help prevent overfitting. Following the successive convolutional and pooling (and possibly other) layers, the results are concatenated into a single-dimensional vector and fed into an MLP neural network to combine these features for classification.

In 2021 alone, deep learning neural networks have been used to classify beef freshness from visible-NIR reflectance spectra [60], to analyze NIR HSI to detect the presence of contamination during food packing [61], and to conduct a series of different food quality analyses from NIR spectra [62].

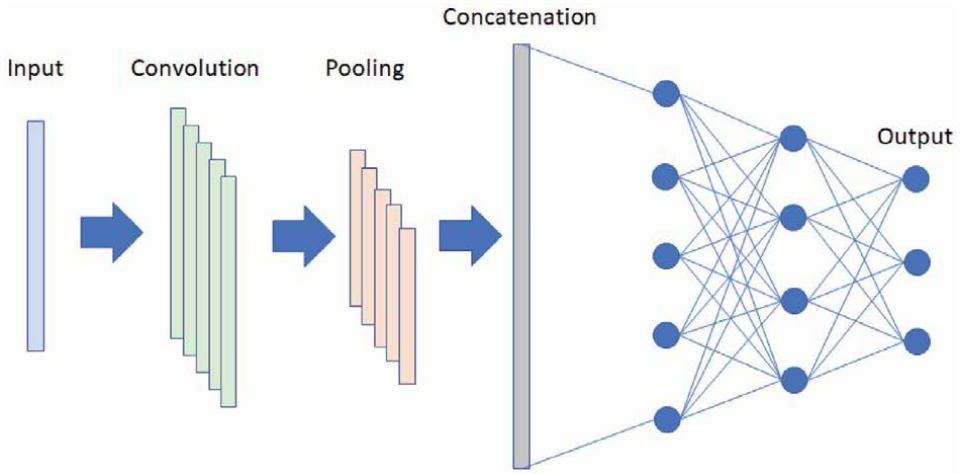

Figure 13.
Basic CNN architecture. Data at the input layer is passed through a convolutional layer which generates feature sets. These are then reduced in size through averaging in the pooling layer and the resulting features are concatenated. The final layers form an MLP-based neural network to yield the final classifications.

4. Food traceability and dynamic pricing

4.1 Inadequacies of existing traceability technologies

Traceability and real time analysis of food products that can help minimize waste will require new tools for quality assurance, authentication and digital supply chain management that can track products from harvest to market. Such tools must be objective, verifiable, provide data on quality, provenance, and freshness, and easy to incorporate at multiple nodes in the supply chain. Current technologies are inadequate to address most of these challenges and address only some components of the most difficult problems.

State of the art seafood traceability platforms provide tools for establishing chain of custody but lack dynamic pricing features or the verifiable and trusted freshness and authenticity data. These current platforms rely on estimates of shelf life based on catch date and storage conditions. These inputs are insufficiently verifiable and quantifiable for digital tools based on them to be broadly trusted and accepted for dynamic pricing, and they do not address authentication and quality metrics or capabilities at all.

As of 2021, dynamic pricing software solutions also lack higher quality verifiable and trusted freshness and authenticity data and are primarily designed for final retail discounting, often integrated only into broader retailer systems. This makes them less effective for application to upstream supply chain node tasks and adding value for each node in the supply chain.

Products for quantitative measurement of fish freshness rely primarily on destructive laboratory-based methods that are not capable of accurate spot checks of individual fish or fish portions or cannot be realistically and easily repeated at low cost at multiple points along the supply chain. Tools are available that measure tissue conductivity through fish skin, primarily to assess moisture, but they are not designed to address broader nutrient content, species, and traceability.

4.2 SafetySpect's quality, adulteration, and traceability (QAT) technology

One approach to addressing the problem of traceability and rapid detection of spoilage is SafetySpect's newly developed handheld QAT scanner that optically

detects previously established chemical signatures of seafood freshness, quality, and species ID. This device integrates several types of spectroscopic data through its fusion-AI algorithm into simple, human readable reports. The handheld scanner will enable spot-checks of quality, species ID, and freshness all along the supply chain. Integration with a mobile device and app can couple the QAT output to blockchain-enabled, cloud-based, supply chain management platforms for tracking product quality, freshness, and species ID from harvest to market. When integrated with a digital platform using blockchain and dynamic pricing technology, these tools will support the modernization of the global fishing and seafood processing industries, supply chains and retail outlets, as well as provide accurate information to consumers about the sustainability, freshness, and quality of their seafood purchases. Specially designed apps can also integrate smallholder producers in developing economies into the broader, emerging digital supply chain platforms in these markets.

By providing trusted product and pricing data at any node of the supply chain, QAT fundamentally changes the business models of seafood processors, wholesalers and retailers, making it practical to (a) identify mislabeled product, and (b) dynamically price perishable seafood at multiple purchase decision points – beyond traditional final-discounting by retailers. The quantitative underlying data provides high confidence and additional visibility and trust in the freshness of such a highly perishable good, and makes intelligent pricing based on quality/freshness practical at all nodes along the supply chain before final retail sale.

With this capability, QAT will spur innovation in the execution of seafood supply chains by providing accountability at each node for maintaining quality. This will drive improvements in purchase decision making, traceability, authentication, and inventory planning. Retailers can use dynamic pricing in both their purchase and final sale decision making. Given the high proportion of seafood sales attributable to the largest retailers in both developed and developing markets, retailers can have significant economic incentive to adopt such technologies, and the power to encourage its adoption by upstream suppliers.

Technologies like SafetySpect QAT will have four major impacts on the global seafood industry: (1) Reduce waste by tracking fish freshness, thus enabling vastly improved freshness-based dynamic pricing tools at multiple supply chain nodes; (2) Increased visibility and trusted information; (3) Increased value of seafood across markets and positive impact on public health by providing assurance of quality, species, and freshness; (4) Positive impact on a number of sustainability goals including better management of at-risk wild fisheries, combatting illegal poaching of fish and wild animal species, improving food security, and providing better economic engagement and access for marginalized smallholder producers to broader digital supply chain systems and platforms, reducing resource consumption and combatting climate change.

5. Conclusion

Food waste is a global problem caused in large part by food spoilage that has gone undetected. This problem exacerbates world hunger issues and affects consumers by causing them to pay too high a price for food products whose shelf lives have been improperly or inadequately estimated. Several technologies have been applied to improve the detection of food spoilage and provide better valorization of food products at all points along the food supply chain, but many of these methods are either unreliable or damage the samples being evaluated. Spectroscopic techniques, on the other hand, offer a reliable and non-invasive means of detecting and

quantifying spoilage. Recent developments in fundamental spectroscopic technologies have enabled the development and productization of portable and handheld devices for conducting analysis of food products in-situ. Furthermore, algorithmic advancements have improved our ability to extract the most relevant features from the spectroscopic data and yield highly accurate classifications and quantifications of spoilage. These technologies, in combination with advancements such as blockchain, used in conjunction with technologies like SafetySpect's QAT scanner, offer the promise to reduce food waste and extend shelf lives through detection of spoilage at earlier points along the food supply chain and will provide the ability to impose intelligent pricing and traceability tracking for the benefit of consumers around the globe.

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Section 3

Innovation in the Food Sector

Chapter 7

Innovation in the Seafood Sector through the Valorization of By-Products

Marzieh Moosavi-Nasab, Najme Oliyaei, Jong-Bang Eun and Armin Mirzapour-Kouhdasht

Abstract

Aquatic, marine and algae, is reservoir of bioactive compounds, which have considerable potential to supply novel ingredients toward the development of commercial functional food products. Meanwhile, several valuable by-products generate during the manufacturing process. Seafood is still an intact reservoir of valuable compounds with significant potential to provide unique compounds applicable in functional food development. Seafood, as an important part of the diet all around the world, can be used as a source of functional components that are positively affecting the human health. Annually, 50–80 percent of the seafood processing is discarded as waste every year. Algae are also the novel natural resources for their biological and pharmacological properties. This chapter will be discussing the innovations in seafood and algae sector through the valorization of their by-products. Firstly, protein production, its characterization and the protein hydrolysates derived from seafood will be reviewed. Subsequently, bioactivity of the peptides obtained from these protein hydrolysates and other bioactive compounds such as carotenoid compounds derived from seafood including fish, shrimp, alga, and so on will be included. Finally, the main components of algae including sulfated polysaccharides, pigments and proteins will be surveyed.

Keywords: seafood by-products, algae by-products, bioactive compounds, protein, pigments, carotenoids, sulfated polysaccharides

1. Introduction

It is well-known that the seafood has been one of the most important parts of the human nutrition for a long time. According to reports obtained from FAO, the annual discard from global marine capture between 2010 and 2014 was 9.1 million tons. This huge amount of by-products represents 10.8% (10.1% –11.5%) of the annual average catch of 2010 to 2014 [1]. Utilizing this discarded part of the fishery industries could be environmentally and economically profitable.

Several value added products can be generated from seafood processing by-products depending on which kind of seafood is processed. Based on this, this chapter is divided into 3 major parts; (I) fish by-products, (II) crustaceans, and (III) seaweeds. This study has provided a review of use of fish by-products to produce some value added products including proteins, peptides, and oil. These products are the most

important major products that have a promising future in global market. During last decades, different efforts have been done to utilize the seafood by-products to generate these value added products [2]. Obtaining proteins and peptides as functional and nutritional compounds from seafood by-products have been the objective of many researches [3–9].

Algae are an important renewable source of food, medicines and fertilizers and their utilization have increased in all around the world. They are considered to possess a high nutritional value and their metabolites, and associated biological activities, have particular significance for multiple nutraceutical, cosmetic and pharmaceutical applications [10, 11]. Seaweed consumption has a long tradition in Asian countries and has increased in European countries in over recent decades, due to increased awareness of their beneficial effects [12]. Thus, development of way for the utilization of marine algae for food, feed, and bioenergy is essential. One of the best way is conversion of biomass into a variety of valuable products which is known as biorefinery [13].

In recent years, numerous compounds with biological activities or pharmacological properties such as antibacterial, anti-inflammatory, anticancer, antiviral and anticoagulant are discovered in algae. Algae by-products can be used for human and animal as food, animal feed and ingredients of dietary supplements. Sulfated polysaccharides, pigments, proteins and lipid are the main by-products of algae [12].

This chapter focuses on important value added bioactive chemicals identified in seafood by products over the last years and describes the range of biological activities as well as industrial applications for which they are responsible.

2. Fish by-products

2.1 Proteins

Fish by-products obtained from seafood processing industries contain huge amounts of head, skin, scales, bones, fins, viscera, and dark muscle. The protein content of these by-products is approximately 15%, which is similar to that of fish fillets. The muscle which is attached to this by-product contains two distinct type of proteins including structural (myofibrillar) (approximately 70–80%) and sarcoplasmic proteins (approximately 20–30%). These high nutritional value proteins (even more than red meat and milk casein) indicate remarkable functional and technological properties like water holding capacity, emulsifying activity, film forming ability, foam forming capacity, and gel forming ability [14–17]. Commercial gelatins are mostly obtained from mammalian (porcine and bovine) skins and bones. As the researches confirm, the substitution of mammalian gelatin with fish gelatin is an appropriate and appealing due to increasing concerns of researchers and consumers about the risks of transmission of the pathogenic vectors such as prions. Albeit, number of committees like the Scientific Steering Committee of the European Union, have stated that consumption of bovine bone gelatin is safe [18], researchers are still debating on this.

Nowadays, researches have become to notice on a unique protein which can be easily extracted from fish by-products especially skin, scales, bones, and fins. This valuable protein is collagen/gelatin. Collagen is the most abundant protein in tissues including skin and bones (approximately 30% of the total protein). The structural investigates show that collagen is a triple helix with three identical polypeptide chains. The primary structure of this protein is continuous repeating of the Gly-X-Y-sequence. The positions of X and Y are mostly proline and hydroxyproline, respectively. Different types of collagen (29 distinct types) have been discovered

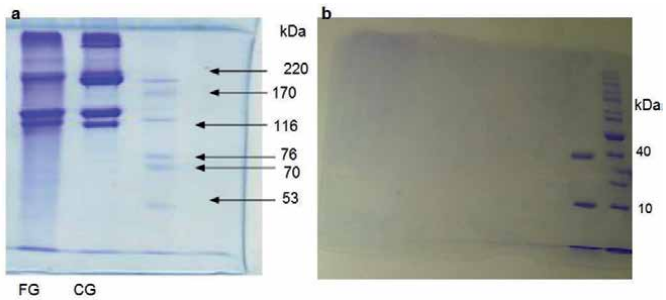

Figure 1.
Molecular weight distribution analysis by SDS-PAGE for gelatins. CG (commercial gelatin) and FG (fish wastes gelatin) (a) and for protease (b). Adapted from [23].

so far, which have right-handed triple helical conformation. The difference among these types is due to the variety in their amino acid sequences as a result of genetic variants [19–21]. Fish gelatin could be extracted from its by-products by a partially denaturation of collagen usually performed by hot water. Before extraction of fish by-products, some pretreatments are needed to ready them for being used as a gelatin source. The pretreatment step is ordinarily an alkaline and/or and acidic swelling process. The alkaline and/or acidic pretreatment is used to partial cleavage of rigid cross-links in the collagen and remove non-collagenous materials. The enzymatic aided chemical pretreatments are those which can be supplemented or replaced by enzymatic reaction. The "conditioning process" is the known name of this step by manufacturers of gelatin. Afterward, the gelatin (warm water soluble) will be extracted from collagen (not soluble) by hot water at a specific temperature and time. There are lots of studies performed in this research area. In a paper authored by Mirzapour-Kouhdasht, Moosavi-Nasab [22], gelatin was optimized at different levels of time and temperature using the response surface methodology (RSM). The responses including yield, protein content, gel strength, and viscosity indicated that the optimum conditions were 70.71°C and 5.85 h. Rheological, structural, and functional experiments showed that the gelatin characteristics were acceptable compared to the commercial bovine gelatin. The pretreatment in these experiments was performed by alkaline solution. In another study [23], gelatin was produced from Common carp wastes using alkaline protease from *Bacillus licheniformis* PTCC 1595. The enzymatic reaction was performed in 5, 10, 15, 20, and 25 units per gram of wastes. The molecular weight distribution of the gelatin (**Figure 1**) showed that this gelatin could be successively replace the commercial gelatin.

In some researches also fish gelatin is modified by some functional groups or chemical agents to improve the functional characteristics. In a study performed by [24], rheological, emulsifying, and structural properties of phosphorylated fish gelatin was investigated. The results of this study revealed that phosphorylation in a short time, enhances gel and rheological behavior of fish gelatin. Phosphorylation could improve the emulsions stability of fish gelatin as well. Authors stated that the structural properties of fish gelatin were significantly affected by this modification **Figure 2**.

2.2 Peptides

Peptides obtained from seafood processing by-products have been reported to have potent biological activities including antioxidant activity [25–31], antihypertensive, anticancer, anti-inflammatory, and anticoagulant properties [22, 32–37]. Among all these researches, the use of gelatin derived from fish by-products has

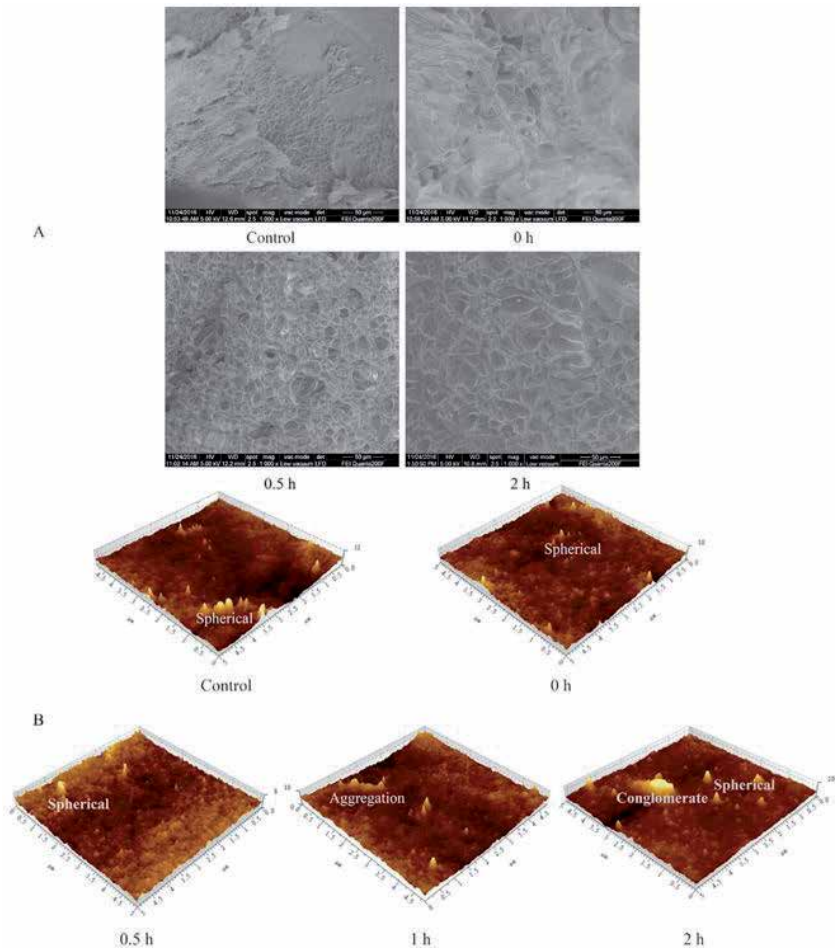

Figure 2.
Micrographs of control and phosphorylated fish gelatin. SEM (A) and AFM (B). Adapted from [24].

been well investigated as a source of bioactive peptides with various biological activities. In a study performed by Jin, Teng [38], salmon skin collagen was hydrolyzed by different proteolytic enzymes including pepsin, trypsin, papain, and Alcalase 2.4 L. Hydrolysates obtained from trypsin hydrolysis reaction indicated the highest dipeptidyl peptidase IV (DPP-IV) inhibitory activity (66.12%). After fractionation and identification processes, a bioactive peptide with sequence of LDKVFR for DPP-IV inhibitory activity was detected to be responsible for this activity (IC50 value of 0.1 ± 0.03 mg/mL). In another research conducted by Mirzapour-Kouhdasht and Moosavi-Nasab [39], gelatin extracted from *Scomberomorus commerson* skin in combination with its hydrolysates obtained by Actinidin from kiwifruit was used to extent the shelf-life of whole shrimp (*Penaeus merguiensis*). The results revealed that the gelatin hydrolysates can be applied as a preservative coating agent for whole shrimp.

2.3 Oil

Nowadays, of the most important nutritional substances which have gained much attention are Omega-3 long-chain polyunsaturated fatty acids (LCPUFA). These LCPUFA are necessary for human and animal physiology due to their

structural and regulatory functions [40]. Fish by-products are a good natural source of LCPUFA, especially EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid). Fish oil is rich in vitamins (E, D, A). Due to these valuable components, fish oil consumption could be a promising way to impede some health risks such as inflammation, coronary heart diseases, obesity, arthritis, autoimmune disorders, and cancer [41–44].

Generally, the extraction of oils from fish by-products can be divided in two categories including conventional and modern methods. Generally, in conventional methods the raw material (fish by-products obtained from fish processing industries) are first cooked. After the cooking, the by-products are sieved followed by pressing for oil extraction. Subsequently the extracted slurry is decanted and the oil is stored in oil storing tanks [45].

In comparison with conventional extraction method, the modern extraction methods such as supercritical fluid extraction (SFE) could be useful for reducing the oxidation of LCPUFA. In a research performed by Rubio-Rodríguez and coworkers [46], SFE method with carbon dioxide under moderate conditions (25 MPa and 313 K) was used to extract oil from different fish by-products. They resulted that SFE is an advantageous method for oil extraction from fish by-products. The authors stated that the SFE can impede lipid oxidation and reduce extraction of impurities. In another study conducted by Sabzipour and others [47], quality of rainbow trout (*Oncorhynchus mykiss*) by-products oil was investigated. However, the aim of this study was to determine the effect of different postmortem processing times and blanching methods. The authors presented that the degradation of fish by-products oil occurs faster than the fish tissue oil. So they surveyed the effect of different treatments on the quality of the fish by-products oil. According to their report, salt blanching could decrease the effects of delayed processing and led to a higher quality.

However, the limitation of fish oil for utilization in food and pharmaceutical industries is related to the low stability and strong fishy flavor. The solution for this problem is to encapsulate the fish oil using different strategies to cover the off-flavor and also increase the stability. In a research performed by Drusch et al. [48], fish oil with was microencapsulated by spray-drying in a matrix of n-octenylsuccinate-derivatized starch and sugars. The results of this study indicated that this protocol can increase the oxidative stability of fish oil without any significant changes in physicochemical properties of the oil such as particle size, oil droplet size, and true density. Another study conducted by Chen et al. [49], the fish oil co-encapsulated with phytosterol ester and limonene, prepared by spray-drying and freeze-drying methods. The wall material used for encapsulation were whey protein isolate and soluble corn fiber. Sensory analysis of the encapsulated fish oil showed that the addition of limonene could cover the fishy flavor. The authors also reported that this procedure could significantly enhance the oxidative stability of the fish oil during 168 h of storage.

3. Crustaceans

3.1 Proteins and peptides

Tremendous amounts of shrimp processing by-products (head and body carapace) are discarded annually, which could be an important source of bioactive molecules. The amount of by-products generated during processing is about 48–56% of the whole shrimp depending on the species. The major composition of these by-products are protein (35–50%), polysaccharide (predominantly chitin) (15–25%),

minerals (10–15%), and a few percent carotenoids [50]. Recently production of bioactive peptides from shrimp by-products has gained attentions. Several researchers found that this source of by-products could be a good one to generate bioactive peptides with especial activities such as angiotensin converting enzyme inhibitory (ACE inhibitory) [51, 52], antimicrobial activity [53], antioxidant activity [52, 54], etc. More investigations are required to characterize the biological and functional properties of these peptides.

3.2 Chitin

The major value added product obtained from crustaceans is chitin which has the second position among frequent and used biopolymers in the world after cellulose [55, 56]. In fact, chitin is a polymer of β-(1 → 4)- *N* -acetyl- D–glucosamine units which is extracted mainly from shrimp and crabs. This polysaccharide could be found in arthropods exoskeleton or in the cell walls of fungi and yeast as the major prominent structural component [57–65]. Chitosan is a linear polysaccharide derived from chitin deacetylation [66]. Chitin and chitosan have attained lots of attentions due to their non-toxicity, biocompatibility, biodegradability, and low cost [56, 67]. Chitosan is known as a biologically active component in many fields such as food and pharmaceutical applications. A number of activities of this polysaccharide such as making delivery systems [68], tissue engineering [69], food packaging and film forming [70, 71], and antimicrobial and wound healing [72] are investigated.

One of the most important characteristics of chitosan which can affect its pharmaceutical and functional properties is the degree of acetylation. In case of designing delivery systems, the molecular weight of this bioactive molecule becomes more important due to changing the encapsulation efficiency [73]. It is very important to know that chitosan has a higher solubility in lower pH values due to protonation of the amino groups of the molecule [74]. Permeation enhancers substances can increase the absorption of encapsulated biological active compounds in the gastrointestinal tract. One of the mechanisms of this action is opening the tight junctions of the epithelium cells [75, 76]. Chitosan has a mucoadhesive nature and capable to open epithelial connections (tight junctions) of the epithelium cells [77, 78]. **Figure 3** shows a schematically the action place of permeation enhancers to increase the absorbance of bioactive components in gastrointestinal tract.

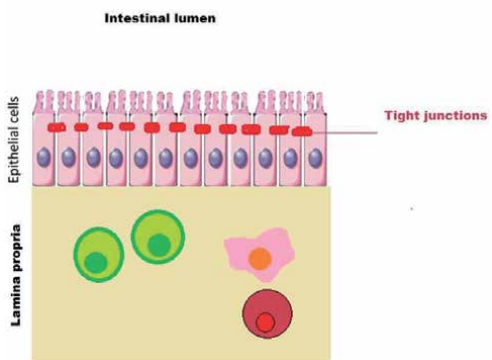

Figure 3.
The action place of permeation enhancers to increase the absorbance of bioactive components in gastrointestinal tract.

4. Algae

4.1 Sulfated polysaccharides

Phycocolloids or hydrocolloids are polysaccharides have been one of the most accessible and widely used in food industry as thickening and gel forming agent. Indeed, numerous sulfated polysaccharides from algae including agars, carrageenans and fucoidan (**Figure 4**) are the main bioactive components that have been determined to possess significant various biological activities [79].

Agar is polysaccharide comprised of two major components, agarose and agaropectin and has been extracted from seaweeds for industrial purposes in pharmaceutical, cosmetics and food industry as gelling and thickening agent [80]. The commercially used seaweeds for the extraction of agar are mainly *Gracilaria* and *Gelidium* species [81].

In addition, carrageenan is another linear sulfated polysaccharides that extracted from red seaweed and exhibits several applications in food industries as gelling, thickening, and emulsifying attributes, clarification of beer and wines. Carrageenan mainly obtain from two algae *Kappaphycus* and *Eucheuma* [82].

Fucoidans, a complex sulfated groups with fucose which found mainly in cell-wall matrix of brown macroalgae [83]. In addition to fucose, fucoidan contain other monosaccharides such as glucose, galactose, rhamnose, xylose, mannose and uronic acids [84]. Numerous brown seaweeds have been used for fucoidan extraction including *Sargassum* [85, 86], *Undaria* [87], *Laminaria* [88], *Cladosiphon* [89], *Fucus* [90], *Saccharina* [91] and *Ascophyllum* [92]. Several investigations have been confirmed the biological activities of fucoidan including antitumor, anticoagulant, antioxidant, immunomodulatory, anti-inflammatory, antiviral, antithrombotic, and hepatoprotective effects [93, 94]. This bioactivity of fucoidan is depend on its molecular weight, the monosaccharide composition, the sulfate content, the position of the sulfate ester group, the extraction technique, and fucoidan structure [94]. Thus, several extraction techniques are used such as conventional methods (hot water) [95] and non-conventional methods such as pressurized liquid extraction [84], ultrasound [96], enzyme assisted [90], microwave assisted [97] and subcritical water [91] extraction.

Subsequently, the green algae *Monostroma nitidum* is the commercial source of a sulfated polysaccharide named rhamnan sulfate [98]. Rhamnan sulfate found in

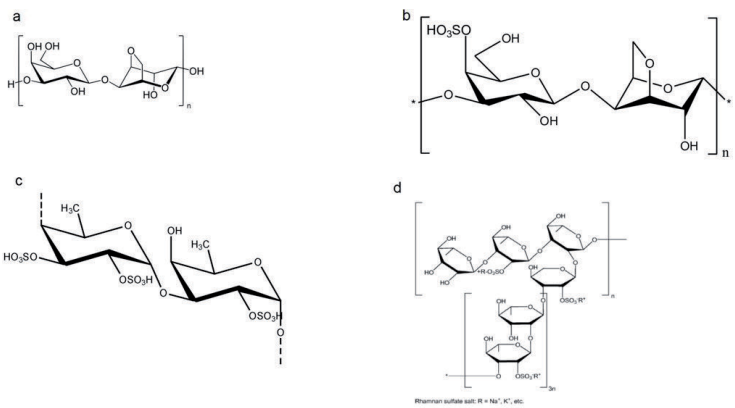

Figure 4.
The chemical structure of (a) agar; (b) carrageenan, (c) fucoidan and (d) Rhamnan sulfate.

cell wall of *M. nitidum* and structurally consists of rhamnose with a sulfate-group substituent that forms main chains with branched side chains [98, 99].

This polysaccharide is extracted by hot water, though is poorly water soluble [100]. Several studies exhibit its biological activities such as antiviral, anticoagulant, antitumor, anti-inflammatory, anti-hypercholesterolemic, anti-obesity and anti-hypertensive properties. Further, *M. nitidum*-derived rhamnan sulfate is considered to promote the human health [100].

Calcium spirulan (Ca-SP) is another novel sulfated polysaccharide isolated from blue-green alga Spirulina platensis. Ca-SP is an attractive candidate therapeutic agent for viral infectious diseases because of its antivirus and antitumor activities [101, 102].

4.2 Pigments

4.2.1 Carotenoids

Carotenoids and chlorophylls are generally wasted together with the residual biomass during the extraction of phycocyanin or sulfated polysaccharide, while can isolate as valuable product from algae [103].

Carotenoids are the most widespread class of pigments that are characterized as natural colorant and antioxidants with healthy effects including anti-cancer, anti-diabetic anti-obesity and eye diseases. The bio-functional properties of algal carotenoids make them potentially to use in nutraceuticals, cosmeceuticals and feed supplements in aquaculture sectors. Carotenoids divided into primary and secondary based on their metabolism and function. Primary carotenoids are structural and functional components in the photosynthetic apparatus, which take direct part in photosynthesis. Secondary carotenoids refer to extra-plastidic pigments produced in large quantities, through carotenogenesis, after exposure to specific environmental stimuli [104].

Microalgae are a potential renewable resource of primary and secondary carotenoids. α-carotene, β-carotene, lutein, fucoxanthin, violaxanthin, zeaxanthin, and neoxanthin, are characterized as primary carotenoids while astaxanthin, canthaxanthin, and echinenone are secondary carotenoids. Astaxanthin, zeaxanthin, fucoxanthin and lutein receive much attention as commercial carotenoids [104].

Seaweeds are the important sources of bioactive compounds which have several human health benefits. The most predominant seaweed carotenoids, such as fucoxanthin, lutein, β-carotene and siphonaxanthin have remarkable biological functions and applications [105]. Pigments are waste during the polysaccharide extraction process. Thus, carotenoids are recovered from microalgae and seaweeds by different approaches including conventional solvent extraction, non-conventional methods including pulsed electric field [106, 107], moderate electric field [108], supercritical fluid extraction [109], pressurized liquid extraction [110], microwave ssisted extraction [111, 112], ultrasound assisted extraction [113], high pressure homogenization [114].

Fucoxanthin ($C_{42}H_{58}O_6$) is the predominant carotenoid in brown algae (*Sargassum angustifolium*, *Laminaria japonica* and *Undaria pinnatifida*) and some microalgae (*Phaeodactylum tricornutum*, *Isochrysis galbana*, *Odontella aurita*) that accounting for more than 10% of the estimated total natural production of carotenoids. This yellowish-brown pigment exhibit remarkable biological properties, including anticancer, anti-inflammatory, antiobesity and neuroprotective activity [115–117]. Moreover, fucoxanthin extraction can be by-product of fucoidan extraction process as Yip et al., [118] obtained the fucoxanthin-rich extract from *S. binderi* with yield of 7.4 ± 0.4 mg/g.

Astaxanthin as king of antioxidant is found in microalgae such as *Haematococcus*. *H. pluvialis* is rich in astaxanthin and provide a natural and inexpensive source of astaxanthin [119]. The antioxidant activity of astaxanthin is 100 and 10 times greater than those of vitamin E and β-carotene. Moreover, astaxanthin has a superior preventive effect toward photo-oxidative compared with canthaxanthin, and β-carotene [120].

4.2.2 Phycobiliproteins

Phycobiliproteins are natural fluorescent dyes which participate in photosynthesis. These pigments are assembled large, distinct granules as phycobilisomes, which are attached to the thylakoid membrane of chloroplast. These pigment-protein complex plays an important role in light-harvesting in cyanobacteria, red algae cryptomonads, glaucophytes and some pyrrophyceae [121, 122]. Phycobiliproteins are divided into two main groups; phycoerythrin (PE –bright pink red), phycocyanin (PC –deep blue). The main components of phycocyanins are C-phycocyanin (C-PC), R-phycocyanin (R-PC), and allophycocyanin (AP – bluish green) [121, 122]. Moreover, there are differences between in their structural position. PE is at the tip of the rod-like phycobilisomes, PC is in the middle, while AP forms a core attached to the reaction and energy transfer proceeds successively from PE to PC to AP and to chlorophyll [123]. The other classification of phycobiliproteins is based on their spectral attributes which including phycoerythrobilin (PEB, A max 560 nm), phycocyanobilin (PCB, A max 620–650 nm), phycobiliviolin (PXB, A max 575 nm) and phycourobilin (PUB, A max 498 nm) [123]. These biopigments have attracted much attention in medicines, foods, cosmetics and fluorescent materials. The recent research has brought attention to the use of phycobiliproteins as food colorant, health drink and coloring agent in confectionary and cosmetics because they are hydrophilic and stable at low temperature with some preservative like citric acid, in acidic and basic solutions [121, 123]. Moreover, phycobiliproteins are used in diagnostic kits in immunology as fluorescent tracer of antibodies [123] and gel electrophoresis and gel exclusion chromatography as marker because of their high molecular absorptivity at visible wavelengths [122].

Phycocyanins have an apparent molecular mass of 140–210 kD and two subunits, α and β [124]. C-Phycocyanin is found in cyanobacteria strains such as *Spirulina* sp. (freshwater), *Phormidium* sp. (marine water) and *Lyngbya* sp. (marine water) [125]. However, the commercial source of this pigment is *Spirulina* which consists of about 20% of the dry weight of this algae [126]. Further, the other new source of phycocyanin is *Anabaena oryzae* SOS13 [124, 127].

Recent studies have demonstrated the role of C-PC as antioxidant, anti-inflammatory, hepatoprotective, and as well as free radical scavenger [128, 129]. Various techniques are used to extract phycocyanin from *Arthrospira platensis* (*Spirulina*) biomass including in various approaches such as physical (freeze–thaw) or an enzymatic (lysozyme) [124], supercritical fluid extraction [130] andsonication and microwave [131].

Phycoerythrin also have numerous health benefits, however, the absorption spectrum of cyanobacteria phycoerythrin is deferent from red algae. The cyanobacteria phycoerythrin exhibits a single peak at 565 nm in the visible wavelength region, while the absorption spectrum of red algae phycoerythrin includes three peaks in the visible wavelength region at 500, 550 and 565 nm (R-phycoerythrin) [123].

Allophycocyanin is a light-harvesting pigment protein complex found mainly in *A. platensis*. This water-soluble pigment is broadly used in biochemical techniques such as a fluorescent probe, especially for flow cytometry. Further, allophycocyanin

has promising applications as antioxidative, anti-inflammatory, antitumor, anti-enterovirus and hepatoprotective [132]. Despite its potential biochemical and therapeutic benefits, there are some challenges in its downstream processing including difficulty in primary extraction and purification, containing lower proportion of phycobiliprotein rather than phycocyanin and the resistance of cell membrane to disruption cause extraction of 50–60% of A-PC by conventional methods. Moreover, the main objective of pigment extraction form *spirulina* is C-PC, consequently, remaining high content of A-PC (about 40–50%) in biomass after C-PC extraction [133].

4.3 Proteins

Algae protein waste is a by-product derived from water-extraction process of microalgae, during algae essence manufacturing. The underutilized algae wastes, containing above 50% protein, have low economical value to be used as animal feed. The pepsin hydrolysate from algae protein waste exhibited antioxidative activity in preliminary experiments, indicating that algae waste might become a new protein source for selection of novel antioxidative peptides [134].

Furthermore, protein hydrolysates from marine sources such as algae by-products, have generally been used to produce seafood flavorings. A high flavor quality is difficult to ensure for seafood flavoring that is produced from marine animal sources because of their high susceptibility to lipid oxidation and the high cost of removing excess fat. Seaweed by-products after agar extraction are good sources of plant protein and contain taste-active amino acids, such as aspartic acid, glutamic acid, arginine, and lysine, in addition to a low fat content [135].

A seaweed protein hydrolysate using 10% bromelain for 3 h, resulted in high level of arginine, lysine, and leucine as free amino acids. These amino acids exhibited an umami taste and a seaweed odor [135].

Most microalgae contain high level of protein which discarded or damaged during biofuels production, while are good candidate for protein extraction and consequently, obtain lipid-rich product as by-product as feedstock for biofuels production. Even though proteins are major algae biomass component, usually they are undervalued compared to minor components such as omega fatty acids, pigments or other possible valuable buy-products [136].

For instance, Garcia-Moscoso et al. [136] extracted more than 60 wt% of nitrogen content of *Scenedesmus* sp. by subcritical water medium then the lipid-rich residue used as suitable feedstock for biofuel production.

There are numerous investigations about algae protein waste and extraction of peptides or amino acids with functional properties. For instance, the antioxidative peptide of VECYGPNRPQF was isolated by pepsin from *Chlorella vulgaris*. This peptide had some bioactivity such as DNA protective effect against hydroxyl radicals, gastrointestinal enzyme-resistance, and strong antioxidant properties. Fractionation of proteins exhibited the high level of aspartic acid, glutamic acid, leucine and lysine [134]. This amino acid sequence (VECYGPNRPQF) can act as cheap and natural anticancer peptide because had antiproliferation and induced a post-G1 cell cycle arrest in AGS cells with no cytotoxicity effect in WI-38 lung fibroblasts cells [137].

Moreover, protein isolation, as valuable by-product, from defatted *Nannochloropsis*, can be obtained after lipid extraction during biofuel production. Defatted and non-defatted *Nannochloropsis* contained 56.9% and 40.5% protein respectively. The protein yields by alkaline (pH 11 and 60 C) extraction method were 16% and 30% respectively. These isolated proteins had a high molecular weight approximately 250 kDa [138].

Macroalgae are also a suitable protein source and rich in protein after extraction of their polysaccharide, lipid and polyphenols. Among three seaweed *Porphyra umbilicalis*, *Ulva lactuca*, and *Saccharina latissimi*, the highest protein isolated using pH- shift method (71%) was related to the *P. umbilicalis*. Furthermore, among different extraction methods including pH-shift method, accelerated solvent extraction and sonication in water and precipitation by ammonium sulfate, pH shift process is promising approach. However, the yield and extraction approach are influence by type and species of seaweed [139].

Brown algae such as *Laurencia filiformis*, *L. intricata*, *Gracilaria domingensis* and *Gracilaria. birdiae* can supply dietary proteins for human and animals because their protein content reported 18.3, 4.6, 6.2 and 7.1% respectively [140].

Combination of acid-alkaline process is another protein isolation from algae. First acid and then alkaline extraction is an alternative extraction by 59% protein recovery from brown seaweed *Ascophyllum nodosum*. The obtained protein had about 2–4 kDa molecular weight [141].

