IntechOpen

Products from Olive Tree

Edited by Dimitrios Boskou and Maria Lisa Clodoveo

PRODUCTS FROM OLIVE TREE

Edited by **Dimitrios Boskou**
and **Maria Lisa Clodoveo**

Products from Olive Tree
http://dx.doi.org/10.5772/61902
Edited by Dimitrios Boskou and Maria Lisa Clodoveo

Contributors

Annette Bongartz, Dieter Oberg, Reinhard Schneller, Martin Popp, Lourdes Gallardo-Guerrero, Beatriz Gandul-Rojas, María Roca, Valentina Domenici, Mario Cifelli, Cristina Lazzerini, Gianluca Bleve, Panagiotis Kalaitzis, Christos Bazakos, Stelios Spaniolas, Jesus Osada, Sara Cicerale, Gianluca Veneziani, Beatrice Sordini, Agnese Taticchi, Sonia Esposto, Roberto Selvaggini, Stefania Urbani, Ilona Di Maio, Maurizio Servili, Manel Hamza, Alessandro Parenti, Lorenzo Guerrini, Sabria Aued-Pimentel, Alegria Carrasco-Pancorbo, Aadil Bajoub, Lucia Olmo-Garcia, Noureddine Ouazzani, Romina Paula Monasterio, Gabriel Beltran, Maja Jukić Špika, Klara Kraljić, Dubravka Škevin, Javier Miguel Ochando Pulido, Zisimos Likudis, Nurcan Degirmencioglu, Riccardo Amirante

Notice

First published in Croatia, 2016 by INTECH d.o.o.
eBook (PDF) Published by IN TECH d.o.o.
Place and year of publication of eBook (PDF): Rijeka, 2019.
IntechOpen is the global imprint of IN TECH d.o.o.
Printed in Croatia

Legal deposit, Croatia: National and University Library in Zagreb

Additional hard and PDF copies can be obtained from orders@intechopen.com

Products from Olive Tree
Edited by Dimitrios Boskou and Maria Lisa Clodoveo
p. cm.
Print ISBN 978-953-51-2724-6
Online ISBN 978-953-51-2725-3
eBook (PDF) ISBN 978-953-51-4164-8

Meet the editors

Dimitrios Boskou received his diploma in chemistry from the School of Chemistry, Aristotle University of Thessaloniki, Hellas; his Philosophy Doctor degree from the University of London, UK; and his degree of Doctor of Science from the School of Chemistry, Aristotle University of Thessaloniki, Hellas. He served as an assistant, lecturer, assistant professor, associate professor, professor and head of the Laboratory of Food Chemistry and Technology, School of Chemistry, Aristotle University of Thessaloniki (1970–2006). From 1986 to 1998, he was a member of the IUPAC Commission on Oils, Fats, and Derivatives. In the years 1995–2005, he served as a member of the Supreme Chemical Council, Athens. From 1995 to 2012, he was a member of the Scientific Committee for Food of the European Commission and a member and expert of the Food Additives Panel of the European Food Safety Authority. His achievements are: over 90 published papers and reviews; author and editor of 8 books; author of 22 chapters in books related to major and minor constituents of fats, natural antioxidants, olive oil and frying of food; and contributor to international scientific encyclopedias and the Lexicon of Lipid Nutrition, a joint IUPAC/IUNS work.

Maria Lisa Clodoveo is an assistant professor with tenure at the University of Bari, Italy. She is the director of the short master in "Health claims of extra virgin olive oil as marketing tool to improve the company's competitiveness" and deputy director of the postgraduate course in "Olive Growing Management." She is a member of the "Accademia dei Georgofili" and of "Accademia Nazionale dell'Olivo e dell'Olio." She is a member of the scientific committee of the international journal *Grasas y Aceites*. She is the founder of the "Research Centre for Olive Growing and Olive Oil Industry" at the University of Bari. She is the winner of the award "Antico Fattore" (2016) assigned by the "Accademia dei Georgofili" for the innovative approach on virgin olive oil technologies and the award for "Innovation and applicability" at the International Congress GENP 2016 for the results obtained in the application of ultrasound technology to the virgin olive oil process. She is the inventor of two patents and the author of more than 100 articles and book chapters in the olive oil sector.

Contents

Preface

The main olive tree products are olive oil and table olives. They are both integral components of the dietary pattern known as Mediterranean diet. In the last decade, they have been the subject of intensive research over a short period of time because of the growing interest in this diet and its health benefits. Today it is universally recognized that many important constituents of olive oil and table oils, both triacylglycerols and non-glyceride components, are related to lower levels of systematic inflammation and lower rates of diseases such as cardiovascular heart disease, certain types of cancer and diabetes; they may also have an effect on cognition. Information accumulated from studies on olive oil and olive fruit composition focuses now mainly on the minor bioactive constituents. In light of new evidence, new proposals have recently appeared. These proposals aim at modifying and improving the technology of production to avoid significant losses of bioactive constituents due to processing and storage. This book presents updated information related to two important olive tree products: **olive oil** and **table olives. Olive-milling wastes** as sources of hydroxytyrosol, an important biophenol, are also discussed.

The book is composed of 17 monographic chapters and organized in 7 sections.

Section 1: Bioactive Compounds in Olive Oil

Section 1 contains Chapter 1 'Squalene, a Trove of Metabolic Actions'. Squalene's concentration is uniquely high in olive oil (up to 0.7 %) as compared to other vegetable oils and animal fats. This important phytochemical and its action may help to explain the protective role of virgin olive oil. New connections between nutrition and gene expression upregulated by squalene administration are highlighted.

Section 2: Olive Oil Production, Composition and Quality

Section 2 has Chapters 2–7.

Chapter 2 'Improvement of Olive Oil Mechanical Extraction: New Technologies, Process Efficiency and Extra Virgin Olive Oil Quality' explores the innovations introduced in the oil extraction, which improve the working efficiency of the production system and preserve volatiles and phenolic compound concentrations that are strictly related to the health and sensory properties of the product.

Chapter 3 'Ultrasound in Olive Oil Extraction' presents ultrasound application and recent innovations in the virgin olive oil extraction process.

Chapter 4 'Stabilization of Extra-Virgin Olive Oil' focuses on the technologies recently proposed for the removal of suspended solids and the water from stored extra-virgin olive oil.

Chapter 5 'Chlorophylls and Carotenoids in Food Products from Olive Tree' is an updated overview about the chlorophyll and carotenoid pigments present in olive fruits and their

products. Chlorophyll and carotenoid pigments present in olive fruits change during the processing of table olives according to the main styles of preparation due to the different reaction mechanisms, which occur during the debittering process. Chlorophyll concentration is a key element in the photo-oxidation of virgin olive oil.

Chapter 6 'Pigments in Extra-Virgin Olive Oil: Authenticity and Quality' concentrates mainly on the analytical methods applied to identify and quantify olive oil pigments. Modern chromatographic and spectroscopic techniques are useful tools in the evaluation of authenticity and quality of extra-virgin olive oil.

Chapter 7 'DNA-Based Approaches for Traceability and Authentication of Olive Oil' provides an overview of methods based on DNA analysis that have gained attention in recent years. The reliability and reproducibility of these techniques depend on the quality of the trace amounts of DNA extracted from oil samples. A significant number of DNA isolation published protocols and commercial kits for olive oil DNA extraction are discussed.

Section 3: Consumers' Perception

Section 3 contains Chapters 8 and 9.

Chapter 8 'Evaluation of the "Harmony-Value" A Sensory Method to Discriminate the Quality Range within the Category of EVOO' proposes an extended and reproducible organoleptic profile sheet, apart from the official panel test, with the aim to discriminate oils between different quality levels within the range of extra-virgin olive oil. This more detailed description is important for the evaluation of an additional quality criterion, the so-called harmony-value.

Chapter 9 'Consumer Perception, Attitudes, Liking and Preferences for Olive Oil' analyzes the factors that influence consumers' perceptions, attitudes and preferences for olive oil. Olive growers and olive oil manufacturers and marketers can utilize these insights in order to develop products in line with consumer needs and demands, especially extra-virgin olive oil (EVOO) and virgin olive oil (VOO).

Section 4: Oils with Protected Designation of Origin

Section 4 contains Chapters 10 and 11.

Chapter 10 'Olive Oils with Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI)' discusses PDO/PGI certification and labelling of olive oil. The need for future analytical studies is stressed to indicate that better cultivation and industrial processes associated with PDO/PGI certification result in lower levels of agrochemicals in the final product.

Chapter 11 'Geographical Indication Labels in Moroccan Olive Oil Sector: Territorial Dimension and Characterization of Typicality. A Case Study of Meknès Region' is an extended study of a Moroccan olive-growing area, which looks forward to acquire a geographical indication label. A four-stage methodological approach is presented, which includes excellent chemical work necessary for the oil's characterization and typicality.

Section 5: Innovations in Table Olives Production

This section contains Chapters 12 and 13.

Chapter 12 'Modern Techniques in the Production of Table Oils' is a general review of processing techniques applied today to improve the quality characteristics of table olives.

Chapter 13 'How Biotechnology Can Improve a Traditional Product as Table Olives' examines how microbial starters, selected for specific technological and safety traits, can be used to improve organoleptic characteristics and ensure the maintenance and/or improvement of nutritional and healthy features of the product. Table olives as a carrier of microorganisms with probiotic characteristics are also discussed.

Section 6: Olive-Processing Wastes

Section 6 contains Chapters 14 and 15.

Chapter 14 'The Possibility of Recovering of Hydroxytyrosol from Olive Milling Waste Water by Enzymatic Bioconversion' analyzes an innovative approach to obtain liquid fractions from olive oil waste water (OMW) rich in hydroxytyrosol, an important bioactive phenol . These fractions are further enriched in hydroxytyrosol by ultrafiltration.

Chapter 15 'A Brief Review on Recent Processes for the Treatment of Olive Mill Effluents': In this chapter, the state of the art of oil mill effluent management is presented, with a focus on biological and advanced oxidation processes.

Section 7: Regional Studies

Section 7 contains Chapters 16 and 17.

Chapter 16 'Olive Oil in Brazil: Economic and Regulatory Control Aspects' is an overview of the economic, regulatory and inspection aspects in Brazil, one of the world's main importers of olive oil. The expansion of the market and the commercial production outlook have intensified efforts to improve control of this product and enable laboratories to monitor quality and authenticity.

Chapter 17 'Tocopherols: Chemical Structure, Bioactivity and Variability in Croatian Virgin Olive Oils' presents research work on the tocopherol content and composition variability in virgin olive oils of the most widespread Croatian cultivar 'Oblica'.

It is hoped that this book will be a source of special value to food scientists, biotechnologists, olive growers and producers, legislators, nutritionists, dieticians, researchers in the area of food chemistry and also members of the general public and especially consumers who are interested in the benefits of a healthy diet .The editors express their gratitude to the contributors. Their experience and research work they have conducted are a guarantee for objective state-of-the-art reviews on the matters discussed.

Dimitrios Boskou
Aristotle University of Thessaloniki
School of Chemistry
Hellas

Maria Lisa Clodoveo
University of Bari
Department of Agricultural and Environmental Science
Italy

Section 1

Bioactive Compounds in Olive Oil

Chapter 1

Squalene: A Trove of Metabolic Actions

Adela Ramírez-Torres, Clara Gabás-Rivera and Jesús Osada

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64384

Abstract

Squalene is present in high concentration in the liver of certain sharks and in small concentrations in olive oil. Previous studies showed that its administration decreases hepatic steatosis in male *Apoe*-knockout mice, but these changes might be complex. Transcriptomics, using DNA microarrays, and proteomics from mitochondrial and microsomal fractions, analyzed by 2D-DIGE and mass spectrometry, were used in these mice that received 1 g/kg/day squalene for 10 weeks. Squalene administration significantly modified the expression of genes such as lipin 1 (*Lpin1)* and thyroid hormone responsive (*Thrsp)*. Changes in methionine adenosyltransferase 1 alpha (*Mat1α*), short-chain specific acyl-CoA dehydrogenase (*Acads*), and thioredoxin domain–containing protein 5 (*Txndc5*) expressions were consistent with their protein levels. Their mRNA levels were associated with hepatic fat content. These results suggest that squalene action involves changes in hepatic gene expression associated with its anti-steatotic properties. This approach shows new connections between nutrition and gene expression since *Txndc5*, a gene with unknown biological function, was upregulated by squalene administration. Overall, this nutrigenomic approach illustrates the effects of squalene and provides further support to the idea that not all monounsaturated fatty acid–containing oils behave similarly. Therefore, selection of cultivars producing olive oils enriched in this compound will be a plus.

Keywords: Apolipoprotein E–deficient mice, Virgin olive oil, Squalene, *Lpin1* , *Thrsp* , *Mat1a* , *Acads* , *Txndc5*

1. Introduction

The "Seven Countries" Study evidenced that cardiovascular mortality was the lowest in Mediterranean countries compared to other regions participating in the study [1]. The

Mediterranean dietary pattern is not only associated with lower cardiovascular mortality but also with total mortality [2]. Dietary interventions using Mediterranean diets have resulted in favorable outcomes either in primary [3] and secondary prevention by reducing the number of coronary events and death toll [4]. All these evidences have provided the scientific background to propose the Mediterranean Diet as an intangible cultural heritage of humanity (http://www.unesco.org/culture/ich/es/RL/00394).

In traditional Mediterranean diet, the main source of fat was olive oil [5]. Virgin olive oil, an example of oil extracted by physical means, is a functional food since it contains several components that may contribute to its overall biological properties. Known for its high levels of triacylglycerols containing monounsaturated fatty acids, it is a good source of phytochemicals such as squalene [6], phenolic compounds [7, 8], terpenes, phytosterols, and alpha-tocopherol [9, 10]. The content of squalene in virgin olive oil shows a great variability, from 1.5 to 9.6 g/kg [11], and may vary according to grove varieties [12]. In spite of this variation, squalene represents the second most abundant component of virgin olive oils and the highest in commonly consumed vegetable oils [13]. In some refinement processes, the loss of squalene may reach a 20 % [6]. However, this molecule remains stable in virgin olive oil heated at 180 °C for 36 h [14]. Its thermal stability makes squalene suitable to ensure its intake when consumed both in cooked and raw food. In vitro, it is a highly effective oxygen-scavenging agent, and it has been shown to be chemopreventive against several tumors [a detailed review of its described properties is found in Ref. [13]].

The average intake of squalene is 30 mg/day in the United States. However, when consumption of olive oil is high, the intake of squalene can reach from 200 to 400 mg/day, as observed in Mediterranean countries [15], or even can amount up to 1 g daily [16]. Despite the fact that plasma squalene levels come from endogenous biosynthesis in addition to dietary sources, its concentration is higher in those human populations consuming virgin olive oil or shark liver [17]. Its stability and bioavailability make squalene an attractive compound to characterize its biological properties.

2. The liver: an organ sensitive to diet nutrients

The liver secretes phospholipids, cholesterol, and triacylglycerols into plasma as lipoprotein complexes, which allow the transport of those lipids into the aqueous medium of blood. Apolipoproteins such as APOB100, APOA1, APOA2, and APOE are the main protein constituents of lipoproteins. Furthermore, this organ also secretes the enzymes (hepatic lipase, lecithin-cholesterol acyltransferase, and phospholipid transfer protein) involved in the plasma transformation of lipoproteins [18].

Apoe-deficient mice lack APOE, and as consequence, the elimination of lipoproteins from blood is impaired. Due to this fact, lipoproteins accumulate into vessel walls contributing to the development of spontaneous atherosclerosis [19]. When fed with high-fat diets, these mice induced changes in plasma apolipoproteins [20], as a result of hepatic apolipoprotein gene expression variations [21]. This adaptive response of the liver as an effect of different olive oil

intakes represents an ideal model to explore changes in diet composition. Using *Apoe*-deficient mice as a model of spontaneous atherosclerosis and baseline steatosis, our group showed that squalene administration decreased atherosclerotic lesion [22] and exhibited an association between hepatic fat content and atherosclerotic progression. In this study, squalene accumulated in the liver and was able to decrease the storage of hepatic triacylglycerols. We concluded that squalene was transported to the liver in an apolipoprotein E–independent way, and its mechanisms of action were complex. To address this complexity, high-throughput approaches of transcriptomics and proteomics have been employed to further characterize squalene action. The livers of *Apoe*-deficient mice fed with chow diets or the same diets supplemented with squalene were analyzed. The chapter will review our experience dealing with squalene and the use of omic technologies to explore its effects.

3. Methodological workflow

Two-month old, male, homozygous Apoe-deficient mice with C57BL/6J × Ola129 genetic background were used. Two study groups of equal plasma cholesterol were established: (a) one received chow diet, and its beverage contained 1 % (v/v) of glycerol solution (n = 8) and (b) the other received the same chow diet, but its drinking solution was supplemented with squalene to provide a 1 g/kg/day dose (n = 9). For 10 weeks, mice were fed with experimental diets, which were well tolerated since there was no incidence on survival, physical appearance, and solid and liquid intakes, as described previously [22]. After this time, animals were sacrificed and the liver removed. One aliquot stored in neutral formaldehyde was used to evaluate the extent of lipid droplets, expressed as the percentage of total liver section, and the remaining, frozen in liquid nitrogen, was used to extract its total RNA and to isolate subcellular fractions.

The changes in expression of 22,690 transcripts represented on the Affymetrix GeneChip Murine Genome MOE430A array were analyzed to find out the effect of squalene. In order to do that, pooled liver samples of eight mice on the chow diet were compared with those receiving the compound, as depicted in **Figure 1**.