5. Conclusions

This chapter indicated that seafood by-products are one of the most important sources of value added products that can play an important role in the global market due to the increasing growth of demands for health beneficiary products. Through this opportunity and based on our research background for many years, we decided to provide important information about some value-added products obtained from seafood by-products. Proteins and peptides are a major part of the seafood by-products composition that can easily provide essential amino acids and bioactive peptides with health beneficent. Fish oil is another valuable product that could be extracted from seafood by-products. This source is rich in LCPUFA and decreases the risks of chronic diseases such as cardiovascular issues, thereby directly related to our health. Marine algae are a versatile, abundant, and valuable source of many compounds that have been widely used for many industries. The presence of bioactive compounds such as sulfated polysaccharide, carotenoid, and protein makes them a suitable candidate in biomedical applications. It seems, they will play an important role in human life because of their broad applications in food, pharmaceutical, and cosmetic industries.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 8

Valorization of Native Nuts from Brazil and Their Coproducts

Renan Danielski, Gabriela Polmann and Jane Mara Block

Abstract

The consumption of nuts as part of a healthy diet and active lifestyle has long been associated with chronic disease prevention. Nuts and their coproducts such as oil, cake, plant-based milk substitutes, flour, and shell are rich in lipids, proteins, phenolics, and other bioactive compounds. Nut flour also presents interesting physical properties, such as water or oil holding capacity, foam properties, emulsifying activity, and emulsion stability. These biological and physical properties make these products commercially attractive as organic ingredients in several foods such as spreads, bakery products, and cereal bars. In this chapter, the nutritional and bioactive profiles, as well as the evidenced health-promoting effects of nuts originating from Brazil, will be discussed. The focus will be on commercial nuts such as cashews, pecan, and Brazil nuts, along with some underexplored and relatively unknown indigenous species, such as sapucaia, chichá, monguba, and pracaxi. The knowledge of these Brazilian native nuts and their coproducts is important for stimulating their consumption among the population and their large-scale commercialization.

Keywords: oilseeds, biodiversity, bioactive compounds, lipids

1. Introduction

The consumption of nuts as part of a healthy diet and active lifestyle has long been associated with chronic disease prevention such as cardiovascular diseases, type 2 diabetes, cognitive function impairment, inflammatory disorders, among others. A closer look into the composition of nuts may help understand their health-promoting effects. They are rich in unsaturated acids such as oleic and linoleic, and have low concentrations of saturated fatty acids. In addition, the oil fraction presents significant amounts of tocopherols and phytosterols. The cake and shell, coproducts obtained from nuts, are rich in polyphenols. Besides the lipids and bioactive substances, nuts and their coproducts are also rich in other macronutrients (protein and fiber) and micronutrients (vitamins and minerals). The composition and concentration of bioactive compounds vary according to the type of nut and its coproducts [1].

Brazil has a great diversity of nut trees distributed in five of its six biomes Amazon, Cerrado, Atlantic Forest, Caatinga, Pampas, and Pantanal. **Figure 1** presents the Brazilian biomes where the nuts discussed in this chapter are found.

Brazil nuts and cashew are commercial nuts native to Brazil. The cashew tree (*Anacardium occidentale* L.), which is well adapted in tropical and subtropical regions, is present in Cerrado, a biome known for long periods of drought. The nut,

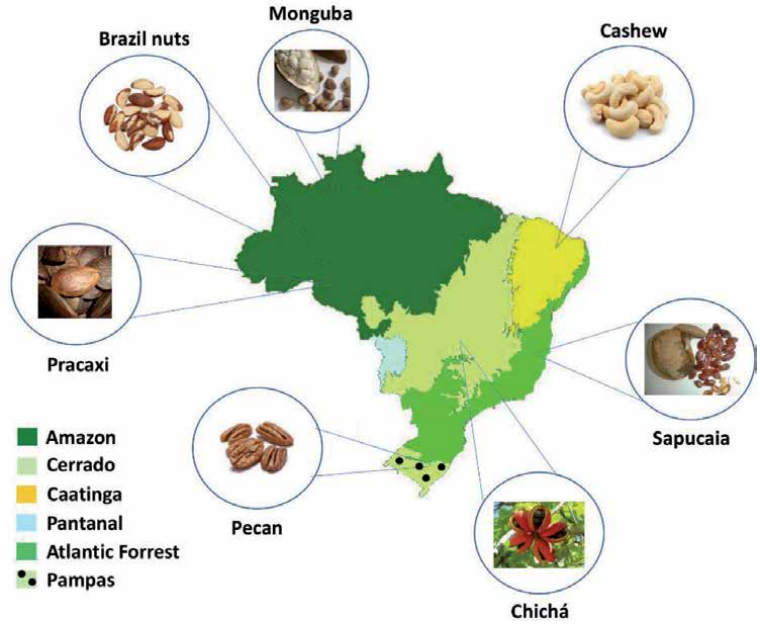

Figure 1.
Distribution of different nut trees in Brazilian biomes.

which is obtained from the cashew fruit, is composed of shell, skin, and almond. The largest producer of cashew nuts in 2018 was Vietnam (863,060 tons), followed by India (745,000 tons), Côte d'Ivoire (711,000 tons), Philippines (222,541 tons) and Brazil (141,418 tons) [2]. The Brazilian state of Ceará led the production with 61.9% of the planted area of cashew trees, followed by Rio Grande do Norte (15.4%), and Piauí (15.2%) [2].

Brazil nut tree (*Bertholletia excelsa*) is present in the Amazon, where it grows in well-drained soil along the Amazon River without the use of pesticides and herbicides. Brazil is the largest producer of Brazil nuts in the world (36,923 tons), followed by Bolivia, Ivory Coast, and Peru (25,749; 19,356; and 6042 tons, respectively) [2].

On the other hand, the pecan nut (*Carya illinoinensis*) is native to northern Mexico and southern United States. It was introduced in Brazil in 1866, and it is cultivated in the biomes Atlantic Forest, and mainly in the Pampas in the southeast and south regions. The tree is large-sized and can grow up to 20-40 m, and its fruit is technically classified as a drupe, characterized by a single pit surrounded by husk [3, 4].

These commercial or named conventional nuts in Brazil are usually consumed in a variety of ways such as raw, roasted and salted, caramelized, and coated. In addition, they can be incorporated into other food products, such as cereal bars, chocolates, bakery goods and spreads, among other foods. They can also be used for the extraction of specialty oils and for obtaining plant-based milk [1, 5–7].

However, the availability of nuts in Brazil is not limited to cashew, pecan, and Brazil nuts. A large variety of nut trees is available, especially in the Amazon region. These relatively unknown indigenous nuts represent a vast potential for the introduction into the diet of Brazilians and other consumers around the world. Sapucaia, chichá, monguba, and pracaxi are some of the nuts, which are not commercialized on a large scale and are usually consumed in their natural form by the local population. Therefore, data on their production is not found in the literature. A few

reports showed that such nuts are also rich in macro and micronutrients, including phenolic compounds [8–10].

Sapucaia (*Lecythis Pisonis*) grows in the Atlantic Forest biome [9], while monguba (*Prachira aquatica*) and pracaxi (*Pentaclethra macroloba* (Wild.) Kuntze) can be found in the Amazon. The monguba tree is cultivated in different regions of the Amazon biome as an ornamental plant. Its fruits are oval-shaped, surrounded by a brown wooden peel where large-sized seeds are contained. The seeds are edible and can be consumed in a variety of ways, such as roasted, boiled, or fried [10]. The pracaxi tree produces a pod-shaped fruit containing edible seeds, from which phenolic-rich oils can be obtained [11]. Chichá (*Sterculia striata*), a nut tree originated from India and Malaysia, has thrived in the semi-arid conditions of the Cerrado, yielding nuts rich in phenolic compounds and lower lipid content [8].

This chapter presents the nutritional composition, phytochemical properties, and bioactive compounds of commercial (cashew, Brazil nuts, and pecan) and non-commercial (sapucaia, chichá, monguba, and pracaxi) nuts found in Brazil. The health benefits associated with their consumption, as well as novel products based on these nuts and their coproducts will be discussed with emphasis on their functional properties and nutritional profile.

2. Nutritional composition of native Brazilian nuts

Table 1 shows the macronutrient composition of conventional and non-conventional nuts native to Brazil. Brazil nuts present the highest lipid content and energy value. On the other hand, cashew nuts have higher protein content followed by chichá, which has the highest content of carbohydrates and the lower content of lipids. The sapucaia nut presents the highest fiber content among all nuts showed in **Table 1**.

Table 2 shows that the conventional and non-conventional nuts are rich in lipids considered beneficial to health, such as monounsaturated fatty acids (MUFAS) and polyunsaturated fatty acids (PUFAS). Pecan nut presents the highest content of the MUFA oleic acid (C18:1, ω9) followed by the cashew and pracaxi. Besides the nutritional benefits, another advantage of oleic acid is related to its higher oxidative stability compared to PUFAS. On the other hand, Brazil nuts show a high PUFA

Component (g/100 g)	Conventional			Non-conventional			
	Brazil nuts [12, 13]	Cashew [14]	Pecan [15]	Chichá [8, 16]	Monguba [10, 17]	Pracaxi [11]	Sapucaia [18, 19]
Ashes	3.3	2.8-4.1	1.8	3.0-3.2	2.3-2.7	1.9	2.9-3.5
Moisture	3.1-3.2	2.7-8.4	3.3	8.2-11.4	5.3-8.3	4.0	4.1-4.2
Protein	14.4-16.2	19.7-24.5	8.6	18.5-22.5	13.3-15.4	15.5	15.5-20.5
Lipids	64.9-67.3	39.8-47.1	62.2	24.5-28.6	41.9-45.6	53.4	58.7-60.8
Total fiber	7.5-8.0	2.5-4.2	10.9	4.6-5.8	4.7-6.1	—	16.5
Carbohydrate	10.9-15.9	27.1-34.9	13.4	40.5-45.8	34.3-36.2	25.2	4.9-13.8
Energy value (kcal/100 g)	659-715	499-707	633.9	456-530	557-677	644	616-665

—: not presented.

Table 1.
Composition of macronutrients of conventional and non-conventional nuts native from Brazil.

Fatty acids (%)	Conventional			Non-conventional			
	Brazil nuts [12, 13, 20]	**Cashew [21, 22]**	**Pecan [4, 15]**	**Chichá [8]**	**Monguba [10]**	**Pracaxi [11, 23]**	**Sapucaia [18]**
Miristic (C14:0)	0-0.1	—	—	—	—	—	0.1
Palmitic (C16:0)	14.9-16.7	10.3	5.4	26.5	60.9	1.4-1.5	12.9-15.2
Palmitoleic (C16:1 cis 9)	0-0.4	0.3	—	2.4	—	—	0.2-0.3
Margaric (C17:0)	—	0.1	—	—	—	—	0-0.1
Stearic (C18:0)	9.9-11.9	9.8	1.4	4.0	1.8	2.5-2.7	7.7-8.4
Oleic (C18:1 cis 9)	28.5-36.3	60.6	71.8	37.8	7.7	53.2-53.5	39.7-44.4
Linoleic (C18:2 cis 9,12)	36-37.5	17.0	20.2	11.2	6.6	12.1-12.2	32.2-40.0
Linolenic (C18:3cis 9,12,15)	0.1-0.2	0.2	0.8	0.3	—	0.1	0.3-0.4
Arachidic (C20:0)	0-0.2	0.7	—	0.7	—	—	—
Gondoic (C20:1 cis 11)	0-0.1	0.2	—	—	—	—	0.1
Behenic (C22:0)	—	0.1	—	0.3	—	16.4-16.5	—
Lignoceric (C24:0)	—	—	—	—	—	11.1-11.6	—
∑ Saturated	24.9-28.9	21.3	7.0	31.5	62.7	33.3-33.6	21.5-23.3
∑Monounsaturated	28.5-36.8	61.1	71.8	40.4	14.2	54.1-54.3	40.1-45.7
∑Polyunsaturated	36.1-37.7	17.2	21.0	12.2	—	12.2-12.3	32.7-40.4
Tocopherol (µg/g)							
α-Tocopherol	72.5	78.4	1.7	16.6	ND	ND	11.2
β-Tocopherol	—	1329.8	—	1.1	ND	ND	ND
γ-Tocopherol	74.4	300.3	26.8	88.5	5.1	416.1	285.0
δ-Tocopherol	5.9	6.3	—	21.0	ND	7.8	2.8
Total	152.8	1714.8	28.7	127.0	5.1	423.9	299.0
Phytosterols (mg/100 g)							
Brassicasterol	1.5	101.4	—	ND	—	—	ND
β-Sitosterol	39.5	7.8	—	184.9	—	—	93.7
Campesterol	4.0	—	—	18.6	—	—	8.6
Stigmasterol	11.3	—	—	54.2	—	—	11.2
Sitostanol	39.5	8.6	—	—	—	—	—
δ5-Avenasterol + δ7-stigmasterol	6.7	—	—	—	—	—	—
Total	47-148	117.8	0.2-0.3	257.7	—	—	113.5

—: not presented.

Table 2.
Lipid composition of conventional and non-conventional nuts.

linoleic acid (C18:2, ω6) content (36-37%), which is an essential fatty acid. It also presents a balanced fatty acid composition containing significant concentrations of MUFAs and saturated fatty acids (SFAs). The main fatty acids present in Brazil nuts

is oleic (C18:1, ω9), linoleic (C18:2, ω6), and palmitic (C16:0) acids. In addition, most of the nuts have a low concentration of saturated fatty acids. The exception is monguba, carrying 63% saturated fatty acid, with palmitic acid being the main fatty acid.

Between the nuts shown in **Table 2**, cashew has the highest tocopherol content (1714.80 μg/g) followed by the pracaxi nuts (423 μg/g). The main tocopherols identified in the cashew nut were β-tocopherol > γ-tocopherol > α-tocopherol > δ-tocopherol. The chichá nut presents the highest content of phytosterols (257.7 mg/100 g), followed by the cashew nut (117 mg/100 g). β-sitosterol and brassicasterol were the main phytosterols found in these nuts. The bioactive composition of Brazilian nuts is presented in item 3 of this chapter.

Table 3 shows that Brazil and sapucaia nuts are a source of selenium. Considering the level of Se detected in Brazil (36.1 μg/g), and sapucaia (46.9 μg/g) nuts, a daily intake of more than one or two nuts can exceed the recommended daily dose of selenium for adults (55 μg/day) [18]. A selenium intake higher than 400 μg/day has been associated with toxic effects, including selenosis, which symptoms are hair loss, skin damage, and nervous system disorders [12]. Sapucaia nuts are also rich in magnesium (1572 μg/g) and calcium (1168 μg/g). Chichá is rich in potassium (8718 μg/g) and presents the highest concentration of zinc (24.2 μg/100 g). On the other hand, cashew is rich in potassium (K) and pecan in phosphorus (P).

Minerals play fundamental roles in several functions in the human body, acting as cofactors in enzymatic processes, structural elements, and participating in the regulation of acid–base balance, nerve impulse, and muscle activity [24]. The consumption of nuts as part of a balanced diet can contribute to a proper consumption of minerals, which play an important role in maintaining good health.

Mineral (μg/g)	Conventional			Non-conventional			
	Brazil nuts [25]	**Cashew [26]**	**Pecan [27]**	**Chichá [8, 16]**	**Monguba [10, 17]**	**Pracaxi [28]**	**Sapucaia [18, 19]**
Al	—	—	—	6.2	—	—	1.7
Ca	7432.8 ± 10.2	35.8 ± 0.7	70.0	149.1	558.9	8.9 ± 0.2	1168.0
Cd	—	—	—	0.4	—	—	0.3
Cr	1.3 ± 0.2	—	—	0.4	—	—	0.5
Cu	59.4 ± 0.5	9.5 ± 0.2	—	7.5	7.5	—	16.9
Fe	74.3 ± 0.5	26.1 ± 0.5	2.5	218.2	4.4	1.7 ± 0.1	—
K	—	1443.3 ± 26.2	4.1	8718.0	—	—	—
Mg	9678.5 ± 68.5	612.7 ± 13.2	121.0	1327.0	875.3	5.6 ± 0.2	1572.0
Mn	3.4 ± 0.4	7.6 ± 0.2	4.5	32.1	2.0	—	41.4
Na	—	148.5 ± 2.6	—	—	11.4	—	0.5
Ni	—	0.6 ± 0.5	—	0.4	—	—	—
P	—	—	277.0	—	—	18.1	—
Pb	—	—	—	0.5	—	—	0.5
Se	36.1 ± 0.4	0.2 ± 0.7	—	—	—	—	46.9
Sn	—	—	—	16.6	—	—	11.1
Zn	110.3 ± 1.3	28.9 ± 1.2	4.5	24.2	9.9	—	20.9

—: not presented.

Table 3.
Mineral composition of conventional and non-conventional nuts.

On the other hand, an excessive consumption of Brazil and sapucaia nuts is not recommended due to the high levels of selenium.

3. Bioactive composition of Brazilian nuts

Tocopherols and tocotrienols are a group of eight compounds widely spread in nature. They are monophenols chemically characterized by a chromanol ring, in which a hydroxyl group is attached. The configuration of the side hydrocarbon chain determines whether the compound is either tocopherol or tocotrienol (saturated side chain or three double bonds, respectively). Both tocopherols and tocotrienols have four homologs each (α, β, γ, and δ), which are defined by the methylation pattern on the chromanol ring. These compounds can act as antioxidants by the donation of a hydrogen atom from the hydroxyl group to free radicals, stabilizing them and reducing oxidative stress. α-Tocopherol presents the highest *in vivo* antioxidant capacity and 100% of vitamin E activity. The activity of α-tocopherol is related to the transfer protein (α-TTP) in the liver, involved in the absorption of tocopherols [29].

Nuts, seeds, and vegetable oils are good sources for tocopherols, and α-tocopherol is the most abundant one in photosynthetic tissues. On the other hand, seeds accumulate about 10–20 times more γ-tocopherol. Tocopherols can be found in considerable amounts in Brazil nuts, where they are concentrated in the oil fraction due to their lipophilic character, as reported by Costa et al. [20]. The authors reported a total tocopherol content of 152.80 μg/g for Brazil nuts, composed of α-tocopherol (72.55 μg/g), γ-tocopherol (74.35 μg/g), and δ-tocopherol (5.90 μg/g). This profile may change according to the region where the nuts are grown. Funasaki et al. [30] reported the tocopherol composition of Brazil nuts from seven different Amazon rainforest areas. α-Tocopherol content ranged from 37.92 μg/g (Manicoré 2-AM) to 74.48 μg/g (Manicoré 1-AM) and γ-tocopherol levels varied between 106.88 μg/g (Manicoré 2-AM) and 171.80 μg/g (Xapuri – AC). The differences in the tocopherol content can be related to climate variations and post-harvest handling.

As natural antioxidants, tocopherols play a role in the protection of nuts against oxidation. Zajdenwerg et al. [31] showed a correlation between the decrease in tocopherol concentration and the appearance of secondary oxidation products in Brazil nuts after a period of storage of 16 days at 80°C γ-tocopherol was depleted by 50%, and α-tocopherol was completely consumed, while aldehydes started to build up from hydroperoxide breakdown. The authors suggested that α-tocopherol acted as a primary antioxidant, which would explain why this homolog was depleted first. α-Tocopherol is less polar than γ-tocopherol due to the presence of three methyl groups on its chromanol ring, which gives it higher antioxidant efficiency in non-polar systems [31].

Cashew nuts, on the other hand, present a lower concentration of tocopherols when compared with Brazil nuts. Ryan et al. [32] reported α-tocopherol and γ-tocopherol contents of 3.6 mg/g and 57.2 mg/g, respectively, for cashew oil. Meanwhile, Brazil nut oil was composed of 82.9 mg/g of α-tocopherol and 116.2 mg/g of γ-tocopherol. However, according to the same study, cashew oil is richer in other bioactive compounds than Brazil nut oil, namely some types of phytosterols, such as β-sitosterol (1768 mg/g against 1325 mg/g from Brazil nut) and campesterol (105.3 mg/g against 26.9 mg/g for Brazil nut). Nevertheless, stigmasterol is more concentrated in Brazil nuts oil (577.5 mg/g) than cashew oil (116.7 mg/g). The consumption of phytosterols has been associated with a decrease in LDL-cholesterol levels. The reason behind this bioactivity relies on the higher hydrophobicity of phytosterols compared with cholesterol, which would give them

an advantage in being transported by micelles and later exerted once humans do not absorb them. This would prevent cholesterol from accumulating in the enterocytes and further reaching the bloodstream [32]. Pecan nut is rich in γ-tocopherol, with 23.8-38.1 mg/100 g [4].

The bioactive composition of cashew, Brazil and pecan nuts goes beyond the presence of tocopherols and phytosterols in their oil fraction. Both are also rich sources of polyphenols, an extensive class of secondary plant metabolites with antioxidant properties. Polyphenols primarily act by donating a hydrogen atom to free radicals in order to interrupt oxidation reaction chains. As a minor antioxidant mechanism, phenolics also can chelate transition metals, preventing these prooxidant agents from initiating the reaction chain that originates from the oxidative process. Based on structural differences, this large group can be further divided into subgroups, with the main ones being flavonoids, hydroxycinnamic and hydroxybenzoic acids, hydrolysable tannins, and proanthocyanidins. In nuts, phenolics can be present as free soluble compounds, esterified to fatty acids (soluble esters), or insoluble-bound to macromolecules (e.g., cellulose, structural protein, pectin) [1].

John et al. [1] reported the phenolic composition and antioxidant activity of Brazil nut. Soluble phenolics (free plus esterified) were found to be the predominant state present in the whole nut (519.11 mg/100 g), as well as the kernel (406.83 mg/100 g) and the brown skin (1236.07 mg/100 g). However, a significant amount (352.48 mg/100 g) of insoluble-bound phenolics were detected in the brown skin of Brazil nuts. Gallocatechin, protocatechuic acid, catechin, and vanillic acid were the main phenolics identified in the bound fraction. Insoluble-bound polyphenols are related to beneficial effects on gut health, once they are associated with a decrease in the colonic pH, preventing the growth of harmful microorganisms [33]. The study of John et al. [1] also demonstrated that the brown skin extract of Brazil nuts showed the highest *in vitro* antioxidant activity, measured by DPPH, hydroxyl radical scavenging activity, reducing power and ORAC assay. The brown skin showed the highest amount of soluble and insoluble-bound polyphenols and this coproduct of Brazil nuts can be considered an economical source of natural antioxidants.

Yang et al. [34] compared the phenolic content and antiproliferative activities of Brazil nuts and cashew. Cashew showed a higher concentration of both soluble (86.7 mg/100 g) and insoluble-bound (229.7 mg/100 g) phenolics when compared to Brazil nuts (46.2 mg/100 g of soluble and 123.1 mg/100 g of insoluble-bound). Brazil nuts did not display antiproliferative activity against HepG2 (human liver cancer cells) at the doses tested in the study (from 1 to 200 mg/mL). On the other hand, cashew displayed the effect at high doses (100-200 mg/mL). In addition, neither of them showed inhibition for human colon cancer cells (Caco-2) proliferation.

The roasting, which is a common process in nut preparation, has shown evidence to impact the phenolic composition of cashew and Brazil nuts in different ways. Özcan et al. [35] reported that the total phenolic content of Brazil nuts significantly decreased from 68.97 mg/100 (raw nut) to 66.47 mg/100 g when oven-roasted (130°C for 20 min). The microwave-roasted (720 W for 5 min) showed the highest impact on the phenolic compounds, decreasing from 68.97 mg/100 to 25.88 mg/100 g. The DPPH radical scavenging capacity of the phenolic extracts also declined from 81.77% in the raw nut to 40.66% in the oven-roasted nut and 34.60% in the microwave-roasted nut. On the other hand, Chandrasekara and Shahidi [21] demonstrated that oven-roasting cashew at 130°C for 33 min increased the total phenolic content (both soluble and insoluble-bound) of the whole nut, as well as the kernel and the testa. At the same time, the levels of proanthocyanidins decreased, except for soluble phenolic extract from the kernel. In particular, the concentrations of the flavonoids (+)-catechin, (−)-epicatechin, and epigallocatechin were

enhanced after the roasting process, which also positively affected the antioxidant capacity of such extracts. According to the authors, these findings could be owed to the release of phenolics because of temperature, making them readily soluble in the extraction solvent. Although relatively high, the temperature was applied for a short period, which prevented an extensive degradation of polyphenols.

Pecan nutshell, a coproduct, have demonstrated great potential due to its rich phenolic composition and high antioxidant capacity. According to Hilbig et al. [36], extracts from this coproduct obtained under optimum conditions yielded total phenolic contents of 426.15-581.9 mg GAE/g, resulting in antioxidant activities of 2574.32-2573 μmol TEAC/g and 1268.03-1287.08 μmol TEAC/g measured by ABTS and DPPH assays, respectively. The extracts showed a variety of 29 different phenolic compounds, with gallic acid as the predominant one.

Demoliner et al. [9, 18] investigated the nutritional and phytochemical composition of sapucaia nut. The oil extracted from the nuts presented a considerable amount of total tocopherols (21.8-29.9 mg/100 g), with γ-tocopherol identified as the primary homolog (19.2-28.5 mg/100 g). α- and δ-Tocopherols were also identified in smaller quantities. Mounting evidence has shown that γ-Tocopherol excels in scavenging reactive oxygen species, which play an essential role in the development of chronic diseases [37–39]. Sapucaia nut oil also showed a significant concentration of phytosterols, namely β-sitosterol (92.8-193.9 mg/100 g), stigmasterol (9.92-13.2 mg/100 g), and campesterol (8.42-9.63 mg/100 g) [9].

Demoliner et al. [9] extracted the phenolic compounds from sapucaia nut and shell and used LC-ESI-MS/MS to identify and quantify the individual polyphenols present. The nut extracts were composed of 14 compounds, mainly phenolic acids, and flavonoids, with myricetin, vanillic, ferulic, and ellagic acid showing the highest amounts. Interestingly, shell extracts demonstrated to carry a greater variety of phenolics, with 22 compounds identified, with high levels of phenolic acids (gallic, protocatechuic, vanillic, ferulic, and ellagic) and flavonoids (epigallocatechin, catechin, epicatechin, taxifolin, myricetin, and vanillin). Shell extracts were also superior to the nut extracts in terms of *in vitro* antioxidant activity, measured by DPPH, FRAP, and ABTS. These outcomes highlight the potential for using nuts coproducts as sources of natural antioxidants.

Similar to sapucaia, the coproducts of chichá, namely the pellicle (26.26 mg GAE/g) and the shell (21.42 mg GAE/g), have been reported to be richer in total phenolic compounds than the nut (16.85 mg GAE/g). The extracts presented a wide variety of phenolic acids, such as ellagic, ferulic, salicylic, protocatechuic, and rosmarinic acid [8].

It has been reported that monguba oil is source of γ-tocopherol (513.5 mg/kg). In addition, ten phenolic compounds have been identified, mainly phenolic acids and flavonoids. The majority of phenolics (74.58%) were in the esterified form, followed by the glycosylated (13.02%), free (8.22%), and insoluble bound (4.18%) forms. Caffeic acid Monguba was the main phenolic compound found in this raw material (57.5%) [10].

Teixeira et al. [11] reported total phenolic contents ranging from 31.92 to 54.05 mg GAE/kg for pracaxi oil extracted by supercritical CO_2 extraction. The highest phenolic content and in vitro antioxidant activity of the extracts was obtained using 200 bar at 40-60°C. The oils obtained under low pressure demonstrated high antioxidant capacity measure by the CUPRAC method, indicating a significant content of both hydrophilic (phenolics) and lipophilic (carotenoids and tocopherols) antioxidants. The presence of natural antioxidants may also have positively affected the stability oxidative index (OSI of 11.38 h at 110°C and 10.83 at 120°C), which suggests a prolonged shelf life for the oil [11].

4. Bioavailability and health-promoting benefits

The nutritional profile and bioactive compounds present in Brazilian nuts are certainly a positive characteristic of these raw materials. However, the presence of these nutrients and minor compounds do not guarantee their conversion into health-promoting benefits. They need to be efficiently released from the food matrix and be absorbed in sufficient amounts to be converted into biologically active metabolites for having and positive impact on human health. In addition, the potentially toxic compounds should be assessed to ensure its safety. That is especially true when considering expanding the commercialization of relatively unknown nuts, like the ones we have been presenting throughout this chapter.

Moreda-Piñeiro et al. [40] assessed the *in vitro* bioavailability of essential and toxic metals in several nuts and seeds, including Brazil nuts and cashew. Essential metals (Ba, Ca, Cd, Co, Cu, K, Li, Mg, Mn, Mo, P, Pb, Se, Sr., Tl, and Zn) consistently presented moderate bioavailability (dialyzability of 2.1-40.7%) among the samples. The exception was iron, which presented a low dialyzability (0.70-3.7%). On the other hand, toxic metals (Al, Ba, Cd, and Hg) demonstrated poor bioavailability (dialyzability ratios between 0.35-16.8%). The results suggest that the consumption of these nuts can be considered as safe and beneficial because of the high bioavailability of the essential metals. The authors also found a positive correlation between carbohydrate content and dialyzability ratio, meaning that the higher the carbohydrate concentration, the greater the bioavailability. On the other hand, nuts with high-fat content were found to present lower mineral bioavailability.

Nascimento et al. [41] reported the *in vitro* bioavailability of Cu and Fe in cashew nuts using simulated gastric and intestinal fluids as well as dialysis procedures. The results showed that 83% of Cu and 78% of Fe were recovered during the experiment, indicating a high level of bioavailability for these minerals in cashew nuts. These two minerals are essential for a variety of physiological and metabolic processes. Cu deficiency can lead to high blood pressure and infertility, while Fe deficiency can cause anemia.

Using an *in vitro* dialyzability approach, Herbello-Hermelo et al. [42] evaluated the bioavailability of the polyphenol fraction of selected nuts and seeds. Brazil nuts and cashew were among the samples with the highest recovery of phenolics in the digested extracts (81 and 89%, respectively). The authors also showed that polyphenol bioavailability was dependent on Cu content. This can be explained by the strong binding ability between Cu^{+} and phenolics, with the latter being reported to reduce Cu^{2+} to Cu^{+}, even though the mechanism by which this happens is not entirely understood yet.

Brazil nuts are one of the main sources of Se in nature, which are constituents of selenoproteins (e.g., glutathione peroxidases – GSH-Px), enzymes that are part of the endogenous antioxidant defenses system. A high blood concentration of such enzymes is associated with a lower risk of cardiovascular diseases. Stockler-Pinto et al. [43] conducted a human trial on the supplementation of Brazil nuts to patients on hemodialysis, which produces a large amount of reactive oxygen species. The administration of one nut per day for three months increased the subjects' GSH-Px activity. Before the supplementation, 11% of the patients presented GSH-Px levels below the normal range. After the supplementation, all subjects showed results within the normal range.

Inflammation processes are essential biological responses when the organism needs to fight intrusive agents. However, inflammatory disorders are extremely damaging and can lead to conditions such as cancer, type 1 diabetes, and rheumatoid arthritis, among others [44]. Colpo et al. [45] monitored the activity of

inflammation markers upon the intake of different portions of Brazil nuts (from 0 to 50 g) by healthy individuals. The trial revealed that the consumption of 20 or 50 g of Brazil nuts was responsible for a decrease in serum levels of inflammation markers (IL-1, IL-6, TNF-α, and INF-γ).

Nanoemulsions prepared from cashew nut shell liquid showed cytotoxicity against human breast cancer cell line MCF-7. The cells treated with the nanoemulsion presented reduced viability, primarily through apoptosis or necrosis [46]. Cashew nut has also demonstrated a positive impact on cholesterol levels and systolic blood pressure. In a trial conducted with 300 type 2 diabetic adult patients, the administration of 30 g of cashew nuts daily for 12 weeks could reduce the subjects' blood pressure while the concentration of plasma HDL cholesterol increased.

Müller et al. [47] reported a potential biological effect of pecan nutshell extracts *in vivo* and *in vitro* studies. The phenolic-rich extracts from pecan nutshell demonstrated protection against liver damage induced by ethanol in rats by increasing the levels of endogenous antioxidant defenses, such as glutathione (by 33%), superoxide dismutase (by 47%) and catalase (by 47-73%). Hilbig et al. [48] showed that aqueous extracts from pecan nutshell were able to reduce the viability of MCF-7 breast cancer cells. The effect was attributed to the high abundance of phenolic compounds in the extract, such as gallic, ellagic, and chlorogenic acid, as well as catechin, epicatechin, epigallocatechin, among others.

5. Nut-based products

Nuts are usually consumed as a snack, in their natural form, as well as toasted, salted, or caramelized. The Brazil nuts, cashew and pecan and are used as ingredients in several industrialized food products such as bakery goods, sweets, chocolates and ice creams, among others. The underexplored nuts, such as chichá, monguba, pracaxi, and sapucaia, although not reaching the same level of commercialization, have shown to be potentially suitable for these types of application.

5.1 Nut oil

Nut oils are usually obtained by pressing, and since they are not refined, they are classified as extra virgin. The main steps involved in the processing of an extra virgin nut oil are harvesting, pre-drying, peeling, drying, oil extraction, and centrifugation. Because of the appreciable sensorial attributes and elevated price compared with other vegetable oils, the nut oils are considered gourmet oils. The price will differ according to the nut type, its availability, and the processing used.

Brazil nut, pecan and cashew nut oils are usually found in specialized stores of natural products, in pharmacies (for use as cosmetics) and are available for online purchase, where their price ranges from 8 US $ to 17 US $. The pracaxi oil can be found for commercialization, mainly as a cosmetic, due to its emollient properties. On the other hand, chichá, sapucaia and monguba oils have not been commercially produced yet. However, studies show that these oils are rich in monounsaturated fatty acids and possess high oxidative stability, thus showing great potential for commercialization [8, 18]. Monguba oil, which is rich in palmitic acid, has a great potential for food applications as a substitute for cocoa butter [10].

5.2 Cereal bars

Cereal bars are consumed mainly as a diet substitute for sugar-dense snacks, as well as an energy and protein source for athletes. In the formulation of cereal bars, it

is important to take into account the cereal choice (oats, wheat, rice, barley, maize). In addition, the selection of the appropriate carbohydrate to maintain a balance between taste and shelf life, the nutrient profile, the dietary fiber, and the processing stability [49] are also important. Cereal bars made with nuts are widely accepted by consumers. Besides their nutritional and sensory quality, they remain stable during product storage. Currently, we can easily find cereal bars containing Brazil nuts and cashew as ingredients.

5.3 Nut flour

Nuts can be ground into flour for use in bakery goods. Cake, the residue left after nut oil extraction, can be used as raw material to produce defatted nut flour. This is considered a sustainable process since it adds value to a coproduct, reducing waste generation [50]. Nut flour is rich in dietary fiber, protein, vitamins, minerals, and bioactive compounds. The application of partially defatted nut flour has been reported to improved consumer acceptance of gluten-free bakery products [50]. The combination of rich nutritional composition with appreciable physical properties makes these flours suitable for bakery products, such as cookies, bread, cakes, sweets, among others.

Physical properties, such as water or oil retention capacity, foam properties, emulsifying activity, and emulsion stability, are very important for the incorporation of flour into bakery products. Sanchez et al. [51] reported that pistachio and cashew nut flours with thermal trapping in autoclave showed interesting emulsifying and water rectifying properties for application in bakery products. In another study, Teixeira et al. [19] reported functional properties of defatted sapucaia flour. The parameters of emulsion formation and stability indicated that it could be applied as an ingredient in emulsified products such as cakes, creams, sauces and sausages, among others.

5.4 Nut-based milk alternative

Plant-based milk alternatives are beverages obtained from crushing a specific feedstock homogenized in water. The resulting particle size distribution should be between 5 and 20 μm to mimic cow's milk in appearance and consistency. Sethi et al. [5] reported that the vegetable-based milks can be divided in five categories according to the raw material used: cereals (oats, rice, corn, spelled), vegetables (soy, peanuts, lupines, cowpea), nuts (almonds, coconut, hazelnuts, pistachios, walnuts), oilseeds (sesame, flax, hemp, sunflower), and pseudo-cereals (quinoa, teff, amaranth).

The improved diagnosis of conditions related to cow's milk consumption, such as milk protein allergy and lactose intolerance, increased the demand for non-dairy milk alternatives. Besides, vegans also prefer these beverages, and it is being considered a continuously growing niche [5]. Nuts, such as walnuts, chestnuts, and almonds, are used to produce plant-based milk alternatives, mainly due to their functionality and better sensory characteristics [5]. The allergenic potential and high cost are some of the limiting factors presented by the nut-based milks.

The addition of Brazil nuts extract in a soybean-based drink positively influenced the sensory characteristics of the product [52]. On the other hand, prebiotic drinks based on cashew nuts and fruit juice have proved to be a viable alternative for the development of functional products [53]. Bruno et al. [6] reported that cashew nut-based milk alternative was a good matrix for the development of probiotics. They showed that *Bifidobacterium animalis*, *Lactobacillus acidophilus* and *L. plantarum* remained viable during 30 days at 4°C. The probiotic drink based on

cashew nuts also achieved a good sensory acceptance, without significant changes in whiteness and microbiological quality.

Plant-based milk of sapucaia nut cake using block freeze concentration, which was done in five consecutive stages, allowed the concentration of the phenolic compounds (gallic, vanillic, ferulic, sinapic and salicylic acids, catechin, taxifolin and sinapaldehyde) and minerals in all the five fractions [54]. Studies with different raw materials and formulations, especially non-conventional nuts, are still scarce. The knowledge about such products should contribute to encouraging their consumption.

6. Nuts shell and cake

Industrial nut processing results in a large amount of shells, a coproduct that can represent approximately 40-50% of the original total mass. The reuse of these coproducts, which are usually discarded, should be encouraged for reducing waste disposal, preserving the environment and adding value to the raw materials.

Some nutshells, such as pecan, are sold in pieces to make tea [4]. The ethnopharmacological use of nutshell tea includes the prevention and treatment of various diseases, such as type 2 diabetes, obesity, hypertension, hypercholesterolemia, cancer and, inflammatory diseases [24]. The therapeutic effects of the tea made with pecan nutshell have been associated with the presence of several phenolic compounds, such as phenolic acids, flavonoids, and proanthocyanidins. The antioxidant, antimicrobial, and antitumor activity of pecan peel extract have been reported [4, 50]. Other nutshells may have health benefits due to their bioactive composition.

Cashew nut is covered with a thin antioxidant-rich layer of reddish-brown color, known as testa. This fraction is an excellent source of hydrolysable tannins and polymeric proanthocyanidins. It comprises of phenolic acids like syringic acid, gallic acid, and *p*-coumaric acid as the major components. The concentration of catechin and epicatechin were found as 5.70 and 4.46 g/kg of dry matter, respectively [55].

The process of mechanical oil extraction generates a partially defatted byproduct known as cake. The agro-industrial use of the defatted nut cake has great nutritional value, based on its high lipid and protein contents and the functional aspect present in this material, adding value to food products. The nuts cake can be used in bakery (as presented in item 5.3), but also in sweets. In the study by Lima et al. [56], the cashew nut cake was used to replace peanuts in the production of a sweet known as *paçoca,* made by mixing ground peanuts with other ingredients, such as corn flour, sugar, honey, and oil. The product showed physicochemical and microbiological stability, as well as good sensory acceptance. In addition, the nut cake presents bioactive compounds. For instance, Maciel et al. [57] demonstrated that pecan nut cake is rich in phenolic compounds with antioxidant activity.

7. Future perspectives

Commercial and non-commercial Brazilian nuts are nutritionally rich in macronutrients and bioactive compounds, with considerable amounts of natural antioxidants. Such substances are related to a myriad of health benefits since they are able to reduce oxidative stress. A significant number of studies have investigated the bioavailability of these bioactive compounds of Brazil nuts and cashew with promising results. The effect of the nuts on health using human trials had positive outcomes.

However, relatively unknown Brazilian nuts, such as sapucaia, chichá, pracaxi, and monguba remain an underexplored topic. Information about their bioavailability aspects and their impact on human health is still necessary. Therefore, more studies should be done in order to stimulate large-scale commercialization.

Finally, nuts are incredibly versatile with great economic potential. The incorporation of the coproducts, which are rich in nutrients and bioactive compounds, is an opportunity to enrich food formulation with these cost-effective ingredients, diminishing the waste generated by nut processing.

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Chapter 9

Bioactive Peptides from Agriculture and Food Industry Co-Products: Peptide Structure and Health Benefits

Jirawat Yongsawatdigul and Ali Hamzeh

Abstract

Co-products from food processing are typically disposed or turned into low value animal feed. Proteinaceous co-products can be converted to bioactive peptides exerting health benefits, which can lead to development of nutraceuticals and functional foods. This is an effective means for valorization of these co-products. The release of encrypted peptides exhibits various bioactivities, including antihypertension, antioxidant, immunomodulatory activities among others, *in vitro*, and some activities have been demonstrated *in vivo*. Structure modification of bioactive peptides occurring under gastrointestinal digestion and cellular transport remains the important factor determining the health benefits of bioactive peptides. Understanding peptide transformation in gastrointestinal tract and in blood circulation before reaching the target organs would shed some lights on its bioavailability and subsequently ability to exert physiological impact. In this chapter, the potential health promoting properties of peptides encrypted in various sources of co-products will be reviewed based on evidence on *in vitro*, *in vivo* and clinical trial studies. Structural changes of bioactive peptides under physiological condition will also be discussed in relation to its bioactivities.

Keywords: bioactive peptide, transepithelial transport, bioavailability, peptide sequence

1. Introduction

A large number of food co-products is annually generated from various plant- and animal-based food processing plants. The Food and Agriculture Organization (FAO) recently reported that 14% of global food production is lost prior to getting the retails [1]. There is also an estimation of generating co-products and waste of 38 percent in food processing industries [2]. These co-products are usually disposed, landfilled, incinerated and/or processed into animal feed and other low value products such as compost and fertilizer [3], which would cause economic losses and environmental concerns.

Bioactive peptide production is a promising approach to fully utilize proteinaceous co-products. These peptides could also exert physiological functions, leading to health benefits, such as antioxidant, antihypertension, antidiabetic,

immunomodulatory activities among others. But these peptides are encrypted in the protein chain and needed to be released to exert bioactivities. Fermentation, enzymatic and chemical (by acid or alkali) hydrolysis could be applied to produce bioactive peptides as well as using solvents which is normally used to extract natural peptides. Among them, enzymatic hydrolysis known to be more effective as it is a mild, ecofriendly, and controllable process. Bioactivities of the released peptides are affected by their size, hydrophobicity, charge, amino acid composition and their sequence which are different based on various enzyme and substrate as well as the hydrolysis conditions. After production of peptides with the certain bioactivity, their structural modification upon gastrointestinal digestion and epithelial transportation and absorption must be taken into consideration to determine their potential bioavailability. Several bioactivities and physiological functions of peptides derived from both animal and plant-based agricultural co-products have been reported, which are mainly focused on their antioxidant, antihypertensive, antidiabetic and antibacterial properties. In this chapter, their main biological activities along with the associated structure will be considered. Besides, the structure-bioavailability relation of the peptides will be demonstrated and procedures to keep them intact upon gastrointestinal digestion and transepithelial transportation will also be proposed.

2. Agricultural co-products as a source of bioactive peptides

Agricultural co-products involve a vast variety of materials with great potential as a substrate for bioactive peptide production due to the low value and high protein content. It is estimated that about 9000 tons in dairy, 3 and 9 million tons in seafood and livestock industries, respectively, are annually discarded [4]. Skins, bones and heads are rich in collagen. Collagen is known as a promising source of peptides with several bioactivities which could exert physiological functions. Collagen comprises of hydrophobic amino acids, particularly proline and hydroxyproline, offering a higher chance of being absorbed through epithelial membrane. These amino acids also make them stable against proteases in gastrointestinal digestion tract and brush border, so that the intact peptides could be absorbed and reach the target organ. Blood is also high-protein co-product obtained in a large volume in a slaughterhouse. It has been reported that about 2.5 billion tons of blood are annually generated only in Europe [5]. Hemoglobin in red corpuscles and albumin, globulins, and fibrinogen in plasma are the major proteins [6]. Whey is a predominant co-product of dairy industry with an annual production of approximately 180–190 million tons [7]. The whey-derived peptides have also lower allergenicity beyond their bioactivities, because β-lactoglobulin (β-lg) as a main whey protein contributing to allergenicity would degrade within hydrolysis process [8].

Production of bioactive peptide from some plan-based materials are restricted due to difficulties in protein recovery from their unique rigid structure of polysaccharides. To meet the challenge, pretreatment of these substrates would improve protein recovery and provide other benefits such as reduction of time and energy consumption, leading to a higher efficacy in bioactive peptide production [9]. Corn gluten meal was immersed in alkali solution, treated with α-amylase and cooked prior to proteolysis [10]. Ultrasound exposure of watermelon seed caused structural changes, leading to production of peptides with higher antioxidant activity than those without any treatment. Ultrasound could degrade interactions in matrix and unfold proteins, so that more hydrophobic residues and reactive sites are exposed, resulting in a higher efficacy in protein hydrolysis [9]. Oat bran polysaccharide was digested by cellulase and viscozyme to ease protein recovery prior to proteolysis and production of antioxidant peptides [11].

Bran from rice and wheat, is a main protein-rich co-product in cereal industry with production of about 50–60 and 90 million tons every year, respectively. Albumin, globulin, prolamin and glutelin are the major proteins in bran with a total protein content of 12–20% [12]. Seeds are also another source of bioactive peptides, among them those harvested for oil production generate a large mass of co-product. Soybean, rapeseed and canola meals with the crude protein contents of about 48, 36 and 38%, respectively, are important cases of protein-rich co-products obtained from oil industries [13]. Corn gluten meal is also a major proteinaceous co-product generated in a large quantity from corn wet milling process in which, zein and glutelin are the main proteins (68 and 28%, respectively). Zein composes of mainly hydrophobic amino acids by which, more hydrophobic peptides are likely produced [14]. Hydrophobicity is an important feature of peptides to exert some bioactivities such as antioxidant and antihypertensive properties.

Thus far, peptides with various bioactivities *in vitro* and *in vivo* levels have been recognized from these low value co-products. Conversion of these co-products to more value-added hydrolysates and/or peptides would ultimately lead to development of health promoting food products.

3. Biological activities

Several bioactivities and physiological functions of peptides derived from both animal- and plant- based agricultural co-products have been reported. These activities include antioxidant, antihypertensive, antidiabetic and antibacterial properties.

3.1 Antioxidant activity

Reactive oxygen species (ROS) are formed during normal cellular metabolism of providing energy, respiration and also when cells are exposed to exogenous oxidative stress [15, 16]. Normally, these products are neutralized by endogenous antioxidant defense systems, such as antioxidant enzymes (superoxide dismutase: SOD, glutathione peroxidase: GPx and catalase), glutathione (GSH) and others. But, the excessive level of ROS could result in many health disorders, such as cancer, cardiovascular, respiratory, neurodegenerative and other diseases [16, 17]. Therefore, antioxidant-containing diet could help to overcome ROS and subsequently their corresponding disorders. Nowadays, attempts have been made to find new antioxidant compounds from natural resources due to their benefits over synthetic compounds. To that direction, antioxidant peptides from agricultural co-products are gaining more attractions, because of their nontoxicity and safety besides their nutritional properties. Antioxidant hydrolysates/peptides production from agricultural co-products including skins, bones, viscera, whey as animal-based and brans, seeds, leaves, gluten as plant-based co-products have been extensively reviewed [17–21].

Lower molecular weight (MW) peptides with hydrophobic and aromatic amino acids (HAAs and AAA, respectively) have been generally reported to exert good antioxidant activities [22]. The HAAs could improve peptides accessibility toward ROS through binding with lipid and reaching to free radicals, so that the peptides could quench them effectively. The AAAs including Trp, Phe and Tyr are also correlated with the strong antioxidant activity via their high electron transferring capacities of their aromatic rings. Besides HAAs and AAAs, some of hydrophilic amino acids, such as His could improve the activity through its imidazole ring which has been indicated as strong electron donator [23]. Presence of charged amino acids in peptide structure could also improve the activity. A higher negatively charged amino acids (NCAA) have been observed in plasma hydrolysates prepared from

chicken blood that showed higher antioxidant activity than those prepared from blood corpuscles with lower NCAAs [6]. Presence of NCAAs are reported to correlate with the strong antioxidant activity, because they can neutralize free radicals by giving their excess electrons.

Although antioxidant activity of agricultural co-product has been widely evaluated, few studies were conducted to assess the activity *in vivo* and there is still a gap for clinical trial. The chemical *in vitro* studies are not able to reflect the activity in biological systems due to their complicated physiological conditions. In addition, cellular evaluation might be able to provide a comparable environment to biological systems. Antioxidant properties of peptides from fish sauce increased with the increasing of peptides concentration based on chemical assays, while these peptides could act as pro-oxidants in higher concentration (>50 μg L-leucine equivalent/ml) in cellular experiments [24]. Hydrolysates prepared from corn gluten meal and ham seed meal as well as ACFL, a peptide from horse mackerel viscera, increased the level of antioxidant enzymes based on *in vivo* models upon exposure to oxidative stress [10, 25, 26]. In a human trial study, a reduction in plasma malondialdehyde and an increase in SOD level was observed after daily ingestion of 4.5 g black soy derived peptides for 8 weeks [27].

3.2 Antihypertensive activity

Hypertension is a major risk for many disorders including coronary heart disease, stroke, heart failure, vision loss, chronic kidney disease, and dementia [28, 29]. Renin-angiotensin system (RAS) is responsible in blood pressure regulation. The liver-released angiotensin is converted to angiotensin-I (Ang-I) by renin, then the Ang-I is easily degraded to Ang-II, a potent vasoconstrictor, by angiotensin converting enzyme (ACE), leading to vasoconstriction and hypertension. Thus, hypertension can be controlled using renin and ACE inhibitors, in which the latter is more important due to its dual functions, catalyzing Ang-II (a potent vasoconstrictor) and inactivation of bradykinin (a vasodilator) [30]. However, controlling blood pressure by peptides from agricultural co-products also reported to be achieved through other mechanisms, such as nitric oxide production and blocking calcium channel and Ang-II receptor. The receptor blockers are able to hinder vasoconstriction and other functions mediated by Ang-II. Calcium channel blocker can avoid calcium availability in blood vessel cell wall and heart which make them to have a lower extent contraction, leading to relaxation and lower blood pressure [27, 31]. Arg-containing peptides have also been reported to suppress hypertension as Arg is a precursor in nitric oxide production. Nitric oxide (NO) is synthesized by the reaction of Arg and oxygen in the presence of nitric oxide synthase as a catalyst. NO is a vasodilator which act against Ang-II and a balanced level of these two compounds could lead to normal blood pressure [32]. Peptides namely, LIWKL, RPYL, RRWQWR, blocking the Ang-II receptor and HRW, a calcium channel blocker, have been reported to exert antihypertensive effects [33, 34].

The antihypertensive activity of peptides depends on several factors including amino acid composition and their position, molecular weight and charge. Normally, peptides with lower molecular weight exert higher activity due to their higher affinity to bind with ACE, in which the most potent reported peptides are di- and tri-peptide. Moreover, small peptides can stay intact through gastrointestinal digestion and epithelial transportation, reaching to blood circulation system and the target organ [31]. The structure–activity relationship of 168 dipeptides and 140 tripeptides with ACE inhibitory activity was assessed [35]. The authors reported that presence of bulky side chain and hydrophobic amino acids in dipeptides could result in higher activity, while presence of aromatic amino acids at C-terminus

and positively charged amino acids in the middle and hydrophobic residues at N-terminus brought them higher ACE inhibitory activity. The interaction of peptides with zinc ion in active site of ACE could effectively deactivate the enzymes. It has been reported that Leu could bind with Zn^{2+} by its carboxyl group and inhibit the enzyme activity [36].

Antihypertensive activity of hydrolysates/peptides from agricultural co-products was extensively explored by *in vitro* and *in vivo* studied, mostly based on their inhibition capacity against ACE and rat with hypertension (either SHR or hypertensive-induced rat), respectively (**Table 1**). However, clinical studies are still needed to confirm their health benefits. Clinical studies on the antihypertensive effect of IPP and VPP showed a controversial result as it reduced systolic and diastolic blood pressure in Asian case studies to a higher extent than Caucasians, while no effect was observed in Dutch and Danish cases [46, 47]. They reported that the difference could be associated to variations in genetics and dietary habit which should be taken into consideration. Kwak et al. [27] reported antihypertensive effect of black soy peptide in human trial, in which systolic blood pressure decreased in hypertensive subjects likely through reduction in ACE activity and increase in nitric oxide production. The authors concluded that the activity of black soy peptides might also be associated with higher arginine content which is a substrate for nitric oxide formation, known as a strong vasodilator.

Source	Enzyme	Hydrolysate/ Peptide	Activity		Ref.
			In vivo	*In vitro*	
Animal based co-products					
Bovine whey lactoferrin	Pepsin	Partially purified hydrolysate (<3 kDa) LIWKI, RPYL and RRWQWR		ACE inhibitory activity, Blocking Ang-II receptor	[33]
Fish (Cobia) skin	Protamex	Hydrolysate WAA, AWW, IWW, WL	Systolic and diastolic blood pressure reduction at 600 mg/kg: −21.9 and − 15.5 mmHg, respectively, after 4 h in SHR	ACE inhibitory activity ACE inhibitory activity IC_{50}[2]: 41, 4.3, 0.2 and 8.5, respectively	[37]
Chicken bone	Pepsin	YYRA	Systolic blood pressure reduction at 10 mg/kg in SHR: −2 mmHg after 6 h	ACE inhibitory activity IC_{50}: 33.9	[38]
Bovine bone gelatin	Alcalase	RGL-(Hyp)-GL and RGM-(Hyp)-GF	Systolic blood pressure reduction at 30 mg/kg: −31.3 and −38.6 mmHg for RGL and RGM after 4 and 6 h, respectively, in SHR	ACE inhibitory activity IC_{50}: 0.9 and 6.9, respectively	[39]
Bovine blood plasma	Flavourzyme	HPY		ACE inhibitory activity IC_{50}: 0.7	[40]

Source	Enzyme	Hydrolysate/ Peptide	Activity		Ref.
			In vivo	*In vitro*	
Poultry viscera	Autolysis	ARIYH, LRKGNLE and RVWCP		ACE inhibitory activity IC_{50}: 8.9, 8.9 and 4.9, respectively	[29]
Whey		IW	ACE activity reduction in human plasma by 32% at 50 mg administration		[41]
Plant based co-products					
Wheat bran	Alcalase	Hydrolysate, peptides <1 kDa including NL, QL, FL, HAL, AAVL, AKTVF, TPLTR	Systolic blood pressure reduction at 100 mg/kg in SHR[1]: −20 and − 35 mmHg after 6 h for hydrolysates and the peptides <1 kDa, respectively	Renin and ACE inhibition activity	[42]
Corn gluten meal	Trypsin	AY	Systolic blood pressure reduction at 50 mg/kg in SHR: −9.5 mmHg after 2 h	ACE inhibitory activity IC_{50}: 3.6	[28]
Cottonseed	Papain	FPAIGMK		ACE inhibitory activity IC_{50}: 46.7	[43]
Flaxseed protein isolate	Thermoase	Partially purified hydrolysate (3–5 kDa)	Systolic blood pressure reduction at 200 mg/kg in SHR: −37 mmHg after 8 h	Renin and ACE inhibitory activity	[44]
Red seaweed (*Porphyra columbina*) by-product	Trypsin and Alcalase	Hydrolysate		ACE inhibitory activity	[45]

[1]*Spontaneously hypertensive rat.*
[2]*IC_{50} based on μg/ml.*

Table 1.
Peptides/hydrolysates derived from agricultural co-products involved in antihypertension activity.

3.3 Antidiabetic activity

The diabetes is a chronic health problem which involved 463 million adults in 2019 and it is estimated to reach 700 million by 2045. In the health disorder, the elevated blood glucose cannot be treated properly due to either pancreas failure in insulin production (type-I) or insulin resistance in the body (type-II), in which the latter comprised the majority of about 90–95% [48].

The diabetes type-I treatment is associated to insulin injection, while the diabetes type-II can be prevented by controlling the pathways, by which the blood glucose elevates. The enzymes, α-amylase and α-glucosidase, play roles in carbohydrate digestion through breaking them down to oligosaccharides and subsequently

to glucose, which is easily absorbed from intestine to blood, leading to hyperglycemia. In addition, dipeptidyl peptidase IV (DPP-IV), a protease located on endothelial, epithelial and some other cells, could easily degrade hormones stimulating insulin secretion during food ingestion, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), and cause dysregulation of blood glucose. Therefore, many studies have attempted to find bioactive peptides to inhibit these enzymes, so that the diabetes type-II would be cured or prevented. Bioactive peptide with *in vitro* enzyme inhibitory and *in vivo* antidiabetic activities derived from agricultural co-products are summarized in **Table 2**.

Source	Enzyme	Hydrolysate/ Peptide	Activity		Ref.
			In vivo	*In vitro*	
Animal based co-products					
Chicken feet	Neutrase	Hydrolysate	Reduction of glycemia in glucose-intolerant rats at 300 mg/kg	DPP-IV inhibitory activity	[49]
Collagen from pig and cattle skin, fish scale and chicken feet	Collagenase	GA-Hyp GPA GP-Hyp		DPP-IV inhibitory activity IC_{50}: >20, 5.03 and 2.51 mM, respectively	[50]
Mare whey	Papain	NLEIILR TQMVDEEIM-EKFR		DPP-IV inhibitory activity IC_{50}: 86.3 and 69.84 μM, respectively	[51]
β-Lactoglobulin from bovine whey	Trypsin	VAGTWY	Reduction of glucose level in mice at 300 mg/kg	DPP-IV inhibitory activity IC_{50}: 210 μM	[52]
	Trypsin	IPAVF		DPP-IV inhibitory activity IC_{50}: 44.7 μM	[53]
Atlantic salmon skin	Flavourzyme	GPAE, GPGA		DPP-IV inhibitory activity IC_{50}: 49.6 and 41.9 μM, respectively	[54]
Cuttlefish viscera	Crude protease extracts from smooth hound and cuttlefish hepatopancreas	Hydrolysate		Stimulation cholecystokinin (CCK) and GLP-1 release in enteroendocrine STC-1 cells, DPP-IV inhibitory activity	[55]
Bovine haemoglobin	*in vitro* GI digestion by pepsin and pancreatin	VAAA KAAVT, YGAE, ANVST and TKAVEH		DPP-IV inhibitory activity, IC_{50}: 141 μM Stimulation of GLP-1 secretion in STC-1	[56]

Source	Enzyme	Hydrolysate/ Peptide	Activity		Ref.
			In vivo	*In vitro*	
Plant based co-products					
Wheat gluten	Debitrase HYW20	Pro-containing peptides		DPP-IV inhibitory activity	[57]
	Protease from ginger	QPQ, QPG, QPF, LPQ, SPQ		DPP-IV inhibitory activity IC_{50}: 79.8, 70.9, 71.7, 56.7, and 78.9 μM, respectively	[58]
Oat globulin	Trypsin	LQAFEPLR		DPP-IV inhibitory activity IC_{50}: 103.5 μM	[59]
Luffa cylindrica seed	Pepsin Trypsin Alcalsae	Hydrolysate		α-amylase and α-glucosidase inhibitory activity	[60]
Rice bran	UmamizymeG Alcalase	IP 13 peptides containing 6–32 amino acids		DPP-IV inhibitory activity IC_{50}: 0.41 mM α-amylase and β-glucosidase inhibitory activity	[61] [62]
Hemp seed meal	Alcalase	LR, PLMLP		α-glucosidase inhibitory activity	[63]

Table 2.
Examples of agricultural by-product peptides and hydrolysates exhibiting antidiabetic activity.

In a clinical study, Goudarzi and Madadlou [64] indicated that hydrolysate prepared from whey proteins stimulated insulin production, so that plasma glucose got back to normal level in postprandial hyperglycaemia cases, while the hydrolysate had no effect in prehypertensive cases. Although studies indicated that hydrolysates/peptides might stimulate secretions of hormones involving in insulin production [55, 56, 64], most studies have focused on major enzymes involving in carbohydrate digestion and DPP-IV. The structure–activity relation of peptides possessing the diabetes-involving-enzyme inhibition has not been completely understood yet. Nongonierma et al. [57] identified di- and tri-peptides inhibiting DPP-IV in wheat gluten hydrolysate. These peptides had some main characteristics including the presence of Pro at carboxyl terminus or penultimate position and Phe or Leu at amino terminus. Li-Chan et al. [54] described that peptides with DPP-IV inhibitory activity required presence of hydrophobic amino acids, particularly Pro, as Pro placed at 1–4 (preferably at second) positions from N-terminal end and bounded with Leu, Val, Phe, Ala and Gly. Dipeptides as X-Pro with X as a small size hydrophobic amino acid would likely be an effective inhibitor. Presence of hydrophobic and aromatic amino acids at N-terminal end of peptides with DPP-IV inhibitory activity was also reported by Lima et al. [65]. Ren et al. [63] evaluated the α-glucosidase inhibitory capacity of peptides from hemp seed and indicated that hydrophobicity of peptides was a prime factor affecting inhibitory activity and molecular weight as a second priority. The authors have also reported that larger molecular weight peptides could also enhance α-glucosidase activity. α-Amylase is another enzyme involving in carbohydrate digestion and it has been reported that presence of branched and aromatic amino acids such as Lys, Phe, Tyr and Trp and positively charged amino acid could help to inhibit the enzyme [60].

3.4 Antibacterial activity

The antibacterial activity of hydrolysates/peptides has been studied to a lesser extent when compared to other aforementioned properties. Hydrolysates/peptides with antibacterial activity have been obtained from co-products of milk, seafood, meat and others which are summarized in **Table 3**. Conventional antibiotics and preservatives are extensively applied to control pathogens, which lead to antibiotic-resistant strains. Therefore, an alternative antimicrobial agent has been sought. Peptides with antibacterial properties could be one of alternative agents as they are non-toxic and could act against both Gram-negative and Gram-positive as well as antibiotic-resistant bacteria [76]. Typically, chemical antibiotics have specific targets and bacteria can develop various defense strategies towards antibiotics. In contrast, antimicrobial peptides target cell membrane and can cause serious damage which make it difficult to develop resistance [77].

The antibacterial activity of these peptides is associated to their molecular weight, charge and hydrophobicity [67]. Peptides are attached by negatively-charged residues of cell membrane, like lipopolysaccharides and lipoteichonic acid on Gram-negative and Gram-positive bacteria, respectively, through electrostatic

Source	Peptide/Hydrolysate	Test bacteria	Activity		Ref.
Animal based by-products					
Rainbow trout Viscera	Hydrolysate prepared by pepsin	*Flavobacterium psychrophilum, Renibacterium salmoninarum*	MIC[1] (mg/ml)	2 5	[66]
Yellowfin tuna viscera	Partially purified (<3 kDa) hydrolysate prepared by Protamex	*Listeria. monocytogenes, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*	MIC (mg/ml)	0.5 0.5 0.5 0.5	[67]
Bovine hemoglobin	TSKYR obtained by pepsin-hydrolyzed hemoglobin	Total viable colonies Coliform bacteria Yeasts Molds	Reduce microbial counts in ground beef stored in refrigerator		[68]
Porcine blood proteins: albumin globulin	Hydrolysate prepared by Alcalase, Flavourzyme, Protamex, trypsin and papain	*Bacillus cereus*	IZ[2] (mm): 2.30–2.55		[69]
Camel whey	Crude hydrolysate prepared by trypsin/ partially purified (<3 kDa)	*E. coli* *S. aureus* *Salmonella typhimurium* *Streptococcus mutans*	MIC (mg/ml)	130/65 260/130 NI[3]/260 NI/260	[70]
Cow whey	Crude hydrolysate prepared by trypsin/ partially purified (<3 kDa)	*E. coli* *S. aureus* *S. typhimurium* *S. mutans*	MIC (mg/ml)	260/130 260/130 NI/260 NI/260	
Snow crab co-products	Hydrolysate prepared by Protamex	*E. coli* *L. innocua*	IZ (mm)	9 7.5	[71]

Source	Peptide/Hydrolysate	Test bacteria	Activity		Ref.
Plant-based co-products					
Palm kernel cake	Hydrolysate prepared by Alcalase	*Lisinibacillus sphaericus* *Bacillus thuringiensis* *B. cereus* *Clostridium perfringens* *B. subtilis*	MIC (μg/ml)	150 150 200 250 450	[72]
Jatropha curcas meal	CAILTHKR obtained by Protamex-hydrolyzed meal	*E. coli* *Shigella dysenteriae* *P. aeruginosa* *S. aureus* *B. subtilis* *S. pneumoniae*	MIC (μg/ml)	29 46 58 45 34 68	[73]
Rice bran	KVDHFPL obtained by bromelain-hydrolyzed bran	*Listeria monocytogenes* *L. monocytogenes* biofilm	MIC (μg/ml)	0.25 8	[74]
	LRRHASEGGHGPHW EKLLGKQDKGVIIRA SSFSKGVQRAAF obtained by pepsin-hydrolyzed bran	*Porphyromonas gingivalis* (PG), *Candida albicans* (CA)	IC_{50}: (PG, CA; μM)	289, ND[4] ND, 75.6 ND, 78.5	[75]

[1]*Minimum inhibitory concentration.*
[2]*Inhibition zone.*
[3]*No inhibition.*
[4]*Not detected.*

Table 3.
Antibacterial peptide/hydrolysate prepared from agricultural co-products.

interactions, by which the structure of cell surface was disrupted. Subsequently, peptides could permeate to the cell and reach to cytoplasmic membrane, causing leakage of cytoplasmic fluid [78].

Antibacterial activity of hydrolysates and/or peptides has mostly evaluated by their directly exposure to pathogens and less studies have conducted to assess their application in food. A peptide, TSKYR, obtained from bovine hemoglobin [68] and hydrolysate prepared from yellowfin tuna waste [67] were added to ground beef and minced fish, respectively. The peptide, TSKYR, could reduce total viable colonies, yeasts, molds and particularly coliform bacteria within 14 days storage in a refrigerator. Moreover, the peptide (0.5% w/w) in ground beef was able to diminish lipid oxidation by 60% which was reported to be comparable to BHT. Pezeshk et al. [67] reported that hydrolysates prepared from yellowfin tuna were able to increase fish (silver carp) mince shelf-life in a refrigerator through inhibition of psychrophilic and total count bacteria as well as prevention of oxidative degradation.

4. Bioavailability

Studies revealed that peptides prepared from agricultural co-products have great potential of health promoting properties. However, bioavailability of these peptides is a challenge, in which they need to stay intact within gastrointestinal tract (GIT) and epithelial transportation to reach their target organs and exert physiological

functions. Proteases and peptidases in GIT, brush border and cytoplasm are able to break down the peptide bond to a higher extent, leading to changes in structure and subsequently the activity. However, some peptides have been reported to be stable within the digestion and transportation. There are several factors affecting the peptides stability, which are associated to proteases specificities in GIT. Lower molecular weight, negatively charged, hydrophilic and acidic amino acid containing peptides are reported to be more stable against GI digestion. Negatively charged peptides from milk are more stable against GI digestion followed by positively charged and neutral peptides [79, 80]. Hydrophobic peptides reported to have less stability, which might be due to pepsin specificity towards hydrophobic amino acids [81]. Peptides containing more acidic amino acids, and also those with lower molecular weight showed more stability against GI digestion [6, 79]. Peptides with the molecular weight of larger than 3 kDa were easily digested by GI proteases, while peptides with <1 kDa mostly survived and no change in their antioxidant activity upon GI digestion [82]. Savoie et al. [83] reported that peptides from animal- (casein and cod fish) and plant- (soy and gluten) based substrates with Pro and Glu showed higher stability. Pro has a rigid ring structure bonded to β-carbon which makes it resistant against proteolytic degradation [80]. Thus, Pro containing peptides, IAGRP and PTPVP, have been reported to stay intact after in vitro GI digestion [84].

Epithelial permeation of bioactive peptides into blood circulation system is another challenge that affects physiological activities. Peptides may undergo some structural modification induced by brush border proteases (**Table 4**). For instance, a peptide with ACE inhibitory activity, KPLL, can be degraded to KP and LL within epithelial permeation, resulting in lower activity than the intact form [96]. The permeation could occur through four pathways, including peptide transporter 1 (PepT1), passive paracellular transportation through tight junctions, transcytosis and simple passive transcellular diffusion. Peptide properties such as size, hydrophobicity, charge and amino acid sequence are important factors affecting their absorption. Briefly, small (di- and tri-) peptides can be transported via PepT1 route, however peptide properties have effects on its efficacy. Non charged and hydrophobic peptides have higher affinity towards PepT1. Hydrophilic and negatively charged low molecular weight peptides can pass through energy-independence paracellular route. Transcytosis is an energy-dependent route, by which long chain peptides, particularly hydrophobic, can be transported. A highly hydrophobic peptide is likely transported through simple passive transcellular diffusion. To evaluate the effect of molecular weight on the permeation, Wang and Li [97] reported that hydrolysates with the molecular weight lower than 500 Da (mostly di- and tri-peptides) showed higher bioavailability and were able to pass through Caco2 cell via PepT1 route, while those with the molecular weight ranging 500–1000 and 1300–1600 Da permeated through paracellular route. Besides molecular weight, peptide sequence also affects its bioavailability. A Pro-containing peptide has more stability towards brush border proteases and peptides with Leu at N-terminus have been reported to be highly susceptible to hydrolysis [86, 90, 91].

Peptides structural changes usually occur in GI tract and transepithelial transportation that would likely have effects on their physiological functions. To meet the challenge, some approaches have been applied to improve the stability of these peptides such as using permeation enhancer, enzyme inhibitor and encapsulation. Sodium caprate has been used to improve the permeability of two antihypertensive peptides, IPP and LKP, through paracellular route in Caco2 cell [98]. The authors reported that sodium caprate could intensify the peptides absorption via paracellular mechanism and inhibited PepT1 route, leading to antihypertensive effect in SHRs model. An antihypertensive peptide, RLSFNP, would degrade to RLSF, SFNP, FNP and F during the epithelial transportation. Permeation enhancers including

Peptide	Bioactivity	Permeated peptides	Route of transport	Ref.
VLPVPQK	ACE inhibitor Antioxidant	VLPVPQK, VLPVPQ	PepT1/ SOPT2	[85]
LHLPLP	ACE inhibitor Antihypertensive	LHLPLP, HLPLP	Paracellular	[86]
IPP, LKP	ACE inhibitor	IPP, LKP	PepT1 and paracellular	[87]
IWHHT	ACE inhibitor Antihypertensive Antioxidant Anti-inflammatory	IWHHT, IWH, IW	Paracellular, PepT1 and paracellular, PepT1	[88]
YQEPVLGPVRGPFPIIV	Immunomodulatory	YQEPVLGPVRGPFPIIV, QEPVLGPVRGPFPIIV, YQEPVLGPVR-GPFPII	Transcytosis	[89]
LSW	ACE inhibitor Anti-inflammatory	LSW, SW	PepT1 and paracellular	[90]
RRWQWR	ACE inhibitory Antihypertensive	RWQ, WQ	Paracellular	[91]
WDHHAPQLR	Antioxidant	WDHHAPQLR, DHHAPQLR, WDHHAP, QLR	Paracellular PepT1	[92]
YWDHNNPQIR	Antioxidant	YWDHNNPQIR, WDHNNPQIR, DHNNPQIR and YWDHNNPQ	Transcytosis	[93]
YFCLT, GLLLPH	Antioxidant	YFCLT, GLLLPH	Paracellular and transcytosis	[94]
WGAPSL	Cholesterol- Lower	WGAPSL, WGAPS, WGAP, GAPSL, GAP, SL	Paracellular	[95]

Table 4.
Peptide modification within epithelial permeation and their transportation route across Caco-2 cell.

sodium glycocholate hydrate, sodium deoxycholate and Na_2EDTA as well as enzyme inhibitors, bacitracin and leupeptin, have been applied to improve the intact peptide bioavailability [99]. Na_2EDTA was the most effective to enhance RLSFNP absorption through enlarging intracellular junctions. They also reported that bacitracin could exert permeation enhancer activity beyond its protease inhibitory effect. Permeation enhancer is believed to cause damages in cell membrane in case of long-term usage, leading to inflammation. However, major destructive effects were not observed by using bacitracin in rat intestine [100]. Besides, encapsulation of RLSFNP by liposome could also facilitate the intact peptide transportation through transcytosis in Caco2 cell [101]. In addition, Li et al. [102] used nano-encapsulation of antidiabetic peptides made by chitosan coated liposome to maintain the stability of peptides.

5. Conclusions

Co-products are inevitably generated in food production, distribution, processing and consumption. These protein rich and low value materials could provide a

great source of bioactive peptides production. Many peptides with various activities have been purified and identified, however, their activities need to be confirmed via *in vivo* studies and human trials, so that they could be further develop to functional food products. The main obstacle of developing these peptides in functional foods is their structural modification after ingestion via proteolytic degradation in gastrointestinal tract and epithelial absorption, which would likely lead to a reduction in bioactivity. Although some peptides, particularly those containing Pro, could stay intact within digestion and absorption, structural modification usually happen in the route. Encapsulation of susceptible peptides or applying protease inhibitor as well as permeation enhancer in epithelial cells could facilitate the intact peptides absorption. Although these strategies might allow peptides to reach the target organ and exert certain physiological effect, their safety, particularly the use of protease inhibitors, needs further investigation regarding their side effects under physiological condition.

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Conflict of interest

The authors declare no conflict of interest.

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Chapter 10

The Potential of Lutein Extract of *Tagetes erecta* L. Flower as an Antioxidant and Enhancing Phagocytic Activity of Macrophage Cells

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Abstract

Marigold flower (*Tagetes erecta* L.) produces lutein compounds which present biological activities such as antioxidant, antiinflammatory, antimutagenicity, and immunomodulatory effects. The study was to investigate the antioxidant activity of the lutein of *T. erecta* L. and the effect of lutein on the activity and phagocytic capacity of macrophage cells. The antioxidant screening was carried out using diphenyl picrylhydrazyl (DPPH), 2,2′-and-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging assay with serial concentrations and ferric-reducing antioxidant power (FRAP) method. For the observation of activity and phagocytic capacity of peritoneal macrophages, twenty-eight mice were used and divided into seven groups each comprising four replicates, i.e., Group (I) normal controls, mice were untreated (II) a negative control, mice were induced by *Staphylococcus aureus* (III) positive control, mice were induced by *S. aureus* and treatment of meniran extract (*Phyllanthus niruri*). The treatment group (IV-VII) mice were induced by *S. aureus* and treated crude lutein, respectively: 0.15 mg, 0.30 mg, 0.60 mg, and 0.90 mg. 20 g^{-1} of body weight. The lutein extracted from *T. erecta* shows an antioxidant activity against DPPH radical with an IC50 value of 53.58 $\mu g.ml^{-1}$, while the antioxidant activity against ABTS has an IC50 value of 72.91 $\mu g.ml^{-1}$. The antioxidant activity test results by the FRAP method at each lutein concentrations of 10, 25, 50, and 75 ppm were obtained respectively of 33, 88, 185.5, and 288.5 µmol Fe^{2+}/g extract. The data were analyzed using one-way ANOVA and Duncan's multiple range test (DMRT) after. The phagocytic activity was 45.5%; 54.75%; 57.50% and 67.0%, respectively, while the phagocytic capacity values were 355; 519; 611 and 767 *S. aureus* bacterial cells per 50 macrophage cells. The lutein from marigolds (*T. erecta* L.) is capable of scavenging free radicals and reducing oxidants. Lutein can increase the activity and capacity of phagocytic of peritoneum macrophage cells in mice.