The huge amount of information provided by microarrays requires further processing in order to get a meaningful and manageable data to work with, such as selecting only the genes with the highest expression changes or those involved in a certain metabolic pathway [23]. In the present work, the first approach has been adopted, and only those genes whose expression was strongly modified (signal $\log_2$ ratio ≥ 1.5 or ≤1.5) were considered highly responders to the intake of squalene. Gene expression was later confirmed by quantitative reverse transcriptase-polymerase chain reaction (RT-qPCR) to reinforce the validity of results.

For the preparation of mitochondrial and microsomal fractions, livers were homogenized in PBS (4 ml/g of tissue) with protease inhibitor cocktail tablets (Roche). Tissue debris was removed by centrifugation at 200 × g for 10 min at 4 °C. The homogenate was spun down at 1,000 × g for 15 min. The supernatant-containing mitochondria were centrifuged at full speed, 13,000 × g for 2 min. The mitochondrial pellets were then washed twice, pelleted, resuspended

in PBS, and spun for 1 min. Microsomal fractions resulted from centrifugation of the post mitochondrial supernatant at 105,000 × g for 90 min. These pellets were washed twice, spun at the same speed, and finally resuspended in 0.5 ml of PBS [24, 25].

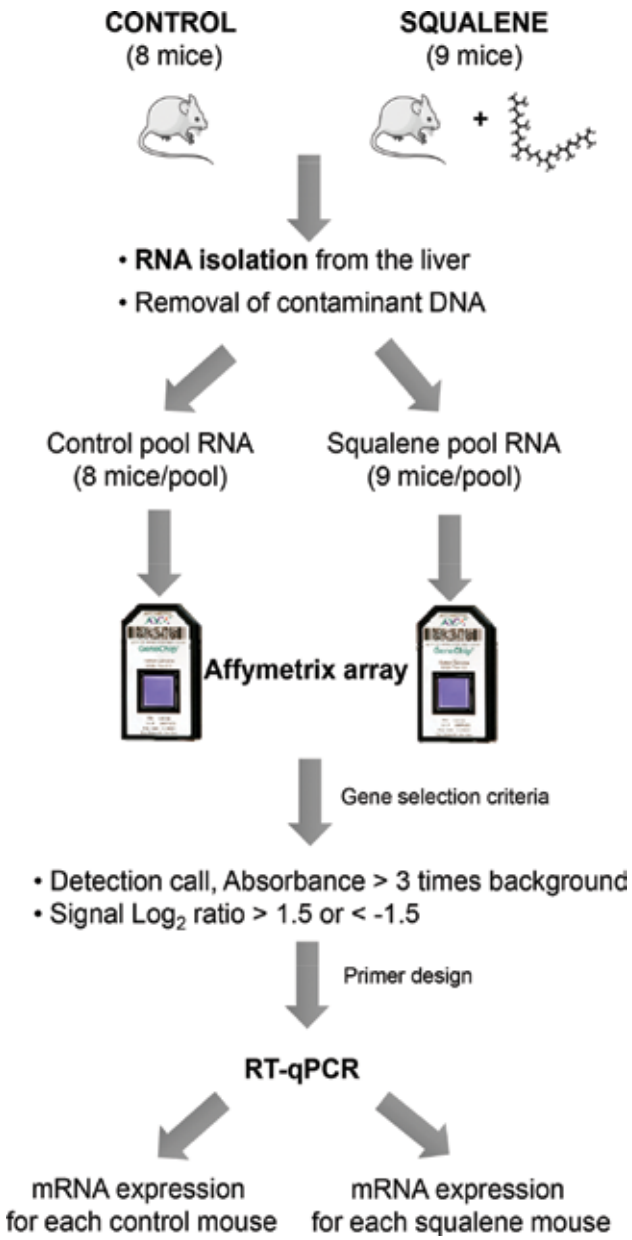


Figure 1. Graphical representation of the used approach. The process encompasses RNA preparation, microarray processing to select expression changes, and confirmation by quantitative reverse transcriptase-polymerase chain reaction (RT-qPCR) of individual samples.

Differential protein expression was analyzed by DIGE analysis. Spots whose densities significantly differed between treatments were excised from the preparative gel and subjected to tryptic digestion and identification by mass spectrometry, as described [24, 25].

4. Squalene-induced global changes in hepatic gene expression

Affymetrix software identified 11,528 transcripts as expressed in the livers of chow-fed mice and 11,187 in those of squalene-fed animals. According to the Mann-Whitney ranking feature of the Affymetrix software ($P < 0.01$), squalene administration increased and reduced the expression of 413 and 428 sequences, respectively. The original data were deposited in the GEO repository (accession number GSE36932).

Biological process	GenBank	Name	Gene symbol	Chow	Squalene	Signal log_2ratio
Upregulated genes						
Cell signaling	NM_011267	Regulator of G-protein signaling 16	*Rgs16*	102	543	2.5
Nuclear protein	NM_009381	Thyroid hormone-responsive SPOT14 homolog (Rattus)	*Thrsp*	238	760	1.7
Lipid metabolism	NM_172950	Lipin1	*Lpin1*	423	1675	1.6
Transcription factor	NM_011575	Trefoil factor 3 intestinal	*Tff3*	200	503	1.5
Transcription factor	NM_016974	D site albumin promoter binding protein	*Dbp*	589	1547	1.5
Downregulated genes						
Cell cycle	NM_008059	G0/G1 switch gene 2	*G0s2*	3012	487	−2.5
Transcription factor	NM_007489	Aryl hydrocarbon receptor nuclear translocator-like	*Arntl*	66	11	−2.3
Cell signaling	NM_019840	Phosphodiesterase 4B	*Pde4b*	82	24	−1.8
Immunity	NM_010378	Histocompatibility 2, class II antigen A, alpha	*H2-Aa*	944	583	−1.5
Immunity	NM_010382	Histocompatibility 2, class II antigen E beta	*H2-Eb1*	357	140	−1.5

Data represent intensity of signal for each condition with the Affymetrix chip.

Table 1. Hepatic genes differentially regulated by the administration of squalene at the level of signal log_2 ratio ≥ 1.5 or ≤ 1.5 in male *Apoe*-deficient mice.

To select the most relevant, only differentially regulated genes with a signal $\log_2$ ratio ≥ 1.5 (for those genes upregulated) or ≤ 1.5 (for those repressed) were taken into account. **Table 1** lists the genes whose mRNAs reflected these changes. Five genes showing increased expression as a response to the administration of squalene. Two of these genes coded for transcription factors (*Dbp* and *Tff3*) and three for proteins with miscellaneous functions (one of them was involved in lipid metabolism [*Lpin1*], the second was a signaling molecule [*Rgs16*], and the third was a nuclear protein [*Thrsp*]). Five genes met the criterion of showing a reduced expression as a response to the administration of squalene (**Table 1**). Of these, two were involved in immunity (*H2-Aa* and *H2-Eb1*), one was a transcription factor (*Arntl*), one was involved in cell cycle (*G0s2*), and finally one coded for an enzyme involved in cellular signaling (*Pde-4b*).

	Chow (n = 8)	Squalene (n = 9)	Fold change	SL_2R
Upregulated genes				
Rgs16	0.91 ± 0.16	11.64 ± 1.5**	12.8	3.7
Thrsp	0.92 ± 0.13	4.00 ± 0.65**	4.3	2.1
Lpin1	0.96 ± 0.19	9.77 ± 2.00**	10.2	3.3
Tff3	0.85 ± 0.11	1.26 ± 0.29	1.6	0.6
Dbp	0.92 ± 0.20	3.76 ± 0.72**	4.08	2.0
Downregulated genes				
G0s2	0.56 ± 0.19	0.13 ± 0.02*	0.2	−2.3
Arntl	1.19 ± 0.25	0.46 ± 0.07**	0.4	−1.3
Pde4b	1.11 ± 0.17	1.22 ± 0.27	1.1	0.1
H2-Aa	0.94 ± 0.18	0.92 ± 0.18	1.0	0.0
H2-Eb1	1.01 ± 0.16	0.75 ± 0.23	0.7	−0.5

Data (means ± SEM) represent arbitrary units normalized to the *Cyclophilin B* expression for each condition with the RT-qPCR. Statistical analysis was carried out by the Mann-Whitney *U* test. $^{**}P \leq 0.01$ vs chow, $^{*}P \geq 0.05$ vs chow.

Table 2. Effect of squalene on the hepatic gene expression in male *Apoe*-deficient mice.

To validate the results obtained with the microarray, the expressions of the above genes—*Arntl, Dbp, G0s2, H2-Aa, H2-Eb1, Lpin1, Pde-4b, Rgs16, Tff3,* and *Thrsp*—that were up- or downregulated were individually analyzed by specific RT-qPCR assays. *Cyclophilin B* was the reference gene used to normalize the results (**Table 2**).

Four out of the five upregulated genes included in the validation analysis—*Rgs16, Thrsp, Lpin1,* and *Dbp*—were confirmed to be significantly increased in their expressions by the squalene administration. Two of the five downregulated genes selected—*G0s2* and *Arntl*—were significantly decreased in male mice receiving squalene. Good agreement between these procedures was obtained ($r = 0.94$, $P < 0.007$), and all samples except the two were correctly classified, although the magnitude of the response differed between both methods. These

results indicate that pooled samples can be successfully used to provide an initial screening of gene expression, with the economic and timesaving benefits and with the limitation of no information on biological variability.

To further explore the significance of these changes, correlation analyses between hepatic fat and gene expressions were studied. Two genes, *Lpin1* and *Thrsp*, showed significant inverse associations (**Figure 2**).

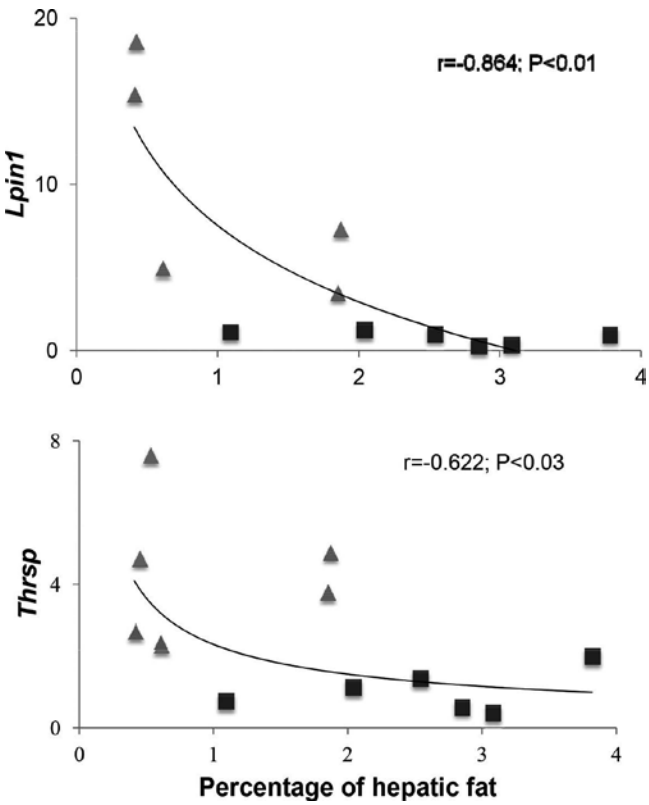


Figure 2. Association analysis among liver fat content and hepatic mRNA levels in male *ApoE*-deficient mice. Correlations were calculated according to Spearman's test, and values corresponding to all experimental groups have been included. Squares and triangles correspond to chow and squalene groups, respectively.

Squalene modulated these genes and could modulate hepatic lipid metabolism. In fact, LPIN1 (LIPIN1) plays a dual function in lipid metabolism by (1) catalysis of the conversion of phosphatidate to diacylglycerol, required for triacylglycerol and phospholipid biosynthesis, and (2) by acting as a transcriptional regulator. Through its 3-sn-phosphatidate phosphatase activity, this protein favors triacylglycerol biosynthesis [26]. Conversely, acting as a transcriptional regulator, it suppresses the lipogenic program [27]. Accordingly, a hypothetical increase in nuclear LPIN1 protein levels induced by the action of squalene may explain the strong negative association with hepatic fat content (**Figure 1**). THRSP is also a nuclear protein that

participates in the regulation of lipid synthesis by modulating the levels of lipogenic enzymes such as ATP citrate lyase, fatty acid synthase, and malic enzyme [28]. However, *Thrsp*-deficient mice showed enhanced lipogenesis, which led to the finding of its paralog, called S14R [29]. THRSP and S14R might have an overlapping role in this metabolism [30]. Interestingly, *Lipin1* and *Thrsp* expressions showed a strong positive association ($r = 0.84$, $P < 0.001$], suggesting that they both play a role in lipid metabolism and are influenced by squalene administration.

5. Squalene-induced changes in mitochondrial proteins

The mitochondrial proteome analysis unveiled caused induction of methionine adenosyltransferase 1 alpha and decreased short-chain specific acyl-CoA dehydrogenase levels [24]. Both changes were associated with lipid droplet area ($r = –0.661$ and 0.721, $P < 0.05$). These changes in proteins were due to changes in their mRNAs (**Figure 3**), and these mRNA changes were associated with lipid droplet content, as well. In fact, squalene reverted changes in ACADS to values present in wild-type mice without baseline steatosis. This protein could be a marker of hepatic steatosis. These results point out that changes in MAT1A and ACADS levels are influenced by squalene, being the former a target of squalene administration, while the latter is associated with its anti-steatotic properties [24].

Two genes, MAT1A and MAT2A, codify for methionine adenosyltransferases, which catalyze the generation of S-adenosyl-L-methionine (SAMe), the main biological methyl donor. The mammalian liver is the main organ in the regulation of serum methionine since more than 85 % of all methylation reactions and up to 48 % of methionine metabolism take place in hepatocytes. MAT1A is the isoform present in adult liver, and mice lacking the *Mat1a* gene exhibit a chronic reduction in hepatic SAMe levels and spontaneous development of nonalcoholic steatohepatitis [31] and hepatocellular carcinoma [32]. Recently, *Mat1a* deficiency has been associated with fatty liver, by regulating phosphatidylcholine-mediated processing of sterol regulatory element-binding protein 1 [33], required for very-low-density lipoprotein (VLDL) assembly and plasma lipid homeostasis in mice. Therefore, the relevant role of MAT1A in VLDL metabolism [34] may explain its increased expression in squalene-treated mice and the significant correlation with hepatic fat content.

A family of acyl-CoA dehydrogenases, including ACADS, whose function is exerted on short-chain acyl-CoA [35], catalyzes the initial step in fatty acid β-oxidation. A genome-wide association study found that some variants of this gene were associated with impaired fatty acid β-oxidation and seemed to be a marker of hepatic steatosis [36]. Thus, ACADS changes may play a role in this condition's amelioration induced by squalene. These findings regarding these two proteins, MAT1A and ACADS, as targets of squalene action and their role in advanced liver diseases suggest that squalene could have a role in preventing these pathologies.

http://dx.doi.org/10.5772/64384

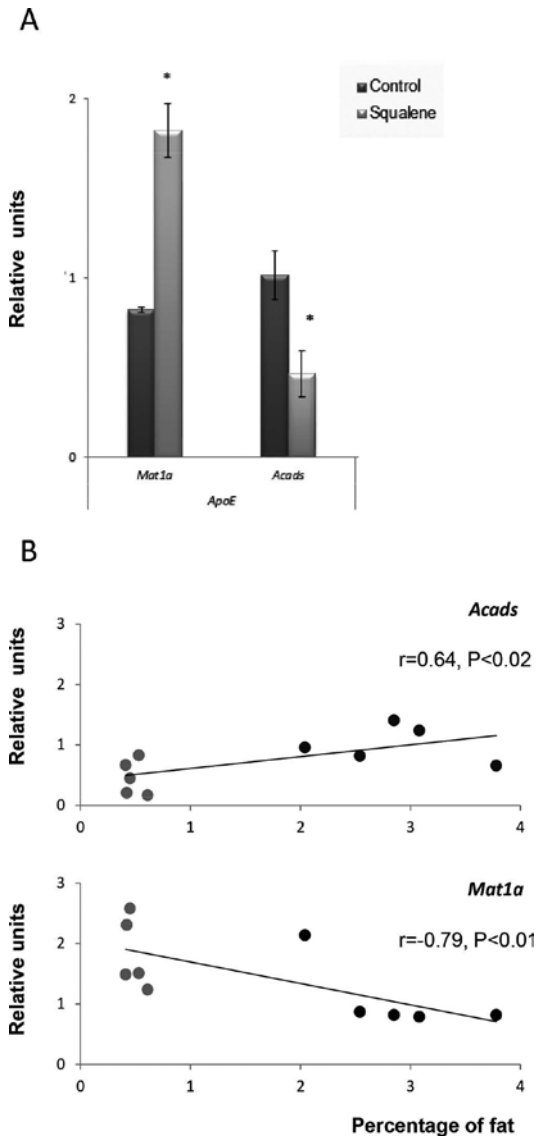


Figure 3. Effect of squalene on hepatic *Mat1a* and *Acads* mRNA levels in male *ApoE*-deficient mice. (A) Hepatic mRNA levels. Data, expressed as arbitrary absorbance units referred to *Cyclophilin B* gene expression obtained by RT-qPCR analysis, are presented as mean ± SEM. Statistical analyses were carried out using the Mann-Whitney *U* test. **P < 0.01; *P < 0.05. (B) Association among hepatic mRNA levels and liver fat content in *ApoE*-deficient mice.