Keywords: *Tagetes erecta* L., lutein, antioxidant, peritoneal macrophages, phagocytic activity

1. Introduction

Marigold flower (*Tagetes erecta* L.) is an annual herbaceous plant commercialized worldwide as an ornamental plant and a natural source of pigment from its yellow/orange flowers. The flower is rich in carotenoids, the extract of which is used as a colorant in a wide variety of food products, including cake mixture, drinks, and ordinary drinks, cereals, chewing gum, dairy analogs, egg products, fats and oils, dairy products, processed fruit and fruit juices, and soups [1, 2]. Marigold flowers are classified as medicinal plants from the Compositae tribe. People empirically use it to treat asthma, bronchitis, fever, ulcers, burns, and swelling [3]. The pigment content in marigold flowers are classified as carotenoids, namely lutein pigments that are yellow to orange [4]. *T. erecta* L. is a major source of lutein for commercial use. In 2010, lutein occupied a $ 233 million share of the world carotenoid market [1, 2, 5]. Lutein is a primary pigment because this pigment is not produced synthetically. It is due to its production, which requires a long process. Lutein from *T. erecta* L. is a pure extract obtained from marigold oleoresin, extracted from the petals of marigold flowers with an organic solvent. After the saponification process, the final product contains the main component lutein and a fraction of zeaxanthin. Lutein (3R, 3'R, 6'R-βε-carotene-3,3′-diol) is a member of the pigment group known as xanthophylls and lacks provitamin A activity. Lutein generally coexists in nature with its stereoisomer zeaxanthin and the double bonds of the isoprene backbone can exist in the all-trans (**Figure 1**). Pure lutein typically appears as a yellow-orange crystalline, lipophilic, solid with the chemical name β,ε-carotene-3,3′-diol (C40H56O2) [3].

Lutein pigments are found in egg yolks, fruits, and vegetables, including tomatoes, carrots, pumpkin, corn, and various green plants [6]. Some research results reported that lutein is efficacious to protect the eyes from macular degeneration and epithelial cancer, and has antioxidant properties [7], and can increase the immune system in the body [8]. Unfortunately, lutein cannot be synthesized in the body; so, it depends on plants [9]. In plants, lutein can be in the form of free lutein such as in spinach, cabbage, and broccoli, or in the form of esters with the fatty acids in the following fruits and vegetables: mango, orange, papaya, red or green peppers, yellow corn etc. [10–12]. Lutein content in natural sources depends on the type, variety, level of maturity, part of the fruit, and also on processing processes such as heating, preservation, or storage [12]. Dried marigold flowers contain 0.1%–0.2% carotenoids with a composition of 80% as lutein ester [4].

Lutein is an antioxidant because it can reduce free radicals and reactive molecules that can inhibit cell damage [13]. Free radicals can be produced from the body's metabolism and also from outside the body, such as the smoke of a cigarette, environmental pollution, radiation, drugs, pesticides, and ultraviolet rays [14]. Free radicals can cause damage because they can react with cell components that are important for maintaining cell life, both structural components (for example, the molecules making up membranes) or functional components (for example, DNA enzymes) [15]. Antioxidants are chemical compounds that can neutralize free

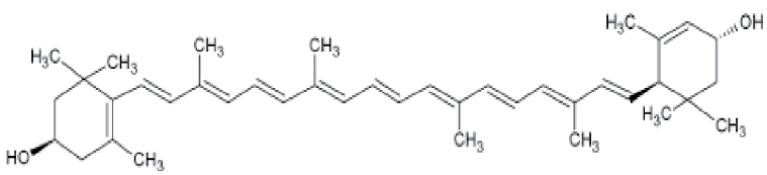

Figure 1.
Structural of all-trans lutein.

radicals by donating one or more electrons to free radicals; so, these free radicals can be suppressed [16]. Lutein is very effective as an antioxidant to protect the eyes because it is able to neutralize free radicals formed by the action of ultraviolet radiation on the retina of the eye and reduce the risk of cataracts due to aging [17]. Humans are unable to synthesize lutein, so they can obtain it by consuming fruits, vegetables, and dietary supplements [12]. Testing the antioxidant potential of lutein compounds from marigold flowers (*T. erecta* L.) can be carried out using DPPH, ABTS, and FRAP methods.

The lutein pigment in marigold flowers (*T. erecta* L.) can act as an immunomodulator, especially to protect the eyes from pathogenic elements such as viruses, bacteria, and infectious diseases intra-ocular inflammation [18]. Humans have resistance to a disease/infection from microorganisms or foreign substances, which is called the immune system. The body's immune system's decreased function can be caused by stress, an unhealthy lifestyle, aging, and chronic disease. The human body's immune system consists of specific and non-specific immune systems. One of the defenses made by the body's non-specific immune system in preventing the entry of antigens is by doing phagocytosis. Phagocytosis is the process of ingesting, digesting, and destroying antigens/microbes. The cells that play their role in carrying out phagocytosis are macrophage cells [19].

Previous research conducted *in vivo* showed that lutein derived from plants with a yellow pigment had an immunomodulatory activity increasing the regeneration of the immune system [8]. Immunomodulators are substances that can restore and repair the immune system, which function is disturbed or to suppress its excessive function [19]. In previous studies, researcher found that lutein activity was able to stimulate an immune response. In addition, it was found that 10 mg of lutein consumed daily by cats for 12 weeks led to an increase in the percentage of CD4+ and CD21+ lymphocytes, plasma IgG concentrations, and NK cell activation. It showed that lutein could stimulate mediator cells and humoral immunity in cats, which was done *in vivo*; so, stimulating the activity and capacity of macrophage cells in the body as an immune response [8]. Testing of immunomodulatory activity was carried out using observing the activity and capacity of macrophage cells from test animals (mice) induced by *S. aureus* bacteria [20].

This research has two objectives. First, this study examined the potential of lutein compounds from marigold flowers (*T. erecta* L.) as antioxidants using the DPPH, ABTS, and FRAP methods. Second, this study examined the possibility of lutein extract as an immunomodulator through a non-specific immune system, namely the activity and capacity of phagocytosis of mice's peritoneal macrophage cells (*in vivo*) induced by *S. aureus*.

2. Experiment

2.1 Material collection

Marigold flower (*T. erecta* L.) belongs to the Asteraceae family obtained from Taman Bunga Nusantara, Cipanas - West Java. Plant determination was carried out at Herbarium Bogorienses, Research Center for Biology, Indonesian Institute of Sciences (LIPI).

2.2 Lutein extract from marigold flowers (*T. erecta* L.)

Dry powder of Marigold flower crown was weighed as much as 20 g into Erlenmeyer to extract of lutein [21, 22]. The weighing was carried out six times.

Figure 2.
Extraction of lutein from the crown of marigold flowers (T. erecta L.).

Each Erlenmeyer was added with 300 ml of n-hexane (Merck) and was shaken out for 24 h. The extracted liquid was filtered with filter paper to separate the filtrate and residue. The residue was remastered by adding 200 ml of n-hexane and was shaken out for 24 h, then was filtered, and the filtrate was combined.

The filtrate was concentrated with a rotary evaporator. The extract was obtained, and it was dried at 40°C until hexane extract was obtained. The n-hexane extract was digested by adding isopropanol solution, then was stirred for one hour at 50°C. At the same temperature, it was saponified with 50% NaOH solution, stirred homogeneously for one hour until two layers were formed, i.e., semisolid and liquid. Next semisolid and liquid were cooled, the semisolid part is separated from the liquid. The semisolid solution was digested with distilled water repeatedly until the other carotenoids were completely separated. The solution was centrifuged for ten minutes at a speed of 3000 rpm; yellow deposits were formed, then the filtrate was removed. The precipitate was dried in a water bath at 40°C; so, the lutein extract was obtained (**Figure 2**).

2.3 Antioxidant activity of lutein extract from *T. erecta* L.

2.3.1 DPPH free radical-scavenging activity

The ability of Lutein extract of *T. erecta* L. to scavenge the DPPH radical was estimated using the method described by Kushwaha and Verma [23]. An aliquot of 50 μL of various sample concentrations was added to a volume of 2 mL from the DPPH methanolic solution (60 μM). The reaction mixture was well shaken and incubated for 20 min at room temperature in the dark and the absorbance was recorded at 517 nm. The blank was constituted by methanol instead of the extract. The percentage inhibition of the DPPH radical by the samples was calculated using the following equation:

$$\%\, inhibition = \frac{Ao - As}{Ao} x100. \quad (1)$$

where A_0 is the absorbance of blank and As is the absorbance of the lutein sample. The sample concentration providing 50% of inhibition (IC50) was determined from the plotted curve of inhibition using several concentrations. Vitamin E were used as a comparative antioxidant compound.

2.3.2 ABTS [2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)] free radical scavenging activity assay

The ABTS● + stock solution was prepared by reacting ABTS (Sigma Aldrich aqueous solution 7 mM) with 2.45 mM aqueous solution of potassium persulfate (Merck) in equal quantities; the mixture was allowed to stand in the dark at room temperature for 12–16 hours before use [24–25]. The working solution of

ABTS● + was obtained by diluting the stock solution in methanol to give an absorbance of 0.70 ± 0.02 at 734 nm. Then, 1.0 mL of ABTS● + solution was mixed with 0.5 mL of the Lutein extracts at different concentrations (25–100 ppm). The mixture was then incubated at room temperature for exactly 10 min in the dark. The control was prepared by mixing 1.0 mL of ABTS● + solution with 0.5 mL of distilled water. The absorbance was measured against a blank at 734 nm using spectrophotometer (Shimadzu UV-160). The percentage of scavenging activity of each extract on ABTS● + was calculated as % inhibition (I%) using the following equation:

$$\% \, Inhibition = \left[\frac{(Ao - As)}{Ao} \right] x\, 100 \qquad (2)$$

Where Ao is the absorption of blank and As is the absorption of the lutein extract solution.

2.3.3 Ferric ion reducing antioxidant power (FRAP) assay

The ferric reducing capacity of extracts was investigated by using the potassium ferricyanide-ferric chloride method [26]. Briefly, Reagents for FRAP assay a) Acetate buffer 300 mM pH 3.6: b) TPTZ (2, 4, 6-tripyridyl-s- triazine): (M.W. 312.34), 10 mM in 40 mM HCl (M.W. 36.46). c) FeCl3. 6 H2O: (M.W. 270.30), 20 mM. The working FRAP reagent was prepared by mixing a, b and c in the ratio of 10:1:1 just before testing. All the reagents were prepared from Merck (Germany) company. FRAP solution (3.6 mL) add to distilled water (0.4 mL) and incubated at 37°C for 5 min. Then this solution was mixed with certain concentration of the plant extract (80 mL) and incubated at 37°C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For construction of the calibration curve, five concentrations of FeSO4, 7H2O (25, 50, 75, 100, 150, 200, 300 μmol) were used and the absorbance values were measured as for sample solutions. The sample concentration providing 0.5 of absorbance (IC50) was calculated by plotting absorbance against the corresponding sample concentration [27, 28]. Vitamin E were used as a comparative antioxidant compound.

2.4 Measurement of *in vivo* phagocytosis activities and capacity of macrophage

Activity and capacity were observed under a 10x100 magnificent light microscope [29]. Animal experimental was performed in the Animal laboratory of the Faculty of Veterinary Medicine, IPB University- Dramaga Bogor. The experimental animal used was male mice (*Mus musculus*) strain DDY. A total of 28 mice were used in 6–8 weeks old, with 18–21 g of body weight. The mice were acclimated for seven days ahead. The mice were randomly divided into seven experimental groups of four mice in each group: (I) Normal control (mice were given vegetable oil), (II) Negative control (mice were given vegetable oil + *S aureus*, (III) Positive control (mice were given immunomodulator stimulator 0.078 mg + *S. aureus*), (IV) Lutein extract dose 0.15 mg/20 g BW + *S. aureus*, (V) Lutein extract dose of 0.30 mg/20 g BW + *S. aureus*, (VI) Lutein extract dose of 0.60 mg/20 g BW + *S. aureus*, (VII) Lutein extract dose 0.90 mg/20 g BW + *S. aureus*. Lutein extract was administered orally for 14 days. On the 15th day, all experimental animals were injected intraperitoneally with 0.5 ml of *S. aureus* bacterial suspension (1 x 10^6 CFU/ml), except the mice in normal control group. Those experimental animals were euthanatized one hour after infection, and then their peritoneal liquid was collected. Swab preparation was made for all samples, and then fixated using methanol for 5 minutes, stained with Giemsa and left for 20 minutes, and then washed using distilled water.

2.4.1 Phagocytosis activity

Phagocytosis activity value is the number of macrophage cells that actively phagocyte *S. aureus* in 100 macrophage cells. Phagocytosis activity was presented in percent, with the formulation [30] as the following:

$$\%\ Activity = \frac{The\ number\ of\ active\ macrophage}{The\ number\ of\ whole\ macrophage} x\,100 \qquad (3)$$

2.4.2 Phagocytosis capacity

Phagocytosis capacity value is the number of bacteria ingested by 50 active macrophages [30]. This parameter was observed by staining with Giemsa and then the bacteria number was counted under microscope. Phagocytosis is a devouring process on bacteria or strange objects by enfolding those things using macrophage cytoplasm.

2.5 Data analysis

Determining of immunomodulatory activity of crude lutein from *T. erecta* L. was through macrophage activity and capacity induced by *Staphylococcus aureus* bacteria by *in vivo*. Data were analyzed by one way ANOVA with 4 replications using SPSS ver 22.0 with P = 0.05. This analysis was then followed by Duncan Multiple Range Test.

3. Results

3.1 Antioxidant activity of lutein extract from *T. erecta* L.

Antioxidant activity is a complex procedure usually happening through several mechanisms and is influenced by many factors, which cannot be fully described with one method. Therefore, it is essential to perform more than one type of antioxidant capacity measurement to consider the various mechanisms.

3.1.1 DPPH free radical-scavenging activity and ABTS radical cation scavenging activity assay

The potential test of lutein extract as an antioxidant using the DPPH method has some advantages, such as it is easy to conduct, and the process is relatively fast. The test method using the DPPH was based on a decrease in absorbance caused by a change in the DPPH solution's color. DPPH will react with hydrogen atoms from free radical absorbing compounds to form DPPH Hydrazine, which is more stable. DPPH reagent that reacts with antioxidants will change color from purple to yellow; the intensity of the color formed depends on the antioxidants' ability [31]. The data processing technique was carried out by comparing the concentration with the percentage value of antioxidant activity for each sample in a regression graph (**Figure 3**).

The results of the marigold flower lutein extract antioxidant test using the DPPH and ABTS methods can be seen in **Table 1**.

IC50 is a number that shows a concentration (μg/ml) which can inhibit the oxidation process by 50%. The smaller the IC50 value, the higher the antioxidant activity is. Based on the IC50 value, if it is less than 50 ppm, the antioxidant is very strong, 50 ppm–100 ppm is strong, 100 ppm–150 ppm is moderate, and 150 ppm–200 ppm is weak [31].

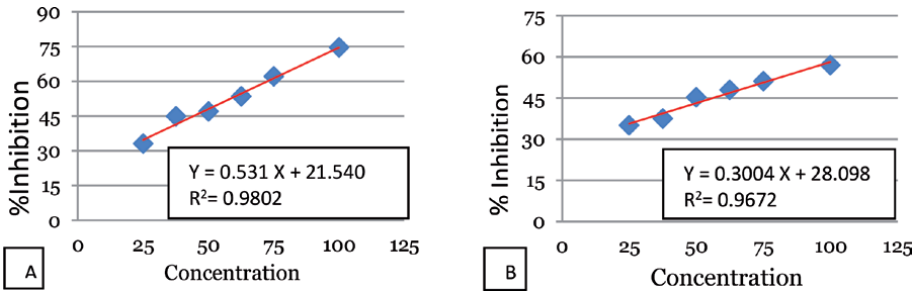

Figure 3.
Relationship between the concentration of lutein extract from Marigold flower (T. erecta L.) (ppm) with % inhibition of (A) DPPH, and (B) ABTS.

Lutein Conc. (ppm)	DPPH		ABTS	
	% Inhibition	IC 50(μg/ml)	% Inhibition	IC 50(μg/ml)
25	33.15	53.58	35.07	72.91
37.5	44.88		37.50	
50	46.96		45.28	
62.5	53.44		47.88	
75	62.07		51.10	
100	74.64		56.92	

Table 1.
Antioxidant activity values of marigold flower (T. erecta L.) using different assays.

Vit E Conc. (ppm)	DPPH		ABTS	
	% Inhibition	IC 50(μg/ml)	% Inhibition	IC 50(μg/ml)
4	34.97	9.60	34.43	12.36
6	40.47		37.81	
8	45.06		42.30	
10	51.14		44.73	
12	56.36		49.76	

Table 2.
Antioxidant activity values of vitamin E using different assays.

The results of testing for antioxidant potential with the DPPH suppression method showed that the lutein extract of flowers (*T. erecta* L.) had strong antioxidant activity with an IC50 value of 53.58 μg/ml (**Table 1**). The IC50 value of Vitamin E, i.e. 9.60, is categorized as a very strong antioxidant (**Table 2**). The antioxidant activity was measured based on the reduction in the purple DPPH intensity which was proportional to the reduction in the concentration of DPPH solution. The color change from purple to reddish purple then to yellow occurred after 30 minutes of giving lutein extract into the DPPH solution. The color change was caused by the reaction between diphenyl picryl hydrazine and the hydrogen atom released by one molecule of the lutein extract component; then diphenyl picryl hydrazine compound was [32].

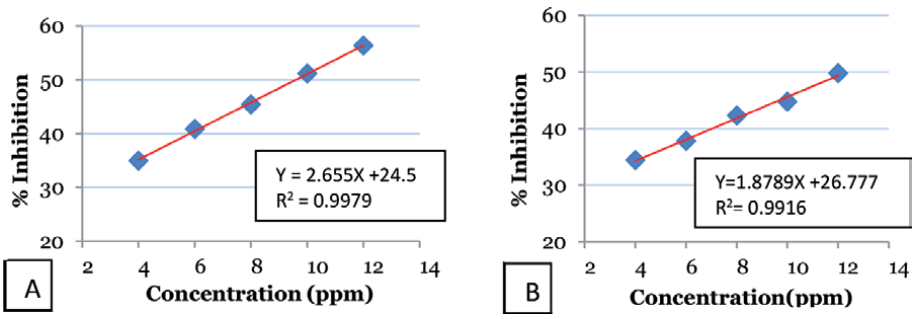

Figure 4.
Relationship between the concentration of vitamin E (ppm) with % inhibition of (A) DPPH and (B) ABTS.

Antioxidant activity testing could be done using the compound 2.2-Azinobic Acid (3-ethylbenzatiazolin-6 sulfonate). ABTS was a water-soluble and chemically-stable compound. This method was used for research on antioxidants that are water soluble, fat soluble, and dissolve in pure compounds. ABTS compounds were converted into radical cations (ABTS ●+) with the addition of potassium per sulfate. Free radical cations were blue green which could absorb light at a wavelength of 734 nm [33].

Maximum absorbance could also occur at other wavelengths. Radical cations were reactive to most antioxidants. ABTS which was blue-green during the reaction turned colorless which was analyzed using a spectrophotometer. From measurements using a UV–VIS spectrophotometer, the absorption value was obtained, and the % resistance was calculated. Furthermore, the relationship between concentration and % inhibition of marigold flower lutein extract (*T. erecta* L.) or vitamin E was plotted and the linear regression was calculated. The results can be seen in **Figures 3** and **4**. Furthermore, from the Eq. 50% activity was plotted, so that the concentration value (IC50) of the marigold flower lutein extract (*T. erecta* L.) was obtained, i.e. 72.91 µg/ml which was included in the strong antioxidant category because it had an IC50 value of around 50–100 µg/ml. The IC50 vitamin E yield of 12.36 µg/ml was in the very strong category. The use of the two DPPH and ABTS methods for testing the antioxidants of lutein and vitamin E extracts showed that there were differences in the IC50 obtained. Thus, the use of the DPPH method was more suitable and showed more sensitive results.

3.1.2 Ferric ion reducing antioxidant power (FRAP) of lutein extracts and vitamin E assay

The FRAP assay is based on the measurement of the ability of the substance to reduce Fe3+ to Fe2+ resulting in the change of color from yellow to blue colored solution of Fe2 + – TPTZ complex (Fe2+ tripyridyltriazine) which has a high absorbance at 593 nm. The FRAP assay provides a reliable method.

FRAP value was obtained by comparing the changes of absorbance values in the sample mixture with those obtained from the increased concentrations of Fe3+. FRAP values were expressed as µmol of Fe2+ equivalents per g sample. **Figure 5** shows the relationship between Fe (II) sulfate concentration and absorption at the wavelength 593 nm. **Table 3** FRAP values of lutein extract of marigold flower and Vitamin E in several concentration. The FRAP value is directly proportional to the concentration of sample. The higher the sample concentration exhibited the higher the FRAP value.

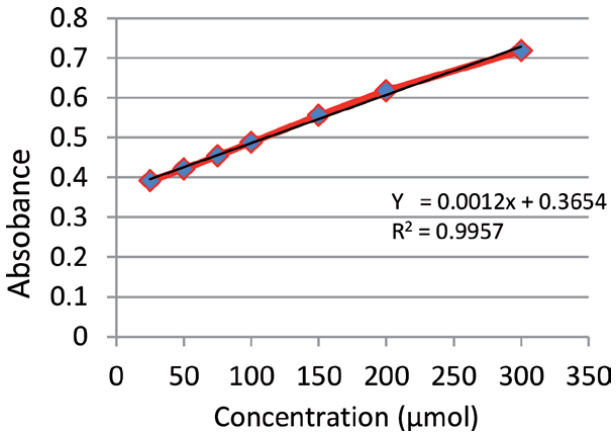

Figure 5.
Standard curve of FeSO4.7H2O.

No	Sample	Concentration of Sample (ppm)	Conc. of Fe(II) (µmol)
1	Lutein Extract of *T. erecta* L.	10	33.0
		25	88.0
		50	185.5
		75	288.5
2	Vitamin E	4	12.16
		6	18.83
		8	25.50
		10	33.83

Table 3.
Antioxidant activity of lutein extracts from marigold flower (T. erecta L.), using FRAP assay.

Generally, the reducing properties are associated with the presence of compounds, which exert their action by breaking the free radical chain through donating a hydrogen atom [34, 35].

3.2 Activity and capacity of phagocytosis of macrophage cells in mice peritoneum liquids

The results of the immunomodulatory activity test upon lutein extract of kenikir flower (*T. erecta* L.) based on the value of phagocytic activity and the phagocytic capacity of mice peritoneal fluid macrophage cells can be seen in **Figure 6** and **Table 4**.

Remarks:

I. Group normal control, mice were given vegetable oil.

II. Group negative control, mice were given vegetable oil+0.5 ml *S. aureus.*

III. Group positive control, mice were given stimuno®0.078 mg/20 g bw + 0.5 ml *S. aureus*.

IV. Group mice were given the extract lutein 0.15 mg/20 g bw/day +0.5 mL *S. aureus.*

V. Group mice were given the extract lutein 0.30 mg/20 g bw/day +0.5 mL *S. aureus.*

VI. Group mice were given the extract lutein 0.60 mg/20 g bw/day +0.5 mL *S. aureus.*

VII. Group mice were given the extract lutein 0.90 mg/20 g bw/day+0.5 mL *S. aureus.*

The results of the Duncan Multiple Range Test on the value of phagocytosis activity for each test group showed that there was no significant difference between the negative control group and the 0.15 mg/20gBW lutein extract treatment group. Treatment of lutein extract 0.30 mg/20gBW, was not significantly different from treatment of lutein extract 0.60 mg/20gBW, while lutein treatment 0.90 mg/20gBW showed a significant difference. The normal control group and the positive control group showed significantly-different phagocytic activity. The lowest phagocytosis activity was found in the normal control group because in the normal control group mice were not given intra-peritoneal *S. aureus* suspension; so, macrophages were not triggered to perform phagocytosis. Macrophages are activated by various stimuli originating from exogenous antigens and microorganisms.

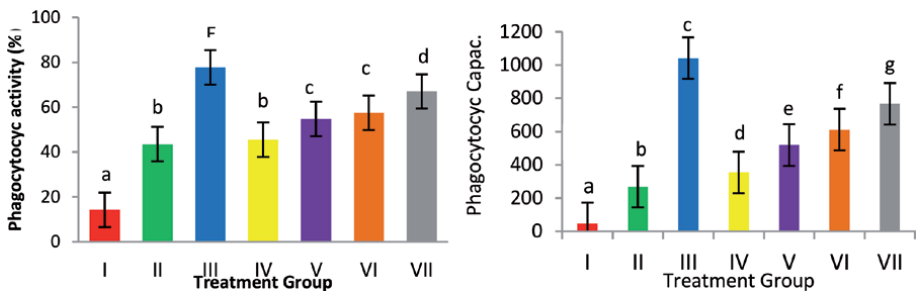

Figure 6.
Histogram of activity and capacity of phagocytosis macrophage cells in mice peritoneum liquids on each test group.

No.	Test Group	Phagocytosis Activity (%)	Phagocytosis Capacity *S. aureus* cells/ 50 macrophage cells
I	Normal Control	$14.25^{a} \pm 2.22$	$46.50^{a} \pm 6.61$
II	Negative Control	$43.50^{b} \pm 2.65$	$268.00^{b} \pm 5.09$
III	Positive Control	$77.75^{e} \pm 4.11$	$1041.50^{c} \pm 15.06$
IV	Lutein Extract 0.15 mg/20 g bw	$45.50^{b} \pm 1.29$	$354.75^{d} \pm 3.59$
V	Lutein Extract 0.30 mg/20 g bw	$54.75^{c} \pm 4.35$	$519.25^{e} \pm 5.12$
VI	Lutein Extract 0.60 mg/20 g bw	$57.50^{c} \pm 2.65$	$611.25^{f} \pm 4.57$
VII	Lutein Extract 0.90 mg/20 g bw	$67.00^{d} \pm 2.58$	$766.75^{g} \pm 6.50$

Note: The numbers followed by the same letter are not significantly different ($P < 0.05$).

Table 4.
Phagocytosis activity of macrophage cell (per 100 cells) and phagocytosis capacity (the number of bacteria devoured per 50 active macrophage cells).

Both will be recognized, then captured, eaten, and digested (phagocytes), and finally degraded and disappeared from the body. Peritoneal macrophages are freely available in the peritoneal fluid, so that pathogens entering the body [19] are possible to be captured. The highest value of phagocytosis activity was found in the immunosuppressive positive control group and in the treatment group lutein extract 0.90 mg/20gBW. Oral administration of lutein with different doses, namely 0.15 mg, 0.30, 0.60, and 0.90 mg per 20 g BW, resulted an increase in phagocytic activity, respectively 45.50%, 54.75%, 57.50%, and 67.0%. The increase in phagocytic activity of peritoneal fluid macrophage cells of mice occurred simultaneously with the increase in lutein dose.

The results of Duncan's test on the value of phagocytosis capacity in each of the 50 active macrophage cells showed that there were significant differences between groups. The lowest macrophage cell phagocytosis capacity was found in the normal control group. It was due to the fact that in a healthy body condition, macrophages became inactive; so, they did not show any activity and capacity to carry out phagocytosis. The highest value of macrophage phagocytosis capacity was found in the normal control group. The lutein extract treatment group with the highest phagocytosis capacity value was found in the lutein treatment group 0.90 mg/20gBW. The administration of lutein extract for 14 days to experimental animals, at a dose of 0.15 mg/20gBW, 0.30 mg/20gBW, 0.60 mg/20gBW and 0.90 mg/20gBW, was able to increase the phagocytosis capacity of every 50 peritoneum macrophage cells, respectively 355; 519; 611 and 767 of *S. aureus* cells.

Phagocytosis is an important process for nutrition in unicellular organisms, while in multicellular organisms it is found in specialized cells called phagocytes. Phagocytes can ingest microbial pathogens, but importantly also apoptotic cells. Phagocytosis becomes essential not only for microbial elimination, but also for tissue homeostasis [36]. Macrophages are one of the cells that play an important role in the phagocytosis process or as antigen presenting cells (APC) [19]. The phagocytosis ability of macrophage cells is a form of the body's immune system response to overcome the incoming antigen. Bacteria *S. aureus* will stimulate macrophages to produce IL-6 which will activate NK cells and then will secrete IFN-β which will activate macrophages. Macrophages will also be stimulated to produce TNF-α which will activate other macrophages. Experiments of administering lutein extract for 14 days resulted the increased activity and capacity of mice peritoneal fluid macrophage macrophages. It showed that lutein could boost the body's immune system or could act as an immunomodulator. The lutein mechanism could increase macrophage phagocytosis activity, namely by inducing ROS (Reactive Oxygen Species), intracellular and iNOS (inducible nitric oxide synthase), activating MAPK (mitogen activated protein kinase), and RAR β (retinoic acid receptor beta) in macrophage cells that could increase the phagocytosis ability of macrophages [37].

4. Conclusions

The work confirms that lutein extract from marigold flower (*T. erecta* L.) had strong an antioxidant potential. The three protocols used i.e. DPPH, ABTS and FRAP provide a good selection method to use for antioxidant measurements. Determination of antioxidant lutein extract will use to developed new drugs in pharmaceutical fields. The results of *in vivo* experiments showed that Lutein extract was able to increase the activity and phagocytic capacity of *S. aureus*-induced rat peritoneal macrophages. Phagocytic processes in immune cells contribute in particular to homeostasis and defense against disease.

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Conflict of interest

The authors declare no conflict of interest

Animal rights

The institutional and international ethical guidelines for the Care and Use of Laboratory Animals were followed. See the experimental parts for details.

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Chapter 11

Utilization of Starch in Food and Allied Industries in Africa: Challenges and Prospects

Akeem Olayemi Raji

Abstract

The shortage of food supply has affected the food situation in most developing tropical countries, resulting into a high incidence of hunger and malnutrition. This has also affected the attainment of self sufficiency in starch production for food, pharmaceutical and industrial usage. The review critically appraised the challenges that food and allied industries are facing on the utilization of starch as their major raw material. Information on various conventional and non conventional starch sources were provided, starch forms, properties and recent advances in starch modification methods were discussed. Starch applications in food and allied industries were mentioned and various challenges facing common starch sources were stated. Possibly, utilization of unconventional lesser known crops as starch sources might broadening the present narrow commonly cultivated starch sources, while value addition and good agricultural practices might improve the productivity of conventional starch sources.

Keywords: starch, sources, utilization, challenges, prospects

1. Introduction

Starch or amylum can be defined as a polysaccharide carbohydrate consisting of a large number of glucose units joined together by glycosidic bonds [1]. It comprises of two main components which are mainly linear amylose and highly branched amylopectin. It exists as a stored discrete semi crystalline granules in higher plants [2]. Starch is an important energy source for humans, produced by all green plant as an energy store [2]. Its production in the chloroplast occurs in the daylight and it was rapidly produced by plants. However, glucose chain produced biochemically by photosynthesis in plant cells is responsible for the synthesis of starch [3].

Amylose contains 1–4 D-glucopyranosyl units and it constitutes about 15–30% of common starch, while amylopectin possesses a large number of short chains linked together at their reducing end side by 1–6 glucosidic linkage [4, 5]. However, the formation of crystalline lamella in starch is linked to amylopectin and their branching points are part of the amorphous nature [6]. Starch is regarded as a semi crystalline entity because of the presence of amorphous and crystalline regions in starch granules [7]. Starch also contains some minor components such as lipids and proteins aside amylase and amylopectin. The sizes of starch granules range from 1 to 100 mm, while its shape and composition depends on their botanical source [8].

Starch is derived from a range of raw materials such as corn, wheat, pea, potato, and cassava roots [9] and it has a wide range of applications beyond the food industry. It is also used in the paper/board sector for wet-end addition, size press, surface coating and in the production of recycled paper. It is also used as a binding agent in the pharmaceuticals sector, as an adhesive in industrial binding sector and as a stiffener in the textile sector [10]. Other non food applications of starch include utilization as alcohol-based fuel, low-calorie substitutes, biodegradable packaging materials, thin films and thermoplastic materials with improved thermal and mechanical properties [5].

Starch is the basis of our food and industrial economy, but the food situation in most developing tropical countries is alarmingly worsening owing to increasing population and shortage of fertile land [11]. The shortage of food supply has resulted into a high incidence of hunger and malnutrition [12, 13]. It also affected the demand for starch as food, pharmaceutical and industrial uses coupled with the need to attain self-sufficiency in starch production. However, the focus of this review is to critically appraise the challenges that food and allied industries are facing on the utilization of starch as their major raw materials and to suggest possible way outs.

2. Sources of starch

Starch is a source of carbon and energy, it is predominantly found in different part of a plant such as stem, roots, flowers, seeds, and fruits [3]. Starch can be derived from tubers and cereals, such as cassava, potatoes, maize, yam, rice, etc. [14]. **Table 1** shows the starch compositions of some crops. The starch contents of cereals and tubers presented in **Table 1** ranged from 20.48–77.90%, with soft wheat having the highest value and bitter yam having the lowest value.

2.1 Cassava

Cassava (*Manihot esculenta* Crantz) is a tropical valuable root crop, used both as food and industrial raw material, due to the high starch content in tubers [22, 23]. It is one of the world's most important food crops, with annual global production at approximately 276 million metric tons in 2013 [24]. Its price is often decided by the industrialists based on the percentage of starch in the tubers. In 2013, the top producing countries globally were: Nigeria (accounting for 19% of the total), Thailand (11%), Indonesia (9%), Brazil (8%) and Democratic Republic of Congo (6%) [24]. It is a source of livelihood for at least 300 million people. Nearly 90% of cassava produced in Africa is used as a staple food for human consumption, which provides calories for 500 million people and it constitutes 37% of the population's dietary energy requirements [24]. Cassava is perceived in most African countries both as a food security crop and also as a raw material for various types of industries. It can be transformed into several types of products ranging from traditional foods and feeds to novel food products [24, 25].

2.2 Potatoes

Potato is one of the top four staple food crops in the world. Majority of its production is meant for human consumption (50–60%), while the rest are used for production of animal feeds, industrial products and as seed tubers [26, 27]. The tuber is a carbohydrate reserve and it also contains high quality protein, substantial amounts of vitamins, minerals and trace elements. Potatoes grow best in moderate

Food Plants	Starch Contents (%)	Sources
Cereals		
Waxy rice	74.76	Moongngarm [15]
Hard wheat	77.40	Ragaee *et al*. [16]
Soft wheat	77.90	Ragaee *et al*. [16]
Barley	53.60	Ragaee *et al*. [16]
Millet	67.40	Ragaee *et al*. [16]
Rye	58.00	Ragaee *et al*. [16]
Sorghum	67.70	Ragaee *et al*. [16]
Fonio	68.00	Cruz *et al.* [17]
Sweet Corn	36.23	Moongngarm [15]
Roots/ Tubers		
Sweet potato	52.54	Moongngarm [15]
Lesser yam	54.70	Moongngarm [15]
White yam	58.02	Moongngarm [15]
Water Yam	31.90	Brunnschweiler *et al*. [18]
Bitter yam	20.48	Ezeocha and Okafor [19]
Potato yam	38.10	Shajeela *et al.* [20]
Yellow yam	41.72	Oladebeye *et al.* [21]
Taro	63.74	Moongngarm [15]
Cassava	65.71	Moongngarm [15]

Table 1.
Starch compositions of cereals and tubers.

climate, where continuous cultivation throughout the year is impossible [26]. Among the potato varieties, sweet potato (*Ipomoea batatas* L (Lam)) is regarded as one of the most economically important species, as it can grow in great abundance on marginal soils [27]. It is rich in starch (58–76% on a dry basis) and the starch is widely used in starch noodles, bakery foods, snack foods and confectionary products [27].

2.3 Yams

Yams belong to the family *Dioscoreaceae* and they are annual or perennial tuber-bearing and climbing plants. It is an important major food crop in Nigeria [28] and ranked as the fourth major root crop in the world after cassava, potatoes and sweet potatoes, having an annual production of above 28 million metric tonnes [29]. However, Nigeria was rated as the world's largest producer of yams, with *Dioscorea rotundata* and *Dioscorea alata* as the two most cultivated yam species [30]. Yam can be explored for commercial starch production because of their high starch content of about 70 to 80% dry weight [31], and it plays a prominent role in ensuring food and livelihood security of at least 60 million people in West Africa. Globally, roughly about 57 million tons of yams (representing 93% of annual global production) are produced on 4.7 million hectares annually in this sub-region which comprise of Benin, Côte d'Ivoire, Ghana, Nigeria, and Togo [24, 32]. The yam species that are majorly grown globally include: *Dioscorea alata* (water yam), *Dioscorea bulbifera* (potato yam), *Dioscorea cayenensis* (yellow yam), *Dioscorea dumetorum*

(bitter yam), *Dioscorea esculenta* (lesser yam), *Dioscorea opposita* (Chinese yam), *Dioscorea rotundata* (white yam), and *Dioscorea trifida* (cush-cush yam) [33]. Among the species mentioned above, *Dioscorea rotundata* and *Dioscorea cayenensis* are the most commonly grown for consumption and commercial production. Yam contains mainly carbohydrates with little amount of proteins, lipids and vitamins, and it can provide around 110 calories per 100 grams of products [34]. Yam possesses high in moisture, dry matter, starch, dietary fiber, vitamin B_6, but low in saturated fat, sodium and vitamin A contents. Yams contain about 5–10 mg/100 g of vitamin C, and the limiting amino acids are isoleucine and sulfur containing amino acids. They also contain a compound called "diosgenin", which can be extracted and used as base for drugs such as cortisone and hormonal drugs. Some species contain alkaloids (e.g. dioscorine $C_{13}H_{19}O_2N$) and steroid derivatives [34]. However, the nutrient content of yam is compared with other crops in **Table 2**.

2.4 Yam varieties

2.4.1 Dioscorea alata

It is widely called "water yam", "winged yam" and "purple yam". It is the most widely cultivated specie globally and it is second to white yam in popularity [36, 37]. Water yam is economically important yam specie which serve as a staple food for millions of people in tropical and subtropical countries, and with a great potential for increase in consumers demand due to its low sugar content necessary for diabetic patients [38]. Aside from being source of carbohydrate, it also possesses higher content of protein and low lipids than *D. cayenensis*, *D. escunlenta*, *D. rotundata* and *D. trifida* [39].

2.4.2 Dioscorea bulbifera

It is usually cultivated in Africa and Asia, with slight differences between those found in each place. It is a large vine of about 6 meters (20 ft.) or more in length and produces tubers (bulbils) which grow at the base of its leaves. It is an important food product and it is about the size of potatoes (hence the name "air potato"), weighing from 0.5 to 2 kg (1 to 5 lbs.) [40]. Some known varieties of air potato can be eaten raw while some need to be detoxified by either soaking or boiling before eating. Its growth for commercial purpose is hampered by its moderately unpleasant

	Crops						
Staple	**Maize/Corn**	**Rice**	**Wheat**	**Potato**	**Cassava**	**Soybean (Green)**	**Yam**
COMPONENT (PER 100G PORTION)							
Water (g)	10	12	13	79	60	68	70
Protein (g)	9.4	7.1	12.6	2	1.4	13	1.5
Fat (g)	4.74	0.66	1.54	0.09	0.28	6.8	0.17
Carbohydrate (g)	74	80	71	17	38	11	28
Fiber (g)	7.3	1.3	12.2	2.2	1.8	4.2	4.1
Sugar (g)	0.64	0.12	0.41	0.78	1.7	0	0.5

Source: Kumar et al. [35].

Table 2.
Nutrient content of white yam in comparison with other crops.

flavor, making other yam to be mostly preferred by consumers [41, 42]. The aerial or air yam is popular in home vegetable gardens, because it produces its first bubils only after four months of growth and thereafter throughout the life of the vine, as long as two years [41, 43].

2.4.3 Dioscorea cayenesis

This yam specie got its common name from its yellow flesh, due to the presence of carotenoids. It is a West African native; it has a longer period of vegetation and a shorter dormancy than white yam [44]. Yellow and white yam were considered in past as two separate species, but now they are been considered as same specie by most taxonomists with about over 200 cultivated varieties between them [44]. They are large and their vines are as long as 10 to 12 meters (35 to 40 feet). The tubers can weigh up to about 2.5 to 5 kg (6 to 12 lbs.) each for an average size, but extra large tubers can weigh as much as 25 kg (60 lbs.) [40]. The maturation stage after planting is 7 to 12 months [45]. It is commonly used in Africa for the traditional popular dish known as "iyan" (pounded yam) [46].

2.4.4 Dioscorea dumetorum

The tuber is commonly referred to as cluster yam, bitter yam or trifoliate yam. It is called *"ona"* by the Igbos of Southeast Nigeria and 'esuru' by the Yorubas in Southern Nigeria [42, 47]. The tuber contains fleshy edible part having a yellowish or whitish color which can be boiled and eaten as snacks. Extract from the tubers can be used for the treatment of *diabetes mellitus* in traditional medicine [48, 49]. It has been reported that the tuber is rich in fiber and contains an alkaloid, dioscorentine, which possesses hypoglycemic activity [50, 51]. It is fairly high in protein and possesses a well balanced amino acid profile, making it the most nutritious of the commonly consumed yam species. It has been established that trifoliate yam contain 15–38% starch on wet weight basis and 70–80% dry weight basis [52].

2.4.5 Dioscorea esculenta

This type of tuber is popularly referred as 'Chinese yam'; it is smaller than other known yam species grown in Africa [24]. It possesses ability to tolerate cold temperature and can be grown in much cooler conditions than other yams. It is commonly grown in China, Korea, and Japan and it was introduced to Europe in the 1800s when the potato crop was affected by diseases [53]. In France, it is still grown for Asian food market. The tubers mature in 6 months and can be eaten immediately after harvest, while others are used as ingredients for other dishes, including noodles and traditional medicines [24, 54].

2.4.6 Dioscorea rotundata Poir (white yam)

This yam specie is native to Africa. It produces edible tubers that possess economic importance [55]. It is among the most important cultivated yam species and it is mostly consumed in West Africa and Cameroon [56]. It has a long shelf-life which makes it available all year round. White yam are subjected to different cooking methods in Western and Central Africa, however, the most common ones are boiling, frying and roasting [29]. It is mostly used in Africa for the traditional popular dish known as "iyan" (pounded yam). Sun-drying of parboiled yam pieces and then milled into a light-brownish powder (*elubo*) is another method of processing

in Nigeria. A thick brown starchy paste known as" *amala*" which is consumed with local soups and sauces can be prepared from the light brown powder [55].

2.4.7 Dioscorea trifida (cush-cush yam)

This specie is a native of Guyana region of South America and it is the mostly referred to as "New World" yam, their growth cycle is less related to seasonal changes than other yam, since they originated from the tropical rain forest. They can be easily cultivated when compared to other yams and also they have good flavor, which may serve as a great potential for increase production [54, 57]. Its starch contains amylose content in the 34.7–43.3% range for white and purple varieties [57].

2.4.8 Dioscorea villosa (wild yam)

It is a perennial vine that grows in moist thickets and hedges [57]. It possesses a reddish-brown stem, having heart-shaped leaves with prominent veins and inconspicuous greenish yellow flowers that flourish from September to October. It is usually cultivated as food source and its roots are harvested in the fall. Its taste is usually bland and then acrid, but under special preparation herbalist use its fresh and dried roots for medicine [58]. Also, it is a common herbal remedy for pains associated with rheumatism and arthritis, colic and intestinal cramps, as well as a reliable antispasmodic and anti-inflammatory [59]. It is also applicable in contraceptive manufacture. High content of saponin in species of *Dioscorea villosa* made them useful for the preparation of steroids in the pharmaceutical industry [60, 61]. Studies also revealed that *Dioscorea villosa* had antioxidant activities [62] and the anti-inflammatory activity could be linked to the antiphlogistic effect of the steroidal saponins.

2.5 Cereals

Cereals are edible grains or seeds that belong to the grass family *Gramineae* (**Figure 1**). Grains are developed from flowers or florets and their structures vary from one to another with some typical features [64, 65]. They possess *embryo* (or germ) which is a thin-walled structure, containing the new plant. The embryo is separated by the *scutellum* (which is involved in mobilization of food reserves of the grain during germination) from the main part of the grain. The endosperm surrounded by thin-walled cells (*aleurone*), packed with starch grains [66, 67]. The *aleurone* layer present in grains consist of one or three cell layers (wheat, rye, oats,

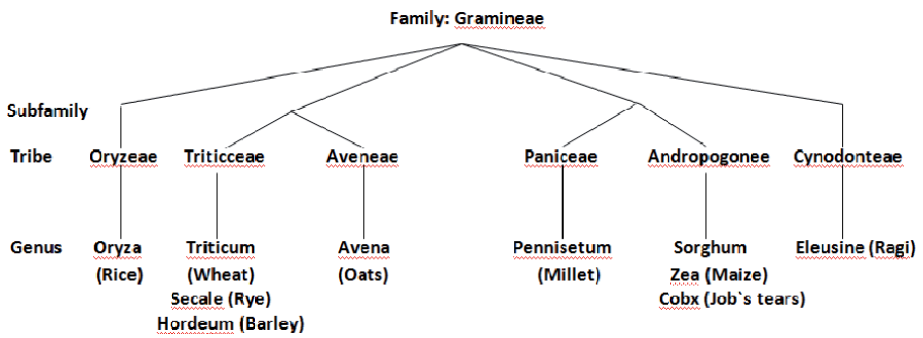

Figure 1.
Taxonomy of the Graminae family. Source: Shewry et al. *[63].*

maize and sorghum have one; rice and barley have three). The pericap is the outer layers of the grain (derived from the ovary of the flower) that surround the *seed coat* (the testa), while the bran is formed from the outer thick-walled structures. They are staple foods both for direct human consumption and indirect through livestock feed [68]. Cereal based foods are good sources of energy, protein, B vitamins and minerals for the world population. Cereals are inexpensive to produce, they are easy to store and transported, and they do not deteriorate readily if kept dry.

However, in cereal products, a proportion of this starch is not digested and absorbed in the small intestine. This is referred to as resistant starch and it appears to act in a similar way to dietary fiber. Four categories of resistance have been defined [65, 69].

- RS1 refers to starch that is physically inaccessible for digestion as it is 'trapped' (e.g. intact whole grains and partially milled grains).
- RS2 refers to native resistance starch granules (e.g. found in high amylose maize starch).
- RS3 refers to retrograded starch (e.g. found in cooked and cooled potatoes, bread and some types of corn-flakes).
- RS4 refers to chemically modified starch (e.g. commercially manufactured starches).

2.5.1 Wheat

Wheat is ranked as a major cereal crop in many parts of the world and belongs to the *Triticum* family of which there are many thousands of species [65, 67]. Among the known species, *T. aestivum* subspecies *vulgare* and the hard wheat *T. durum* are the most commercially viable [65, 70]. Wheat is grown in both winter and spring but their cultivation in winter or spring is dependent on the species, varieties and their adaptability. However, wheat is grown in many countries around the world, but the most prominent great wheat-producing countries are USA, China and Russia. Wheat grains are also present extensively in India, Pakistan, the European Union (EU), Canada, Argentina and Australia. Estimation showed that 556.4 million tons of wheat might have been produced in 2003; resulting to 30% of the world's cereal production [71]. The wheat grain is sandwiched between the lemma and the palea of each spikelet, possessing an elliptical, oval or ovate shape, and has short or long brush hairs.

2.5.2 Rice

Rice is an important staple food crop for many of the world's population, especially those living in Asia. Rice is cultivated mainly for human consumption and this include utilization as breakfast cereals, and its use in Japan as brew saké [65, 67]. There are a lot of rice varieties but only a few are grown widely (*e.g.* varieties of the improved semi-dwarf plant type with erect leaves). The rice grain possesses an outer protective coating which is referred to as the hull or husk and the edible rice caryopsis. Also, the brown rice contains an outer layer called pericarp (which contains pigment), seed coat, the embryo and the endosperm. The endosperm comprises of the aleurone layer which encloses the embryo, sub aleurone layer and the starchy or inner endosperm. However, wild rice is less common and it is the grain of a North American plant, *Zizania aquatic*. It is difficult to harvest and it is more expensive than other grains. It possesses higher protein content than rice [64].

2.5.3 Maize

Zea mays L., alternatively referred to as corn is a native of the Western Hemisphere and its production in the USA exceeds that of others countries [72]. The kernel which is the reproductive seed of the plant has four main parts, and these are the germ, the endosperm, the pericarp and the tip cap. It is an inexpensive source of starch and it is a major source of energy for animal feed [65, 70]. Among the hundreds of different varieties of wheat grains, only four varieties listed below are of commercial importance:

1. Dent maize (identified by the dent in the crown of the kernel);
2. Flint maize (hard, round kernels);
3. Sweet corn (a dent-type maize);
4. Popcorn (flint-type maize which expands when heated).

2.5.4 Barley

Barley is a resilient plant that can tolerate a wide range of conditions, which might have been cultivated since 15,000 BC [72]. The head or spike of barley is made up of spikelets that are attached to the rachis in an alternating pattern. The barley kernel's outer layers consist of the husk, the pericarp (to which the husk is tightly joined in most species), the testa or seed coat and the aleurone which completely covering the grain. Barley (*Hordeum vulgare*) is mainly cultivated for malting and brewing in the manufacture of beer and for distilling into whisky manufacture [65, 70]. The spent grains from the brew are used as animal feed, especially for pigs. However, a small amount of barley is used for food. Pearled barley is used in cooking soups and stews in the UK and in the far Middle East. Barley flour is also used in bread production and can be cooked as porridge in some countries [67].

2.5.5 Oats

Among the several different species of oats, spring or white oat (*Avena sativa* L.) is the most important cultivated form. *Avena byzantine* is a red-oat type or alternatively known as a winter oat is cultivated in warmer climates [65, 70]. A spikelet of oat consists of oat kernels. A hall which is made up of two layers called the lemma and palea, enclosed each kernel, the hall is only loosely attached to the groat. However, the groat is made up of 65–85% of the oat kernel and it is enveloped by bran layers (pericarp, seed coat and aleurone cells) [65]. It grows well on poor soil and in cool, moist climates and has mainly been grown for animal feed. Its cultivation for human consumption is at minimal level, products made from oats are oatmeal for porridge and oatcakes, rolled oats for porridge and oat flour for baby foods and for ready-to-eat (RTE) breakfast cereals [67]. Non-food uses of oats include their utilization in the production of cosmetics and adhesives [70].

2.5.6 Rye

Rye is generally a hardy plant and it is wildly cultivated in cool temperature zones, where other cereals can merely survive. Rye can also be grown in semi-arid areas and at high altitudes. It is cultivated as a winter crop, which is sown in early autumn and harvested in early summer [65]. The plant varies in height from 30 cm

to more than 2 m. It is a major crop grown in Russia, Poland, Germany and the Scandinavian countries for crispy bread, alcohol animal feed production [67]. The grain is protected with a bearded *glume* husk, arranged in an alternating pattern along the rachis. Rye is thinner and more elongated than wheat; it possesses a grayish yellow color and varies from 1.5 mm to 3.5 mm in size. The grain comprises of the starchy endosperm, the pericarp and the testa. Other constituents include the bran, accounting for 10% of the grain with the remainder consisting of the germ (the embryo and scutellum).

2.5.7 Millet

Millet is an annually cultivated cereal which possesses different species of small grains [64, 70]. Among the various species of millet, pearl millet is the only one that is mostly of economic concern. Other known species of millet include, finger (or ragi), proso and foxtail, but the minor millets account for less than 1% of the grains produced for human consumption, they are less important in terms of world food production [65, 70]. Cultivation of millet is of utmost importance in certain locations in Africa and Asia where major cereals cannot be solely depended on to provide sustainable yields [73]. The species type influence climatic and soil requirements, length of growing period, grain consistency, size and taste of millet [64].

2.5.8 Sorghum

Sorghum (*Sorghum bicolor* L. Moench) is wild regarded as a warm season crop, intolerant of low temperatures but fairly resistant to serious pests and diseases. The known varieties are great millet and guinea corn in West Africa, kafir corn in South Africa, jowar in India and kaoliang in China. It is one of the staple foods in many parts of Africa, Asia, and parts of the Middle East [65, 70]. However, most of the sorghum grains produced in North and Central America, South America and Oceania are used for animal feed [73]. The grain comprises of a naked caryopsis, which is made up of a pericarp, endosperm and germ. Based on huge range of physical diversities, such as the color of the pericarp (white, yellow or red) and presence/absence of pigmented testa (with/without tannins), sorghums are classed into one of four groups and these are (1) grain sorghum, (2) forage sorghum, (3) grass sorghum or (4) Sudan sorghums and broom corn [70].

2.5.9 Acha

Acha, which is also referred to as "fonio", "findi", "fundi", "pom, and kabug" "hungry rice" and "petit mil", is a small-grained cereal that is native to West Africa, which is generally classified among the millet [74, 75]. It is cultivated in various parts of Nigeria, Sierra Leone, Ghana, Guinea Bissau and Benin Republic on less fertile sandy soils that could not support the growth of other more demanding cereals. There are two known varieties of acha which are *Digitaria exilis Kippis stapf.* and *Digitaria iburua Kippis Stapf.* It is regarded as one of the lost crops in the West Africa sub region. Its production is important to West African Farmers, though hindered by several factors among which are poor agronomic performance because of unimproved seeds and husbandary practices. Its West Africa annual production is about 250,000 tonnes [17]. The annual yields of 3098 metric tonnes, 112,000 metric tonnes and 126,000 metric tonnes of fonio were reported in Nigeria [76]. The economic returns of acha when compared with other crops like rice, sorghum, and cowpea showed that it was profitable to grow the crop on a commercial scale [77]. Fonio are the most nutritious and testiest of all grains [77] and it contains 7%

crude protein that is high in leucine (19.8%), methionie and cystine (7%) and valine (5.8%) [78]. Fonio grains are mostly consumed wholly, they are also milled into flour and constitute a versatile raw material for preparation varieties of food such as gruels, porridges, couscous, bread, beer, and beverages [79]. Starch extracted from fonio possesses good disintegrant and binding properties [80] and it also has good glidant properties [81].

2.6 Food starch properties

The starch granules sizes obtained from different crops vary in properties, because of their sources, extraction methods and cultivars [82]. The purity as well as the granules size can be determined using scanning electron microscopy [83]. The amylose content of starch is one of the most important factors influencing the cooking and textural qualities of whole storage root, and quality of starch-based foods [84]. *Dioscorea* starch granules possess varying shapes, which are spherical, oval and polygonal, depending on the species with granule size varying from 2 to 50 mm [85]. Also the X-ray diffraction pattern of the *Dioscorea* starch granules range from the B to C-type, depending on the *Dioscorea* specie [18]. *Dioscorea* starches contain18 to 30% amylose contents and their gelatinization temperature vary from 70 to 92°C [84].

Starch particle sizes obtained from white and yellow yam varieties showed similar patterns with a single symmetrical distribution centered at approximately 32 and 35 mm respectively. Sources, varietal differences and growing conditions significantly influence size and shape of starch granules. However, due to better mouth feel, small sized starch granules has been suggested as possible lipid substitute in food systems [86]. They are also used as laundry-stiffening agents because they possess good fabric penetration ability, better glossiness and stiffness, which are required in textile industries [87].

Sweet potato starch granules possess spherical, oval, and polygonal shapes, and they are about 2–46 mm in size. The X-ray diffraction patterns of the sweet potato starch granules are of type A and they also exhibit 38% crystallinity [88]. The starch in sweet potato is made of 16.1–24.4% amylose, having a swelling power of 80% at 90°C and gelatinization temperature of 64.6–84.6°C [84]. Cassava starch possesses small spherical granules, having an average granule size of 14.7 mm. Its amylose content ranges from 13.6 to 23.8% [84, 89] and its crystallinity has been reported to be 38% [84], while the gelatinization temperature varies from 59.6 to 87.2°C [84, 89]. The X-ray diffraction patterns of the cassava starch granules depict type A [90]. The cocoyam granules (Taro) are small rounded and ellipsoidal-truncated, with their sizes varying from 0.5 to 5 mm in diameter, making them to be more easily digestible [84]. Taro starch has been used in the preparation of some baby foods and diets of people who are allergic to cereals [91]. The X-ray diffraction patterns of the taro starch granules exhibit the typical A-type pattern, while the starch contains 14.0–19% amylose with the pasting temperatures varying from 81 to 85°C [92]. Interestingly, starch from Tannia (*Xanthosoma sagittifolium*) comprises of small rounded and large truncated ellipsoidal-shaped granules, which possesses granular diameters which range from 2 to 50 mm [91]. The amylose contents of different cultivars range from 21.3 to 25.4% [93]. Tannia starch possesses a type A X-ray diffraction pattern, higher pasting temperatures and lower paste viscosity than those of other starches, such as potato starch [89]. Also, it has higher swelling power and solubility at relatively high temperatures than sweet potato starch granule [84]. The maize starch granules exhibit polyhedral granule shapes and differences in their mean granule size range from 2.3 to 19.5 μm. The starch samples show A-type diffraction pattern with strong reflection at 15.25, 18.11,

and 23.33° [94]. The gelatinization temperatures of maize starch range from 69.16 to 76.98°C [95] and the amylose content varies from 24.74 to 30.32% [94]. Fonio starch granules are polygonal in shape with diameter ranging from 2.0–14.3 mm. Their X-ray diffraction patterns are of the type-A crystalline form [96], with the amylose contents ranging from 18.7% to 19.6%. The millet starch granules are small, spherical to polygonal in shape, but may vary from specie to another. The granular sizes range from 0.8 to 10 mm. The gelatinization temperatures of millet starch ranged from 75.8 to 84.9°C and the amylose content vary from 16.0 to 27.1% [84]. The starch granules obtained from sorghum are typically 3–27 mm and the gelatinization temperature range 61.1–81.2°C [97]. The starch showed the A-type crystalline diffraction pattern and its amylase content varies from 11.2–28.5%, depending on both genetic and environmental factors [98].

2.7 Forms of starch

2.7.1 Native starch

Native starches are crude starches that are extracted directly from their sources and they are mainly used as food, but irrespective of their sources they are undesirable for many applications because of their inability to withstand processing conditions. Each starch has unique functional properties, and much of the starch used industrially is modified before use, giving a wider range of useful products [99]. Starch can be readily converted chemically and biologically into many useful and diverse products such as paper, textiles, adhesives, beverages, confectionery, pharmaceuticals, and plastics, so as to improve desirable functional properties in order to meet the requirements of specific industrial processes [13].

2.7.2 Extraction techniques

Starch granules' settling is often prevented by presence of various components like mucilage and latex, which may lead not only to loss of starch, but also reducing the quality of extracted starch. However, microbial growth can also be promoted if the extraction residence time is prolonged than necessary, which may also result into breakdown of starch and resultant loss of starch quality. It also affects the color of the starch limiting its utilization in food and industrial applications. Therefore, optimum recovery of starch having physicochemical and functional qualities coupled with economical extraction of starches from cereals and tubers is important. Extraction of starch with water is the most common form of starch extraction, but this has been improved upon over time. The Central Tuber Crops Research Institute, Trivandrum, India research on various chemicals that could improve the yield of starch from various tubers [100, 101]. It was discovered that ammoniacal solutions gave the best results. However, when aqueous ammonia (0.03 M) was used for starch extraction, the yield, paste viscosity and swelling capacity of the extracted starch improved. Ammonia formed a complex with the mucilagenous material in the slurry, thereby releasing the starch granules and promoting faster settling of starch in less viscous slurry, which prevents microbiological damage of the starch due to short residence time. Moorthy [101] observed that lactic and citric acids improved the yield and color of starch from sweet potato tubers. Interestingly, an enzymatic method for enhancing the recovery (26% increase) of starch from cassava tubers using pectinase and cellulase enzymes [101]. The aforementioned enzymes work by altering the integrity of the pectin-cellulosic matrix of cell membranes and thereby promoting the release of the starch granules. The same technique was used to promote starch recovery from sweet potatoes by 20% without affecting starch properties [101].

2.7.3 Modified starch

Modified starches are native starches whose physical and chemical characteristics have been altered in order to improve their functional characteristics [102]. However, modification of starch is done to tailor starch to a specific food application, to stabilize starch granules during processing and make it suitable for many foods and industrial applications. During modification, starch properties are altered, including solution viscosity, gelatinization properties, pasting properties, retrogradation behavior, association behavior, and shelf-life stability in final products [102, 103]. Modification can be achieved through etherification, esterification, cross-linking/grafting of starch, decomposition (acid or enzymatic hydrolysis and oxidization of starch) and physical treatment of starch using heat or moisture [104].

Modified starches produced from different methods and sources are usually used as thickening agents to provide desired structures in food products [105]. Acid-thinned starch is starch that has been treated in acid slurry [1]. This starch possesses faster gelatilization, low viscosity, and could produce a weak gel (Abbas *et al*., 2010). Oxidized starch is a starch which has undergone oxidation and it has a low hot paste viscosity. Both of acid-thinned and oxidized starches are suitable for confections because they allow rapid and efficient cooking of starch solution in the presence of concentrated sugar syrups [106]. Stabilized starch is a type of modified starch that possesses a resistant property against acid degradation under dry acidic storage. Stabilized starch can be produced by adding buffer (pH 6–9) to starch slurry and drying the starch-buffer slurry in a conventional oven [107]. Stabilized starch has a reduced starch gelatinization temperature; it's easier to cook and allows for the formation of stronger gel with increased clarity and longer self-life. Converted starch, such as dextrin, which possesses a good film forming capacity, can be used with high-sugar solutions to produce stable and flexible coatings [108]. Thin boiling starch is a modified starch that can be produced by treatint the starch with amylase enzyme to hydrolize the α-1, 4 glycosidic bonds. This starch possesses gelling properties and low hot paste viscosity, which make it suitable for gum drops since this type of starch allows better evaporation and pouring [104].

Octenyl succinic anhydride (OSA) starch is a recent type of chemically modified starch, possessing surface active properties [109]. Octenyl succinic anhydride (OSA) starch is produced by esterification of different sources of starch with anhydrous octenyl succinic acid under alkaline conditions [110]. The adsorption of the OSA starch molecules at the oil/water (O/W) interface might be as a result of hydrophobic short octenyl succinate side chains in octenyl succinic anhydride (OSA) starch molecules and the long amylopectin backbone which protect the droplets against flocculation by the mechanism of steric stabilization [111]. OSA behaves like a typical surfactant and forms a strong film at the O/W interface which provides a good resistance against re-agglomeration [111]. The increase in the viscosity of the continuous phase in conjunction with its ability of adsorption at interfaces enables OSA starch to behave as a stabilizer and also as an emulsifier in O/W emulsion systems. OSA starch type of modified starch has been approved as a food additive by the Food and Drug Administration (FDA) and European Union [112].

2.8 Utilization of starch in food industries

Starch plays a crucial role in food systems by stabilizing and creating the food structure. Starch also co-exists with other components to deliver or maintain nutrient and flavor [113]. However, starch importance in some food applications are elaborated below.

2.8.1 Snack foods

Starch is generally used in the production snack foods to achieve desired textural and sensory attributes by improving crispiness, oil binding properties, expansions, and overall eating quality [113]. The properties of the amylopectin and amylose of starch are important for the texture creating of this kind of foods. The highly branched amylopectin might increase dough expansion and viscosity, which could result to production of light, crispy, and expanded products. The amylase on the other part strengthens the dough and improves its forming and cutting properties. Consequentially, a more crunchy final texture could be obtained [114]. Furthermore, a high quality fiber-fortified snack could be developed by incorporating modified starch [108].

2.8.2 Baked products

Starch contribution to baked products quality is through its important properties, such as gelatinization, water absorption, and retrogradation [115]. The gelatinization property of starch is very important in building the structure and texture of baked products. However, starch ability to bind water could reduce the stickiness of dough, improve handling, and increase cake volume. It could also improve the moistness and softening the texture of baked products [108].

2.8.3 Confectionery

Starch behaves as a structure builder in coatings and also acts as a medium for molding to support desired shapes in confectionaries. Starch is used in the production of pastes, gums, and molds; it is also in the manufacture of dusting sweets to prevent them from sticking together. Starch is selected because of its ease of cooking in high-sugar environments and also based on its ease of handling during processing [108]. In most cases, starch is modified to possess specific properties that suit certain applications.

2.8.4 Gravies, soups, and sauces

Starch application in gravies, soup, and sauces depends on the production process, which is usually influenced by pH of products and heat during processing [114]. Compared to neutral products, high acid products (pH < 4.5) require a higher-degree of cross-linking starch. The production of cross-linked starch is achieved by the reaction in which a small number of hydroxyl groups on the glucose units of amylose and amylopectin, mostly in the amorphous are modified without destroying the granular nature of starch [116]. Since sterilization of acidic products require shorter processing time and lower temperature, other factors such as shelf-life requirements, fill-viscosity, and heat penetration also influence the suitability of starch used in these types of food products. Hydroxypropylated starch which possesses high freeze thaw stability is suitable for chilled and frozen foods [108]. Hydroxypropylated starch is produced by reaction of starch with propylene oxide. Aside its better freeze thaw stability, modification improves hydroxypropylated starch shelf-life, cold-storage stability, past clarity and texture properties of its paste. Generally, gravies, soups, and sauces require starch with opaque paste [108].

2.8.5 Mayonnaise and salad dressing

The function of starch in these products is mainly to thicken and stabilize the dispersed phase [108]. Basically, these food products are produced under acidic

condition and the process involves application heat and shear thinning. Therefore, starch which has ability to tolerate acidity, heat, and shear is suitable for these products. Also, lipophilic starch which has potential to stabilize emulsions, other modified starches such as cross-linked starch and stabilized starch are the most commonly used for these products [117]. Lipophilic starch is produced by esterification with n-octenyl succinic anhydrate, which led to a starch structure comprising both hydrophilic and lipophilic properties. This starch can be used for mayonnaise and salad dressing, lipophilic starch might be used to replace animal-derived sodium caseinate and gum Arabic [102].

2.8.6 Emulsion stabilizer

A scientific study suggested that starch could be used as a stabilizer in oil-in-water emulsions [117]. The study further revealed that emulsion prepared with equivalent volume ratio of water and paraffin could be stabilized by adding an aliquot of starch dispersion [117]. Kim *et al*. [117] established that the introduction of starch nano particles of more than 0.02% wt kept the emulsion longer than 2 month of storage without coalescence of oil droplets. Starch might be used in various emulsions not only for foods but also for cosmetics and pharmaceuticals [117].

2.9 Utilization of starch in allied industries

Some non food uses of starch in allied industries are described below:

2.9.1 Packaging component

Nanoparticle from starch can be used as a material in barrier films for food packaging. The primary focus of the barrier properties of the packaging films is on water vapor transmission and oxygen permeability [117]. However, a decrease in water vapors permeability was observed when the maize starch was used. Kristo and Biliaderis [118] revealed addition of 30–40% waxy maize starch significantly decreased the water vapor permeability in the component. Similarly, García *et al*. [119] observed a 40% decrease in the permeability of cassava starch film, reinforced with 2.5% starch nanoparticles.

2.9.2 Drug carrier and tablet binder

Starch has been described as a nontoxic, biodegradable, and biocompatible polymer [117]. Its nanoparticles possess the ability to deliver an ample range of molecules to any location in human body for sustained periods of time. Starch has been useful in tablet formulation and binding due to its relative inertness, abundance, low cost, and suitable physicochemical properties [31].

2.9.3 Binders in paper making and paper coating

Starch possesses a binding property and can be used as a binder in papermaking and paper coating. Cooked starch is widely used as paper-making additive and the retention of the cooked starch on the paper matrix is based on starch absorption [108]. However, the amount of starch absorbed is limited by the cellulose substrates absorption saturation. Another problem which might cause operational problems is the high viscosity of the starch paste after cooking. Bloembergen *et al*. [120] revealed that the modified starch possessed better paper binding capacity than cooked starches.

2.9.4 Starch as energy source

Starch bioconversion into ethanol is a two-step process. Saccharification is the first step, which involves conversion of starch into sugar using an amylolytic microorganism or enzymes such as glucoamylase and α-amylase. Fermentation is the second step, which involves conversion of sugar into ethanol using *Saccharomyces cerevisiae* [121]. An alternative to the conventional multistage process of starch fermentation which offers poor economic feasibility is the use of amylolytic yeasts for the direct fermentation of starch. Despite the fact that there are over 150 amylolytic yeast species, they possess limited industrial use because of their low ethanol tolerance [121]. Therefore, most research effort has been geared towards the development of genetically engineered amylolytic strains of *S. cerevisiae*, and in these strains, heterologous genes encoding α-amylase and glucoamylase from various organisms have been produced including their products and their products [121].

3. Challenges facing common starch sources and way outs

Starch is the basis of our food and industrial economy, but the food situation in most developing tropical countries is alarmingly worsening owing to increasing population, fragmented farms with rudimentary technologies, poor pre-harvest and postharvest farm practices and shortage of fertile land [11, 24, 108]. The shortage of food supply has resulted into a high incidence of hunger and malnutrition [12, 13]. It also affected the demand for starch as food, pharmaceutical and industrial uses coupled with the need to attain self-sufficiency in starch production.

However, self-sufficiency in starch production could be attained if the following suggestions described below are given utmost attention:

i. Some lesser known and unconventional crops could be good sources of nutrients and starch, and even have the potential of broadening the present narrow food base of the human species (Viano *et al*., 1995). *Dioscorea villosa* L (common name wild yam or atlantic yam) is one of the underutilized starch sources, and it is less explored due the presence of anti nutritional factors. Processing techniques can reduce the anti-nutritional factors in food and make them readily available for consumption.

ii. Efficient and effective development of the various starch based crop value chains such as cassava in order to increase their productivity [24].

iii. Encouraging small and medium scale investments in order to enhance production, processing and delivery of high quality and quantity of cassava products to the larger industries [24].

iv. Promotion of effective pest/pathogens integrated management programs and reduction of the occurrence of mycotoxin in starch based crops, to prevent emerging and endemic pests and diseases as well as reduction of mycotoxin contamination in starch [108].

v. Access to high yielding, climate-resilient and affordable quality seeds, advances in breeding technology using genomic approaches that is cost-effective and field-relevant high throughput phenotyping approaches will accelerate and contribute significantly to mass production starch-based crops [24].

4. Conclusion

Conclusively, suggested way outs should be given utmost attention, most especially research efforts should be geared towards utilization of lesser known non conventional crops as sources starch such as wild yams. Sufficient to say, the current level of research on wild yams starches, such *Dioscorea villosa*, *Dioscorea bulbifera* and other lesser known starchy crops is limited. Therefore, the properties and potential utilization of starch obtained from *D. villosa*, *D. bulbifera* and other lesser known starchy crops should be established in order to broadening the present narrow commonly cultivated starch sources. Also, value addition and conceptual efforts towards good agricultural practices might enhance the productivity of conventional starch sources such as cassava and maize.

Conflict of interest

The author declared that there is no conflict of interest.

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Chapter 12

Genes Involved in Lipid Metabolism in Coconut

Wei Xia

Abstract

Coconut palm (*Cocos nucifera* L) is an economically important monocot plant grown in tropical and subtropical regions. Coconut oil is stored in a solid endosperm and has 47.48–50.5% fatty acid component as lauric acid (C12:0). Present research showed that acyl-acyl carrier protein thioesterases (FatA/B) and lysophosphatidic acid acyltransferase (LAAPT) are key enzymes determining medium-chain fatty acid accumulation in coconut oil. Among five *CnFatB* genes, *CnFatB3* expressed specifically in endosperm and *in vitro* experiment showed that this gene made mainly lauric acid (C12:0) and tetradecenoic acid (C14:1). Overexpression of *CnFatB3* in *Arabidopsis* increased the amounts of C12:0 and C14:0 in transgenic plant. *CnLPAAT* gene that is expressed specifically in coconut endosperm showed a preference for using acyl-CoAs containing C10:0, C12:0, and C14:0 acyl groups as acyl-donor substrates. Coconut and oil palm are closely related species with approximately 50% lauric acid (C12:0) in their endosperm. The two species have a close evolutionary relationship between predominant gene isoforms and high conservation of gene expression bias in the lipid metabolism pathways. Moreover, since no stable transformation system has been constructed in coconut palm, gene function validations have been done in vitro, or genes transformed into a heterologous system.

Keywords: medium-chain fatty acid, lipid metabolism, coconut endosperm, gene evolution, de novo fatty acid synthesis, TAG biosynthesis

1. Introduction

Coconut palm (*Cocos nucifera* L), belonging to the Arecaceae family, is an economically important monocot plant grown in tropical and subtropical regions. Coconut kernels have approximately 63.1% oil content in a solid endosperm (copra) [1]. A noticeable feature of coconut oil is that 47.48–50.5% of its fatty acid component is lauric acid (C12:0), which is a type of medium-chain fatty acid (MCFA) [2]. A closely related species of coconut, the African oil palm, also contains 50% lauric acid in its kernel oil [3]. The lauric acid content of coconut oil makes it useful for a range of edible and nonedible purposes. A number of genes differentially expressed in coconut endosperm have been identified by suppression subtractive hybridization [4]. *Arabidopsis* has more than 600 genes involved acyl-lipid metabolism, and Xiao et al. [5] identified 806 orthologous genes of these *Arabidopsis* genes in coconut palm based on the first version of coconut genome sequences [1, 2]. A better understanding of lipid biosynthesis and tissue-specific transcription could help breeding efforts to improve the content and composition of coconut oil used

Coconut gene ID	Protein	Annotation	Gene validated in reference
CCG009120.1	PDH-E1α	E1-alpha component of pyruvate dehydrogenase complex	
CCG003104.1	PDH-E1β	E1-beta component of pyruvate dehydrogenase complex	
CCG004328.1,CCG016344.1,CCG020484.1,CCG022353.1	LTA1	Dihydrolipoamide acetyltransferase, E2 component of pyruvate dehydrogenase complex	
CCG022878.1	LTA2	Dihydrolipoamide acetyltransferase, E2 component of pyruvate dehydrogenase complex	
CCG016999.1	LPD2	Dihydrolipoamide dehydrogenase, E3 component of pyruvate dehydrogenase complex	
CCG004885.1	CT-α	Carboxyltransferase-alpha; subunit of heteromeric ACCase	
CCG016556.1	BCCP	Biotin carboxyl carrier protein of heteromeric ACCase	
CCG014874.2,CCG016422.1	BC	Biotin carboxylase of heteromeric ACCase	
CCG000475.1,CCG019561.1	MCMT	Malonyl-CoA: ACP malonyltransferase	
CCG001191.1,CCG001193.1,CCG001194.1,CCG024029.1,CCG026381.1	KASI	Ketoacyl-ACP Synthase I	[6]
CCG000907.1,CCG015780.2,CCG023608.3	KASII	Ketoacyl-ACP Synthase II	
CCG003289.1,CCG025932.1	KASIII	Ketoacyl-ACP Synthase III	
CCG006105.2,CCG025988.1,CCG014527.1,CCG024266.1	KAR	Ketoacyl-ACP Reductase	
CCG007292.1,CCG001741.1	HAD	Hydroxyacyl-ACP Dehydratase	
CCG019022.2,CCG019145.2	ENR1	Enoyl-ACP Reductase	
CCG001923.1	ACP1	Acyl carrier protein	
CCG000806.1,CCG000980.1,CCG017093.1,CCG026999.1,CCG027016.1 ,CCG027238.1	ACP4		

Coconut gene ID	Protein	Annotation	Gene validated in reference
CCG025689.1	DES6	Stearoyl-ACP desaturase	[7]
CCG005175.1,CCG011462.1,CCG019622.1	FAB2		
CCG017191.1,CCG017192.1,CCG017193.1,CCG021345.1	DES5		
CCG005178.1,CCG012754.1	FatA	Acyl-ACP thioesterase A	
CCG006479.1,CCG007799.1,CCG011598.1,CCG015192.1,CCG019705.1	FATB	Acyl-ACP thioesterase B	[8, 9]
CCG005500.1	HACPS	Holo-ACP synthase	
CCG001744.2,CCG007290.1,CCG007291.1	LACS9	Long-chain Acyl-CoA synthetase	

Table 1.
Coconut genes belong to de novo fatty acid biosynthesis.