6. Squalene-induced changes in microsomal proteins

Analysis of microsomal proteome showed showed that squalene induced the expression of proteins involved in lipid (MUP8 and SCP2) and vesicular transport (NIPSNAP1 and VCP),

protein quality control (PSMA7, PDIA3, HYOU1, and HSPA5), calcium storage (CALR), and redox homeostasis (TXNDC5 and PYROXD2). While the role of PDIA3 in intracellular dynamics of VLDL has been proved, this is not the case for proteins such as GRP78/HSPA5 and TXNDC5 [25]. However, TXNDC5 protein and mRNA levels showed an inverse and statistically significant correlation with the area of lipid droplets, as reflected in **Figure 4**.

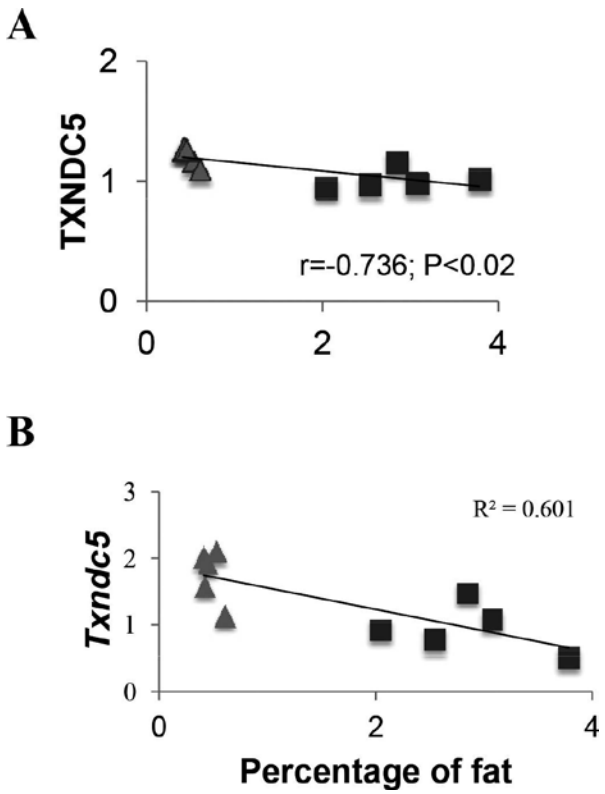


Figure 4. Association analyses among liver fat content and hepatic mRNA and protein levels of control and squalene-treated *Apoe*-deficient mice. (A) Correlation analysis between liver fat content and protein levels, (B) correlation analysis between *Txndc5* mRNA levels and liver fat content. Black squares denote chow-fed mice and gray triangles squalene-treated mice.

TXNDC5, a member of the thioredoxin family, is considered to catalyze disulfide formation in protein folding, to protect proteins against oxidative damage, and to prevent endoplasmic reticulum stress [37]. A decrease in oxidative stress, evaluated as 8-isoprostaglandin $F_{2\alpha}$, was found after squalene administration in mice [22], in agreement with other authors [38]. In this study, the observed TXNDC5 changes could contribute to lower oxidative stress. Considering that the latter is a factor inducing APOB degradation [39] and consequently decreases VLDL secretion, the increase in TXNDC5 could stabilize APOB and favor VLDL secretion. This hypothetical mechanism could explain the observed association between TXNDC5 levels and the degree of fatty liver and represents a new role for this protein. Furthermore, the action of

squalene was exerted at mRNA level. TXNDC5 seems to be a marker of the hepatic steatosis developed in the absence of APOE and may play a role in this condition's amelioration induced by squalene. This role of TXNDC5 in terms of lipid metabolism and lipid droplets needs to be defined.

7. A tentative model of squalene action

Overall, squalene is decreasing the hepatic content of lipids by facilitating the output of triacylglycerols in VLDL and promoting fatty acid oxidation, as displayed in **Figure 5**. These mechanisms were observed in male mice showing basal hepatic steatosis, as is the case of apolipoprotein E deficiency.

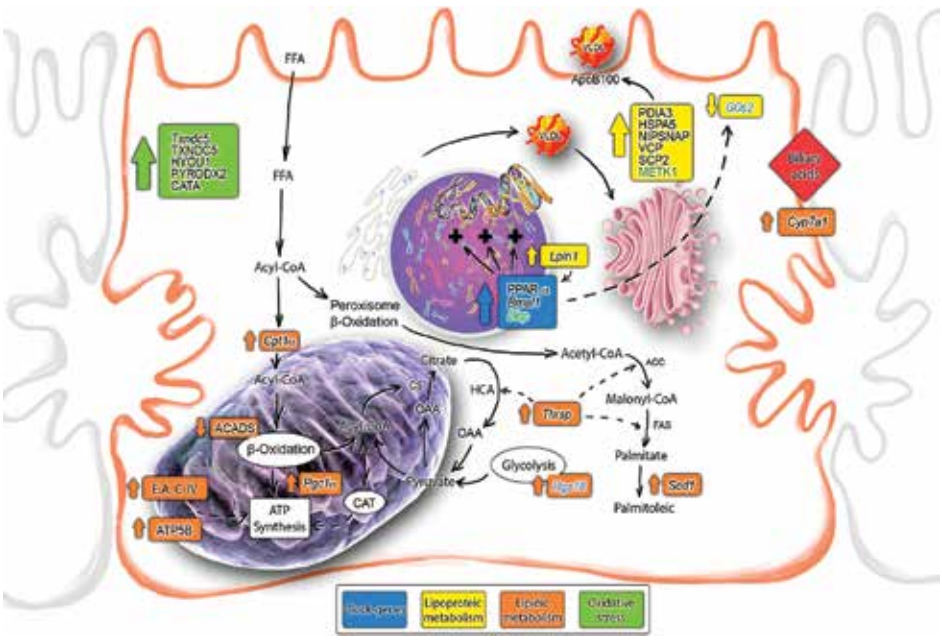


Figure 5. Squalene action in hepatocytes of *Apoe*-deficient mice. Squalene decreases fatty liver extent by favoring the secretion of VLDL and stimulating mitochondrial β-oxidation.

In addition, the complex role of dietary administered squalene is contributing to better understand hepatic lipid dynamics. The action of squalene may help to explain the protective role of virgin olive oil, where steatosis was observed with lower oxidative stress [40] and lesser atherosclerosis development compared to mice receiving palm oil [41].

In acute toxicology, a no-observed-adverse-effect level (NOAEL) of 58 g/kg was detected after a single oral dose and of 29 g/kg after intramuscular administration in mice [42]. Using 20 g/kg/day for four days, Gajkowska et al. reported the development of encephaloneuropathy in rats [43]. In mice, the lethal dose 50 is considered 5 g/kg/day [44], and a NOAEL of 2 g/kg/day

was found in 10-day administration regimen [42]. The 1 g/kg/day squalene dose used in our work is perfectly safe, and in fact, no secondary effects were noted. As mice display a higher metabolic rate than humans [45], this dose would correspond to a human dose of 100 mg/kg/day. Clearly, this dose is higher than the reported in human nutritional studies (15 mg/kg/day) [46] but does not reach the doses of 185 and 385 mg/kg/day used in women [47]. Therefore, the present study explores an attractive dose able to be reached in fortified foods and suggests a potential squalene dose to be used as functional food or therapy in fatty liver.

Acknowledgements

This research was funded by grants: 2013-41651-R from the Spanish Ministerio de Economía y Competitividad, European Regional Development Fund; B-69 from the European Social Fund, Gobierno de Aragón; and CB06/03/1012 from CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), an initiative of ISCIII.

Author details

Adela Ramírez-Torres[1], Clara Gabás-Rivera[1] and Jesús Osada[1,2,3*]

*Address all correspondence to: Josada@unizar.es

1 Department de Biochemistry and Molecular and Cellular Biology, Veterinary School, Health Research Institute of Aragón-University of Zaragoza, Zaragoza, Spain

2 Agriculture and Food Institute of Aragón, CITA-University of Zaragoza, Zaragoza, Spain

3 CIBER of Physiopathology of Obesity and Nutrition, Health Institute Carlos III, Madrid, Spain

References

[1] Keys A, Menotti A, Karvonen MJ. The diet and 15-year death rate in the Seven Countries Study. Am J Epidemiol. 1986;124:903-15.

[2] Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med. 2003;348(26):2599-608.

[3] Estruch R, Ros E, Salas-Salvadó J, Covas M-I, Corella D, Arós F, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med. 2013;368(0):1279-90.

[4] de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. Circulation. 1999;99(6):779-85.

[5] Pérez-Martínez P, López-Miranda J, Delgado-Lista J, López-Segura F, Pérez-Jiménez F. Olive oil and cardiovascular prevention: more than fat. Clin Invest Arterioscl. 2006;18:195-205.

[6] Owen RW, Mier W, Giacosa A, Hull WE, Spiegelhalder B, Bartsch H. Phenolic compounds and squalene in olive oils: the concentration and antioxidant potential of total phenols, simple phenols, secoiridoids, lignans and squalene. Food Chem Toxicol. 2000;38(8):647-59.

[7] Visioli F. Biological activities and metabolic fate of olive oil phenols. Eur J Lipid Sci Technol. 2002;104:677-84.

[8] Arbonés-Mainar JM, Navarro MA, Lou-Bonafonte JM, Martínez-Gracia MV, Osada J. Olive oil phenolics as potential therapeutic agents. 2009; Vasallo N, editor. Nova Science, New York.

[9] Gutfinger J, Letan L. Studies of unsaponifiables in several vegetable oils. Lipids Magazine. 1974;9:658.

[10] Perona JS, Cabello-Moruno R, Ruiz-Gutiérrez V. The role of virgin olive oil components in the modulation of endothelial function. J Nutr Biochem. 2006;17:429–45.

[11] Murkovic M, Lechner S, Pietzka A, Bratacos M, Katzogiannos E. Analysis of minor components in olive oil. J Biochem Biophys Methods. 2004;61(1-2):155-60.

[12] Uceda M, Hermoso M. The quality of olive oil. In: Barranco D, Fernández-Escobar R, Rallo L, editors. Olive cultivation. 3 ed. Madrid: Ediciones Mundi-Prensa; 1999. p. 572–96

[13] Ramírez-Torres A, Gabás C, Barranquero C, Martínez- Beamonte R, Fernández-Juan M, Navarro MA, et al. Squalene: Current Knowledge and Potential Therapeutical Uses. 1st ed. New York: Nova; 2010.

[14] Allouche Y, Jimenez A, Gaforio JJ, Uceda M, Beltran G. How heating affects extra virgin olive oil quality indexes and chemical composition. J Agric Food Chem. 2007;55(23): 9646-54.

[15] Smith TJ. Squalene: potential chemopreventive agent. Expert Opin Investig Drugs. 2000;9(8):1841-8.

[16] Liu GC, Ahrens EH, Jr., Schreibman PH, Crouse JR. Measurement of squalene in human tissues and plasma: validation and application. J Lipid Res. 1976;17(1):38-45.

[17] Miettinen TA, Vanhanen H. Serum concentration and metabolism of cholesterol during rapeseed oil and squalene feeding. Am J Clin Nutr. 1994;59(2):356-63.

[18] den Boer M, Voshol PJ, Kuipers F, Havekes LM, Romijn JA. Hepatic steatosis: a mediator of the metabolic syndrome. Lessons from animal models. Arterioscler Thromb Vasc Biol. 2004;24(4):644-9.

[19] Osada J, Joven J, Maeda N. The value of apolipoprotein E knockout mice for studying the effects of dietary fat and cholesterol on atherogenesis. Curr Opin Lipidol. 2000;11(1):25-9.

[20] Arbones-Mainar JM, Navarro MA, Guzman MA, Arnal C, Surra JC, Acin S, et al. Selective effect of conjugated linoleic acid isomers on atherosclerotic lesion development in apolipoprotein E knockout mice. Atherosclerosis. 2006;189(2):318-27.

[21] Arbonés-Mainar JM, Navarro MA, Acín S, Guzmán MA, Arnal C, Surra JC, et al. Trans-10, cis-12- and cis-9, trans-11-conjugated linoleic acid isomers selectively modify HDL-apolipoprotein composition in apolipoprotein E knockout mice. J Nutr. 2006;136:I353-9.

[22] Guillen N, Acin S, Navarro MA, Perona JS, Arbones-Mainar JM, Arnal C, et al. Squalene in a sex-dependent manner modulates atherosclerotic lesion which correlates with hepatic fat content in apoE-knockout male mice. Atherosclerosis. 2008;196:558-64.

[23] Osada J. The use of transcriptomics to unveil the role of nutrients in mammalian liver. ISRN Nutr. 2013;2013:403792.

[24] Ramirez-Torres A, Barcelo-Batllori S, Fernandez-Vizarra E, Navarro MA, Arnal C, Guillen N, et al. Proteomics and gene expression analyses of mitochondria from squalene-treated apoE-deficient mice identify short-chain specific acyl-CoA dehydrogenase changes associated with fatty liver amelioration. J Proteomics. 2012;75(9): 2563-75.

[25] Ramirez-Torres A, Barcelo-Batllori S, Martinez-Beamonte R, Navarro MA, Surra JC, Arnal C, et al. Proteomics and gene expression analyses of squalene-supplemented mice identify microsomal thioredoxin domain-containing protein 5 changes associated with hepatic steatosis. J Proteomics. 2012;77:27-39.

[26] Kim DK, Kim JR, Koh M, Kim YD, Lee JM, Chanda D, et al. Estrogen-related receptor gamma (ERRgamma) is a novel transcriptional regulator of phosphatidic acid phosphatase, LIPIN1, and inhibits hepatic insulin signaling. J Biol Chem. 2011;286(44): 38035-42.

[27] Finck BN, Gropler MC, Chen Z, Leone TC, Croce MA, Harris TE, et al. Lipin 1 is an inducible amplifier of the hepatic PGC-1alpha/PPARalpha regulatory pathway. Cell Metab. 2006;4(3):199-210.

[28] Kinlaw WB, Church JL, Harmon J, Mariash CN. Direct evidence for a role of the "spot 14" protein in the regulation of lipid synthesis. J Biol Chem. 1995;270(28):16615-8.

[29] LaFave LT, Augustin LB, Mariash CN. S14: insights from knockout mice. Endocrinology. 2006;147(9):4044-7.

[30] Aipoalani DL, O'Callaghan BL, Mashek DG, Mariash CN, Towle HC. Overlapping roles of the glucose-responsive genes, S14 and S14R, in hepatic lipogenesis. Endocrinology. 2010;151(5):2071-7.

[31] Lu SC, Alvarez L, Huang ZZ, Chen L, An W, Corrales FJ, et al. Methionine adenosyltransferase 1A knockout mice are predisposed to liver injury and exhibit increased expression of genes involved in proliferation. Proc Natl Acad Sci U S A. 2001;98(10): 5560-5.

[32] Martinez-Chantar ML, Corrales FJ, Martinez-Cruz LA, Garcia-Trevijano ER, Huang ZZ, Chen L, et al. Spontaneous oxidative stress and liver tumors in mice lacking methionine adenosyltransferase 1A. FASEB J. 2002;16(10):1292-4.

[33] Walker AK, Jacobs RL, Watts JL, Rottiers V, Jiang K, Finnegan DM, et al. A conserved SREBP-1/phosphatidylcholine feedback circuit regulates lipogenesis in metazoans. Cell. 2011;147(4):840-52.

[34] Cano A, Buque X, Martinez-Una M, Aurrekoetxea I, Menor A, Garcia-Rodriguez JL, et al. Methionine adenosyltransferase 1A gene deletion disrupts hepatic VLDL assembly in mice. Hepatology. 2011;54(6):1975-86.

[35] Musso G, Gambino R, Cassader M. Recent insights into hepatic lipid metabolism in non-alcoholic fatty liver disease (NAFLD). Prog Lipid Res. 2009;48(1):1-26.

[36] Illig T, Gieger C, Zhai G, Romisch-Margl W, Wang-Sattler R, Prehn C, et al. A genome-wide perspective of genetic variation in human metabolism. Nat Genet. 2010;42(2): 137-41.

[37] Horna-Terron E, Pradilla-Dieste A, Sanchez-de-Diego C, Osada J. TXNDC5, a newly discovered disulfide isomerase with a key role in cell physiology and pathology. Int J Mol Sci. 2014;15(12):23501-18.

[38] Buddhan S, Sivakumar R, Dhandapani N, Ganesan B, Anandan R. Protective effect of dietary squalene supplementation on mitochondrial function in liver of aged rats. Prostaglandins Leukot Essent Fatty Acids. 2007;76(6):349-55.

[39] Uchiyama S, Shimizu T, Shirasawa T. CuZn-SOD deficiency causes ApoB degradation and induces hepatic lipid accumulation by impaired lipoprotein secretion in mice. J Biol Chem. 2006;281(42):31713-9.

[40] Arbones-Mainar JM, Ross K, Rucklidge GJ, Reid M, Duncan G, Arthur JR, et al. Extra virgin olive oils increase hepatic fat accumulation and hepatic antioxidant protein levels in APOE−/− mice. J Proteome Res. 2007;6(10):4041-54.

[41] Arbones-Mainar JM, Navarro MA, Carnicer R, Guillen N, Surra JC, Acin S, et al. Accelerated atherosclerosis in apolipoprotein E-deficient mice fed Western diets containing palm oil compared with extra virgin olive oils: a role for small, dense high-density lipoproteins. Atherosclerosis. 2007;194(2):372-82.

[42] Toxicology International Journal. Final report on the safety assessment of squalane and squalene. Int J Toxicol. 1982;1(2):37-56.

[43] Gajkowska B, Smialek M, Ostrowski RP, Piotrowski P, Frontczak-Baniewicz M. The experimentalsqualeneencephaloneuropathyintherat.ExpToxicolPathol.1999;51(1):75-80.

[44] Merck. Merck Index. 13th ed. O'Neil MJ, Smith A, Heckelman PE, Budavari S, editors. New York, NY. John Wiley & Sons; 2001.

[45] Demetrius L. Of mice and men. When it comes to studying ageing and the means to slow it down, mice are not just small humans. EMBO Rep. 2005;6:S39-S44.

[46] Gylling H, Miettinen TA. Postabsorptive metabolism of dietary squalene. Atherosclerosis. 1994;106(2):169-78.

[47] Cho S, Choi CW, Lee DH, Won CH, Kim SM, Lee S, et al. High-dose squalene ingestion increases type I procollagen and decreases ultraviolet-induced DNA damage in human skin in vivo but is associated with transient adverse effects. Clin Exp Dermatol. 2009;34(4):500-8.

Section 2

Olive Oil Production, Composition and Quality

Chapter 2

Improvement of Olive Oil Mechanical Extraction: New Technologies, Process Efficiency, and Extra Virgin Olive Oil Quality

Gianluca Veneziani, Beatrice Sordini,
Agnese Taticchi, Sonia Esposto, Roberto Selvaggini,
Stefania Urbani, Ilona Di Maio and Maurizio Servili

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64796

Abstract

Most of the recent technological innovations applied to the mechanical oil extraction process are aimed at improving virgin olive oil quality and yield. Extra virgin olive oil (EVOO) quality is mainly based on the qualitative/quantitative composition of monounsaturated fatty acids, volatile and phenolic compounds that are strictly related to the health and sensory properties of the product, with particular attention given to the fraction of secoiridoid derivatives and C_5 and C_6 volatile compounds. The different levels of concentration of these compounds are due to some important variables: agronomic and technological. The chapter explains the recent approaches and innovations introduced in the oil extraction process to improve the working efficiency of the production system and to obtain high-quality extra virgin olive oils.

Keywords: EVOO quality, phenols, volatile compounds, EVOO processing, technological innovations

1. Introduction

Extra virgin olive oil (EVOO) is the main source of lipids in the Mediterranean diet. The marketable, healthy, and sensory quality of an EVOO has been ascribed to the presence of bioactive components such as monounsaturated and polyunsaturated fatty acids (MUFAs and PUFAs), squalene, phytosterols, phenolic, and volatile compounds [1–5]. Several factors such

as the genetic and geographical origin of the olive fruit as well as agronomic practices and technological strategies affect the phenolic content and aromatic profile of EVOO.

Olives, EVOO, and the by-products of the mechanical extraction system such as olive vegetation water and pomace contain several phenolic compounds with recognized biological and health properties. These substances are considered the principal bioactive compounds of EVOO, showing a high antioxidant activity with an important role in the ratio between EVOO consumption and chronic degenerative events, mainly inflammatory and age-dependent diseases such as cardio-brain-vascular diseases and cancer [4–11].

Major phenolic compounds found in EVOO are phenolic acids, phenolic alcohols such as tyrosol (*p*-HPEA) and hydroxytyrosol (3,4-DHPEA), hydroxy-isocromans, flavonoids, lignans, and secoiridoids. This latter class of compounds is represented by the dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA or *p*-HPEA (3,4-DHPEA-EDA or *p*-HPEA-EDA), an isomer of oleuropein aglycon (3,4-DHPEA-EA) and the ligstroside aglycon (*p*-HPEA-EA). They arise from the secoiridoid glycosides (oleuropein, demethyloleuropein, and ligstroside) through the enzymatic action of β-glucosidase during the mechanical extraction process. Secoiridoids are exclusive compounds of olive leaves, fruits, EVOO, and milling by-products (olive vegetation water and pomace). The secoiridoid derivatives, along with lignans ((+)-1-acetoxypinoresinol and (+)-1-pinoresinol), are the most abundant hydrophilic phenols of EVOO [6, 12, 13].

The geographical and genetic origin of olive fruits, the choice of agronomic practices, and the technological conditions of EVOO production affect the wide variability in its phenolic and volatile composition and, therefore, its healthy and sensory quality. The variability range of the content of total phenols and oleuropein derivatives in over 700 industrial EVOO samples analyzed is illustrated in the box and whiskers plots of **Figure 1**. Based on these results, the contents of the total phenols and oleuropein derivatives show a median of 534 and 398 mg/kg, with values ranging between 187–997 and 77–112 mg/kg, respectively [14].

Health-promoting effects and organoleptic properties of EVOO have been mainly ascribed to its phenols content (hydroxytyrosol and secoiridoids, in particular) [5, 15]. Several epidemiological studies have in fact fully demonstrated the inflammatory, antioxidant, antimicrobial, anti-proliferative, antiarrhythmic, platelet antiaggregant and vasodilatory effects of EVOO phenolic compounds [4–6]. Furthermore, based on scientific evidence, Regulation (EU) No 432/2012 granted the health claim to the EVOO polyphenols, fixing the quantity of 5 mg as the daily amount of hydroxytyrosol and its derivatives (e.g. oleuropein complex and tyrosol) that should be ingested, with a moderate consumption of olive oil (20 g/day) to reduce cardiovascular disease [16].

It has been clearly known that phenolic compounds also have antioxidant activity; therefore, they play a pivotal role in the prolonging of EVOO shelf life [6]. Furthermore, from a sensory perspective, EVOO phenols are the compounds responsible for the characteristic notes of "bitterness" and "pungency". They stimulate the receptors of taste and the free endings of trigeminal nerve, which elicit the former the bitterness perception, the latter pungency and astringency interaction [15].

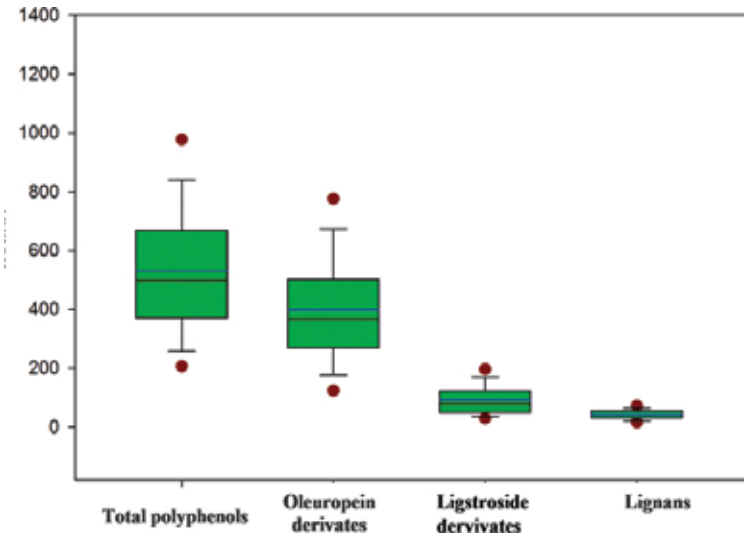


Figure 1. Box and whisker plots of variability (mg/kg) of phenolic compounds evaluated over 700 extra virgin olive oil samples analyzed [14]. Percentile limits in: box = lower 25th, upper 75th; whiskers = lower 10th, upper 90th; the red points = lower 5th, upper 95th; blue and black lines in the box represent the median and the average, respectively.

Another important part of EVOO flavor is characterized by many different olfactory notes, such as "cut grass," "floral," "green apple," "tomato" and "almond," which are often related to many volatile substances such as aldehydes, alcohols, esters, and hydrocarbons. In particular, C_6 and C_5 compounds, especially C_6 linear unsaturated and saturated aldehydes, alcohols, and esters, represent the key odorants responsible for those perceptions of positive aroma [17–19]. When the olive is intact, the concentration of those volatile compounds is still low. They greatly increase when the cell structures rupture during the mechanical extraction process and with the consecutive activation of the lipoxygenase (LOX). The C_6 and C_5 compounds are synthesized from linoleic (LA) and linolenic (LnA) acids by the enzymatic activities included in the lipoxygenase (LOX) pathway, and their concentrations depend on the level and the activity of each enzyme involved in this LOX pathway. **Figure 2** depicts a schematic illustration of the LOX pathway, which was extensively studied and discussed [18, 19].

However, it is worth mentioning that even though the main pathways are known for the formation of olive oil volatiles, the only correlation that has been proved is that between the "cut grass" aroma and C_5 and C_6 aldehydes (saturated and unsaturated) [18, 20].

EVOO processing includes a series of mechanical operations for extracting the oil from olive drupes by physical means only, according to Regulation (EU) No 1348/2013 [21]. Among them, the most important as regards quality is the crushing of the olives, which allows the release of the droplets of oil from the vacuoles, breaking down the cellular structure of the olive fruit; the malaxation of the olive paste, which promotes the coalescence of the oil droplets, with the simultaneous release of phenolic compounds into the oil phase and the increase of EVOO aroma; the mechanical recovery of the oil by centrifugation (continuous process) or pressing (discontinuous process); and lastly, filtration, used for removing suspended particles and

eliminating residual water responsible for EVOO oxidation and the onset of off-flavors during its shelf life.

Many studies have been already developed during the last 10 years in order to optimize all the mechanical extraction steps that play a crucial role in the qualitative/quantitative composition of phenolic and volatile profile and, consequently, the sensory characteristics of the resulting EVOOs. Technological innovations have led to new extraction plants designed to improve the quality of oils obtained from olives with different genetic, geographical, and agronomic characteristics.

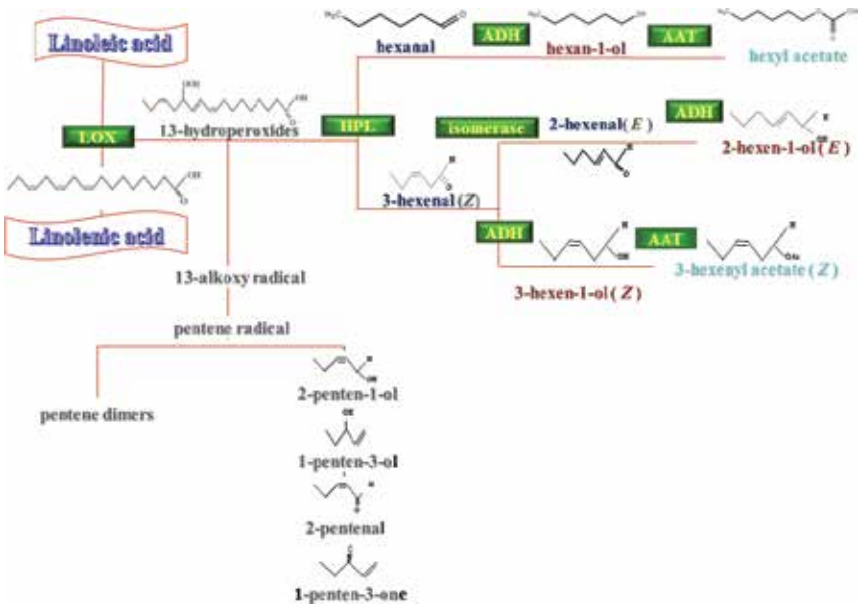


Figure 2. The lipoxygenase pathway on polyunsaturated fatty acids for the synthesis of main volatile compounds in EVOO according to Angerosa et al. [18] and Servili et al. [19]. LOX, lipoxygenase; HPL, hydroperoxide lyases; ADH, alcohol dehydrogenase; AAT, alcohol acetyl transferases.

One of the major challenges in the olive oil field will be the improvement of plant engineering performance in terms of sustainability, efficiency, modularity, and flexibility, reducing production costs, with attention to EVOO quality and yield.

In this regard, the next chapter emphasizes the effects of the recent new approaches and innovations in the olive oil field.

2. Crushing impact on minor components of EVOO

In recent years, several studies have been performed to elucidate the impact of technological operations applied during the mechanical extraction on EVOO yield and on its minor compounds, in particular [14, 22–26].

http://dx.doi.org/10.5772/64796

Traditional stone mills, hammers, blade and disc crushers, and destoning machines are the crushers currently available for industrial equipment operations [25]. Every crusher exerting different mechanical action to break down the olive tissue elicits several effects due to variation induced in the olive paste, in terms of temperature, granulometry of fragments, and exposure to atmospheric oxygen. These variations play a crucial role for endogenous enzymatic activities, affecting the final amount of the EVOO as well as its phenolic and volatile profile [27, 28]. During this phase, in fact, the entire heritage of olive fruit endogenous enzymes (cellulases, hemicellulases, pectinases, polygalacturonases, lipase, β-glucosidase, polyphenoloxidase (PPO), peroxidase (POD), and lipoxygenase (LOX)) is activated and involved in the subsequent phases of the extraction process and the formation and transformation of phenolic and volatile compounds in EVOO [29, 30]. Among them, the most important endogenous enzymes involved in EVOO quality are β-glucosidase, PPO, POD, and LOX. On the other hand, temperature and time become key parameters (to be checked continuously) when oxygen availability is not a limiting factor for the enzymatic activity of oxidoreductases [17, 20]. Many studies have been focused on the thermal stabilities of PPO, POD, and the enzymes pool of the LOX pathway [26, 29, 30]. According to the data found by Taticchi et al. [30] for the Moraiolo cultivar, the activity of PPO and POD varies according to temperature, with a maximum at 50°C and at 34.7°C, respectively. The maximum LOX activity has been observed at 30°C [20]. Hyperoxide lyase (HPL) is a heat-labile enzyme characterized by maximum activity at 15°C, while at 30°C its activity shows a partial inhibition [31].

Therefore, olive crushing is not just a simple physical process; it also represents an EVOO quality key factor.

Several studies carried out on the distribution of olive fruit endogenous enzymes in its constitutive parts (pulp, stone and seed) have shown that the seed is particularly rich in POD, while the phenolic compounds are most concentrated in the pulp [28, 32]. In particular, in olive pastes and the produced oil the decrease of phenols is due to their oxidation catalyzed by the POD and the PPO together. Whereas the LOX, contained in the seed, through the aforementioned cascade pathway, produces only a small amount of volatile compounds, which are mainly generated by the same enzyme of the pulp responsible for the production of C_5 and C_6 saturated and unsaturated aldehydes, alcohols and esters [14]. These findings are fundamental for the innovative use of a hammer with a selective effect on the different constitutive parts of the olive. Blade or tooth crushers, pre-crusher or destoning machines, for example, reduce the degradation of seed tissues, limiting the release of POD in the pastes. This involves in an increase in phenolic concentration, because their enzymatic oxidation is prevented (**Figure 3**) [32, 33]. As concerns aroma, the use of a hammer mill or other crushers causes the pulp tissues to be ground more violently, bringing about an increase of the olive paste temperature and a reduction of HPL activity [18, 27] (**Table 1**). Olive stoning in pre-crushing increases the phenolic concentration in EVOO [32, 34] and, at the same time, positively modifies the composition of the volatile compounds [27, 32].

Nowadays, strategic approaches in the olive oil field are based on the choice of plant engineering systems and operating conditions aimed at the control of these endogenous enzymes [26, 30].

Inarejos-García and co-workers [22] evaluated the effect of stronger (e.g. hammer crushers using small hole grid diameter and high rotation speed) or milder (e.g. hammer crushers using large hole grid diameter, blade crusher, etc.) crushing conditions on minor components (phenolic and volatile fraction, in particular) of olive paste and EVOO. The results obtained showed that the stronger crushing produced an increase of phenols and a decrease of volatile compounds in olive pastes and corresponding EVOO, while milder crushing produced an opposite effect [22]. These findings are in agreement with results reported by other authors [23, 24, 28]. The relationship between pressure and an overheating of the olive paste can explain these data: energetic milling action yields significant shredding of the olive oil tissues. The crushing conditions produce deep changes in the composition of EVOO hydrophilic phenols. This aspect is due to the activity of enzymes such as β-glucosidase and esterase that catalyze the transformation of secoridoids into aglycon forms, which are characterized by greater solubility in the oily phase. In EVOO, the oleuropein derivatives are the most abundant phenols due to both their partition coefficients between the oil and water phases and their different stability [22].

Further, comparative investigations have indicated that the hammer crusher or a partial destoner produces a significantly larger amount of small oil droplets, extending the malaxation time, hence promoting the loss of phenols and volatile compounds [24, 35].

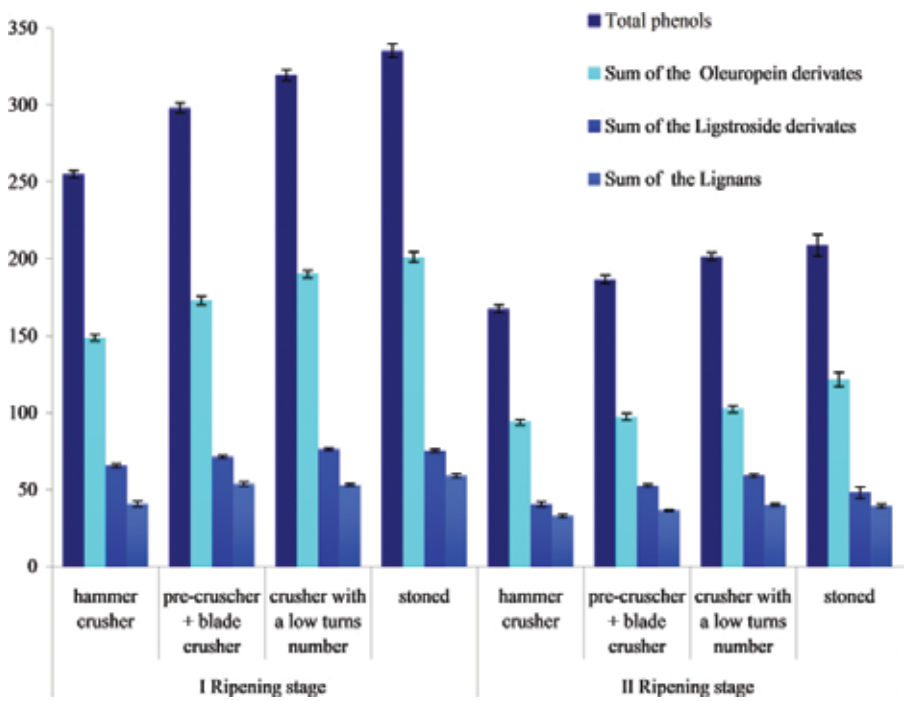


Figure 3. Phenolic composition (mg/kg) of EVOOs (*Frantoio* Cv.) obtained by different crushing methods [32]. The phenols' concentration was evaluated by HPLC previously reported by Montedoro et al. [36]. Results are mean value of three independent determinations ± standard deviation.