Coconut gene ID	Protein	Annotation	Gene validated in reference
CCG004869.3,CCG020141.3,CCG023968.1,CCG027042.1	GPDH	NAD-dependent glycerol-3-phosphate dehydrogenase	
CCG019614.2	GPAT9	Glycerol-3-Phosphate acyltransferase (mammalian homolog)	
CCG006531.1,CCG015599.1,CCG016821.1	LPAAT2	1-Acylglycerol-3-phosphate acyltransferase	[6, 10–13]
CCG022695.1	PAH1	Phosphatidate phosphatase	
CCG009829.1,CCG016247.2	PAH2	Phosphatidate phosphatase	
CCG007725.1,CCG026806.1	LPP-β	Phosphatidate phosphatase	
CCG003641.1,CCG010800.1	LPP-δ	Long chain base 1-phosphate phosphatase	
CCG015429.1,CCG019248.1	DGAT1	Acyl-CoA:diacylglycerol acyltransferase	
CCG004186.1,CCG026159.1	DGAT2	Acyl-CoA:diacylglycerol acyltransferase	[14]
CCG015380.1	DAcT	Wax synthase-like	
CCG005217.1	PDAT1	Phospholipid:diacylglycerol acyltransferase	
CCG019998.1,CCG019999.1,CCG020055.1	PDAT-related?	Phospholipid:acyl acceptor acyltransferase	
CCG011285.1,CCG021291.1	LPEAT1	1-Acylglycerol-3-phosphoethanolamine acyltransferase	
CCG000909.1,CCG000910.1	LPEAT2		
CCG002335.2,CCG015142.3	LPCAT	1-Acylglycerol-3-phosphocholine acyltransferase	
CCG017936.1	PDCT/ROD1	Phosphatidylcholine:diacylglycerol cholinephosphotransferase	
CCG019021.1,CCG019148.1	FAD2	Oleate desaturase	
CCG003640.4,CCG010801.1	CDP-DAGS	CDP-DAG synthase	
CCG021791.1	DAG-CPT	Diacylglycerol cholinephosphotransferase	
CCG009590.1,CCG024101.3,CCG025115.1	CK	Choline kinase	
CCG007754.1,CCG019356.1,CCG026050.3	CCT2	Choline-phosphate cytidylyltransferase	

Coconut gene ID	Protein	Annotation	Gene validated in reference
CCG021844.1	ACBP2	Acyl CoA binding protein	
CCG005041.1,CCG008659.2,CCG018700.1	ACBP3		
CCG009417.1,CCG020854.1,CCG026758.2	ACBP4		
CCG000884.2,CCG026958.1	ACBP6		
CCG009767.1,CCG016753.1,CCG016754.2	LACS4	Long-chain Acyl-CoA synthetase	
CCG027986.1,CCG027990.1	NMT1	Phosphoethanolamine N-methyltransferase	
CCG009861.1	PIS2	Phosphatidylinositol synthase	
CCG026466.1	PSD1	Phosphatidylserine decarboxylase	
CCG023785.2	PSD3		
CCG005386.2,CCG012449.1,CCG015191.4	PSS	Base-exchange-type phosphatidylserine synthase	
CCG001187.2,CCG026384.1	EK	Ethanolamine kinase	
CCG000220.1,CCG001400.4,CCG005823.1,CCG026528.2	PECT1	CDP-ethanolamine synthase	

Table 2.
Coconut genes involved in TAG biosynthesis.

for food and other applications. The most noticeable feature of coconut oil is that the major components of fatty acids are medium-chain fatty acid. This feature has attracted the attention of researchers and become the focus of coconut oil research. What genes related with the accumulation of medium-chain fatty acid in endosperm? How these genes were evolved and related to a closely related species—oil palm (*Elaeis guineensis*), which also has MCFA as its main fatty acid component in endosperm?

We had reviewed three parts of research related to coconut lipid metabolism in this chapter. Firstly, we summarized key genes related to MCFA accumulation in coconut endosperm. Secondly, we summarized the evolutionary relationship between coconut palm and oil palm for MCFA accumulation. Thirdly, we include descriptions of in vivo and in vitro gene validation experiments. Two tables provide coconut genes related to de novo fatty acid biosynthesis (**Table 1**) and triacylglycerols (TAG) biosynthesis (**Table 2**).

2. Genes related to lipid metabolism in coconut palm

Coconut palm stores oil in endosperm tissues, and its fatty acid composition changes in different developing stages of endosperm [4, 5]. The proportion of lauric acid increases with the maturing process of coconut fruit and reaches the peak when the fruit matures. The comparison of gene expression for different developing stages of endosperm indicated that the expression levels of stearoyl-acyl carrier protein desaturase, acyl-ACP thioesterase B (FatB), and lysophosphatidic acid acyltransferase (LPAAT) arose along with the endosperm development [4]. Xiao et al. [5] identified 71 genes belonging to plastidial fatty acid synthesis pathway in coconut, and 62 enzymes catalyze the conversion of pyruvate to fatty acid (**Table 1**). Moreover, the 17 plastidial proteins involved in the conversion of pyruvate to fatty acids were five- to sixfold higher in the endosperm than in the leaf or embryo tissue, such as acyl carrier protein (ACP), ketoacyl-ACP reductase (KAR), hydroxyacyl-ACP dehydratase (HAD), and pyruvate dehydrogenase complex (PDHC). TAG is a compact molecule for energy and carbon storage in organisms. Thus, another key pathway for oil storage—triglycerides (TAG) synthesis is analyzed for coconut palm and 69 genes were identified (**Table 2**). Key genes in the two pathways were deeply analyzed through in vivo and in vitro assays, including FatB, LPAAT, and orthologs of *Arabidopsis WRINKLED 1* (*WRI 1*) [9, 11, 14, 15].

2.1 Genes related to MCFA accumulation in coconut endosperm

2.1.1 Acyl-acyl carrier protein thioesterases

Acyl-acyl carrier protein thioesterases (acyl-ACP TEs) terminate acyl chain elongation during de novo fatty acid biosynthesis. This reaction is the biochemical determinant of the fatty acid compositions of storage lipids. There are two classes of acyl-ACP TEs—FatA and FatB. Since 1996, researchers have cloned acyl-ACP TEs from California bay laurel (*Umbellularia californica*) and validated its role in accumulating MCFA by transforming it into rapeseed (*Brassica napus*). Further research has classified FatB genes into three classes based on their specificities: class I acyl-ACP TEs act primarily on 14- and 16-carbon acyl-ACP substrates; class II acyl-ACP TEs have broad substrate specificities, with major activities toward 8- and 14-carbon acyl-ACP substrates; and class III acyl-ACP TEs act predominantly on 8-carbon acyl-ACPs.

Coconut palm has two acyl-ACP thioesterase A (FatA) genes in coconut palm and five FatB genes, which were CnFatB1 (CCG011598.1), CnFatB2–1 (CCG006479.1), CnFatB2–2 (CCG007799.1), CnFatB3 (CCG019705.1), and CnFatB4 (CCG015192.1). Three FatB genes were highly expressed in more than one analyzed tissue: CnFatB2–1 (leaf and embryo), CnFatB2–2 (leaf, embryo, and endosperm), and CnFatB3 (embryo and endosperm). Three acyl-ACP TEs of coconut (CnFatB1, CnFatB2, and CnFatB3) indicated divergent specificity: CnFatB1 (JF338903) and CnFatB2 (JF338904) produced major fatty acids as myristic acid (C14:0) and palmitoleic acid (C16:1); CnFatB3 (JF338905) made mainly lauric acid (C12:0) and tetradecenoic acid (C14:1) [14]. Yuan et al. transformed and overexpressed CnFatB3 in *Arabidopsis*, and the transgenic plants increased the amounts of 12:0 (lauric acid), 14:0 (myristic acid), 16:0 (palmitic acid), and 18:0 (stearic acid) by 30-, 80-, 4-, and 2-fold, respectively [6].

2.1.2 Lysophosphatidic acid acyltransferase

Coconut oil has 92% saturates and most of its TAGs are trisaturated. Moreover, laurate is found enriched at sn-2 position, which is catalyzed by membrane-bound lysophosphatidic acid acyltransferase (LPAAT) enzyme. Davies et al. detected an enzyme from coconut endosperm, which is a laurate-CoA-preferring LPAAT and active during endosperm maturation [9]. The LPAAT enzyme prefers acyl-CoAs containing C10:0, C12:0, and C14:0 acyl groups as acyl-donor substrates [9]. Knutzon et al. [11] performed the LPAAT protein purification and cloned the corresponding cDNA of this gene from coconut. The gene was then transformed and expressed in *Escherichia coli*, and substrate activity profile of this gene matched that of the coconut enzyme. This copy of LPAAT is the gene named as CCG001603.1 in the first version of coconut genome sequence [5]. Knutzon et al. transformed this gene into a rapeseed transgenic gene line which is expressed of a California bay laurel (*Umbellularia californica*) 12:0-acyl carrier protein thioesterase (BET) and contained up to 50% laurate in its developing seeds [11]. In this transgenic rapeseed with BTE, laurate is found almost exclusively at the *sn-1* and *sn-3* positions of the triacylglycerols. Coexpression of the coconut *LPAAT* gene in the transgenic rapeseeds facilitates efficient laurate deposition at the *sn-2* position and caused the accumulation of trilaurin [11].

Xu et al. cloned the promoter sequence of the LPAAT gene and characterized the promoter by constructing a series of plasmids with promoter sequences with varied length of deletions to promote a β-glucuronidase (GUS) gene. The plasmids were transformed into rice, and the transgenic plants showed that reporter genes with these promoter fragments tend to express specifically in rice endosperm [12]. Yuan et al. transformed *CnLPAAT* into yeast, and tested fatty acid composition indicated that the gene increased the levels of C12:0 and C14:0 in a CnLPAAT-pYES2 transformant [16]. However, heterologous overexpression of CnLPAAT in tobacco (*Nicotiana tabacum* L.) decreased the contents of C12:0 and C14:0 in transgenic tobacco seeds, which could result from low contents of short- and medium-chain FAs (0.22%), which are available in tobacco seeds of the total FAs.

2.1.3 Diacylglycerol acyltransferase

Besides genes important for MCFA accumulation, there are key genes in TAG biosynthesis pathway that influence oil contents and FA composition. Diacylglycerol acyltransferases (DGAT) and phospholipid:diacylglycerol acyltransferases (PDAT) catalyze diacylglycerol (DAG) to form TAG as the final step in TAG synthesis, using either acyl-CoAs or phospholipids. DAG is an important branch

point between storage and membrane lipid synthesis. Coconut palm has three orthologs of AT2G19450 (*AtDGAT1*) and two orthologs of AT3G51520 (*AtDGAT2*). Coconut *DGATs* genes had higher expression level in coconut endosperm than in the leaf and embryo, especially for *DGAT1* isoform CCG007098.3 and *DGAT2* isoform CCG026159.1 [5].

Zheng et al. cloned a DGAT2 gene from coconut pulp and transferred the gene into the deficient yeast H1246 and *Arabidopsis* [13]. The DGAT2 gene that is expressed in the deficient yeast had DGAT catalysis activity and restored TAG synthesis in the yeast. Further lipid composition analysis showed that *CnDGAT2* has a substrate preference for two UFAs (C16:1 and C18:1) in yeast and linoleic acid (C18:2) in transgenic plants. These results provide knowledge on CnDGAT2 and offer new insights into TAG assembly in coconut.

2.2 Transcription factors regulating fatty acid biosynthesis

WRINKLED1 (WRI1, AT3G54320) directly controls the transcriptional activation of the fatty acid biosynthetic pathway in *Arabidopsis* and belongs to the APETALA2-ethylene-responsive element-binding protein (AP2-EREBP) family [17]. The ortholog of *AtWRI1* in oil palm was validated as a key transcription factor associated with lipid synthesis [3]. In coconut, three AT3G54320 orthologs were found—CCG005292.1, CCG012597.1, and CCG019741.1. CCG005292.1 and CCG012597.1 were expressed in the endosperm but had low expression in leaf and endosperm, while CCG019741.1 has no expression in leaf, embryo, or endosperm [5]. The *CnWRI1* gene copy (CCG012597.1) validated its interaction with the promoter sequence of acetyl-CoA carboxylase by yeast one-hybrid system [15]. Overexpression of *CnWRI1* (CCG012597.1) specifically in *Arabidopsis* seed showed an increase of palmitic acid (C16:0) and linolenic acid (C18:3) but a decrease in oleic acid content [15].

3. Evolutionary relationship between coconut palm and oil palm

Coconut and oil palm are important oil trees grown in tropical region and closely related species with approximately 50% lauric acid (C12:0) in their endosperm. There are 806 and 840 lipid-related genes annotated for coconut and oil palm, respectively [13]. The majority of lipid-related genes between coconut and oil palm were homologous genes, while 72.8% (438/601) of genes in coconut palm were located in homologous segments with oil palm. The two species have a close evolutionary relationship between predominant gene isoforms and high conservation of gene expression bias in the lipid metabolism pathways.

Since coconut and oil palm have high lauric acid (C12:0) in their endosperm, key genes responsible for MCFA also shared high homology in gene copy and expression pattern. Both coconut and oil palm have five FATB genes, but only three *EgFatB* genes highly expressed in oil palm mesocarp or endosperm and four *CnFatB* genes were highly expressed in endosperm or embryo. Homologous gene pair—*CnFatB3* and *EgFatB3*—were both highly expressed in their endosperms, which were validated as key genes for MCFA biosynthesis [3, 6]. Another key enzyme—LPAAT, three AtLPAAT1, or AtLPAAT2 orthologs were found in each of coconut and oil palm [5]. The LPAAT1 genes were clustered into class I and class II, and the class I genes of both species had higher expression levels in endosperm tissue. Moreover, the LPAAT2 genes were also clustered into two classes, and genes in class II had low or no expression.

For the key transcription factor associated with lipid synthesis—*WRI1*, Xiao et al. [5] identified three *WRI1* genes in coconut and six in oil palm and classified

the genes into three groups based on conserved amino acid sequences. The coconut and oil palm *WRI1* genes in the same group indicated the same expression pattern: group I was highly expressed both in the coconut endosperm and the oil palm endosperm/mesocarp; group II or III has very low or no expression.

4. Methods used in validation gene function in coconut palm

Coconut palm has a long life cycle and takes 5–10 years to start reproductive stage. Since that, using gene overexpression or knockout to analyze gene function in its own plant system will take years to observe the traits related to fruits. At present, no stable transformation system has been constructed in coconut palm. The convenient ways to validate gene function in coconut are testing biochemical feature of proteins *in vitro* or transforming gene into a heterologous system, such as *Arabidopsis*, rice, yeast or *Escherichia coli (E. coli)*.

4.1 Testing enzyme activity in vitro

Lipid metabolism is composed of more than 120 enzymatic reactions. Validation of gene function related to lipid metabolism could be done by testing enzyme activity in vitro. Davies et al. have isolated CnLPAAT protein from immature coconut seeds and tested the LPAAT activity by adding Acyl-CoA and LPA as substrates [10].

Laurate is found enriched in sn-2, which indicates that a laurate-CoA-preferring LPAAT is active during endosperm maturation. Davies et al. were able to detect such an enzyme from this tissue, which allowed Knutzon et al. [11] to perform protein purification and cloning of a cDNA encoding the 299-amino acid CLP protein from coconut. When expressed in *E. coli*, and using 12:0-LPA as an acceptor, this enzyme preferred medium-chain CoAs over 18:1-CoA as acyl donors. This is a direct evidence that in coconut endosperm, not only had the common fatty acid biosynthesis pathway been modified to produce almost entirely saturated medium chains but at least one enzyme of lipid biosynthesis (LPAAT) had been modified.

4.2 Testing enzyme activity in vivo

Gene function validation has been conducted through gene overexpression in heterologous plant systems which have stable gene transformation system, such as *Arabidopsis*, rice, and tobacco. Functional characterization of *CnWRI1* was done by gene overexpression in *Arabidopsis* and rice [15]. Overexpression of CnWRI1 in *Arabidopsis* seeds caused fatty acid composition changes but not for oil content, while overexpression of the gene in rice endosperm increased the starch content and decreased the protein contents [15]. For gene function validation of CnLPAAT (CCG001603.1), this gene was overexpressed in a transgenic oilseed (*Brassica napus*) plant, which expressed a 12:0-ACP thioesterase from California bay laurel (*Umbellularia californica*). The transgenic lines that coexpressed a 12:0-ACP thioesterase and CnLPAAT had increase laurate content from 50 mol% to total laurate levels, which suggested that CnLPAAT facilitates efficient laurate deposition at the sn-2 position [11].

Transient transgenic expression system of tobacco is also widely used for gene function analysis. Genes belonging to lipid metabolism were also validated by this system, investigating the possibility of oil production in non-sees biomass [18].

Escherichia coli (*E. coli*) strains are commonly used in molecular biology, because the introduction of DNA into *E. coli* is convenient. Since lipid metabolism is basic in all living cells, specific *E. coli* strain with gene mutation could be used for analyzing enzyme functions. Knutzon et al. [11] cloned the CnLAAPT gene copy (CCG001603.1) from coconut endosperm and tested enzyme activity by introducing the gene into *E. coli* strain K27 that has a mutation in the *fadD* gene as well as β-oxidation of fatty acids. Overexpression of this *CnLAAPT* gene copy caused the accumulation of free fatty acids in the growth medium. Enzymic specificity of three acyl-ACP TEs of coconut (CnFatB1, CnFatB2, and CnFatB3) have been tested by transforming and expressing in *E. coli* K27 and analyzing free fatty acids accumulated in the medium [14].

5. Conclusions

Coconut palm (*Cocos nucifera* L) is an economically important monocot plant grown in tropical and subtropical regions. Coconut oil is stored in a solid endosperm and has 47.48–50.5% fatty acid component, which is a medium-chain fatty acid (MCFA) such as lauric acid (C12:0). Present research showed that acyl-acyl carrier protein thioesterases and lysophosphatidic acid acyltransferase are key enzymes determining MCFA accumulation in coconut oil (**Figure 1**). In this chapter, we reviewed three aspects of research related to coconut lipid metabolism. Firstly, we summarized key genes related to MCFA accumulation in coconut endosperm. Secondly, we summarized evolutionary relationship between coconut palm and oil palm for MCFA accumulation. Thirdly, we described studies using in vivo and in vitro gene validation experiments in coconut palm.

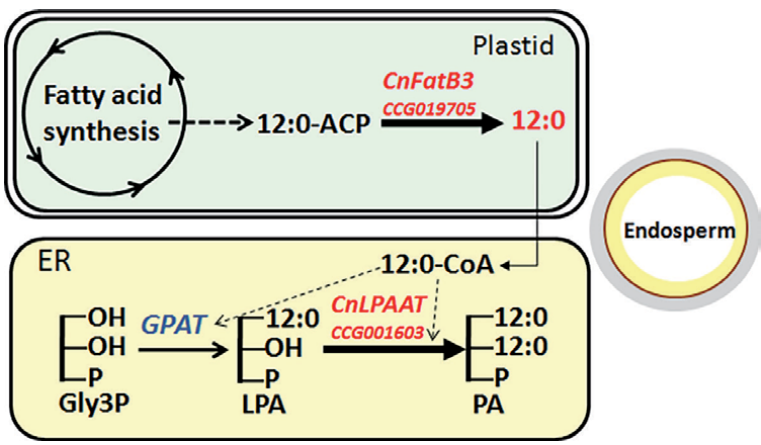

Figure 1.
The diagram of key genes involved in medium-chain fatty acid accumulation in coconut endosperm.

Conflict of interest

The authors declare no conflict of interest.

Appendices and nomenclature

ACP	acyl carrier protein
DGAT	diacylglycerol acyltransferases

FA	fatty acid
FATA	acyl-ACP thioesterase A
FATB	acyl-ACP thioesterase B
HAD	hydroxyacyl-ACP dehydratase
LPAAT	lysophosphatidic acid acyltransferase
PDHC	pyruvate dehydrogenase complex
TAG	triacylglycerols
WRI1	WRINKLED1

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Chapter 13

Gums—Characteristics and Applications in the Food Industry

Diego Aires da Silva, Giselle Cristine Melo Aires and Rosinelson da Silva Pena

Abstract

Gums, or polysaccharides, are complex carbohydrates, soluble in water, which can form gels and mucilages. They have high molar mass and can be formed by galactose, arabinose, rhamnose, xylose, galacturonic acid, among others. They have gelling characteristics, thickening, moisture retention, emulsification and stabilization. Polysaccharides are widely used in the formulation of food products, due to their wide versatility. Its diversity of applications is closely linked to its chemical structures. The characterization of structural molecules allows the knowledge of the properties of polysaccharides or glycoconjugates. In this sense, this chapter addresses knowledge about chemical, molecular, rheological, thermodynamic characteristics that are extremely important to identify the use and applications of polysaccharides in the context of elaboration and innovation in the food industry.

Keywords: gum, hydrocolloids, carbohydrate

1. Introduction

The food and beverage industries face increasingly challenging scenarios, as they need to meet consumers' desires, and use ingredients that are natural, and that fulfill their technological roles in processed foods. Among these ingredients, gums and hydrocolloids are the compounds most widely used as agents of innovation in the food industry.

Gums, also known as hydrocolloids or polysaccharides, are very versatile biopolymers, extensively used in the food sector as ingredient or additive, which fulfill several technological and, sometimes, nutritional functions. This versatility is intrinsically related to their molecular composition, which gives these polysaccharides certain properties such as gelling, thickening, moisture retention, emulsification, and stabilization. In the food industry, they are widely used in confectionery, as ice cream stabilizers, food emulsions, in the microencapsulation of flavors and dyes, clarifiers, and beverage stabilizers.

Therefore, information on the molecular structure, thermal stability, interaction with water, and rheological behavior are essential knowledge for prospecting and developing applications for each type of polysaccharide, whether isolated or in mixtures.

Another important fact, in this sense, is the constant search for new sources of polysaccharides that might have similar and/or better effects than those already known. This is important because it also shows regional valorization, source of income, and new business opportunities.

Thus, this chapter aims to discuss the physical, chemical, and molecular knowledge of polysaccharides, in addition to their versatility of applications in the food industry.

2. Gums: origin and definition

The term gum is generally used to define hydrophilic or hydrophobic molecules of high molar mass, which have colloidal properties [1]. Classified according to origin, behavior, and chemical structure, gums can be derived from plant seed endosperm (guar gum) [2], plant exudates (tragacanth), shrubs or trees (gum arabic, karaya gum, cashew gum) [2–5], algae extracts (agar) [6], bacteria (xanthan gum), animal source (chitin), and others [7–10].

Vegetable exudates are fluids that flow spontaneously from trees, due to adaptations to climatic conditions (physiological gummosis) or in response to any injury suffered, whether mechanical, such as cutting, or by the action of microorganisms, which dry out when exposed to air [11].

Hillis [12] describes in detail the differences between exudates from tree trunks, specifically the differences between resins and gums, and their formation. The author defines resins as materials composed largely by terpenoids, and that may contain phenolic compounds (coumaric, caffeic, and ferulic acids), with few fatty acids and glycerides. They may be formed within plastids present in epithelial cells of plants [13] or even synthesized in spherosomes, both in resin duct cells and in parenchymal cells [14].

Hillis [12] also defines gums as products composed mainly of complex carbohydrates, soluble in water, which can form gels and mucilages. They have high molar mass and can be formed by galactose, arabinose, rhamnose, xylose, galacturonic acid, and other compounds. In some species, they are secreted by organelles present in the bark or between barks, whose main function is protecting the plant from injuries caused by cuts or microbial attack [15–17].

The interest in gums exuded from plants is due to their structural properties and respective functions in food, pharmaceutical, cosmetic, textile, and biomedical products [18]. Water-soluble gums, also known as hydrocolloids, can have various applications such as: dietary fibers, texture modifiers, gelling agents, thickeners, stabilizers, emulsifiers, coatings, films, and as encapsulants [19, 20]. There has been a strong trend towards replacing synthetic materials by natural gums due to their non-toxicity, low cost, safety, and availability [21].

3. Structural aspects of gums

All the properties and applications of gums are closely linked to their chemical structures. Gums can be formed by numerous sugars, in their main chains and/or side chains, and can be more or less branched, which determines, in general, their complexity [15].

Among the most well-known and commercialized gums [22], the gum arabic, produced by the species *Acacia senegal*, presents in its structure a main chain formed by β-D-galactopyranose joined by bonds (1➔3), alternated by highly branched bonds (1➔6), and shows lateral chains constituted by 4-O-methyl-glucuronic acid (1.5%), glucuronic acid (17.5%), galactose (39%), arabinose (28%), and rhamnose (14%) [23]. Anderson; Hirst; Stoddart [24] proposed the structure presented in **Figure 1** for acacia gum. The authors indicated, as possible replacement units, those represented by the radical "R": (L-Araf); (L-Araf 1➔3 L-Araf); (β-L-Arap 1➔3 L-Araf);

(L-Araf 1➔3 L-Araf 1➔3 L-Araf); (β-L-Arap 1➔3 L-Araf 1➔3 L-Araf); (β-D-Galp 1➔3 L-Araf). Arabinofuranoside is Araf, arabinopyranoside is Arap, and galactopyranoside is Galp. The radicals "R" are not shown in **Figure 1B**.

Gum ghatti is also important among exudate gums because of its high emulsifying capacity [25]. It is extracted from the trunk of *Anogeissus latifolia*, an abundant tree in India [26]. Its molecular structure is formed by a main chain of (1➔6)-β-Galactose bonds, whose branches at positions O-3 and O-4 are replaced, consisting of ➔2)-Araf-(1➔4)-GlcpA-(1➔6)-Galp-(1➔6)-Galp-(1➔. The terminal

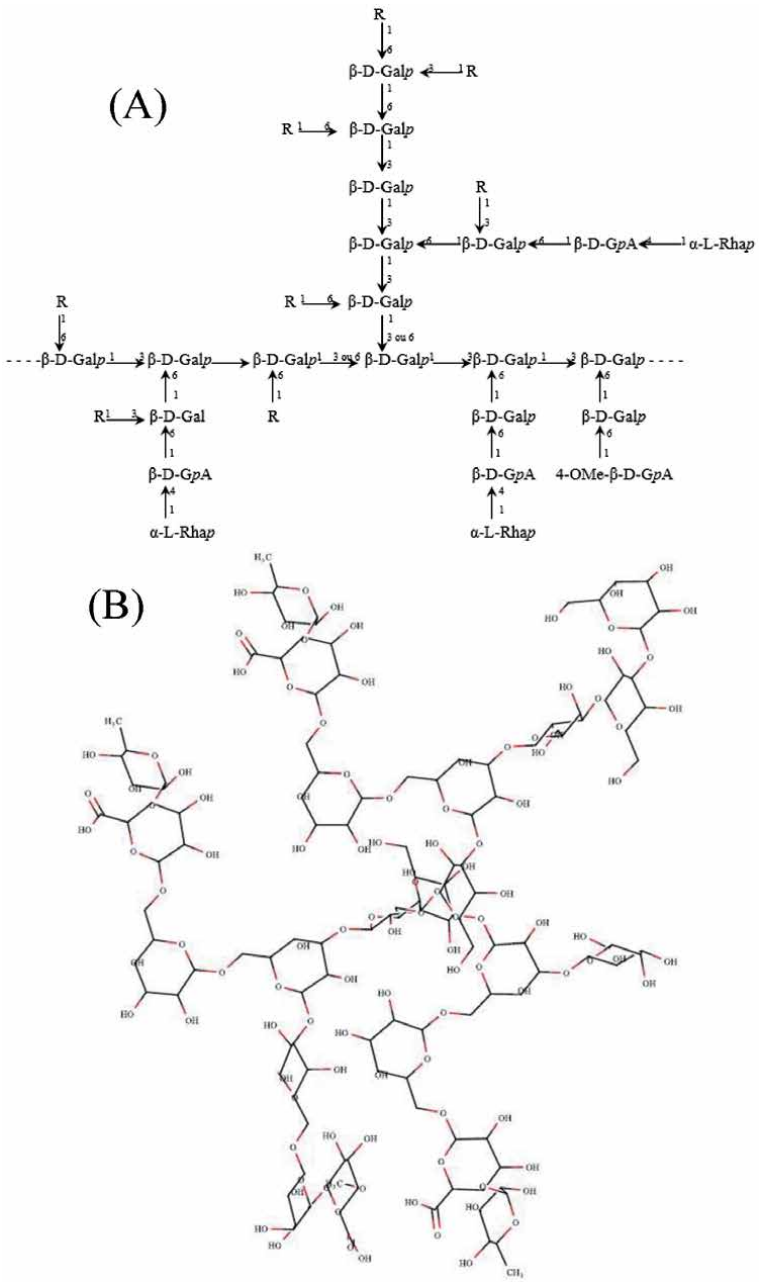

Figure 1.
*Structural fragment of gum arabic (*Acacia senegal*). (A) Scheme and (B) three-dimensional structure referring to the fragment shown.*

lateral chains are formed by residues of arabinofuranoside (Araf) and occasionally by rhamnopyranoside (Rhap), arabinopyranoside (Arap), galactopyranoside (Galp) or glucuronopyranoside (GlcpA) [27, 28]. The structure of gum ghatti is shown in **Figure 2**.

Karaya gum is also on the list of exudates from commercially interesting plants, and is extracted from *Sterculia urens* tree. Structurally, it is a complex, partially acetylated polysaccharide, composed of 55–60% of rhamnose and galactose, 8% of acetyl groups, and 37–40% of uric acid residues (galacturonic and glucuronic acids) [29]. Its structure can be seen in **Figure 3**.

3.1 Gum structure of exudates from arecaceae family species

The Arecaceae (Palmae) family consists of a large variety of monocot plants found predominantly in tropical and subtropical environments, mostly in South America, and contains 457 palm species distributed in 50 genera [30, 31].

Nussinovitch [26] described, in general, three types of gum from plants of the Arecaceae family, with sensory information about them. According to the author, *Borassus flabellifer* palm gum is a black glassy exudate, which swells and is insoluble in water; *Cocos nucifera* L. gum has coloration ranging from light brown to red, and in water, it presents certain insolubility, forms gel, and has low adhesiveness; *Corypha utan Lam*. gum has sweet odor and brown coloration, being used in medicine.

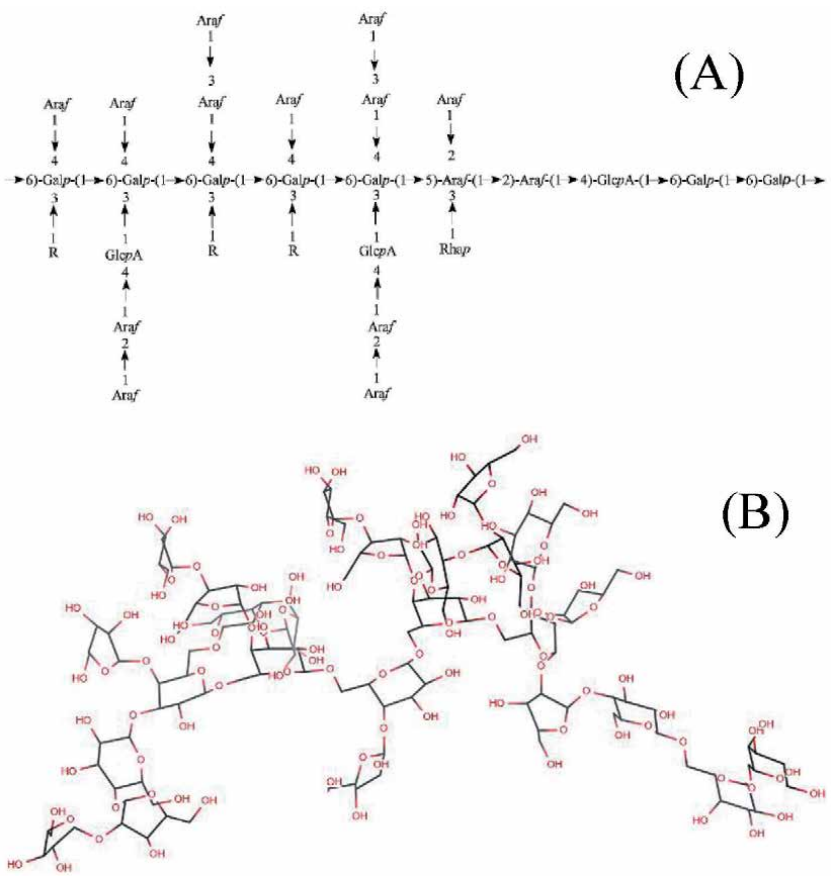

Figure 2.
Structural fragment of gum ghatti. (A) Scheme and (B) three-dimensional structure referring to the fragment shown.

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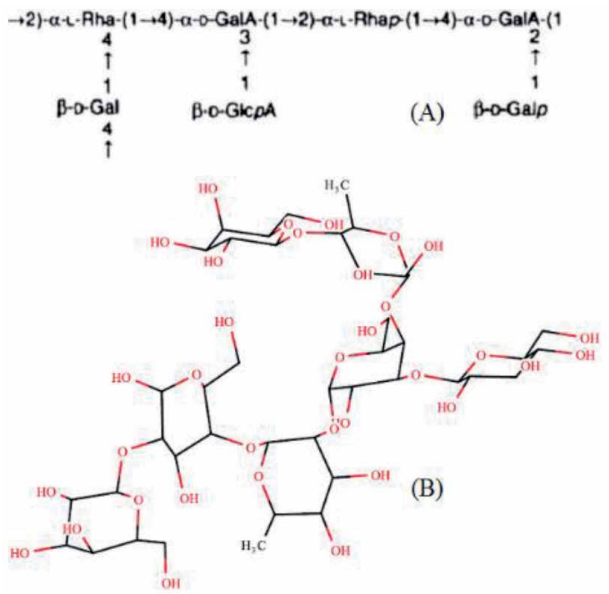

Figure 3.
Structural fragment of karaya gum. (A) Scheme and (B) three-dimensional structure referring to the fragment shown.

Gums from exudates of Chinese fan palm trunk (*Livistona chinensis*) [32] and jerivá (*Syagrus romanzoffiana*) [33] were presented as heteroxylans, whose main chain is joined by β-(1➔4) bonds, highly substituted at O-2 and O-3 positions by units of arabinose, xylose, and terminal fucose, as shown in **Figure 4**.

The exudate from Uricuri palm (*Scheelea phalerata*) was also identified by Fernanda F. Simas et al., [34]. The authors found a water-insoluble polysaccharide with a branched structure. Units of Xylp (~8%) were replaced at O-2, whereas Araf units (12%) were replaced at O-3. They also found non-reducing units of Araf (15%), Fucp (fucopyranose - 10%), Xylp (4%), and Arap (6%) as side chains attached to the main chain composed of Xylp units joined by β-(1➔4) bonds, which were replaced at 3-O-(9%), 2-O-(13%), and 2,3-di-O-(13%) positions.

The structure of the gum obtained from coconut tree trunk exudate (*Cocos nucifera*) was elucidated by Simas-Tosin et al., [35]. This gum is a glucurono-arabinoxylan composed of Fuc, Ara, Xyl, and GlcpA at molar ratio of 7:28:62:3.

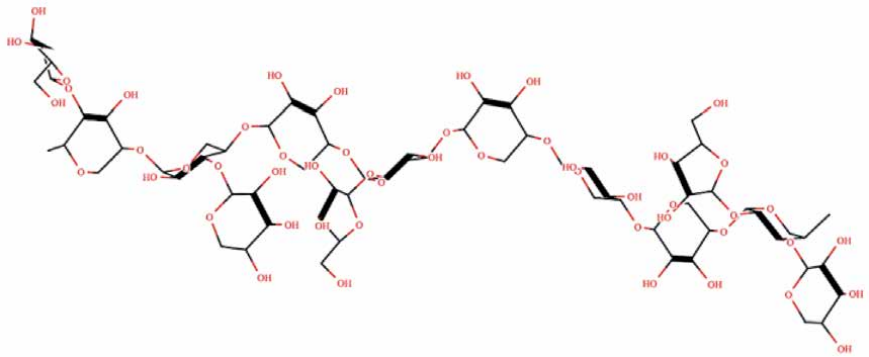

Figure 4.
Three-dimensional representation of the heteroxylan present in Scheelea phalerata *(Uricuri) palm gum, with β-(1➔4) bonds. Main chain branches are substituted at O-2 or O-3 positions by arabinose and xylose units.*

Non-reducing units substituted at 3-O (Araf - 8%); 3,4-di-O-(15%); 2,4-di-O (5%); and 2.3.4-tri-O (Xylp 17%) positions were also found, attached to a main chain composed of Xylp joined by β-(1➔4) bonds.

4. Gum characterization

4.1 Spectroscopic methods for gum characterization

"Structure is the key to everything in chemistry. The properties of a substance depend on the atoms it contains and how these atoms are bound. Less obvious, but very powerful, is the idea that someone with knowledge of chemistry can look at the structural formula of a substance and say several things about its properties" [36]. "Looking at the structural formula" inevitably refers to the use of techniques that assist in the chemical and structural knowledge of organic molecules, and in this context, spectroscopic techniques can be a very important tool to fulfill such function [37].

In order to know the properties of polysaccharides or glycoconjugates, it is essential to elucidate and characterize the structural and dynamic aspects of their molecules [38]. Carbohydrate chemistry can rely on one of the most efficient spectroscopic techniques for investigating organic compounds in solution: Nuclear Magnetic Resonance (NMR), which has advanced methods, and becomes essential in the characterization of polysaccharides with complex structures [39, 40].

The commonly used NMR techniques are hydrogen (^{1}H), carbon-13 (^{13}C), homonuclear correlations (^{1}H-^{1}H), COSY (homonuclear Correlation Spectroscopy), and ^{13}C-^{1}H HMQC (Heteronuclear Multiple Quantum Coherence) [41].

The elements that are most common in organic molecules (carbon and hydrogen) have isotopes (^{1}H and ^{13}C) capable of providing NMR spectra rich in structural information. A proton nuclear magnetic resonance spectrum (1H NMR) provides information about the environments of the various hydrogens present in a molecule. A carbon-13 nuclear magnetic resonance spectrum (^{13}C NMR) does the same for carbon atoms [36, 38].

NMR spectrum of coconut trunk gum (*Cocos nucifera*), obtained by alkaline extraction, presented approximately 10 signs in the anomeric region, which reveals a complex structure. The signals made reference to the presence of L-Araf (δ 108.6–107.0); α-Arap (δ 103.1); β-Xylp (δ 101.6), and also α-Fucp and α-Glcp units (δ 100.5–99.2), bonded to C-4. Reducing terminals were bonded to C-5 [35].

Peach gum (*Prunus persica*) was also considered as a complex molecule, as it shows 8 signs in the anomeric region (δ 110–90). The main sign in δ 103.2 refers to β-D-Galp units in the main chain, and the sign in δ 102.8 suggests the presence of β-D-GlcAp. In the substituted carbon region, the signs in δ 84.1 and δ 82.0–82.5 refer to C-3 of the replaced units α-L-Araf and β-D-Galp 3-O-, respectively [42]. These are examples that demonstrate that the NMR technique is an indispensable tool for the knowledge of polysaccharides and their properties.