	Hammer		Blade + pre-crusher		Low turn number crusher		Stoned	
Aldehydes*								
Pentanal	236.5	(4.0)	273.4	(2.1)	17.9	(1.0)	66.5	(6.7)
Hexanal	280.0	(2.9)	511.4	(35.7)	533.7	(0.3)	579.6	(5.3)
2-Pentanal *(E)*	10.7	(0.3)	13.2	(0.9)	94.8	(1.8)	16.6	(1.0)
2-Hexanal *(E)*	43600.6	(327.0)	44718.9	(207.8)	39811.6	(587.4)	52228.1	(521.0)
2,4-Esadyenal *(E,E)*	19.4	(0.1)	42.0	(3.5)	341.6	(14.4)	88.9	(5.4)
2-Heptanal *(E)*	0.0	(0.0)	0.0	(0.0)	158.2	(10.0)	72.0	(3.7)
Alcohols								
1-Pentanol	167.0	(5.2)	94.5	(4.7)	23.3	(0.7)	62.6	(1.4)
2-Penten-1-ol *(E)*	166.0	(11.3)	91.4	(5.1)	52.4	(3.5)	104.0	(7.4)
1-Penten-3-ol	960.3	(53.2)	899.0	(43.3)	522.0	(49.2)	300.0	(28.2)
1-Hexanol	1788.0	(57.0)	2152.0	(74.0)	521.0	(41.0)	1501.0	(56.0)
3-Hexan-1-ol *(Z)*	88.4	(22.2)	103.6	(10.1)	49.2	(2.3)	77.0	(5.1)
3-Hexan-1-ol *(E)*	22.2	(0.2)	20.2	(0.1)	9.9	(0.2)	20.4	(0.5)

The volatile compounds were determined in duplicate by HS-SPME-GC-MS as reported by Esposto et al. [37].

*Results are the mean value of three independent determinations; standard deviation is reported in parentheses.

Table 1. Volatile composition (μg/kg) of EVOOs (*Frantoio* Cv.) obtained by different crushing methods [32].

3. Malaxation impact on minor components of EVOO

In recent years, the impact of malaxation on EVOO minor compounds has been investigated at length by several authors [30, 38]. During this phase, a slow, continuous mixing up and a gradual temperature increase of olive paste take place. This has the effect of breaking up the water-oil emulsion formed in the previous step and favoring, at the same time, the coalescence of the oil droplets into drops of greater sizes. Furthermore, a reduction of the product viscosity and endogenous enzymatic activities also occurs. Malaxation and the related selective control of enzymes such as PPO, POD and LOX are therefore other critical points of the mechanical extraction process of EVOO [14, 26].

Recently, the optimization of the best operative parameters for the malaxation process, such as time, temperature and oxygen concentration, that can guarantee the correct balance between the yield and the quality of extracted EVOOs (in terms of the amount of phenolic and volatile compounds) has been extensively investigated [39].

The newest malaxer machines ensure a hermetic sealing. To avoid the negative effects of POD and PPO activities on phenols, the O_2 availability in the malaxer headspace is modulated by

valves for inert gas (nitrogen or argon). Other authors have proposed to exploit the emission of carbon dioxide (CO_2) from olive paste coupled with the oxygen depletion during malaxation under sealed conditions, in order to solve the problem of oxidative phenomena without using inert gases [40–42]. The saturation of the malaxation chamber with CO_2 offers a two-fold benefit for the cost of inert gas and for the POD and PPO inactivation. However, the amount of O_2 incorporated during crushing of the olive pastes seems to be for developing volatile compounds from LOX pathway [28].

Through the control of O_2 concentration in the malaxer headspace, it is possible to regulate the content of phenolic and volatile compounds in the end products (**Figure 4** and **Table 2**).

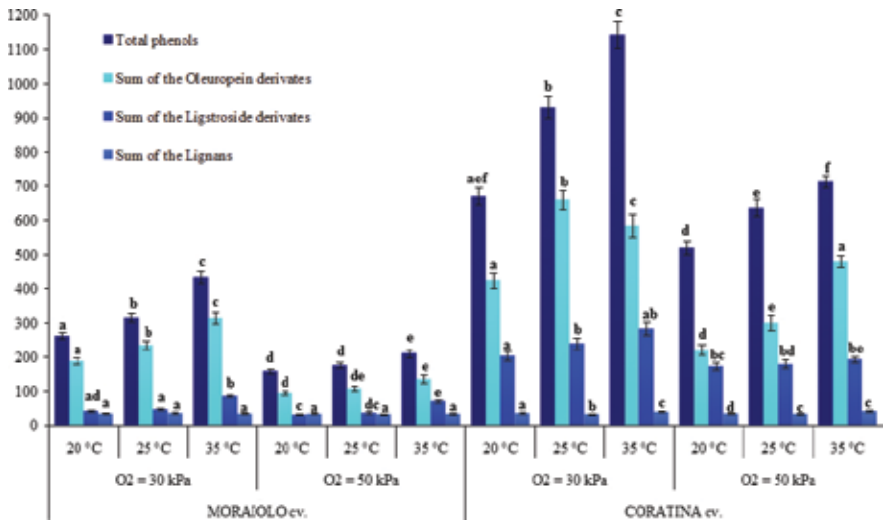


Figure 4. The phenolic composition (mg/kg) of EVOOs obtained malaxing at different temperatures and in different initial atmosphere compositions [30]. The phenols' concentration was evaluated by HPLC previously reported by Montedoro et al. [36]. Data are the mean value of two independent experiments analyzed in duplicate ± standard deviation. The values in each row having different letters (a–f) are significantly different from one another ($p < 0.05$).

This opportunity of regulating the content of phenolic and volatile compounds, obtained by using adequate amounts of O_2 during malaxation, is an important aspect to be taken into account. The influence of malaxing temperature on the olive PPO and POD activities has been studied by Taticchi et al. [30]. The outcomes showed that PPO is characterized by a lower thermal stability than the POD, which explains the variation of phenolic concentrations in the olive paste during processing as a function of temperature. The malaxation temperature promotes the release of phenols from cell wall polysaccharides catalyzed by endogenous hemicellulases and polygalacturonases and improves the solubility of phenols in the oil phase [43]. However, for many Italian autochtone cultivars, temperatures above 30°C led to a strong decrease in the aromatic profile of oils. Hence, the enzymes involved in the LOX pathway are characterized by different temperature optima between 15 and 30°C, while a reduction in their activity level is observed above 30°C [15]. The processing temperature affects the concentration of aldehydes: the lowest amounts have been observed at 35°C, while the highest concentration

occurs at 25°C. Esters also showed the same behavior as the aldehydes; in fact, high malaxation temperatures provoke a decay of esters and of *cis*-3-hexen-1-ol, which are responsible for positive sensorial notes [17, 19, 20].

With regard to the alcohols, their concentration increased with the malaxation temperature. Several authors observed an accumulation of hexan-1-ol and *trans*-2-hexen-1-ol, both associated with odor not completely agreeable [17, 19, 20]. These results suggested that, according to the genetic characteristics of the cultivar, the optimal malaxation temperature could be set at about 28 or 30°C [15]. Other studies have demonstrated that the extending malaxation time shows a decrease in the typical "green" sensory note and all the other pleasant sensations [18, 20].

Therefore, particular emphasis has been placed on optimizing temperature and oxygen concentration during malaxation for obtaining high-quality EVOOs, but always taking into account the olive cultivar [39].

	O_2 = 0 kPa		O_2 = 30 kPa		O_2 = 50 kPa		O_2 = 100 kPa	
Aldehydes*								
2-Pentanal *(E)*	548.5	(16.3)ab	509.7	(5.8)b	636.7	(17.9)c	613.0	(51.2)ac
Hexanal	1187.0	(9.9)a	1624.3	(30)bc	1532.1	(27.3)b	1744.0	(121.2)c
2-Hexanal *(E)*	51565.0	(827.3)a	52900.0	(565.7)ab	54340.5	(355.7)b	53920.0	(333.1)b
Alcohols								
1-Pentanol	40.0	(5.7)a	54.3	(5)b	39.4	(5)a	48.0	(3.2)ab
2-Penten-1-ol (E)	87.5	(0.7)a	67.0	(0.2)b	105.8	(5.7)c	105.0	(8.3)c
1-Penten-3-ol	890.0	(2.8)a	82.0	(1.2)b	1093.5	(33.5)c	1185.0	(91.2)c
1-Hexanol	2326.0	(49.5)a	3694.2	(2)b	1788.0	(57.2)b	2170.0	(123.1)a
3-Hexan-1-ol *(E)*	25.5	(0.7)ab	31.6	(3.8)a	20.0	(1.9)b	21.0	(1.9)b
3-Hexan-1-ol *(Z)*	561.0	(4.2)a	513.6	(9.6)b	486.3	(11.1)b	498.0	(31.2)b
2-Hexan-1-ol *(E)*	3654.5	(30.4)a	5905.0	(321)b	3350.1	(80.5)c	4185.0	(35.6)d

The volatile compounds were determined in duplicate by HS-SPME-GC-MS as reported by Esposto et al. [37].

* Data are the mean values of three independent experiments; standard deviation is reported in parentheses. Values in each row having different letters (a–d) are significantly different from one another at $p < 0.01$.

Table 2. Volatile composition (μg/kg) of EVOOs (*Coratina* Cv.) obtained after malaxation in different initial atmosphere compositions [42].

4. New approaches: emerging techniques

In the olive oil field, the current scientific research is focused on the improvement of its quality, with particular attention given to the optimization of the working efficiency of the extraction plant and to reducing malaxation time. Attempts have been made for converting the traditional malaxation batch process into a continuous one, obtaining a simultaneous positive effect on both the oil yield and the working times [38]. The traditional malaxer is a heat exchanger

characterized by a low thermal transfer efficiency, because the ratio of surface area to volume is disadvantageous. In principle, malaxation is distinguished into two steps, with the "pre-heating" phase, defined as the time required for the olive paste to reach the process temperature (about 27°C), and "actual malaxation". The duration of the "pre-heating" phase is about 45% of the total process time [44]. In order to reduce this phase and optimize the phenolic and volatile compounds related to EVOO healthy and sensory properties, different technological solutions can be adopted during olive paste conditioning: microwave energy (from 300 MHz to 300 GHz) [45, 46], mechanical vibrations (under 200 Hz) [47], pulsed electric field (PEF) [48, 49], ultrasounds (US) [50-52], and heat exchangers [37, 53, 54].

4.1. Microwave technology

Leone and co-workers [45] have built and adjusted a new apparatus in an industrial olive oil extraction plant that is based on microwave technology for the continuous conditioning of the olive paste. It replaces the malaxer, with the purpose of assuring the continuity of the process. The capability of microwaves to generate a thermal and a non-thermal effect on the olive paste is exploited for the purpose of increasing the temperature and the vacuole desegregation, respectively. These combined effects promoted the coalescence that is directly related to extraction efficiency. Tamborrino et al. [46] investigated in a subsequent study the impact of the microwave apparatus on phenolic and volatile compounds of olive oil. The microwave technology is responsible for a reduction in the olive paste conditioning time of around 88% compared to the conventional system, and a significant increase in the extraction yields, without compromising the EVOO marketable parameters. However, the authors observed that the EVOOs obtained from microwave treatment were characterized by low amounts of secoiridoid derivatives, specifically 3,4-DHPEA-EDA, *p*-HPEA-EA, and *p*-HPEA-EDA, compared to the EVOOs extracted with traditional systems. By reducing the time needed for the activation period of the depolymerizing enzymes, the microwave treatment led to a decrease of phenolic concentration in EVOOs. This does not occur with the traditional, slow malaxing process. On the other hand, the EVOOs obtained from rapid microwave conditioning compared with those obtained from traditional malaxation showed the largest increase of volatile compounds due to the shorter overall conditioning time. At the same time, a positive effect on the different activity levels of the LOX pathway is seen. These results are in good agreement with the findings of Angerosa et al. [18] and Esposto et al. [37]. One advantage of exploiting a shorter conditioning time is the reduction of the partial inactivation of the HPL, promoting an accumulation of the C_6 aldehydes [55]. Therefore, microwave technology could be potentially used in olive oil extraction plants to improve the olive oil extraction process and to overcome the bottlenecks of malaxation by guaranteeing a continuous process [45, 46].

4.2. Mechanical vibrations

A further innovative approach based on a vibration system to reduce the malaxation time, always overlooking the EVOO yield and quality, has been recently developed by Gallina Toschi and co-workers [47]. The influence of mechanical vibrations, at frequencies between 5 and 200 Hz, applied in the resonant conditions of the olive paste as pre-treatment and in combination

with traditional malaxation, was investigated. The results obtained suggested that this technological approach breaks down the olive cells to improve the next phase of the EVOO extraction process. Gallina Toschi et al. [47] found that the optimal frequencies of excitation of the olive pastes fall between 50 and 80 Hz.

4.3. Pulsed electric field (PEF)

Among the emerging techniques recently proposed, the application of pulsed electric fields (PEF) during olive oil extraction could be also interesting in the olive oil technological panorama [48, 49]. The study at the pilot scale in an industrial oil mill was conducted by Puértolas and Martínez de Marañón [48] on Arroniz cultivars to assess the effect on the extraction yield and oil quality obtained through the application of a PEF treatment (2 kV/cm; 11.25 kJ/kg) on the crushed olive paste before malaxation. The exposure to electric fields for microseconds causes the formation of pores in cell membranes. This electroporation mechanism increasing the permeability of the vegetable cells promoted the diffusion of solutes through their membranes. This leads to an increase of 13.3% in the yield extraction. The PEF treatment not only showed no negative side effects on the sensory and chemical characteristics of EVOO but also increased the amount of human-health-related compounds, such as phenols, phytosterols, and tocopherols, assuring the EU marketable parameters of highest quality EVOO [49]. Hence, the application of PEF could also represent a good alternative for enhancing the phenolic content in EVOO.

4.4. Ultrasound (US) technology

With the aim of exploiting the technological environmental sustainability for improving EVOO extraction yields, the application of ultrasound (US) technology in olive paste pre-treatment has been tested in the laboratory by Jiménez et al. [51]. The effects of high-power ultrasound, applied directly by probe horn (105 W, 12 cm, and 24 kHz) and indirectly by ultrasound-cleaning bath (150 W and 25 kHz), on olive pastes were observed and compared to conventional malaxation from a sensory and chemical characteristic perspective.

The results of research focusing on the use of new ultrasonic extraction technologies in the EVOO industry should lead to meaningful technological advances in EVOO production. Following experimental trials, the results were employed to suggest innovative scaling-up solutions of the EVOO mechanical extraction process [50]. Briefly, the ultrasound produces mechanical and thermal effects. The mechanical effect is due to the cavitation phenomena, causing the rupture of a part of the uncrushed oil cells. The thermal effect is related to ultrasonic energy: as an acoustic wave propagates through a plant tissue, a part of it is absorbed and converted to heat in the olive paste [52].

More recently, in a pilot-scale plant, Clodoveo and Hachicha Hbaieb [44] compared ultrasound and microwave treatments of olive paste with traditional malaxation to evaluate their capability to increase environmental sustainability by improving EVOO extraction yields. The results demonstrated that a significant reduction of the malaxation pre-heating stage and improvement in the extraction yield were observed in EVOOs obtained from both ultrasound

and microwaves systems [44]. Furthermore, in terms of energy consumption, the ultrasound technology was more sustainable than microwaves and the traditional system, with energy efficiencies of 93.05% (ultrasounds), 42.3% (microwaves), and 49.41% (traditional system). [44].

4.5. Flash thermal conditioning (FTC)

Veneziani et al. [53] carried out a line of research previously studied by Esposto et al. [37], evaluating the impact of a new technology, based on heat exchangers placed after the crashing phase of extraction process, on the quality of EVOO [37, 53, 54]. Crushed olive pastes from five Italian cultivars—Coratina, Ottobratica, Moraiolo, Peranzana, and Cellina di Nardò—were immediately brought to 25 or 30°C by flash thermal conditioning (FTC) by means of a tubular heat exchanger with counter current flow of hot water. After this phase, 15 or 20 minutes of malaxation was applied at the same temperature as the treated pastes (25 and 30°C) [53]. A conventional extraction was done on the same cultivar applying traditional malaxation at temperatures of 25 and 30°C for 30 minutes for non-pretreated crushed pastes. The results obtained indicated that the FTC treatment brings about an increase in phenols in the EVOO for each cultivar studied, as shown in **Figure 5**. Furthermore, the main differences in terms of phenolic composition with respect to the traditional malaxation have been observed in EVOOs

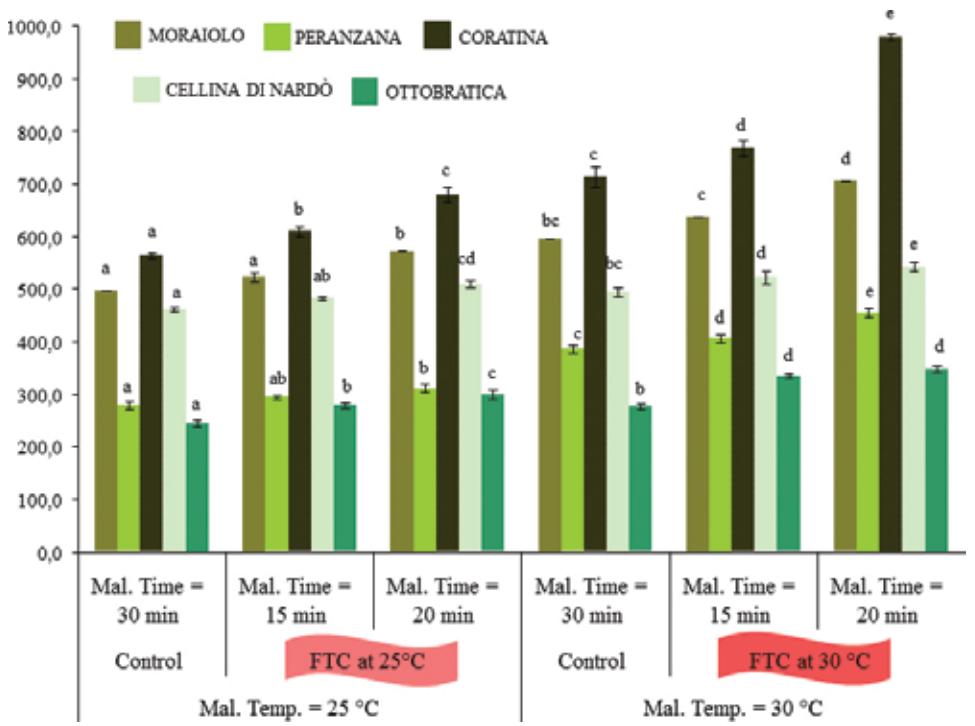


Figure 5. Evaluation of phenolic composition (mg/kg) of EVOO Control and FTC obtained at different temperatures and times of malaxation[a] [53]. The evaluations were carried out by HPLC according to the method reported by Selvaggini et al. [55]. [a] Data are the mean of three independent analytical determinations ± SD. The values in each row having different letters (a–d) are significantly different from one another ($p < 0.05$).

http://dx.doi.org/10.5772/64796

when a lengthy malaxation (20 minutes) followed the FTC treatment, in both the temperature range investigated (25°C and 30°C). These findings were then repeated also for the Peranzana and Coratina cultivars, with the latter characterized by the highest levels of phenolic concentrations [53]. Ottobratica EVOOs were less affected by the FTC pre-treatment of the crushed pastes [53].

The FTC treatment always allowed an increase of total phenols, which ranged from 3.7 to 21.5%, with the higher values obtained when malaxation was longer than 15 minutes. These results confirmed those previously found by Esposto et al. [37]. The minimum time required to determine the cell wall degradation catalyzed by the endogenous depolymerizing enzymes, which are involved in the release of phenols in the oily phase. The differences in terms of phenolic compounds are mainly related to the oleuropein derivatives (3,4-DHPEA-EDA, and 3,4-DHPEA-EA) and to a lesser extent *p*-HPEA and *p*-HPEA-EDA. These increases appear to be influenced not only by the heat treatment but also by the genetic origins of five cultivars tested [53].

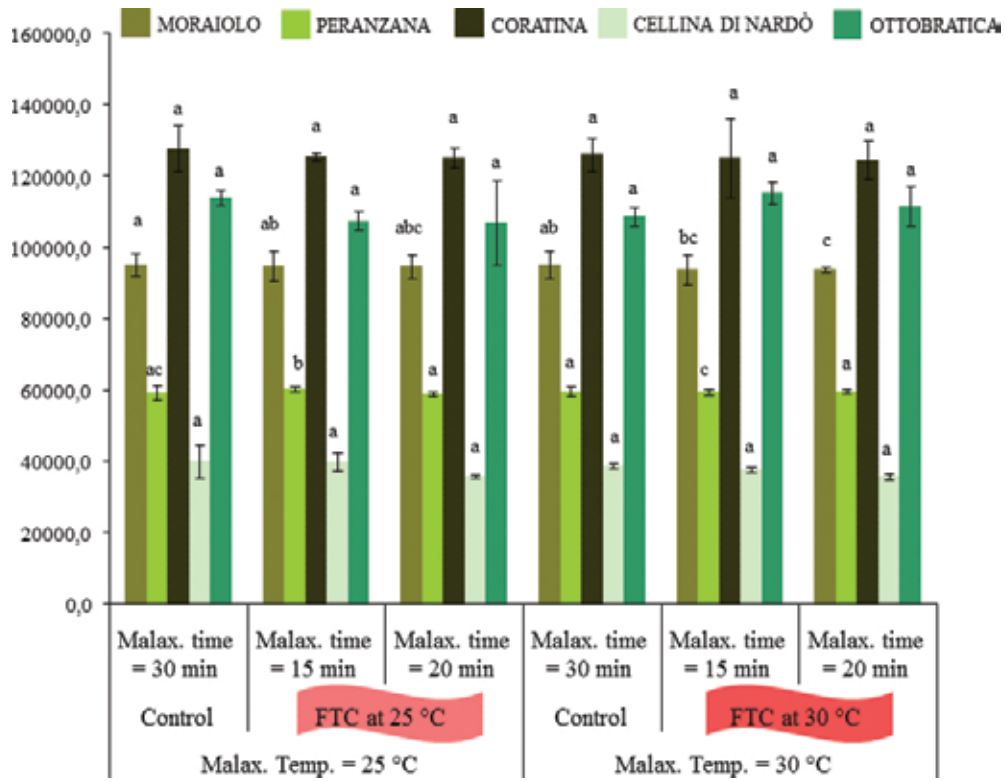


Figure 6. Evaluation of C_5–C_6 volatile compounds (μg/kg) of EVOO Control and FTC obtained at different temperatures and times of malaxation[a] [53]. The evaluations were carried out by HS–SPME–GC/MS according to the method reported by Esposto et al. [37]. [a]Data are the mean of three independent analytical determinations ± SD. The values in each row having different letters (a–c) are significantly different from one another ($p < 0.05$).

The profile of volatile compounds, which highly influence the positive attributes of EVOO aroma, did not show univocal behavior ascribable to the application of FTC treatment to the crushed pastes, according to the cultivars. In Moraiolo cv., significant differences were observed only for EVOOs receiving FTC treatment and subsequent malaxation at 30°C. In Peranzana cv. EVOO, only small variations were seen for EVOO from FTC malaxed for 15 minutes (**Figure 6**) [53]. For the other three cultivars studied, non-significant differences were observed. These results obtained by Veneziani et al. [53] make it possible to highlight the variability according to the cultivar in the formation of volatile compounds during processing, linked to the different activity levels of each enzyme involved in the LOX pathway. A higher accumulation of C_6 saturated and unsaturated aldehydes was observed in the EVOO treated with FTC compared to that in the traditional process (**Figure 6**). The elimination of the stop period at the maximum temperature could account for the reduction of HPL partial inactivation [56].

5. Separation systems

This last phase provides for the separation of oily must from the malaxed paste by different extraction technologies, such as pressure, centrifugation, and selective filtration (i.e., "surface tension" or "percolation"). Over time, the innovation of the oil extraction phase has led to the replacement of traditional discontinuous lines using the pressure system extraction with the continuous lines, using different generations of decanters: two-phase, three-phase, and three-phase with water-saving system decanters [57–60].

The different decanters play an important role particularly in the hydrophilic composition of EVOO. Most of the phenols are effectively flushed away with the olive mill vegetation waters (OMVW) produced, instead of remaining in the oil. In general, the three-phase decanter provides a dilution of malaxed pastes with water (0.2–0.5 m^3/t of olives) producing 50–90 l of OMVW/100 kg of olive paste and 50–60 kg of olive pomace/100 kg of olive paste [15, 28]. However, the addition of water prior to oil separation in order to reduce the paste viscosity can explain the decrease in the phenols and C_6 alcohols, hexan-1-ol and *trans*-2-hexen-1-ol, in particular [18, 28]. In fact, adding water to olive paste gives rise to a loss in oleuropein and ligstroside derivatives, while lignans seem not to be affected by water addition. Indeed, the secoiridoids are amphiphilic in their nature, with a higher solubility in water than in the oil phase [61]; thus, when partitioned, most of them end up in olive oil co-products such as OMVW and/or pomaces.

The development of this machine has led to two-phase and three-phase centrifugal separations with low water consumption. The two-phase system is generally characterized by greatly reduced water consuming during the extraction process, producing 70 kg of olive pomace/100 kg of olive paste [28]. The new decanters produce EVOO characterized by a higher phenolic concentration compared with those extracted by the traditional centrifugation process, and the loss of these hydrophilic compounds in OMVW is reduced [58].

6. Filtration

Filtration is the last step of the EVOO mechanical extraction process before bottling. In the EVOO industry, several filtration systems have been applied: conventional filtration systems (filter tanks and filter presses) and cross-flow filtration (tangential flow filtration). Recently, the new filtration systems, based on a filter bag and inert gas (nitrogen or argon) flow, have been proposed as innovative techniques [62].

Filtration is required to remove the residual water present as water-in-oil emulsions or dispersions, and solid particles deriving from olive pulp and peel. These suspended particles are rich in enzymes and sugars, which can lower EVOO quality by promoting hydrolysis and/or oxidation reactions. They also form unpleasant volatile components responsible for the muddy defect due to microbial fermentation, with consequent shortening of EVOO shelf life [18, 63, 64]. The effects of the filtration on EVOO shelf life are not unique. Several investigations have been carried out in order to evaluate the benefits and drawbacks of filtration in terms of changes of phenolic and volatile composition of olive oil and its stability over time [65, 66]. A number of research papers have shown that in filtered oils, the levels of hydroxytyrosol and tyrosol, which are formed by the hydrolysis rate of their secoiridoid derivatives, had decreased, and these oils showed a more rapid loss in total phenolic compounds compared to unfiltered oils [28, 66]. This change in the profile of the EVOO phenolic fraction implies a lowering of end-product stability [66]. EVOO filtration could help extend shelf life, thus reducing the rate of hydrolysis of the triacylglycerol matrix, especially in oils with higher initial free fatty acidity [66]. From the sensory point of view, filtration removes unwanted particles that cause hydrolysis, lipid oxidation, and microbial fermentation, which would lead to the producing of sensory defects during storage [18]. Positive attributes such as pungency, fruitiness, and bitterness may also be affected, depending on the type of filtration system. Research results differ greatly on the sensory impacts of filtration, which are highly dependent on the sensorial attributes of the unfiltered oil, the type of filtration used, and the time in storage [62].

7. Conclusion

The new technologies, applied to mechanical olive oil extraction systems, are process innovations adaptable to most of the oil extraction plants commonly used, characterized by different purchase costs and different results of application. The increase in oil yield, the transformation into a continuous extraction process, the reduction of processing times, and the greater versatility of some new plants, united by the same goal of high-quality virgin olive oil production, are all elements that aim toward greater economic growth in the olive oil industry.

The application of new technologies properly dimensioned according to the producer's necessities is a valid tool for reaching an optimal compromise between plant performance and VOO quality, according to the olives' characteristics.

Author details

Gianluca Veneziani, Beatrice Sordini, Agnese Taticchi, Sonia Esposto, Roberto Selvaggini, Stefania Urbani, Ilona Di Maio and Maurizio Servili*

*Address all correspondence to: maurizio.servili@unipg.it

Department of Agricultural, Food and Environmental Sciences, University of Perugia, Perugia, Italy

References

[1] Ghanbari R, Anwar F, Alkharfy KM, Gilani AH, Saari N. Valuable nutrients and functional bioactives in different parts of olive (Olea europaea L.)—a review. Int. J. Mol. Sci. 2012; 13: 3291–3340. DOI: 10.3390/ijms13033291

[2] Lopez S, Bermudez B, Montserrat-de la Paz S, Jaramillo S, Varela LM, Ortega-Gomez A, Abia R, Murian FJG. Membrane composition and dynamics: a target of bioactive virgin olive oil constituents. Biochim Biophys Acta. 2014; 1838: 1638–1656. DOI: 10.1016/j.bbamem.2014.01.007

[3] Naziri E, Consonni R, Tsimidou MZ. Squalene oxidation products: Monitoring the formation, characterisation and pro-oxidant activity". Eur. J. Lipid Sci. Technol. 2014; 116: 1400–1411. DOI: 10.1002/ejlt.201300506

[4] Obied HK, Prenzler PD, Omar SH, Ismael R, Servili M, Esposto S, Taticchi A, Selvaggini R, Urbani S. Pharmacology of olive biophenols. In: Fishbein JC, Heilman JM, editors. Handbook of Advances in Molecular Toxicology. ed. Amsterdam, (Netherlands): Elsevier; 2012. p. 195–223. DOI: 10.1016/B978-0-444-59389-4.00006-9

[5] Servili M, Sordini B, Esposto S, Urbani S, Veneziani G, Di Maio I, Selvaggini R, Taticchi A. Biological activities of phenolic compounds of extra virgin olive oil. Antioxidants. 2014; 3: 1–23. DOI: 10.3390/antiox3010001

[6] Servili M, Selvaggini R, Esposto S, Taticchi A,. Montedoro GF, Morozzi G. Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil. J. Chromatogr. A. 2004; 1054: 113–127. DOI: 10.1016/j.chroma.2004.08.070

[7] Servili M, Esposto S, Fabiani R, Urbani S, Taticchi A, Mariucci F, Selvaggini R, Montedoro GF. Phenolic compounds in olive oil: antioxidant, health and organoleptic activities according to their chemical structure. Inflammopharmacology. 2009; 17: 1–9. DOI: 10.1007/s10787-008-8014-y

[8] Casaburi I., Puoci F., Chimento A., Sirianni R., Ruggiero C., Avena P., Pezzi V. Potential of olive oil phenols as chemopreventive and therapeutic agents against cancer: a review of in vitro studies. Mol. Nutr. Food Res. 2013; 57: 71–83. DOI: 10.1002/mnfr.201200503

[9] Rosignoli P, Fuccelli R, Fabiani R, Servili M, Morozzi G. Effect of olive oil phenols on the production of inflammatory mediators in freshly isolated human monocytes. J. Nutr. Biochem. 2013; 24(8): 1513–1519. DOI: 10.1016/j.jnutbio.2012.12.011

[10] Covas MI, Nyyssönen K, Poulsen HE, Kaikkonen J, Zunft HF, Kiesewetter H, Gaddi A, de la Torre R, Mursu J, Bäumler H, et al. The effect of polyphenols in olive oil on heart disease risk factors—a randomized trial. Ann. Intern. Med. 2006; 145: 333–341. DOI: 10.7326/0003-4819-145-5-200609050-00006

[11] Castañer O, Covas MI, Khymenets O, Nyyssonen K, Konstantinidou V, Zunft HF, de la Torre R, Muñoz-Aguayo D, Vila J, Fitó M. Protection of LDL from oxidation by olive oil polyphenols is associated with a downregulation of CD40-ligand expression and its downstream products in vivo in humans1–3. Am. J. Clin. Nutr. 2012; 95: 1238–1244. DOI: 10.3945/ajcn.111.029207

[12] Brenes M, Hidalgo FJ, García A, Rios JJ, García P, Zamora R, Garrido A. Pinoresinol and 1-acetoxypinoresinol, two new phenolic compounds identified in olive oil. J. Am. Oil Chem. Soc. 2000; 77: 715–720. DOI: 10.1007/s11746-000-0115-4.

[13] Owen RW, Mier W, Giacosa A, Hull WE, Spiegelhalder B, Bartsch H. Olive-oil consumption and health: The possible role of antioxidants. Food Chem. Toxicol. 2000; 38: 647–659. DOI: 10.1016/S0278-6915(00)00061-2

[14] Servili M, Esposto S, Taticchi A, Urbani S, Di Maio I, Veneziani G, Selvaggini R. New approaches to virgin olive oil quality, technology and by-products valorization. Eur. J. Lipid Sci. Technol. 2015; 117: 1882–1892. DOI: 10.1002/ejlt.201500138

[15] Taticchi A, Esposto S, Servili M. The basis of the sensory properties of virgin olive oil. In: Monteleone E, Langstaff S, editors. Handbook of Olive Oil Sensory Science. Ed. Chichester (UK): John Wiley & Sons; 2014; p. 33–54. DOI: 10.1002/9781118332511.ch2

[16] European Union. Commission Regulation (EU) No 432/2012 of 16 May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health. Off. J. Eur. Union 2012, L136, 1–40.

[17] Angerosa F, Mostallino R, Basti C, Vito R. Influence of malaxation temperature and time on the quality of virgin olive oils. Food Chem. 2001; 72: 19–28. DOI: 10.1016/S0308-8146(00)00194-1

[18] Angerosa F, Servili M, Selvaggini R, Taticchi A, Esposto S, Montedoro GF. Volatile compounds in virgin olive oil: Occurrence and their relationship with the quality. J. Chromatogr. A. 2004; 1054: 17–31. DOI: 10.1016/j.chroma.2004.07.093

[19] Servili M, Esposto S, Taticchi A, Urbani S, Di Maio I, Sordini B, Selvaggini R, Montedoro GF, Angerosa F. Volatile compounds of virgin olive oil: their importance in the sensory

quality. In: Berti L and Maury J, editors. Handbook of Advances in Olive Resources. Ed. Kerala (India); 2009; p. 45–77. ISBN: 978-81-7895-388-5

[20] Kalua CM, Allen MS, Bedgood Jr DR, Bishop AG, Prenzler PD, Robards R. Olive oil volatile compounds, flavour development and quality: a critical review. Food Chem. 2006; 100 (1): 7641–7651. DOI: 10.1016/j.foodchem.2005.09.059

[21] European Commission Implementing Regulation EU No 1348/2013 of 16 December 2013 amending Regulation No 2568/91/EEC on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Off. J. Eur. Community. 2013; L338: 1–37.