Another technique widely used for the structural identification of polysaccharides, even before the advent of NMR, is the Fourier-Transform Infrared Spectroscopy (FTIR) [36]. Although NMR gives more information about the structure of an unknown compound, infrared is important because it can identify certain functional groups. Structural units, including functional groups, vibrate in characteristic ways, and this sensitivity to group vibrations forms the basis of infrared spectroscopy [43].

Molecular movements are described by two types of vibrations: deformation and stretching (**Figure 5**). The deformation causes a bond angle change that can occur in or out of the molecular plane of symmetry; and the stretching is a linear

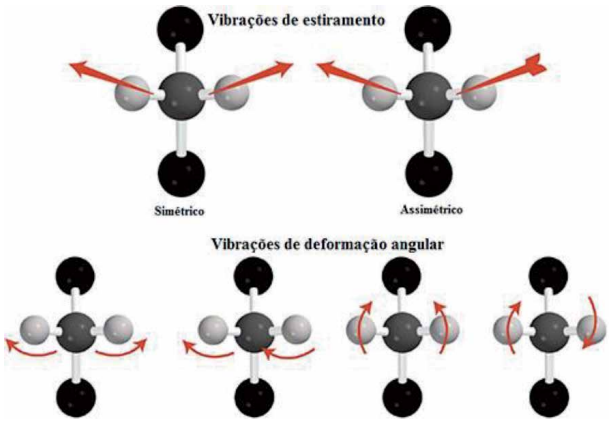

Figure 5.
Aspects of the molecule vibrations observed in infrared spectroscopy.

intermittent movement so that the interatomic distance changes constantly. It can be symmetrical or asymmetrical [44].

When irradiated by infrared light, the atoms of the molecular structure of a given sample absorb it. The vibration or rotation will depend on the type of chemical bond formed by these atoms B [45, 46]. **Table 1** shows some bands of infrared

Bands	Associated vibrations	Possible assignments to bands	References
≈1650 and 1550 cm^{-1}	ν(C=O) γ(CN) δ(NH) $(CCN)_{deform.}$ ν(C=C) $ν(COO^-)$	Amide I and II of proteins, respectively	[47–50]
1640–1600 and 1420 cm^{-1}		Carboxylic acids deprotonated in uronic acid	[48, 50]
1444, 1371, 975–978, and 923 cm^{-1}	$δ(CH_3)$ $(CH)_{deform.}$ δ(CO) δ(NH) δ(C–O) $δ(OH)_{COOH}$ ν(C–O–C) ν(CN)	Methyl ester groups (CH_3) in pectins	[51]
1280 and 1220 cm^{-1}		Methyl ester groups (CH3) in pectates	[51]
1280–1260 cm^{-1}		Phenolic esters bonded to cell walls groups	[52]
≈1230 cm^{-1}		Amide III of protein secondary structures	[49, 52]
Fingerprint region in polysaccharides			[53]
1155–1038 cm^{-1}	ν(C–O–C) ν(C–OH) ν(C–O) ν(C–C) $ν(O–CH_3)$ (CH_3) $(C_1–H)$ δ(OH) δ(CCH) δ(COH)	Galactan attached to main chain β 1➔6 Galp	[53]
1141–1039 cm^{-1}		Arabinans connected to the main and side chains of Araf	[53]
1139–985 cm^{-1}		Arabinogalactans linked to the main chain of β 1➔3 Galp, and side chain of α 1➔3 Araf (8%) and β 1➔6 Galp (92%)	[53]
1140–975 cm^{-1}		Arabinogalactan-rhamnoglycan attached to the main chain β 1➔6 Galp (24%) and α 1➔4 Rhap (42%), and side chain of α-Araf and α 1➔5 Arap (34%)	[53]
900–870 cm^{-1}		B-type bonds between monosaccharides	[54, 55]

Table 1.
Infrared Fourier transform bands in plane (δ); out of plane (γ) and stretching (ν), and assignments related to functional groups.

spectroscopy and their respective functional groups present in polysaccharides. It is also possible to see that FTIR can provide information on important functional groups in polysaccharides in the fingerprint region [44, 46].

In polysaccharides, the infrared spectroscopy can be used to qualitatively observe possible structural changes. Quelemes et al., [56] demonstrated the structural change in cashew gum when submitted to quaternary ammonium reagent, which also improved some properties such as biocompatibility and antimicrobial action. FTIR was also efficient to demonstrate that the interaction of gum arabic and chitosan was formed by electrostatic complexes, a result of the interaction between functional groups ($NH3^{+}$ and —COO-) of both macromolecules. Also, it improved viscoelastic characteristics at different pH's, demonstrating its complex versatility for use as food additives [57].

4.2 Thermal analysis of gums

Most polymers, synthetic or natural, suffer degradation when subjected to thermal stress [58]. This is attributed to chain depolymerization, point splits, or even the elimination of low molecular weight fragments, which cause mass loss due to the increase in temperature [59]. They cause thermal effects related to physical or chemical changes, and are associated with thermodynamic events [58]. These changes in energy and mass can be measured by thermogravimetry (TG), derivative thermogravimetry (DTG), differential thermal analysis (DTA) and differential scanning calorimetry (DSC), which make it possible to obtain information such as changes in the crystalline structure, reaction kinetics, melting and boiling point, glass transition, and others [60]. Changes in mass as a function of temperature and/or time [61] and continuous registration of mass subjected to heating or cooling [62] are definitions attributed to thermogravimetry.

Being the combination of an electronic microbalance and an oven, associated with a linear temperature programmer, thermogravimetric analysis consists of submitting a known mass of sample inside a crucible, suspended by a platinum wire, to a programmed temperature gradient, for a predefined time, which is automatically registered, simultaneously with the sample mass [63].

In DTG, the mass variation derivative (dm/dt) is registered as a function of temperature or time. In this method, the levels observed in TG are replaced by peaks that delimit areas which are proportional to the changes in mass suffered by the sample and can indicate the exact initial temperatures and maximum speed of reactions. DTG allows a clear distinction of successive reactions (not detected by TG), by quantitative determinations of loss or gain of mass which are associated with the peak areas [60].

DSC and DTA are analyses that measure energy gradients between the sample and a reference material subjected to controlled temperature. DSC is a calorimetric method in which energy differences are measured, whereas in DTA, temperature differences between the sample and the reference material are registered [59]. DTA provides a qualitative analysis of the thermal events experienced by the sample, whereas DSC is able to quantify such events because it measures the heat flow through a temperature gradient [64].

Changes in composition, food processing temperatures or ingredients result in changes in phase transitions of the product [65]. Quantifying the variables involved in these phenomena, such as temperature or thermodynamic quantities, is important for understanding processes such as evaporation, dehydration, and freezing [66]. Being the responsible for plasticizing effects and important component of food, water and its state transitions (gaseous or crystalline) guide such processes, and can also be used to describe the effects of temperature on physical properties [59].

4.3 Gum rheology

Natural polymers are of particular interest in rheological studies [67]. Their thickening, emulsifying, gelling, and stabilizing properties, which enable them to be used in food, pharmaceutical, and cosmetic industries are supported by a series of inter and intramolecular association mechanisms inherent to each polymer. Such mechanisms lead them to particular applications in different processes and products [68].

Gum arabic *(Acacia senegal)* 3% (m/v), originating from African regions such as Sudan, Senegal, and Mali, has typical behavior of a liquid. Sanchez, Renard, Robert, Schmitt, & Lefebvre, [69] investigated G' and G" in gum arabic, where G' is the storage modulus and indicates the portion of energy (from the applied voltage) that is temporarily stored during the test, and it provides information on the elastic characteristic of the fluid. On the other hand, G" is the loss modulus, which indicates the portion of energy used to initiate flow. It is irreversibly transferred in the form of heat and provides information on the viscous characteristics of the fluid [70]. The authors state that gum arabic presented a viscous modulus (G') greater than its elastic modulus (G'), but after 5 hours of rest, gel characteristics were identified, consequently showing a more elastic structure [69].

Acacia tortuosa gum, originating from species located in South America (Venezuela) (15% m/v), presented elastic modulus (G') greater than its viscous modulus (G"), indicating the occurrence of a gel material that became progressively weaker with increasing temperature [71]. In both studies, gums showed transition from Newtonian to non-Newtonian behavior with increasing concentration. Also, the influence of inter and intramolecular structural interactions as agents responsible for rheological changes was observed [69, 71].

The emulsifying and rheological characters of chemically modified gum arabic (*Acacia senegal*) (esterified with octenyl succinic anhydride (OSA) at different concentrations) was measured by [72]. The study revealed that the gum presented an increase in its emulsifying capacity and a gradual increase in apparent viscosity with increasing OSA content, indicating satisfying emulsion stability and potential use as microencapsulant. The electrostatic interaction between gum arabic and soy protein β-conglycinin was the mechanism that improved the flocculating action of *Acacia senegal*, in addition to providing greater elasticity at the oil/water interface of the gum, consequently improving its emulsifying capacity [73]. The interaction of gum arabic with native tapioca starch also provided improved product elasticity and adhesiveness [74]. Chenlo, Moreira, & Silva, [75], studied the rheology of aqueous dispersions of tragacanth gum and guar gum (10 g/L) during storage for 47 days. In general, the apparent viscosity decreased significantly ($\alpha = 0.05$) for both systems at low values of $\dot{\gamma}$ ($< 10s^{-1}$) and remained constant above this value. The decrease in viscosity was lower for tragacanth gum and lasted until the 15th day, whereas for guar gum, the decrease occurred until the 20th day.

Mixtures of corn starch (5% m/m) and locust bean gum (0; 0.125; 0.25; 0.50; and 1% m/v) were rheologically evaluated by Hussain, Singh, Vatankhah, & Ramaswamy, [76], who found that the addition of locust bean gum at low concentrations (0.125%) made the mixture behave as a liquid at low oscillatory frequencies (0.1 to 10 rad/s). It also presented increased elasticity, with typically solid behavior at concentrations of 0.5 to 1%, at higher frequencies (15 to 100 rad/s). Thus, locust bean gum has potential to specifically modify the structure and texture of corn starch products.

The research results showed that there are many variables that influence the rheological characteristics of gums. Among them, the fine chemical structure of the polysaccharide, their interactions, and molecular conformations can be highlighted, which confirms the importance of characterizing the structure of new gums.

5. Thermodynamic relations between gums and water

The functions derived from the physical and chemical properties of gums are closely related to the interactions of polysaccharides with water. The relationship between the water content of a product and its relative humidity at equilibrium, at constant temperature, can be expressed by characteristic curves called moisture sorption isotherms [77, 78]. In fact, the thermodynamic properties of sorption, such as water-solute affinity and spontaneity of the sorption process provide a better understanding of the water-solute equilibrium that is present in the product [79]. In addition, they facilitate the definition of order and disorder existing in water-solute systems [80].

The differential enthalpy or isosteric heat of sorption defines the amount of heat released or absorbed in the sorption process at constant pressure, and is used as an indicator of the binding force between the water and solutes of the product [81]. When the free water latent heat of vaporization is added, the integral isosteric heat of sorption is obtained, which is the total energy necessary to transfer the water molecules in the vapor state to a solid surface, or vice versa [79, 82]. Also, the differential entropy of a material is proportional to the number of available sorption sites, corresponding to a specific energy level, and indicates the mobility state of the water molecules present in the product [81]. Entropy describes the degree of disorder and randomness in the movement of water molecules, and has been used to explain how water sorption in biological materials occurs [83].

Thermodynamic properties, such as enthalpy and entropy, are necessary to design a process and to qualitatively understand the water state at a certain food surface. Alterations in enthalpy provide the energy variation of the interaction between water molecules and the adsorbent. Entropy, in contrast, may be associated with the binding or repulsion of forces and, consequently, with the spatial arrangement of the water-adsorbent relationship. Thus, entropy characterizes the degree of order or disorder existing in the water-adsorbent system [84]. Gibbs free energy, in turn, is influenced by the thermodynamic properties enthalpy and entropy, and indicates the energetic spontaneity of the water-adsorbent interaction, providing the availability of process energy. If the value of this property is negative, the process is spontaneous, and if it is positive, the process is nonspontaneous. In systems with many constituents, such as food and polysaccharides, Gibbs-free energy depends not only on pressure and temperature, but also on the amount of each component [80].

6. Gum applications

The applications of gums from plant exudates are very diversified, and can be present in various areas of the food industry: confectionery (lollipops, chocolates, jelly beans, pastilles, and others), in which there is a high sugar content and low humidity; to prevent sugar crystallization; in salad dressings (thickeners and emulsion stabilizers) [85]; in frozen products (pasta, popsicles, ice cream) [1]; in dehydrated products, such as juices obtained by spray drying, protecting important compounds such as vitamin C, anthocyanins, and improving solubility, or also as microencapsulants for colors, flavors, and oils [86]; in wine clarification; flavor fixatives and emulsifiers; and in beverages and meat products [87, 88] (**Table 2**).

In adhesion functions, gums are used as fixatives of skin bioelectrodes, dentures, ostomy devices, and transdermal membrane systems, which perform controlled release of drugs through the skin [7, 121, 122]. They are used as adhesive materials in wood-based industry, and obviously, in adhesive industries in general [123]. Gums have applicability in the pharmaceutical area as emulsifiers and reducing agents for suspended particles, laxatives, in the preparation of antiseptics, binders for tablets and pills, and

Common name	Scientific name	Main chemical compounds	Application	Reference
Gums from fruits				
Date palm mucilage	*Phoenix dactylifera*	Fructose, sucrose, mannose, glucose, and maltose	Anti-cancer action	[89]
"Erva Baleeira" Mucilage	*Cordia obliqua*	Arabinose, galactose, and pyrralinose	Expectorant, tablet binder, emulsifier	[90]
Jackfruit	*Artocarpus heterophyllus*	Galactomannan, starch	Suspension stabilizer, emulsifier, binder, mucoadhesive	[91, 92]
Gums from seeds				
Tamarind gum	*Tamarindus indica*	Glucose:xylose:galactose (3:2:1)	Tablet formulation, biodegradable support for controlled drug release (colon), bioadhesive	[93, 94]
Fenugreek mucilage	*Trigonella foenum-graceum*	Galactomannan	Textural and sensory properties of soup powder/ anthocyanin encapsulation	[95, 96]
Locust bean gum	*Ceretonia Siliqua*	D-galacto-D-manoglycan, cellulose, galactomannan	Superdisintegrant in controlled drug delivery system	[97, 98]
Tara gum	*Caesalpinia spinosa*	Mannose:Galactose (3:1)	Smart food packaging	[99]
Gleditsia triacanthos gum	*Gleditsia triacanthos*	Galactomannan	Matrix formulation for tablets	[100]
Cassia tora Mucilage	*Cassia tora*	Arabinose and glucose	Suspension stabilizer, binder	[101]
Flamboyant gum	*Mimosa scabrella*	Mannose:Galactose (3.65:1)	Dietary fiber, probiotic viability in milk drink	[102]
Guar gum	*Ocimum americanum*	Xylose, arabinose, rhamnose, and galacturonic acids	Guar gum nanocomposite films	[103]
Gums obtained from tree trunks exudates				
Albizia stipulata Boiv. gum	*Albizia stipulata* Boiv.	Arabinose, galactose, and rhamnose	Antioxidant properties	[104]
Almond gum	*Prunus amygdalus*	Aldobionic acid, L-arabinose, L-galactose, and D-mannose	Emulsifier, suspension stabilizer, binder, thickener	[105]
Cashew gum and cashew nut gum	*Anacardium occidentale*	Galactose, arabinose, rhamnose, glucose, glucuronic acid	Encapsulation of a lipid shrimp waste extract, anti-inflammatory effect	[86, 106]

Common name	Scientific name	Main chemical compounds	Application	Reference
Cherry gum	*Prunus avium*	Arabinogalactan	Coating film	[107]
Raphia hookeri gum	*Raphia hookeri*	Mannose and galactose	Aluminum anti-corrosion agent in acid medium	[108]
Tragacanth gum	*Astragalus gummifer*	D-galacturonic acid, D-galactose, L-fucose (6-deoxy-L-galactose), D-xylose, L-arabinose, and L-rhamnose	Catalyst in the production of nanoparticles	[109]
Gum kondagogu	*Cochlospermum gossypium*	Rhamnogalacturonan	Production of biocompatible and antimicrobial scaffold for bandages	[110]
Gums obtained from leaves				
Cocculus hirsutus mucilage	*Cocculus hirsutus*	Polysaccharides and gelatinous materials	Binding agent, gelling agent (drugs)	[111]
Hibiscus mucilage	*Hibiscus rosa-sinensis*	L-rhamnose, D-galactose, Dgalactouronic acid, and D- glucuronic acid	Controlled drug release	[112, 113]
Gums obtained from microorganisms				
Curdlan gum	*Agrobacterium* spp.	Glucose	Food additive, thickener, gelling agent	[114]
Gellan gum	*Sphingomonas* spp.	Glucose, rhamnose, and glucuronate	Emulsion stabilizer, ophthalmic hydrogel	[115, 116]
Cholic acid	*Escherichia coli*	Fucose, glucose, glucuronate, and galactose	Viscosity enhancer	[114]
Xanthan gum	*Xanthomonas spp.*	D-glucose, D-mannose, and glucuronic acid	Carotenoid encapsulation for use in yogurts	[117]
K30 antigen	*Escherichia coli*	Mannose, galactose, and glucuronate	Viscosity enhancer/ controlled drug release	[114]
Gums obtained from tubers				
Konjac glucomannan	*Amorphophallus konjac*	D-Glucose and D-mannose	Gelling agent, controlled drug release	[118, 119]
Taro	*Colocasia Esculenta*	Galactose and arabinose	Gelling agent, mucoadhesives	[120]

Table 2.
Applications of gums from various origins.

in the cosmetics area (perfume fixers, skin cleansers, and repellents) [124–127]. Also, in the medical field, gums are used to control osmotic pressure, in addition to having activity against *Leishmania amazonensis* and antifungal properties [128].

The most recent studies have shown that the versatility of gum use has increased. The beverage industry, for instance, is always seeking products with greater stability. Some polysaccharides are excellent stabilizers, such as tara gum, which is often used to stabilize casein aggregation in dairy drinks, improving phase separation. This occurs because tara gum makes it difficult to approach casein molecules, providing greater stability and improving the sensory acceptance of the product [125].

Carrageenan gum, xanthan gum, guar gum, sodium alginate, carboxymethyl cellulose, gum arabic, and pectin were tested to prevent the formation of turbidity, caused by protein-polyphenol complexation, in packaged beverages. Among them, pectin, xanthan gum, and guar gum showed the best results [126]. These polysaccharides, when present in low concentrations: 0.5, 0.05, and 0.01 mg/mL, compete with proteins to bind polyphenols, which decrease protein-polyphenol aggregation; or they can form a ternary complex (protein-tannin-polysaccharide) to increase the solubility of protein- polyphenol systems. This mechanism promotes the reduction of unwanted turbidity in such products [127].

The use of gums and polysaccharides in film production is also an area of great concentration of studies. Active, functional, and biodegradable packagings are examples which may have antibacterial activity.

Tragacanth gum, for instance, showed excellent results in the production of nanocomposite biofilms, and can be applied in the prevention of lipid oxidation in high-fat foods, with antimicrobial action and excellent responses to biodegradability tests [128, 129]. In addition, chemically modifying the gums to improve their hydration control, gel formation, and swelling can also be an interesting way to use these polysaccharides to produce biodegradable films, which have a good response in prolonging food quality [130].

Gums can offer great innovation opportunities for the food sector. Its use is reported in wastewater treatment and in the production of nanoemulsions, and micro and nano encapsulation of dyes, essential oils, and probiotics [131–136].

Therefore, it is important to encourage the search for new sources of gums and polysaccharides from biodiversity, as their applicability and benefits can and, obviously, should be explored.

7. Conclusion

Gums have incredible versatility and are a rich source of innovation in food formulations and elaborations in the industry. They can be used both in isolation and in mixtures and can be modulated to deliver not only taste and nutrition, but also a new consumption experience, whether due to texture or applied technology. It is important that new sources of these carbohydrates are increasingly known, as there is still much to explore in this area.

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Chapter 14

A Review on Effects of Pseudo Cereals Flour on Quality Properties of Biscuit, Cookies and Cake

Abu Saeid and Maruf Ahmed

Abstract

Gluten free products are currently highly demandable by those with different gluten intolerances. Pseudo cereals are a category of non-grass seeds used to manufacture various gluten free products, including bread, biscuits, cakes, and cookies. Pseudo cereal seeds contain high-quality proteins, rich quantities and unique characteristics of starch, vast amounts of micronutrients such as minerals, vitamins along with diverse bioactive compounds. This chapter is focused on other resentful research work on the characteristics of pseudo cereals seeds and pseudo cereals flour. It also reveals different effects of pseudo cereal flour on physical-chemicals properties of biscuit, cake, and cookie. We think that this study will have a significant influence on product developers and customers on the use of pseudo cereal seeds and pseudo cereals flour.

Keywords: pseudo cereals seeds, pseudo cereals flour, biscuits, cake, cookies

1. Introduction

Pseudo cereal is a category of non-greases that can be ground into flour and then used as cereals. Most of the pseudo cereals are amaranth (*Amaranthu* spp.), quinoa (*Chenopodium quinoa*), and buckwheat (*Fagopyrum esculentum* and *Fagopyrum tartaricum*). Pseudo cereals have high-quality proteins, rich in starch, minerals, vitamins, and bioactive compounds. Pseudo cereals could be the alternative option for developing gluten free food products for people suffering from various gluten intolerances. That is why interest in pseudo cereals has increased enormously since the turn of the century, and research has intensified [1].

Buckwheat (*Fagopyrume sculentum*) is nutritionally enriched due to its high amount of vitamin B_1 and B_2, proteins with significant amount of essential amino-acid. It is a rich source of flavonoids, phytosterols, soluble carbohydrates, and other substances like D-chiro-inositol, fagopyritols, or thiamine-binding proteins [2]. Buckwheat also contains a higher amount of rutin (quercetin-3-rutinoside) than other crops with significant antioxidant, anti-inflammatory, and anticarcinogenic properties. Food products made from buckwheat which have many different biological effects, include promoting intestinal microbiota and growth support of colonies of lactic acid bacteria in the gastrointestinal tract, inhibiting proteases' scavenging ability of free radical, glucose- and cholesterol-lowering effects [3].

Amaranth (*Amaranthus* L.) has a higher amount of protein content (14–19%) than that of other traditional cereal crops with almost an acceptable proportion of essential amino acids which are rich in lysine and methionine [4]. The quality of starch content is low and there is no amylose (approximately 10% of starch, while amylopectin is 90%). Amaranth contains a good source of flavonoids and tocotrienols. Besides, lipid content is essential in amaranth seed included 6-7% of squalene compounds can reduce cancer risk, lipid metabolism control, anti-aging effects on the skin, and positive implications on the human immune system. Amaranth is also a rich source of magnesium, potassium, phosphorous and zinc minerals [5].

Quinoa (*Chenopodium quinoa* Willd) is rich in macronutrients, especially proteins which are analogous to the quality of the casein. Quinoa contains protein that is gluten free because of the lack of prolamins. Quinoas possess useful levels of lipids, such as monounsaturated fat (as oleic acid) and small quantities of omega-3 fatty acids such as alpha-linolenic acid, which are safe for health. It also contains higher fiber, mineral and carbohydrates such as polysaccharides that have a low glycemic index. Quinoa also is a pioneer in phytochemicals, antioxidants such as tocopherols and flavonoids such as quercetin and kaempferol [5–7]. Quinoa contains saponins and other valuable micro- and macronutrients [8].

Pseudo cereals have been widely recognized for many years due to their nutritional value by food scientists and food producers [1]. Many studies have investigated the use of pseudo cereals in the production of gluten free products rich in nutrients such as bread, pasta and confectionary products [8]. Flour, soup, cereal breakfast as well as beer are made using quinoa. Quinoa flour is used with wheat flour or corn meal to make biscuits, bread and processed food such as spaghetti [9]. On the other hand, buckwheat is used as a food supplement that can have a positive health impact and avoid foods being oxidized during processing. Buckwheat is recognized and recorded as part of wheat bread [10, 11]. Amaranth grain has high-quality protein, and flour is used in non-gluten formulations to obtain decent quality bread and cookies [12]. Hozova et al. [13] also proposed the use of amaranth flour to manufacture high-protein/energy-value gluten-free crackers and biscuits.

2. Types and characteristics of pseudo cereals

Pseudo cereals are non-grass species, eaten as grains, and having nutritional value is competitive or in most cases even better [14]. Amaranth (*Amaranthus L.*), quinoa (*Chenopodium quinoa*) and buckwheat (*Fagopyrum spp.)* are the main cereal-referential species (**Figure 1** and **Table 1**). Pseudo grain is not actual grain; it is dicotyledonous and pseudo grain is equivalent to true grain composition (**Table 2**). Hull (whether glossy or dull, brown, black or gray), testa, aleurone and starchy endosperm that occupy most of the seed (**Figure 2**) are the principal components of the buckwheat kernels (where there is perisperm absent). Pseudo cereal grain constitutes a healthy protein source, amino acids, vitamins, minerals and fatty acids [24]. In amaranth and quinoa seeds higher protein and fat levels are observed compared to common cereals due to high concentrations of amaranth and quinoa bran [25].

3. Nutritional compositions and application of pseudo cereals

3.1 Buckwheat

Buckwheat (BW), which belongs to the Polygonaceans families, is a typical Central and Eastern Europe and Asia crop. BW is widely used as a pseudo cereal

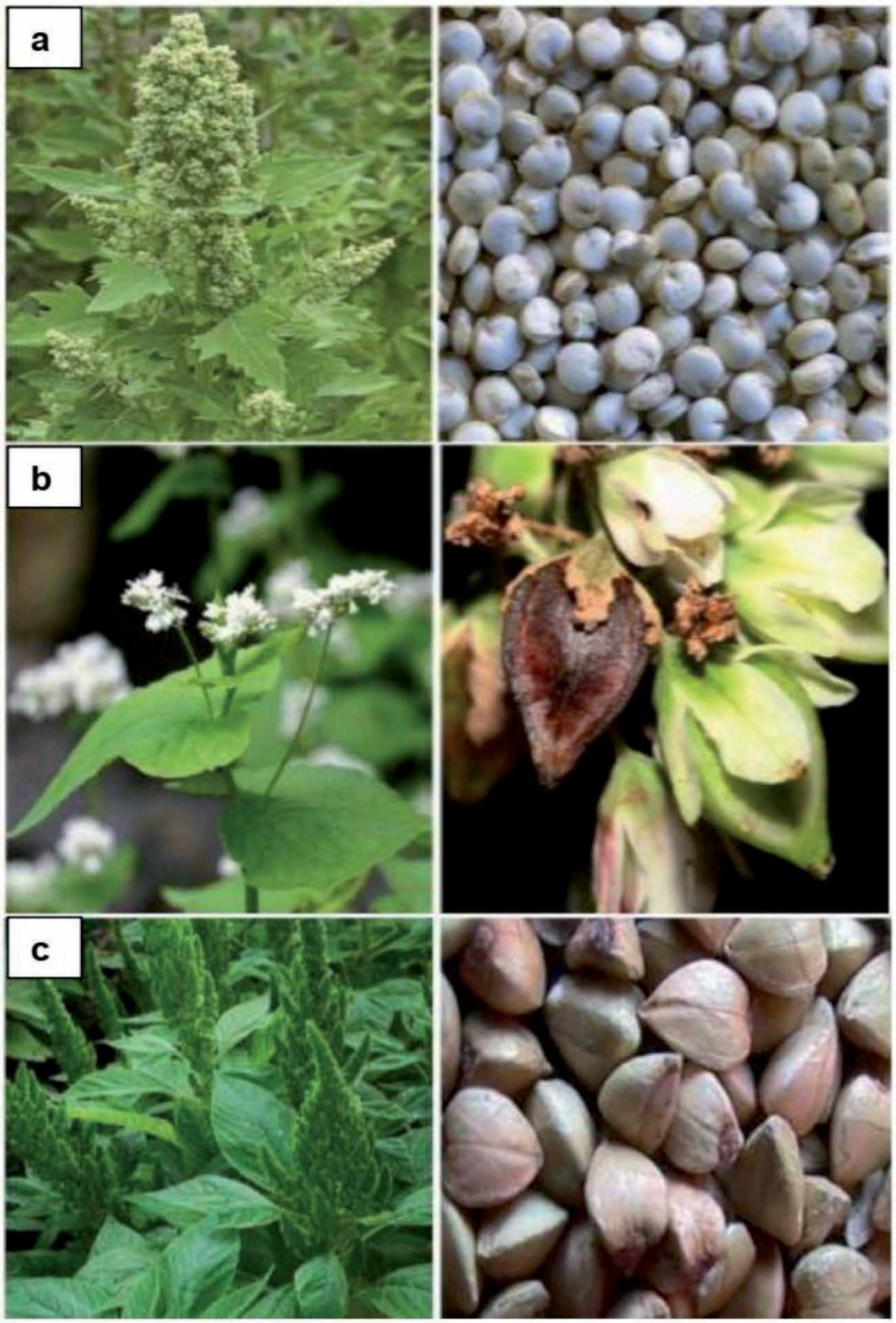

Figure 1.
Different types of leaves and seeds pseudo cereals: (a) quinoa; (b) buckwheat; (c) amaranth.

as an essential functional food. Grains of BW provides various valuable vitamins (B_1, B_2, B_6, and E) and minerals (P, Fe, Zn, K, and Mg) [26]. The biological value of BW proteins is high, but their digestibility is relatively low. The BW protein amino acids are well-balanced and rich in lysine [27] (**Table 2**). Besides, the contents of rutin, catechins, and polyphenols and their potential antioxidant activity are also of

	Cereals							Pseudo cereals			
Name	**Wheat**	**Rye**	**Barley**	**Oat**	**Rice**	**Corn Sorghum**		**Millet**	**Amaranth**	**Quinoa**	**Buckwheat**
Class				*Monocotyledoneae*						*Dicotyledoneae*	
Order					*Poales*					*Caryophyllales*	
Family					*Poaceae*			*Amaranthaceae*			*Polygonaceae*
Subfamily		*Pooideae*		*Bambusoideae*				*Panicoideae*	*Amaranthoideae*	*Chenopodioideae*	*Polygonoideae*
Tribe	*Triticeae*		*Poeae*		*Oryzeae Andropogoneae*			*Paniceae Amarantheae*		*Chenopodieae*	*Fagopyreae*
Genus	*Trtiticum*	*Secale*	*Hordeum*	*Avena*	*Oryza*	*Zea*	*Sorghum*	*Pennisetum Panicum Setaria Paspalum*	*Amaranthus*	*Chemopodium*	*Fagoryrum*
Species	*T. aetivum, T.aestivum* spp., *spelta, T. durum*	*S. cereals*	*H. vulgare*	*A. sativa*	*O. sativa*	*Z. mays*	*S. bicolor*	*P. glaucum (pearl millte) P. meliaceum (proso millet) S. italica (foxitail millet) P. sacrobiculatum (kodo millet)*	*A. caudatus, A. cruentus, A. hypochondriacus*	*Ch. quinoa Wild, Ch. pallidicaule Aellen (kanigua/ kanihua/ canihua) Ch. nuttalia Safford*	*F. tartarum F. esculentum Moench*

Table 1.
Botanical classification of cereals and pseudo cereals [15, 16].

Parameters	Wheat flour	Quinoa flour	Buckwheat flour	Amaranth flour
Moisture (%)	10.12	9.97	9.72	6.21
Ash (%)	0.48	2.28	1.92	2.53
Protein (%)	11.2	12.4	11.5	17.0
Cellulose (%)	0.60	2.6	1.3	5.54
Fat (%)	0.70	5.3	2.4	4.90
Phytic acid/Phytate (mg/100 g)	196.0	1574	1335	237.75
Phytate phosphorus (mg/100 g)	38.35	443.88	376.48	—
Minerals (mg/100 g)				
Ca	20.05	30.5	17.96	1533.0
Cu	0.31	0.81	0.78	0.52
Fe	1.38	4.10	2.87	1.40
K	181.4	681.1	479.8	200.0
Mg	29.9	174.3	190.6	68.5
Mn	0.37	1.6	1.23	1.02
P	208.7	504.3	444.8	257.74
Zn	1.23	4.04	3.01	0.21

Table 2.
Chemical composition of wheat and pseudo cereals flours [17–21].

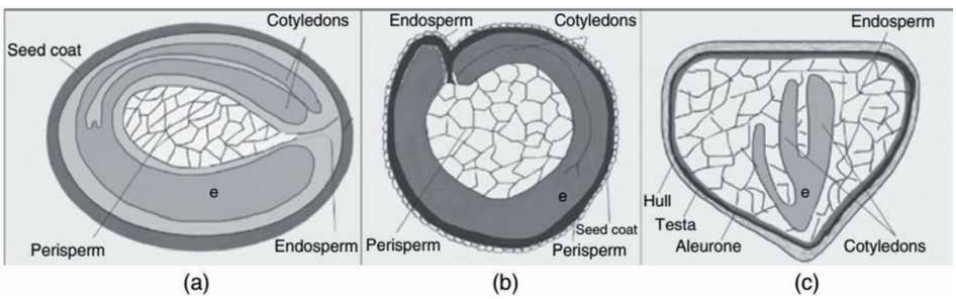

Figure 2.
Longitudinal sections of seed structures of the three major groups of pseudo cereals: (a) amaranth; (b) quinoa; (c) buckwheat [22, 23].

great significance [28]. BW does not contain gluten and can be used in celiac disease patients. Resistant starch content is a significant factor in BW 's preparation of low glycemic index food [28]. Some early studies also showed that BW flours and bran could prepare different bakery products, pasta-noodle, cookie, cake, crepe, breakfast cereal and soap formulations [11, 29, 30].

3.2 Amaranth

Amaranth (*Amaranthus* spp) is an indigenous pseudo grain domesticated in South America and has potential worldwide agronomic value [22]. Amaranth is a highly nutritious pseudo cereal that does not contain gluten. Due to its high protein content and its similar composition of essential amino acid [31], amaranth seed nutrient quality is superior to that of most cereal grains (**Table 2**). Additionally,

lysine is abundant in amaranth grain, which is typically deficient in cereal grains. The overall content of minerals, especially calcium and magnesium, is generally more significant than observed in the grains [8]. Amaranth grain can be toasted, popped, extruded or milled into flour, thus eaten as various cereal products, including bread, cakes, muffins, cookies, dumplings, crepes, noodles and crackers. Some studies have found that amaranth grain could be used in gluten free goods such as crackers, maize tortillas, chips and bread [32, 33]. It is also used in foodstuffs to increase nutritional supplies that typically lack a celiac diet [34].

3.3 Quinoa

Quinoa belongs to the *chenopodiaceae* family, genus *chenopodium* is a pseudo cereal of the Andean regions of South America [35]. Quinoa seed can constitute a rich source for essential fatty acids, including linolenic (18:2n-6:52%) and linolenic (18:3n-6:40%) [36]. Quinoa is a good protein source, provided the nutritional profile of the material (12-18 g/100 g in dry weight), fiber, vitamins (such as C, E and B complex), calcium, magnesium, iron, copper and zinc have powerful content [35]. Several antinutrients, including saponins, phytic acid, tannins, protease inhibitors and others, have been found in quinoa [37]. The amino acid balance of quinoa is higher than the lysine based wheat and maize [38]. Quinoa flour can be added as a substitute for wheat flour as in bread (10–13% quinoa flour), noodles and pasta (30–40% quinoa flour), and sweet biscuits (60% quinoa flour) [39].

4. Impact of pseudo cereals flours on quality parameters of biscuits, cookies and snacks

4.1 Buckwheat biscuits and snacks

Increased market demand for composite flour based bakery products such as biscuits, snacks, or cereals has recently been noted. Buckwheat flour biscuits and corn snacks were produced by Wójtowicz et al. [40] and Baljeet et al. [41], using back wheat up to 20% and 30%, respectively. Buckwheat flour biscuits are nutritionally rich (**Table 3**) [41]. Filipčev et al. [43] have successfully integrated buckwheat flour (up to 50%) and made biscuits of ginger nuts and have found higher nutritional and bio-functional properties compared to control (**Table 4**). Biscuit thickness increased while the spread ratio and percent spread decreased due to decreased diameter with the inclusion of buckwheat flour (**Tables 3** and **4**) [41]. On the other hand, with the integration of buckwheat flour, biscuits' texture decreased in terms of (fracture strength) due to the decreasing gluten content in the buckwheat flours, the biscuits became soft with increasing BWF content [41]. The increase in the weight of biscuits was possibly due to buckwheat flour's ability to hold oil during baking [44]. Baljeet et al. [41] found that an improvement in the percentage of buckwheat flour in composite flour decreases the biscuit's sensory ranking.

4.2 Amaranth snacks and cookies

The protein and ash content of defatted amaranth snacks was higher, while the carbohydrate and lipid content were lower than maize snacks [45]. Compared to cookies made from wheat flour, the spread of cookies made with amaranth flour decreased significantly at 10-20%. Cookie thickness increased by up to 20% with the addition of amaranth flour, with marginal changes in thickness were observed.

Parameters	Control	Buckwheat		
		30%	40%	50%
Moisture content, g/100 g	9.47	8.94	8.56	8.70
Protein content, g/100 g	7.22	7.73	7.84	8.12
Fat content, g/100 g	8.12	8.43	8.45	8.59
Starch content, g/100 g	45.80	43.64	43.77	43.21
Total reducing sugar content	29.37	25.33	25.90	27.29
Total dietary fibers content, g/100 g	3.87	5.49	5.37	7.61
Zn mg/100 g	0.34	0.78	1.00	1.16
Cu, mg/100 g	0.07	0.15	0.16	0.22
Mn, mg/100 g	0.61	0.88	0.88	0.93
Fe, mg/100 g	0.57	1.39	1.66	1.72
Rutin content, mg/100 g	ND*	3.96	5.24	6.57
Quercetin content, mg/100 g	ND*	0.087	0.143	0.214
Total phenolics, mg GAE/100 g	157.06	196.35	202.58	238.92

ND-Not detected.*

Table 3.
Chemical composition of buckwheat supplemented ginger nut biscuits (dry basis) [42].