[22] Inarejos-García AM, Salvador MD, Fregapane G. Effect of crushing on olive paste and virgin olive oil minor components. Eur. Food Res, Technol.. 2011; 232: 441–451. DOI: 10.1007/s00217-010-1406-4

[23] Fregapane G, Salvador MD. Production of superior quality extra virgin olive oil modulating the content and profile of its minor components. Food Res. Int. 2013; 54: 1907–1914. DOI: 10.1016/j.foodres.2013.04.022

[24] Leone A, Romaniello R, Zagaria R, Sabella E, De Bellis L, Tamborrino A. Machining effects of different mechanical crushers on pit particle size and oil drop distribution in olive paste. Eur. J. Lipid Sci. Technol. 2015; 117: 1271–1279. DOI: 10.1002/ejlt.201400485

[25] Frankel E, Bakhouche A, Lozano-Sánchez J, Segura-Carretero A, Fernández-Gutiérrez A. Literature review on production process to obtain extra virgin olive oil enriched in bioactive compounds. Potential use of byproducts as alternative sources of polyphenols. J. Agric. Food Chem. 2013; 61: 5179-5188. DOI: 10.1021/jf400806z

[26] Clodoveo ML, Hachicha Hbaieb R, Kotti F, Scarascia Mugnozza G, Gargouri M. Mechanical strategies to increase nutritional and sensory quality of virgin olive oil by modulating the endogenous enzyme activities. Compr. Rev. Food Sci. Food Saf. 2014; 13: 135–154. DOI: 10.1111/1541-4337.12054

[27] Servili M, Montedoro GF. Contribution of phenolic compounds to virgin olive oil quality. Eur. J. Lipid Sci. Technol. 2002; 104: 602–613. DOI: 10.1002/1438-9312(200210)104:9/10<602::AID-EJLT602>3.0.CO;2-X

[28] Servili M, Taticchi A, Esposto S, Sordini B, Urbani S. Technological aspects of olive oil production. In: Muzzalupo I, editor. Handbook of Olive Germplasm— The Olive Cultivation, Table Olive and Olive Oil Industry in Italy, InTech. Ed Riijeca (Croatia): InTech; 2012; p. 151–172. DOI: 10.5772/52141

[29] Hachicha Hbaieb R, Kotti F, García-Rodríguez R, Gargouri M, Sanz C, Pérez AG. Monitoring endogenous enzymes during olive fruit ripening and storage: Correlation with virgin olive oil phenolic profiles. Food Chem.. 2015; 174: 240–247. DOI: 10.1016/j.foodchem.2014.11.033

[30] Taticchi A, Esposto S, Veneziani G, Urbani S, Selvaggini R, Servili M. The influence of the malaxation temperature on the activity of polyphenoloxidase and peroxidase and

on the phenolic composition of virgin olive oil. Food Chem.. 2013; 136: 975–983. DOI: 10.1016/j.foodchem.2012.08.071

[31] Salas JJ, Sánchez J. The decrease of virgin olive oil flavor produced by high malaxation temperature is due to inactivation of hydroperoxide lyase. J. Agric. Food Chem. 1999; 47(3): 809–812. DOI: 10.1021/jf981261j

[32] Servili M, Taticchi A, Esposto S, Urbani S, Selvaggini R, Montedoro GF. Effect of olive stoning on the volatile and phenolic composition of virgin olive oil. J. Agric. Food Chem. 2007; 55: 7028-7035. DOI: 10.1021/jf070600i

[33] Inglese P, Famiani F, Galvano F, Servili M, Esposto S, Urbani S. Factors affecting extra-virgin olive oil composition. In: Janick J, Editor. Handbook of Horticultural Reviews, Ed. Hoboken (NY):John Wiley & Sons; 2011; p. 83–147. DOI: 10.1002/9780470872376.ch3

[34] Angerosa F, Basti C, Vito R, Lanza B. Effect of fruit stone removal on the production of virgin olive oil volatile compounds. Food Chem. 1999; 67: 295–299. DOI: 10.1016/S0308-8146(99)00138-7

[35] Leone A. Romaniello R, Zagaria R, Sabella E, De Bellis L, Tamborrino A. Machining effects of different mechanical crushers on pit particle size and oil drop distribution in olive paste. Eur. J. Lipid Sci. Technol. 2015; 117: 1271–1279. DOI: 10.1002/ejlt.201400485

[36] Montedoro GF, Servili M, Baldioli M, Miniati E. Simple and hydrolyzable phenolic compounds in virgin olive oil. 1. Their extraction, and quantitative and semiquantitative evaluation by HPLC. J. Agric. Food Chem. 1992; 40: 1571–1576. DOI: 10.1021/jf00021a019

[37] Esposto S, Veneziani G, Taticchi A, Selvaggini R, Urbani S, Di Maio I, Sordini B, Minnocci A, Sebastiani L, Servili M. Flash thermal conditioning of olive pastes during the olive oil mechanical extraction process: impact on the structural modifications of pastes and oil quality. J. Agric. Food Chem. 2013; 61: 4953–4960. DOI: 10.1021/jf400037v

[38] Clodoveo M.L. Malaxation: influence on virgin olive oil quality. Past, present and future—an overview. Trends Food Sci. Technol. 2012; 25(1): 13–23. DOI: 10.1016/j.tifs.2011.11.004

[39] Selvaggini R, Esposto S, Taticchi A, Urbani S, Veneziani G, Di Maio I, Sordini B, Servili M. Optimization of the temperature and oxygen concentration conditions in the malaxation during the oil mechanical extraction process of four Italian olive cultivars. J. Agric. Food Chem. 2014; 62: 3813-3822. DOI: 10.1021/jf405753c

[40] Parenti A, Spugnoli P, Masella P, Calamai L. Carbon dioxide emission from olive oil pastes during the transformation process: technological spin offs. Eur. Food Res. Technol., 2006; 222: 521–526. DOI: 10.1007/s00217-005-0003-4

[41] Parenti A, Spugnoli P, Masella P, Calamai L, Pantani OL. Improving olive oil quality using CO_2 evolved from olive pastes during processing. Eur. J. Lipid Sci. Technol. 2006; 108: 904–912. DOI 10.1002/ejlt.200600182

[42] Servili M, Taticchi A, Esposto S, Urbani S, Selvaggini R, Montedoro GF. Influence of the decrease in oxygen during malaxation of olive paste on the composition of volatiles and phenolic compounds in virgin olive oil. J. Agric. Food Chem. 2008; 56 (21): 10048–10055. DOI: 10.1021/jf800694h

[43] Vierhuis E, Servili M, Baldioli M, Schols HA, Voragen JAG, Montedoro GF. Study of the effect of enzyme treatment during the mechanical extraction of olive oil on phenolic compounds and polysaccharides. J. Agric. Food Chem. 2001; 49 (3): 1218–1223. DOI: 10.1021/jf000578s

[44] Clodoveo ML, Hachicha Hbaieb R. Beyond the traditional virgin olive oil extraction systems: searching innovative and sustainable plant engineering solutions. Food Res. Int. 2013; 54: 1926–1933. DOI: 10.1016/j.foodres.2013.06.014

[45] Leone A, Tamborrino A, Romaniello R, Zagaria R, Sabella E. Specification and implementation of a continuous microwave-assisted system for paste malaxation in an olive oil extraction plant. Biosyst. Eng. 2014; 125: 24-35. DOI: 10.1016/j.biosystemseng.2014.06.017

[46] Tamborrino A, Romaniello R, Zagaria R, Leone A. Microwave-assisted treatment for continuous olive paste conditioning: impact on olive oil quality and yield. Biosyst. Eng. 2014; 127: 92–102. DOI: 10.1016/j.biosystemseng.2014.08.015

[47] Gallina Toschi T, Berardinelli A, Cevoli C, Iaccheri E, Di Lecce G, Bendini A, Ragni L. Effectiveness of the mechanical excitation applied to the olive paste: possible improving of the oil yield, in malaxation phase, by vibration systems. J. Agric. Eng. 2013; 44 (4): 166–169. DOI: 10.4081/jae.2013.225

[48] Puértolas E, Martínez de Marañón I. Olive oil pilot-production assisted by pulsed electric field: impact on extraction yield, chemical parameters and sensory properties. Food Chem. 2015; 167: 497–502. DOI: 10.1016/j.foodchem.2014.07.029

[49] Abenoza M, Benito M, Saldaña G, Álvarez I, Raso J, Sánchez-Gimeno AC. Effects of pulsed electric field on yield extraction and quality of olive oil. Food Bioprocess Technol. 2013; 6: 1367–1373. DOI 10.1007/s11947-012-0817-6

[50] Clodoveo ML, Durante V, La Notte D. Working towards the development of innovative ultrasound equipment for the extraction of virgin olive oil. Ultrason. Sonochem. 2013; 20: 1261–1270. DOI: 10.1016/j.ultsonch.2013.02.001

[51] Jiménez A, Beltran G, Uceda M. High-power ultrasound in olive paste pretreatment. Effect on process yield and virgin olive oil characteristics. Ultrason. Sonochem. 2007; 14: 725–731. DOI: 10.1016/j.ultsonch.2006.12.006

[52] Mizrach A. Ultrasound for fruit and vegetable quality evaluation. In: Chen D, Sharma SK, Mudhoo A. Editors Handbook of Applications of Ultrasound Sonochemistry for Sustainability, Ed. Boca Raton (Florida): Press/Taylor 2011; p. 129–159. DOI: 10.1201/b11012-8.

[53] Veneziani G, Esposto S, Taticchi A, Selvaggini R, Urbani S, Di Maio I, Sordini B, Servili M. Flash thermal conditioning of olive pastes during oil mechanical extraction process: cultivar impact on the phenolic and volatile composition of virgin olive oil. J. Agric. Food Chem. 2015; 63(26): 6066–6074. DOI: 10.1021/acs.jafc.5b01666

[54] Leone A, Esposto S, Tamborrino A, Romaniello R, Taticchi A, Urbani S, Servili M. Using a tubular heat exchanger to improve the conditioning process of the olive paste: Evaluation of yield and olive oil quality. Eur. J. Lipid Sci. Technol. 2015; 117: 1–10. DOI: 10.1002/ejlt.201400616

[55] Selvaggini R, Servili M, Urbani S, Esposto S, Taticchi A, Montedoro GF. Evaluation of phenolic compounds in virgin olive oil by direct injection in high-performance liquid chromatography with fluorometric detection. J. Agric. Food Chem. 2006; 54: 2832–2838. DOI: 10.1021/jf0527596

[56] Sánchez-Ortiz A, Pérez AG, Sanz C. Synthesis of aroma compounds of virgin olive oil: significance of the cleavage of polyunsaturated fatty acid hydroperoxides during the oil extraction process. Food Res. Int. 2013; 54(2): 1972–1978. DOI: 10.1016/j.foodres.2013.03.045

[57] Kalogeropoulos N, Kaliora AC, Artemiou A, Giogios I. Composition, volatile profiles and functional properties of virgin olive oils produced by two-phase vs three-phase centrifugal decanters. Food Sci. Technol. 2014; 58: 272–279. DOI: 10.1016/j.lwt.2014.02.052

[58] Klen TJ, Vodopive BM. The fate of olive fruit phenols during commercial olive oil processing: traditional press versus continuous two- and three-phase centrifuge. Food Sci. Technol. 2012; 49: 267–274. DOI: 10.1016/j.lwt.2012.03.029

[59] Caponio F, Summo C, Paradiso VM, Pasqualone A. Influence of decanter working parameters on the extra virgin olive oil quality. Eur. J. Lipid Sci. Technol. 2014; 116: 1626–1633. DOI: 10.1002/ejlt.201400068

[60] Altieri G, Di Renzo GC, Genovese F. Horizontal centrifuge with screw conveyor (decanter): optimization of oil/water levels and differential speed during olive oil extraction. J. Food Eng. 2013; 119: 561–572. DOI: 10.1016/j.jfoodeng.2013.06.033

[61] Rodis PS, Karanthanos VT, Mantzavinou A. Partitioning of olive oil antioxidants between oil and water phases. J. Agric. Food Chem. 2002; 50: 596–601. DOI: 10.1021/jf010864j

[62] Lozano-Sanchez L, Cerratani L, Bendini A, Gallina Toschi T, Segura-Carretero A, Fernandez-Gutierrez A. New filtration systems for extra-virgin olive oil: effect on antioxidant compounds, oxidative stability, and physicochemical and sensory properties. J. Agric. Food Chem. 2012; 60: 3754-3762. DOI: 10.1021/jf205353b

[63] Sacchi R, Caporaso N, Paduano A, Genovese A. Industrial-scale filtration affects volatile compounds in extravirgin olive oil cv. Ravece. Eur. J. Lipid Sci. Technol. 2015; 117: 2007–2014. DOI: 10.1002/ejlt.201400456

[64] Bubola KB, Koprivnjak O, Sladonja B. Influence of filtration on volatile compounds and sensory profile of virgin olive oils. Food Chem. 2012; 132: 98–103. DOI: 10.1016/j.foodchem.2011.10.038

[65] Lozano-Sanchez L, Cerratani L, Bendini A, Segura-Carretero A, Fernandez-Gutierrez A. Filtration process of extra virgin olive oil: effect on minor components, oxidative stability and sensorial and physicochemical characteristics. Trends Food Sci. Technol. 2010; 21: 201–211. DOI: 10.1016/j.tifs.2009.12.004

[66] Fregapane G, Lavelli V, Leon S, Kapuralin J, Salvador MD. Effect of filtration on virgin olive oil stability during storage. Eur. J. Lipid Sci. Technol. 2006; 108: 134–142. DOI: 10.1002/ejlt.200501175

Chapter 3

Ultrasound in Olive Oil Extraction

Riccardo Amirante and Antonello Paduano

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64765

Abstract

Each olive oil extraction system should combine the best product quality and the highest efficiency. At the same time, the innovative technologies can develop only if they provide sustainable processes. To reach these goals, academic and industrial researchers need to understand the key elements that allow to modulate the events that occur during oil extraction. In the past years, many emerging technologies, that is techniques perceived as capable of changing the present situation, have been developed. Among these, ultrasounds applications seem to be the most promising for their mechanical and slightly thermal effects, without affecting sustainability. In order to explain the maturity of this emerging technology, the main effects of the ultrasounds application in the olive oil extraction process are discussed, the developed plants are presented, and the patents are reported.

Keywords: emerging technologies, ultrasound-assisted extraction, pressure, viscosity, hydrophobic effect, emulsion breaking, olive oil quality, ultrasound patents

1. Introduction

Emerging technologies are techniques perceived as capable of changing the *status quo*. Food emerging technologies include a variety of technologies, such as high pressure, high intensity pulsed light, radio frequency electric fields, ohmic heating, microwave, ultrasound (US), and many others. Significant advances have been made in the research, development, and application of these technologies in food processing [1]. In the olive oil sector, ultrasound technology seems to be the most promising [2–6] due to its mechanical and slightly thermal effects. From a technological point of view, the entire virgin olive oil process has changed very little over the past 20 years. Crushing and malaxation are the most important critical points of the oil mechanical extraction process [7]. Crushing is the first step in the olive oil extraction.

The fruits are ground up into a paste that will have different characteristics depending on the mill type, the olive variety and ripening stage and the type of the crusher employed. The next step is malaxation, a continuous slow stirring of the olive paste that increases the amount of free oil, more easily separable by centrifugation, by helping the oil droplets to merge into large drops and breaking the oil/water emulsion. The efficiency of malaxation depends on the rheological characteristics of olive paste and on technological parameters, such as time and temperature of the operation. However, the malaxer, actually is a machine working in batch, located between two continuous apparatuses, the crusher and the centrifugal decanter; consequently, it represents the bottleneck of the continuous extraction process.

The ultrasound waves applied to olive paste, before or during malaxation, could reduce the process time through two different mechanisms: a thermal effect and a mechanical effect. The thermal effect occurs when kinetic energy of the ultrasound waves is absorbed by a medium, and it is converted into the thermal energy. The mechanical effect is due to the cavitation phenomena. The cavitation is the formation, growth, and implosion of gas bubbles at high negative pressure. This process promotes the release of soluble compounds from the plant tissue by disrupting cell walls and improves mass transfer also in the olive tissues [3].

High-power ultrasound represents an efficient tool for large-scale food processing [8]. For each industrial application, this technology has to be developed and scaled up. When ultrasound is applied on a continuum fluid, it causes an acoustic pressure in addition to the hydrostatic pressure already acting on the medium. This acoustic pressure is a periodic wave dependent on time, frequency and the maximum pressure amplitude. There is a proportional relationship between the maximum pressure amplitude of the wave and the power input of the ultrasonic transducer.