Parameters	Control	Buckwheat		
		30%	40%	50%
Antioxidative activity (AOA)	32.51	26.71	25.37	23.83
Reducing activity	29.36	28.46	28.00	26.2
DPPH scavenging activity	23.06	10.79	9.66	5.25
Chelating activity	11.24	11.84	11.35	11.21

Table 4.
Antioxidant potential profile (IC_{50}, mg/ml) of buckwheat supplemented ginger nut biscuits [42].

The breakage of the cookies decreased significantly with the addition of amaranth flour (**Tables 5** and **6**). Similar conclusions were observed in cookies from sorghum-wheat and oat-wheat mixtures. Hoseney and Rogers [47] reported that cookies' hardness is caused by protein and starch interactions with hydrogen bonding systems. It was noted that the diameter of composite cookies shows a rising trend along with the increasing degree of substitution of amaranth flour. This may be attributed to the lower viscosity of amaranth flour than wheat flour, as the viscosity decreases with the increase of the volume of amaranth flour and the spread rate. The results reveal that the spread ratio of the composite cookies displayed an increasing trend along with the increasing substitution level of amaranth flour. The decreased durability of amaranth flour replacement in cookies could be due to changes in gluten content. The delayed production of gluten matrices, which has contributed to an enormous decline in hardness, is also attributable to gluten reduction in cookie dough by the substitution of Amaranth flour [46]. Chauhan et al. [48] no major change was observed in color, aroma and texture of cookies made from mixtures with up to 100% amaranth flour. The sensory score for the taste decreased after addition of amaranth flour of 60%. This may be attributed to the bitter aftertaste of the amaranth flour. The overall acceptability score indicated that the cookies prepared up to 60% amaranth flour

Amaranth flour (%)	Weight (g)	Spread "W" (mm)	Thickness "T" (mm)	Spread ratio "W/T"	Breaking strength (kg)
0	19.46c ± 0.11	85.6a ± 0.66	10.9d ± 0.08	7.82a ± 0.03	4.935a ± 0.19
10	18.90d ± 0.21	81.30b ± 0.55	12.2a ± 0.08	6.69b ± 0.03	4.990a ± 0.28
20	19.72b ± 0.23	79.0bc ± 0.81	12.1ab ± 0.09	6.52c ± 0.14	4.887a ± 0.21
25	19.90a ± 0.13	77.6 cd ± 0.72	11.9b ± 0.11	6.52c ± 0.09	4.021bc ± 0.22
30	19.70b ± 0.18	75.9 dc ± 0.81	11.6bc ± 0.09	6.52c ± 0.10	3.948c ± 0.25
35	19.72b ± 0.15	75.0 dc ± 0.95	11.5c ± 0.13	6.52c ± 0.04	3.0293d ± 0.32
SEM (±)	0.10	0.15	0.13	0.08	0.12
Df	18	18	18	18	54

Note: Values for a particular column differ significantly when followed by different letters ($p < 0.05$); SEM, standard error of meant at 30 degrees of freedom.

Table 5.
Effect of replacement of wheat flour with amaranth flour on the sensory characteristic of cookies [46].

Amaranth flour (%)	Surface color (10)	Surface cracking (10)	Texture (10)	Mouthfeel (10)	Flavor (10)	Overall quality (50)
0	8.0c	7.8d	8.5b	8.5b	8.4c	40.7d
10	8.0c	8.0c	8.2c	8.5b	8.5b	41.2c
20	8.5b	8.2b	8.1 cd	8.4bc	8.5b	41.7c
25	8.8a	8.5a	8.6a	8.6a	8.7a	43.2a
30	8.9a	8.4a	8.5a	8.5b	8.6ab	42.9ab
35	8.7a	8.5a	8.5a	8.6ab	8.6b	42.4b
SEM (±)	0.10	0.12	0.11	0.14	0.14	0.23

Note: Values for a particular column differ significantly when followed by different letters ($p < 0.05$); SEM, standard error of meant at 30 degrees of freedom.

Table 6.
Effect of replacement of wheat flour with amaranth flour on the physical characteristic of cookies [46].

had most acceptable sensory attributes. This was against Sindhuja et al. [46], which showed that cookies with 25% amaranth flour were most acceptable for panelists. It has been reported that, no significant difference was demonstrated in color, smell, texture of biscuits made from 20, 30, 40 and 50% of amaranth flour but biscuit made from 40 and 50% of amaranth flour had significantly higher value than wheat flour (control). Overall acceptability score showed that biscuit made from maximum 40% amaranth flour best good sensory attributes [49].

4.3 Quinoa cookies and biscuits

The Demir and Kılınç study [50] that cookie samples have significantly increased ash, crude protein and crude fats ($p < 0.05$) with the addition of quinoa meal. In cookies made with different amounts of quinoa flour, the meaningful effect ($p < 0.05$) for the total content of K, Mg, Ca, Fe and Zn has been observed. Calcium, magnesium, iron and zinc are usually greater in quinoa than ordinary cereals, and their iron contents are very high [51, 52]. The use of quinoa flour was stated to lead to a slight increase in product thickness, but the cookie samples' spread ratio and diameter decreased [50]. When quinoa flour added, the hardness of cookies increased by up to 30% [53]. The sensory characteristics of cookie

samples were influenced by quinoa flour. Added quinoa flour had statistically significant color, taste, crispness and total acceptability except odor ratings. Biscuits made of 100% quinoa flour ($p < 0.05$) vary considerably from the controls.

5. Conclusion

Pseudo cereal is a house of high-quality proteins with essential amino acids. It can be used to formulate gluten free food items as an alternative to wheat proteins in subjects suffering from celiac disease, due to the absence of gluten. In addition to the excellent nutrient profile, pseudo cereals are promising sources of phytochemical substances with significant health-promoting properties. Today pseudo cereals like buckwheat, quinoa and amaranth are incorporated successfully in bakery items such as biscuits, cookies, breads and snacks. Up to 50% buckwheat flours were used to produce nutritionally rich ginger-based biscuits with wheat flour. There was no loss of customer acceptance for the 60% amaranth flour used in the cookies production. Besides, the hardness of cookies increased by up to 30% of quinoa meal. This chapter also highlighted the actual color, taste, texture, and nutritional properties of pseudo cereal flour on biscuits, cookies, and the cake quality. Therefore, we assume that this study would considerably affect developers and customers and extensive understanding of pseudo cereal seeds and pseudo cereal flour.

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Chapter 15

Valorization Options of Strawberry Extrudate Agro-Waste. A Review

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Abstract

This review summarizes and critically analyzes the different types of potential valorization options for strawberry extrudate in order to have a broader overview of the potential management of this waste. Animal feed is commonly used as a management option for the strawberry extrudate; however, most of the strawberry extrudate is disposed in landfills. Strawberry extrudate contains different bioactive compounds that encourage the use of an alternative management approach than landfilled. The present review offers a complete comparative, including the advantages and drawbacks of each reviewed technique, to facilitate the selection of the most suitable technology for the different valorization scenarios. This review has been structured in three sections: 1. Composition of the strawberry extrudate and strawberry especially focused on their content in bioactive compounds. 2. The different techniques of extraction and purification of bioactive compounds. 3. The handling and management of the resulting biomass after the extraction process of bioactive compounds.

Keywords: strawberry extrudate, bioactive compounds, bioproducts, extraction techniques, purification

1. Introduction

In 2016, 8 million tons of strawberry were produced in the world with a value of agricultural gross production of 17,739 million US$ [1]. Besides its market as fresh product, strawberry is also used to produce many types of by-products, due to its peculiar flavor and aroma. Strawberry by-products are mainly formulated from a strawberry concentrate. The most common technology to obtain the strawberry concentrate is by extrusion. Strawberries are extruded by twin-screws up to several sieves with different mesh sizes. The sieves retain a residual fraction formed by the fibrous part and the achenes, named strawberry extrudate, which accounts about 7% of the manufactured strawberry [2].

Animal feed is commonly used as a management option for the strawberry extrudate, however, most of the strawberry extrudate is disposed in landfills, contributing to greenhouse emissions due to its high organic load [3]. Alternatives for strawberry extrudate management are required to avoid severe environmental

impacts that cause landfills, such as negative effects on agricultural soil quality, polluting of aquatic ecosystems and atmospheric contamination [4].

Similar to strawberry, strawberry extrudate contains substances of high interest such as bioactive compounds. Some of these bioactive compounds have beneficial health effects on cardiovascular, neurological or cancerous disorders [5]. Due to their health benefits, bioactive compounds have an economic interest for different commercial sectors, such as the pharmaceutical, food and chemical industries [6]. Added to bioactive compounds, strawberry extrudate could be used to obtain other types of resources such as bioenergy [7, 8]. It is also well known the high phenolic composition in the achenes and in the pulp of the strawberry [9].

A general biorefinery scheme as a management option for the strawberry extrudate should look for synergies between unitary processes of extraction of bioactive compounds, purification and the management of the final biomass of the strawberry extrudate after extraction (**Figure 1**).

The extraction of bioactive compounds in agro-waste materials, such as the strawberry extrudate can be performed through various extraction techniques [10]. The main objective at this step consists of solubilizing the compounds of interest, with less possible impurities and making it an economically profitable technique. Extraction techniques in literature can be clustered into two groups: conventional extraction techniques, e.g. hydrothermal treatments, which are widely used at lab and full scale [11], and in recent years, more innovative techniques, e.g. enzyme assisted extraction. Additionally, combined extraction techniques between conventional and innovative techniques are being carried out to achieve high extraction yield [12]. All these extraction techniques will be revised and analyzed in the present chapter.

Any of these extraction techniques usually generate a liquid phase, with the bioactive compounds of interest, and a solid phase with a high amount of organic matter. After purification process of the liquid phase, a new liquid phase remains without the extracted bioactive compounds. Therefore, just the recovery of compounds of interest from the strawberry extrudate does not solve the problem of stabilization of the biomass of the strawberry extrudate and the use of a subsequent

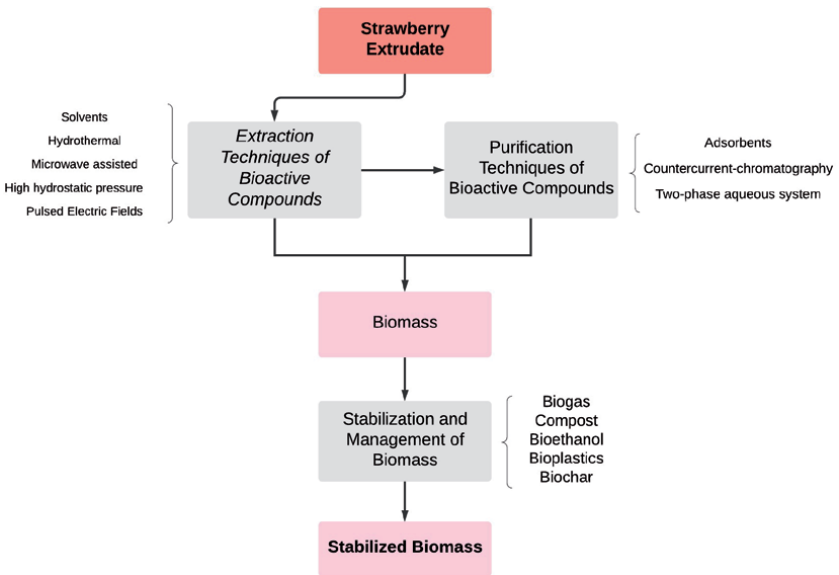

Figure 1.
General scheme of a biorefinery approach as a valorisation option for strawberry extrudate.

treatment is necessary for its stabilization [13]. The liquid phase after purification and the solid phase must undergo a new treatment for stabilization. In addition, extraction and purification processes consume energy which should be valued. The main options for assessing and stabilizing biomass after the extraction and purification process of the bioactive compounds should be focused on obtaining bioenergy and other bioproducts of interest [14].

The present chapter aims to summarize the bioactive compounds present in strawberries, to summarizes and critically analyzes the different extraction and purification techniques for the recovery of these bioactive compounds, as well as the different options for the management and stabilization of the strawberry extrudate after the extraction process.

2. Bioactive compounds in strawberry extrudate and strawberries

2.1 Nutrients

Strawberry extrudate presents similar nutrients composition than strawberry [15]. The strawberry has high concentration of dietary fibrous (2 g fibrous/100 g raw strawberry), such as lignin, hemicellulose, cellulose, and pectin, containing small amounts of protein (0.4–0.5 g protein/100 g raw strawberry) and fat (0.1 g fat/100 g raw strawberry) [16, 17].

The strawberry contains high concentrations of vitamin C, contributing to 24% to the antioxidant capacity of strawberries [16]. The recommended daily intake of vitamins (100–150 mg/day) can be satisfied with an average of 100 g of strawberries per day [18]. Furthermore, strawberry is a source of many other vitamins in smaller amounts, such as vitamin E, vitamin A, vitamin B6, vitamin K, thiamine, riboflavin, folate acid, and niacin (0.01–0.4 g vitamin/100 g raw strawberry) [5, 19]. Strawberry is also rich in manganese, potassium, and a good source of iodine, magnesium, copper, iron, and phosphorus [5, 16].

The sugar composition of strawberries varies with the degree of maturity of the fruit [20], being glucose, fructose, and sucrose the main sugars in strawberries [5]. Sugars in strawberries are involved in the taste of the fruit and are responsible for the caloric value of the strawberries. Organic fatty acids such as citric acid, malic acid, succinic acid, tartaric acid, oxalic acid, and fumaric acid are ones of the response of the taste, texture, pH, and color of the strawberry, and can alter the sensory quality of this fruit [6].

2.2 Phytochemicals

Figure 2 shows a general scheme for the classification of phytochemical compounds that can be founded in the strawberry extrudate. Phytochemicals are widely studied, mainly due to the extensive types of compounds that have potential biological benefits in humans. The main phytochemicals in strawberries are the flavonoids, followed by the hydrolysable tannins and the phenolic acids and, as minor constituents, the condensed tannins [5].

2.2.1 Flavonoids

Flavonoids are divided into two groups, i.e. anthocyanins, and anthoxanthines. Anthoxanthines, in turn, are grouped into five subclasses, i.e. flavones, flavonols, flavanones, flavanols, and isoflavones [21]. The three main classes of flavonoids in strawberries are anthocyanis, flavonols, and flavanols [22].

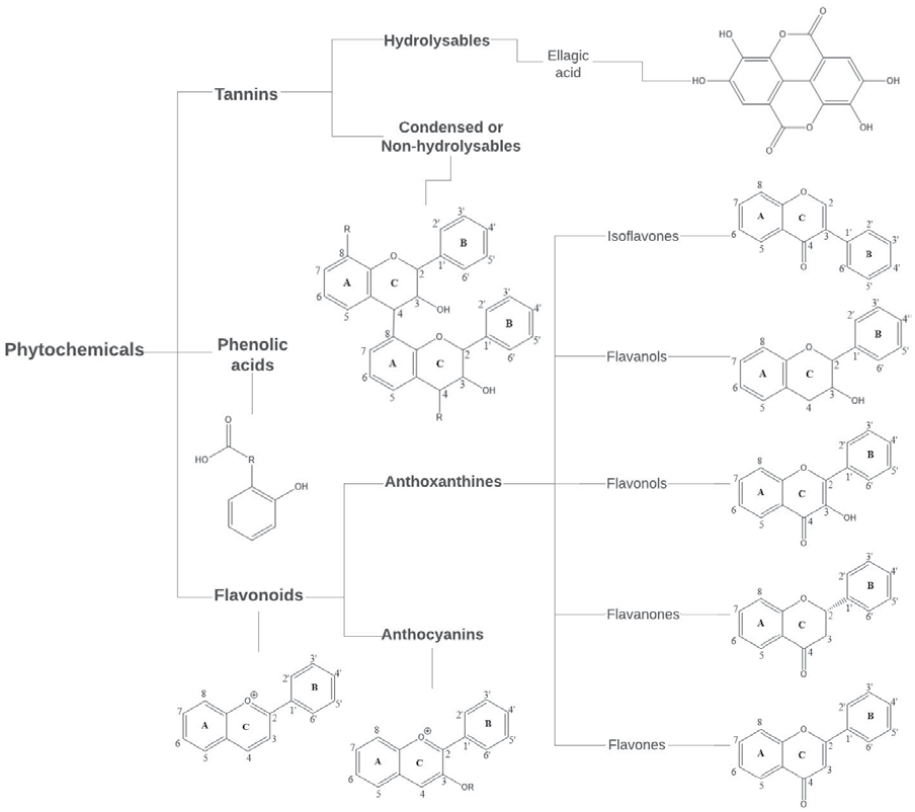

Figure 2.
General scheme of phytochemicals contained in strawberries.

The most relevant flavonoids present in strawberries are the anthocyanins due to their high concentration, approximately 20–47 mg/100 g raw strawberry [16]. More than 25 different pigments of anthocyanins have been described in the different varieties of strawberries [5]. Anthocyanins are responsible for the red color in strawberries [16]. The most important anthocyanins of the strawberry belong to the family of pelargonidin aglycones and cyanidin aglycones [23–25]. According to several studies, pelargonidin-3-glucoside is the dominant anthocyanin in strawberries [16, 24, 26–28]. The interest in anthocyanins have recently increased because of its pharmacological and therapeutic properties [5]. Anthocyanins have shown to positive effect toward reduction of coronary diseases, anticancer, antitumor, anti-inflammatory and anti-diabetic effects; as well as improving visual acuity and cognitive behavior [29]. These therapeutic effects of anthocyanins are connected to their high antioxidant activity [29]. In addition, anthocyanins can be used as a pigment in the food industry [29].

The second most important group of flavonoids in strawberries are flavonols, with approximately 1.5–3.4 mg/100 g raw strawberry [5, 16, 30, 31]. The most important flavonols of the strawberry belong to the family of quercetin and kaempferol, being the quercetin-derivatives the most abundant flavonols in strawberries [5, 17, 25–27]. Quercetin, in particular, is a potent antioxidant, cytoprotective, and anti-inflammatory [30].

Finally, the third group of flavonoids in strawberries are flavanols. Flavanols are the only class of flavonoids that do not naturally occur as glycosides. They are found in strawberries as monomeric compounds, such as catechins, and in

polymeric form, which are called condensed or non-hydrolysable tannins [5, 25]. These compounds can be difficult to measure in the strawberry because they are usually presented as part of a complex mixture of phenolic substances. Because of this, the amount of catechins present is sometimes overestimated [31]. At low concentrations flavanols particularly the catechins, are used as sweetening and/or flavoring additives. These flavonols improve taste and sweetness but are not substitutes for sweeteners and flavorings as they do not have taste and are a little astringent. Some authors have pointed out that their role is to make the receptors in the mouth more sensitive to sweeteners, thus lowering the levels of the sweeteners and flavorings used [21, 32].

2.2.2 Tannins

Tannins are classified into two groups: non-hydrolysable or condensed tannins and hydrolysable tannins (**Figure 2**). The condensed tannins are also called proanthocyanins, and are bound to the flavanols [33]. The content of condensed tannins in strawberries is approximately 54–163 mg/100 g raw strawberry [16]. In strawberries, the most relevant condensed tannins are procyanidins from catechin and its polymers. Condensed tannins are commonly found in the pulp of strawberries and achenes [5]. Due to the variety of physiological activities, they have been reported to possess, directly and indirectly, antioxidant, antimicrobial, anti-allergic and antihypertensive properties, as well as to inhibit the activities of some enzymes and physiological receptors [34].

The most common hydrolysable tannins in strawberries are ellagitannins, specifically sanguiin H-6 and ellagic acid [5, 26–28]. The content of ellagitannins in strawberries is approximately 10–23 mg/100 g raw strawberry [16]. Ellagic acid is an ellagitannin present in the secondary metabolism of vegetables, its main characteristic is its antioxidant, antimicrobial, antimutagenic, anticarcinogenic and antiviral capacity [16]. The content of ellagic acid in strawberries is approximately 1–2 mg/100 g raw strawberry [16]. Due to the phenolic nature of ellagic acid, this compound tends to react by forming complexes with other molecules of proteins, alkaloids, and polysaccharides, so that it is usually found as ellagitannins esterified with glucose, because of this it is difficult to find it free [20]. The properties of ellagic acid are also exploited in the food industry, so it is used in the manufacture of nutraceutical drinks and food supplements. Likewise, the application of ellagic acid for food preservation is of great impact for the perishable food industry, using its antioxidant activity for microorganisms inhibition [35].

2.2.3 Phenolic acids

Strawberries contain a variety a of phenolic acids which are presented as derivatives of the hydroxycinnamic acid, such as caffeic acid, and hydroxybenzoic acids such as gallic acid [5]. The content of phenolic acids in strawberries is approximately 0.8–6.7 mg/100 g raw strawberry [16]. The major hydroxycinnamic acid in strawberries is p-coumaroylhexose, but ferulic acid and caffeic acid glycosides have also been identified in strawberries [26, 27]. Hydroxycinnamic acid derivatives are responsible for the bitter taste of the strawberry, and it is used in the manufacture of creams [33, 36]. The primary derivative of hydroxybenzoic acid is p-hydroxybenzoic glycoside [28]. The p-hydroxybenzoic glycoside is widely used in the synthesis of organic compounds and their esters, known as parabens, which are used as preservatives in cosmetics [37].

3. Extraction and purification of bioactive compounds in strawberry extrudate

3.1 Extraction techniques

3.1.1 Solvents extraction

The extraction with solvents is a technique to isolate a substance from a solid or liquid mixture. This technique is currently used in combination with other techniques such as microwaves and ultrasound since the solvent only extracts soluble compound. Due to the strawberry extrudate nature, the solid–liquid extraction can be carried out with a Soxhlet extractor, which is one of the most commonly used conventional extraction techniques [38]. The extraction efficiency depends mainly on the choice of solvents [39]. The polar character of the bioactive compounds allows their solubility in various solvents, such as water, alcohols, and acetone [40].

Recently, numerous studies have explored the extraction of bioactive compounds using deep eutectic solvents from various groups of natural sources [41]. The formation of eutectic solvents is the result of the complexation of a halide salt, which acts as a hydrogen bond receptor, and a hydrogen bond donor [41]. Some eutectic solvents have been developed from the combination of primary metabolites and bio-renewable starting materials, e.g., sugars, alcohols, amino acids and organic acids [41]. Eutectic solvents produce less adverse effects on the environment, allowing to replace conventional chemical methods [41].

There is a long variety of studies on solvent extraction focusing on the extraction of bioactive compounds. An evaluation of the effect of different solvents and acids in the extraction of anthocyanins from strawberry fruits concluded that acetone provided an efficient and reproducible extraction, avoiding problems with pectins and allowing the concentration of the sample at low temperature [42]. In another study, it was observed that the acetone/acetic acid mixture (99:1, v/v) reached good results for the qualitative and quantitative evaluation of polyphenols present in strawberries [43].

3.1.2 Hydrothermal extraction

Hydrothermal extraction is a process in which the matter is treated by adding hot water or water vapor [38]. Steam explosion is another kind of hydrothermal treatment where the matter is treated with saturated water vapor at high pressure followed by rapid depressurization [44]. The disadvantage of using hydrothermal treatments is that they affect thermosensitive compounds and might form undesirable compounds [45].

Hydrothermal extraction at low temperature, i.e. ranging between 50 and 90°C, mainly induces the de-flocculation of macromolecules [46]. Hydrothermal extraction at medium temperature, i.e. ranging between 150 and 180°C, solubilizes cellulosic and hemicellulose biomass [47]. The steam explosion treatment, with temperature ranging between 180 and 260°C and increase in pressure of 0.69–4.83 MPa, it is able to solubilize lignocellulose biomass [44].

Several studies confirm the successful extraction of bioactive compounds by these hydrothermal extractions [48, 49]. Extraction of bioactive compounds in strawberry extrudate has been studied by applying hydrothermal treatments in the range of 90–200°C, [15]. Thermal treatment at 150°C for 60 minutes was the most efficient process based on the solubilization of sugars and phenols as well as the antioxidant capacity of the liquid phase produced [15].

3.1.3 Microwave assisted extraction

Microwaves are electromagnetic fields in a frequency range of 300 MHz to 30 GHz, which are generally operated at a frequency of 2.45 GHz [10]. Microwaves can access biological matrices and interact with polar molecules, such as water, which vibrate or rotate by the effect of microwaves and generate heat and can enhance the processes of extraction of bioactive compounds [10, 50]. Microwave assisted extraction has been successfully applied in anthocyanin extraction processes in grape skins [51], the recovery of pectins from press residues of various berries, i.e. red and black currant, raspberry and elderberry [52] and to extract phenolic antioxidants from peanut skins [53].

3.1.4 High hydrostatic pressure extraction

High hydrostatic pressure extraction is a method that works at high pressures ranging from 100 to 1000 MPa. These high pressures cause cell deformation, cell membrane damage, protein denaturation, deprotonation of charged groups, and the breakdown of bonds, making bioactive compounds more accessible for extraction [54]. High hydrostatic pressure extraction is considered to be a faster and more efficient technique than other conventional extraction methods [55, 56]. In addition, high hydrostatic pressure extraction has the advantage of not increasing the temperature during the processing time, so it would be an ideal method to extract thermosensitive compounds.

Several high hydrostatic pressure extraction studies have been carried out with strawberries for the extraction of bioactive compounds. The impact of high hydrostatic pressure extraction on total strawberry puree phenols was observed by Patras et al., [56], which reported that the amount of total phenols increases as the pressure in high hydrostatic pressure extraction increases [56]. In another study, the change in kaempferol, and quercetin quantity in strawberries pulps were tested at different pressures and for different processing times [57]. According to this study, the change in the amount of kaempferol was not very significant and the amount of quercetin increased with increasing pressure [57]. Another study showed that the nutritional and sensory qualities of strawberry puree after high-pressure processing at 500 MPa and 50°C for 15 min were much better than after a heat treatment at 90°C for 15 min [58].

3.1.5 Pulsed electric fields extraction

Pulsed electric fields or high intensity pulsed electric fields consist of a short time electrical treatment, between nanoseconds to milliseconds, in which the material located between two electrodes is exposed to a strong electric pulse of intensity field of 100 to 300 V/cm Pulsed Electric Fields or 20 to 80 kV/cm high intensity pulsed electric field, the operation parameters being the duration and number of pulses [59, 60]. Pulsed electric fields can produce the electrical rupture of the cell membranes producing the formation of pores, what is known as electroporation [60, 61]. Pore formation improves cell permeability allowing the recovery of bioactive compounds [62]. Compared to other non-thermal treatments such as the high hydrostatic pressure extraction method, pulsed electric field extraction methods require a much shorter processing time, higher extraction efficiency and these techniques can be easily applied in continuous operation [59]. Therefore, pulsed electric fields is a promising technique for different applications in the food industry because they can improve extraction capacity and recovery of nutritionally valuable compounds as well as the bioavailability of micronutrients and compounds in a wide range of foods [59].

Several studies on the extraction of antioxidant compounds in agri-foods show enhanced yields with pulsed electric fields. For instance, a comparison study between a heat treatment at 90°C for 60 or 30 seconds and high intensity pulsed electric field in strawberries juice, showed that strawberry juice treated with high intensity pulsed electric field maintained greater amount of phenolic acids and total anthocyanins than thermally treated juices [63, 64]. Likewise, the recovery of phenols from the shell of the pomegranate by pulsed electric field has been assayed, resulting in a similar antioxidant extraction yields and an energy saving of 50% compared to an ultrasound extraction technique [65].

3.1.6 Extraction techniques comparison

After reviewing the different extraction techniques that have been applied to the strawberry and strawberry extrudate, a summary describing their most interesting aspects is shown in **Table 1**. The aspects that have been compared are: the specificity of the extraction techniques with the bioactive compounds, the possibility of combining with other extraction techniques, the ability to release bioactive compounds, the potential degradation of bioactive compounds, possibility of intracellular attack, bonds breakage and whether the technique has a high operational and investment cost. The choice of the best technique for the strawberry extrudate is a tailor-made solution for each situation that will depend of the investment capacity, target compounds to be recovered or the required extraction yield.

3.2 Purification techniques of bioactive compounds

3.2.1 Adsorbents

There are many studies that show the properties of adsorbents to separate, concentrate and purify various compounds [66, 67]. Functionality, porosity, irregularities, surface area, tightly bonded impurities, internal porous structure, particle size, ionic strength, pH, and temperature all influence physical adsorption [66]. The temperature influences the adsorption in two ways, increasing the transport

Extraction technique	Specificity	Possibility of combination	Ability to release compounds	Degradation of bioactive compounds	Intracellular attack	Breaks of bonds	High cost
Solvents extraction	x	x					
Hydrothermal extraction		x	x	x		x	
Microwave assisted extraction		x	x	x	x	x	
High hydrostatic pressure extraction		x	x	x	x	x	x
Pulsed electric fields extraction		x	x	x	x	x	

Table 1.
Summary table of characteristics for comparing extraction techniques.

speed through the outer boundary layer and inside the pores due to the decrease in the viscosity of the solution, and changing the capacity of the adsorbent. However, high temperatures can promote irreversible interactions [67]. Another important parameter for purification with adsorbents is pH. For example, at acid pH, the adsorption of phenolic compounds by different adsorbents increases because the phenols are not dissociated and dispersion interactions predominate [66]. At alkaline pH, the adsorption decreases due to the dissociation of hydroxyl groups and carboxyl groups [66]. There are many types of adsorbents such as activated carbons, mineral adsorbents, synthetic polymeric adsorbents, ion exchange resins, lignin and lignocellulosic materials, adsorbents based on polysaccharides and others [66]. Among the available adsorbents Amberlite XAD adsorbents are widely used in the concentration of polyphenols [68]. Zhang et al. [69] reported the isolation and structural characterization of 10 phenolic compounds from strawberry extracts using a combination of Amberlite XAD-16 and C18 columns, HPLC-UV, and nuclear magnetic resonance spectroscopy methods.

3.2.2 Countercurrent-chromatography

Countercurrent chromatography is a technique widely used in the purification of natural products [70]. Countercurrent chromatography is a liquid–liquid partition chromatography process in which both the mobile phase and the stationary phase are liquid [70]. The main advantage of countercurrent chromatography, when compared to equivalent techniques such as low pressure liquid chromatography, is that there are no adsorption losses in the stationary phase [70]. The range of selectivity offered by chromatographic resins is equivalent to the range of selectivity offered by the different solvent systems [70].

Several studies have shown the importance of countercurrent chromatography for the purification of bioactive compounds from strawberry. The compound 2,5-dimethyl-4-hydroxy-3[2H]-furanone 6′O-malonyl-β-d-glucopyranoside was isolated from a strawberry glycosidic extract (Fragaria × *ananassa*, cv. Senga Sengana) by countercurrent chromatography [71]. Peonidin-3-glucoside and malvidin-3-glucoside were obtained from grapes in a single step, while in a second step, cyanidin-3-glucoside was isolated [72]. In another research, the separation of anthocyanin monomers of high purity from mulberry fruits was developed [73].

3.2.3 Two-phase aqueous system

Two-phase aqueous system is a liquid–liquid fractionation technique that is usually formed by mixing two polymers in aqueous media, for example, polyethylene glycol and dextran or maltodextrin, or by a polymer and a salt, such as polyethylene glycol and salts of phosphates, citrates or sulphates [74–76]. This method has advantages over other purification techniques due to a comparatively low consumption of energy and time, as well as the possibility to be designed for a continuous operation. Moreover, two-phase aqueous systems are effective for many types of substances, especially for the concentration and purification of bioactive compounds [74, 75]. Several studies have demonstrated the suitability of this technique for the purification of bioactive compounds such as phenolic compounds from fig fruits (*Ficus carica* L.) [76], or the purification of gallic acid from natural matrices with ionic liquids [77]. Furthermore, two-phase aqueous system has been applied for the purification of polyphenols from a model solution of gallic acid and three real samples of red and white wine, and orange juice in combination with macro and micro extractors [78]. Polyphenols have been also extracted from *Aronia melanocarpa* berries, using ultrasound-assisted extraction in combination with the two-phase aqueous system [79].

4. Stabilization of biomass by obtaining bioenergy and bioproducts

4.1 Biogas production

Anaerobic digestion is a microbiological process, in absence of oxygen, where organic matter is progressively degraded by an heterogeneous bacterial population to methane (55–70%) and carbon dioxide (30–45%) [80]. Anaerobic digestion presents some fundamental advantages such as the possibility of working at high rates of organic load, and the produced methane can be used as an energy source due to its heating value (35,793 kJ/m^3, at 1 atm, 0°C), which equals to 1 kg of raw coal or 0.76 kg of standard coal [3, 11]. The use of biogas for energy supply reduces deforestation, soil erosion and environmental pollution [81, 82]. Also, it can improve the energy efficiency of various production processes due to the energetic contribution that provides [82]. In addition, a wet waste called digestate, which is a mixture of partially degraded organic matter, microbial biomass, and inorganic compounds, is produced during biomethanization and could be used as a base for fertilizers or organic amendments [82, 83].

Several studies on anaerobic digestion of strawberries extrudate have been carried out. The results of one these studies reveal that strawberries extrudates have a high level of anaerobic biodegradability (90% in VS, (total volatile solids)) and that a substantial amount of methane can be obtained in this way (312 mL CH_4 STP/g added VS) (STP: standard temperature and pressure conditions, i.e. 0°C, 1 atm) at an organic loading rate range of 2.04 to 3.51 kg VS/m^3·d [84]. In another study of anaerobic digestion of strawberry waste from supermarkets, using an organic loading rate of 0.55–4.4 (g/L·d), the experimental biogas and methane yields were 0.588 and 0.231 L/g, respectively [85]. It has been observed that sometimes it is necessary to co-digest strawberry extrudate with a substrate that provides alkalinity, such as sewage sludge [86, 87]. Co-digestion studies of strawberry extrudate with other substrates such as fish waste [3] and glycerol [83] have also been studied. Anaerobic digestion of strawberry extrudate is a promising technique but it should be further studied since low alkalinity of the extrudate together with formation of inhibitory compounds caused by the extraction process could negatively affect the digestion process.

4.2 Compost production

Composting has been proposed for a long time as a quite cheap option for agricultural waste management [2]. Composting has also been proposed as a post-treatment for the produced digestate after anaerobic digestion [88]. Composting is the bio-oxidative conversion of organic waste into an organic amendment. According to Gutiérrez et al. (2017) [2], the cost of composting varies in a wide range from $40 to $500 per throughput ton depending on the technology. Composting costs vary widely depending on the type of operation, which ranges from the most simple ones, such as opening windrows, to more complex procedures like in-vessel aerobic composting that allows smell emissions to be controlled and prevents environmental pollution [2]. The great disadvantage is that a considerable amount of offensive odors can be emitted during the process due to the generation of volatile organic compounds [89]. Other disadvantages are the long process time and the necessity of a proper monitoring [90]. Co-composting of a waste mixture containing strawberry extrudate, fish waste, sewage sludge and bulking agent has been successfully proven [2, 89].

4.3 Bioethanol production

Bioethanol is one of the most produced alcohols from the fermentation of sugars found in fruits and vegetables [7, 91–93]. Theoretically, any organic product with a

high content of sugars and starch, such as strawberry extrudate, may be susceptible to obtaining bioethanol [91]. Inedible sources from the strawberry extrudate such as lignocellulosic biomass, which mainly comprises cellulose, hemicellulose, and lignin, can be hydrolysed to produce a mixture of pentoses and hexoses that can be transformed into bioethanol [94]. Bioethanol from agro-waste, such as strawberry extrudate, could be a promising technology that involves four processes, pre-treatment, enzymatic hydrolysis, fermentation and distillation, this final step is crucial for the process to be economically viable on a commercial scale due to high energy consumption in the form of steam to increase the yield of bioethanol production when lignocellulose materials are used as raw material [93]. These processes have several challenges and limitations, such as the efficient pre-treatment process to eliminate lignin from the lignocellulosic agro-residues. The proper pre-treatment process can increase the concentrations of fermentable sugars after enzymatic hydrolysis, thus improving the efficiency of the entire process [92].

4.4 Bioplastics production

Fossil fuel depletion, global warming, and problems of pollution of the environment that provoke plastics in its life cycle are encouraging the development of biodegradable plastics [95, 96]. Agri-food waste are usually rich in many useful substances such as lipids, polysaccharides, and aromatics, which could be used for the manufacture of biodegradable polymeric materials. Bioplastics already play an important role in the sectors of packaging, agriculture, consumer electronics and motoring, but still have a very low share in the total production of plastics. Currently, about 1% of the annual tons of plastic are bioplastics [97]. Examples of such bioplastics are exopolysaccharides, polycaprolactone, polybutylene succinate, polybutylene adipate terephthalate, polyhydroxyalkanoates or polyhydroxybutyrates [97, 98]. For obtaining bioplastics from agri-food waste, the waste must be treated to extract or isolate specific macromolecules, such as cellulose, lignin, suberin, starch, or monomers, such as vegetable oils, tannins and terpenes [96, 99]. A study conducted on the production of bioplastics from Murta fruit extract, that is a native Chilean berry, showed the feasibility of using berries for bioplastic production [8].

4.5 Biochar production

Biochar is the solid carbonaceous residue produced through organic waste and used as a soil improver [100, 101]. Biochar is produced through several types of methods such as pyrolysis, torrefaction or hydrothermal carbonization [100, 101].

There are no studies reported in the literature dealing with the production of biochar from strawberry extrudates. However, the above-mentioned techniques (pyrolysis, torrefaction and hydrothermal carbonization) could be potentially applied for this substrate. Several studies have been carried out on the hydrothermal carbonization of other agri-food waste, such as olive cuttings and olive pulp [102]; grape marc [103]; olive mill waste, canned artichoke and orange waste [104].

5. Conclusions

This chapter has reviewed up-to-date literature on the bioactive compounds contained in strawberries, which have an important health and market value. Different extraction and purification techniques to obtain valuable compounds from strawberry extrudate have been reviewed and analyzed. The reviewed techniques present different advantages and drawbacks that were analyzed to facilitate the selection of the most suitable process for each valorisation scenario. Finally,

different stabilization options for the biomass remaining after extraction have also been reviewed. Stabilization is required to avoid severe environmental impacts, and additionally could be an economically beneficial aid for balancing the cost of the extraction of high value-added compounds. As usually for any waste management option, selection of the best extraction, purification and stabilization technique for the strawberry extruded is a tailor-made solution for each situation.

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Conflict of interest

The authors declare no conflict of interest.

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