When the intensity (amplitude) is low, the pressure wave causes an acoustic streaming within the fluid, consisting of a mixing action. When the intensity is higher, tiny gas bubbles grow within the fluid because the local pressure in the expansion phase of the cycle falls below the vapour pressure of the liquid. If the intensity increases, it can generate a negative transient pressure into the fluid that causes the bubble growth and produces new cavities due to the tensioning effect on the fluid [9]. If the bubble growth reaches a critical size, it implodes causing the phenomenon of cavitation, the most important effect in high-power ultrasound. Cavitation produces very high shear energy waves and turbulence into the medium, coupled with a localized increment of pressure and temperature. The combination of these factors causes the rupture of the biological cells accelerating the mass transfer.

Regarding the ultrasound frequency, the number of vibrations per second affects the bubble size in an inversely proportional manner. Consequently, low frequency ultrasound (i.e. power ultrasound 16–100 kHz) can generate great cavitation bubbles, which cause an increment of temperatures and pressures in the cavitation zones.

The industrial application of ultrasound requires two main conditions: a liquid medium (at least 5% of the overall substrate) and a source of high-energy vibrations (the ultrasonic device). The ultrasonic source is named "transducer." There are two main types of transducers: piezoelectric and magnetostrictive. Piezoelectric transducers utilize the piezoelectric property

of a material to convert electrical energy directly into mechanical energy. Magnetostrictive transducers utilize the magnetostrictive property of a material to convert the energy in a magnetic field into mechanical energy.

Many parameters can be useful to describe an ultrasonic process, such as amplitude, pressure, temperature, viscosity, and concentration of solids. However, the main effects are function of the specific energy per kilogram (J/kg), that is the total amount of vibrational energy being delivered per mass unit, that are independent of scale. This is the reason of the scalability of ultrasonic technology.

If the pressure increases, the cavitation threshold and thus the number of cavitation bubbles is reduced [9]. The increment of the back pressure represents an effective tool to intensify the process maintaining low the amplitude.

Vapour pressure, surface tension, and viscosity of the liquid medium are parameters able to influence the cavitation phenomena and are temperature dependent. Increasing temperature causes an increase in the number of cavitation bubbles but their collapse results "amortized" due to the higher vapour pressure. On the contrary, decreasing temperature is followed by the viscosity decrease, allowing a more violent collapse. Thus, there is an optimum temperature, which enables to obtain both a low viscosity value, enough to create a violent cavitation collapse, and to avoid the dampening effect caused by a high vapour pressure.

2. Useful ultrasound effects in the virgin olive oil extraction process

2.1. Cell disruption

Oil is contained inside the olives in small cellular bags, the elaioplasts, specialized leucoplasts protected by a cellular membrane, responsible for the storage of lipids. Crushing is not effective in terms of oil separation, because it breaks olives but only few cellular membranes. Ultrasound disrupts the tissue structures, including membranes freeing the trapped oily phase.

2.2. Hydrophobic effect

The coalescence phenomenon of oily drops inside the olive paste is due to the hydrophobic interactions. During the malaxation, the oil droplets in the olive paste combine to form larger drops [10, 11]. This phenomenon, called cohesion, is related to the presence of water and its extensive hydrogen bonding. The water molecules tend to stick to each other in a regular pattern. Non-polar oil molecules are neither repelled nor attracted to each other. Water tends to squeeze non-polar molecules together, so oil droplets tend to form larger drops. In reference [12], Jordan observed that ultrasound positively affects collision and attachment of droplets and the coalescence phenomena. In reference [13], Filippov et al. confirmed that hydrophobic particles that are characterised by a low speed can be accelerated and activated by ultrasounds which promote the separation of the slow fraction. Ultrasound cavitation can increase the hydrophobic character of the water and modify the surface of hydrophobic particles by the formation of micro- or nanobubbles, facilitate the bubble-particle attachment and increase

kinetics. Moreover, the ultrasound can enhance the probability of particles collision leading to an increase of oil recovery.

2.3. Emulsion breaking

Sometimes olive fruits can give, after milling, a kind of olive paste defined "difficult" or "very difficult" due to the formation of a strong oil/water emulsion that is hard to break even after malaxation. In these cases, the separation of the oil phase during centrifugation is more laborious. This kind of olive paste cannot give acceptable oil yields because of the fruit characteristics: very high moisture content (50–60%), high oil content (about 30%) and very low level of dry extract (10–15%).

If ultrasound is applied at high frequencies (<30 kHz), it can split the emulsion into its component, aqueous and oily phases [14].

3. Olive oil quality

Many authors have studied the effect of ultrasound applications during the extraction of oil from olives on oil quality parameters, nutritional and sensory characteristics [2]. Each of them, using different experimental conditions and olive varieties, reached similar conclusions. In 2007, Jiménez, Beltrán and Uceda applied, for the first time, high-power ultrasound on olive paste at laboratory scale during the EVOO extraction process. They observed no change in quality parameters (free acidity, peroxide value, K_{270} and K_{232}) in the resulting oils. The ultrasounds showed significant effects on the levels of bitterness and minor compounds. Oils from sonicated pastes showed lower bitterness and higher content of tocopherols (vitamin E), chlorophylls and carotenoids. Sensory evaluation by panel test showed higher intensity of positive attributes in sonicated oils.

Clodoveo et al., in 2013, tested at pilot scale the ultrasound treatment both on olive fruits submerged in a water bath (before crushing) and on olive paste before the malaxation (immediately after crushing). The aim of the experimental plan was to investigate the possibility of reducing the malaxing time. The ultrasound technology allowed a reduction in the duration of malaxing phase improving oil yields and its minor compounds content. Better results were obtained by sonicating the olives in a water bath than sonicating the olive paste.

A new experiment conducted by Bejaoui et al. (2015) confirmed the results obtained by Clodoveo et al. in 2014. They studied the employment of a continuous high-power ultrasound treatment on the olive paste before malaxation at pilot scale with the aim to study its effects on oil yield and quality. They observed that the ultrasound treatment caused an improvement of the oil yield of about 1% and the oil extractability equal to approximately 5.7%.

Recently, Bejaoui et al. (2016), testing high-power ultrasound on olive paste (before the malaxation), developed a response surface model to predict the olive paste temperature based on olive characteristics, olive paste flow rate and high-power ultrasound intensity. More-

over, they confirmed that ultrasound treatment did not cause changes in the oil quality indexes (free acidity, peroxide value, K_{232}, K_{270}) and in the volatile compounds, assuming that the fatty acid autoxidation was not accelerated by this treatment. The oils obtained from olive pastes treated with high-power ultrasound showed higher content of tocopherols, chlorophylls and carotenoids, while a reduction in phenolic content and bitterness intensity was observed. The constructive characteristics of the ultrasonic device, which caused a large exposition of the olive paste to the atmospheric oxygen, could explain these results. Furthermore, the heating of olive paste induced by high-power ultrasound was faster than that induced by the traditional warming system based on the conductive and convective systems. Tests conducted comparing the time required to warm the olive paste up to 27°C, a temperature useful to promote the coalescence phenomena, with the ultrasonic device and with the traditional malaxer, showed that ultrasound allows to reduce the pre-heating time by 66%. The reduction of the malaxation time represents a first step towards the conversion of malaxation in a continuous phase [6]. In reference [3], Clodoveo et al. used an ultrasound (US) treatment of olive paste before the malaxation step on *Coratina* and *Peranzana* cultivars. Different duration of US treatments was tested (0, 2, 4, 6, 8, 10 min), and free acidity, peroxide value, K_{232}, K_{270}, sensory analysis, tocopherols, total carotenoids, chlorophylls and total polyphenols were determined. Results showed that the US treatment caused a quick heating of the olive paste as a function of the US treatment time, allowing the reduction in the malaxation phase from 60 min to about 40 min. The ultrasound technique increased the antioxidants content in both oils, except for phenols that decreased due to the exposition to the atmospheric oxygen. This negative effect could be reduced or eliminated by designing a ultrasound devices equipped with a system able to modulate the atmosphere composition in contact with the olive paste.

The high-power ultrasounds do not affect oil quality parameters, while nutritional and sensory characteristics can be significantly influenced. The biophenols content, as well as tocopherols (vitamin E), chlorophylls and carotenoids content can be improved. Sensory evaluation by panel test showed higher intensity of positive attributes and lower of negative characteristics in oils from sonicated pastes compared to those untreated. Off-flavours were not detected in oils from sonication treatments

3.1. Aromatization

Clodoveo et al. [15] in 2016 studied a new aromatization technique based on the ultrasound treatment of olive paste mixed with aromatic plants (thyme and oregano). The aim of the work was to develop a system applicable in the industrial sector to obtain aromatized oils in a shorter time and with an antioxidant content higher than those obtained by traditional methods (infusion of herbs in olive oil or crushing the herbs together with the olive fruits). They found that ultrasound can inhibit the olive polyphenol oxidase, the endogenous enzyme responsible for olive oil phenol oxidation. Moreover, the ultrasound treatment of olive paste with herbs was the most suitable method to obtain aromatized olive oil, due to the best efficiency, reduced time consumption and labour and the enhancement of the product quality.

4. US prototypes

The US employment, in industrial scale, for VOO extraction process is only in prototype versions. One of them is the full-scale plant realized and tested in a full scale in Italy on October 2014, when Amirante and Clodoveo et al. placed a so-called sono-exchanger immediately after the crusher, in order to transform the discontinuous malaxing step in a continuous phase. The main goals of the research project were to improve the working capacity of the industrial plants and to increase the extraction of the main virgin olive oil minor compounds. The industrial plant was designed on the basis of previous experiences and tests performed by means of a fluid dynamic analysis and reported in [1, 4, 9] by the same authors. A continuous plant was modified by adding a "sono-exchanger" between the crusher and the malaxer, as shown in **Figure 1**.

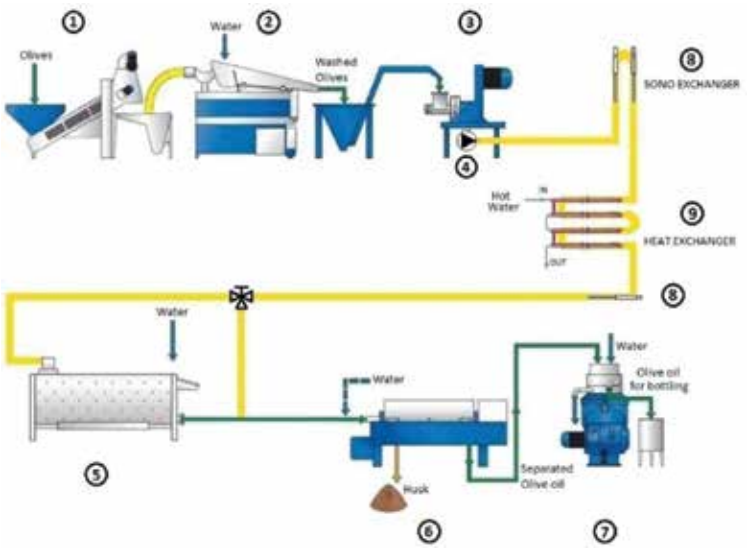


Figure 1. US prototype layout: 1. reception stage; 2. washing stage; 3. crushing stage; 4. pump; 5. malaxing stage; 6. separation stage; 7. clarification stage; 8. ultrasonic probes; 9. heat exchanger.

The sono-exchanger was made of two straight pipes connected by an elbow (cfr. **Figures 1** and **2**). Two ultrasonic rod-style transducers (Sonopush Mono® 30–1500 W–30 kHz) were plugged into the straight pipes through the bend. At last, a third ultrasound transducer (30–1400 W output power) was placed downstream the heat exchanger.

The ultrasonic probes inside the pipe provided a vibrational energy transfer to the olive paste flowing through the sono-exchanger. A specific energy of 12÷15 kJ/kg provided the best results [4]. A series of two annular heat exchangers, suitable for non-Newtonian products with high viscosity and for products that contain particulates, was used to fine-tune the temperature of the olive paste before it reached the malaxer. The heat exchanger consists of four concentric tubes.

The product medium flows in between two service channels and is heated from the inside and outside at the same time. The media fluid was water and flowed in opposite direction to the olive paste.

Figure 2. The sono-exchanger system and the three drivers, which provide the periodic signals (Colletorto, Italy, Aloia farm).

The plant is very easy to set-up for each technical staff, and it gives the opportunity of selecting the process parameters to extract virgin olive oil in relation to the heterogeneity of the fruit characteristics.

5. Ultrasound patents the edible oil industrial sector

The manufacturing industry of olive oil extraction plants and the research institutes have a positive view of the ultrasound technology, patenting its uses.

Jimenez et al.'s researchers of the Andalusian Institute of Agricultural Research and Training, Fisheries, Food and Organic Production registered a patent in 2007 entitled "Apparatus and method for the continuous heating and uniform ultrasonic treatment of olive paste" [16]. The equipment is composed of the following elements:

- a temperature sensor for the inflow of the olive paste;
- an ultrasonic piezoelectric transducer (power comprised between 120 and 1200 W);
- a temperature sensor for the output of the olive paste;

- a system for the control of applied ultrasound and inflow ground olive mass based on the information provided by the temperature sensors.

Pieralisi Srl, one of the main manufacturers of olive oil extraction plants in Italy, registered a patent in 2011 named "Installation for extraction of oil from olive paste" [17]. The purpose of the invention is to eliminate the drawbacks of the prior art by disclosing an installation for extraction of olive oil that considerably reduces kneading time, while improving the quality of the oil without impairing the extraction yield. The installation comprises:

- a crushing station to crush olives in such a way to obtain a paste composed of pulp and olive pit;
- a kneading station (malaxer) comprising at least one basically cylindrical tank with rotating blades supported by a shaft disposed in axial position in the tank to knead the paste;
- a centrifuge for the separation of the oil from olive paste;
- a heater-conveyor disposed between the crushing station and the kneading station, comprising a cylindrical tubular structure with air space with circulation of hot water and a worm conveyor axially disposed inside said cylindrical tubular structure in such a way to generate an auger conveyor with product inlet and outlet.

In order to additionally accelerate the oil extraction process, ultrasounds can be applied in direct contact with the olive paste. The synergic effect of the ultrasound treatment that causes the breakage of the membranes and the release of oil and the heater-conveyor allows a considerable reduction of the kneading time, ensuring high yield without impairing the quality of the oil.

The ultrasound treatment device can be installed upstream the heater-conveyor. In such a case, the application of ultrasounds to the olive paste favours the breakage of the pulp cells, allowing the release of oil from the vacuoles. The above makes the paste more oily and slicker, thus reducing friction on the internal walls of the heater-conveyor. Therefore, the synergetic effect of the ultrasound treatment and the piston pump favours the passage of the olive paste to the heater-conveyor, avoiding possible deposits of paste on the internal walls that may overheat and damage the quality of the extracted oil.

Femenia et al. [18] registered a patent in 2012 with a method for preventing total or partial crystallization of olive oil during storage at low temperature. It includes the application of ultrasonic energy to the olive oil allowing to retain the physical/chemical and sensory properties of the product even if subjected to low temperature during storage.

Besides these patents, specifically related to the virgin olive oil industry, other patents for the industrial application of ultrasound have been registered. The declared effects of these patents could be exploited also to improve the virgin olive oil extraction process.

Bates and Bagnall [19] registered in 2009 a patent entitled "Viscosity reduction". It is a method for reducing a product viscosity by applying highly propagating ultrasonic energy. The method requires the contact of at least a portion of the product with an assembly that propagates highly emission of ultrasonic energy

Bates and Bagnall [20] registered in 2012 the patent "Methods for isolating oil from plant material and for improving separation efficiency". This invention is based in part on the finding that high velocity microliquid streaming, created when cavitation bubbles collapse within a liquid, causes oils to be separated from cellular materials more easily, efficiently and quickly, with higher extraction yield. In this patent description, the inventors employed standing waves at high ultrasonic frequencies, >400 kHz to facilitate the separation of oil from solids. At frequencies >400 kHz, it is practical to produce large area standing waves at low amplitudes. Plate transducers are employed to create standing waves, because they operate at specific amplitudes, very much lower than those accomplished by horn transducers. Acoustic separation by standing waves is in principle quite rapid, separating particles breaking down to submicron size in seconds. Sonication can also reduce the pressure head required to pump liquid and minimize clogging and consequent maintenance costs.

It offers a means of further segregating particles on the basis of their density and compressibility. Moreover, ultrasonic waves have the ability to alter the interaction between fat globules through acoustic pressure. Under the appropriate conditions, they can cause aggregation of fat globules/fine particles, which induce the separation on the basis of the relative specific gravities of the phases and recovery of these particles.

Adnan et al. [21] registered the patent entitled "Improving oil recovery and reducing the oxygen demand of palm oil mill effluent". This method allows to increase the oil recovery and to reduce the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of palm oil mill effluent reducing the amount of palm oil in the effluent.

6. Conclusion

The application of new emerging technologies, such as ultrasounds, in the virgin olive oil production process could offer an interesting number of advantages due to their mechanical and thermal effects. However, many of them are not sustainable because of their "energy vampire" attitude or their very high price. The most promising emerging technology appears to be the ultrasound (US) application. In the recent years, the use of the ultrasound conditioning in adsorptive bubble separation process has been expanding due to the evidence that this technology increases the efficiency of the hydrophobic particles separation. Furthermore, the US technology is able to induce the rupture of cell walls and facilitate the recovery of the oil and minor compounds trapped in the uncrushed olive tissue; this increases the work capacity of the extraction plant and reduces the process time. In recent years, many papers and patents have been presented with concordant results on the use of ultrasound, applied in different ways, by different universities and research institutions. Analysing each activity, it is possible to conclude that one of the most important future challenges is to design and build ultrasound machines to improve the working capacity of the industrial plants and to perform a real continuous process able to increase quality as well as extraction yield. At the same time, it is necessary to reduce production and investment costs and optimise the plant working capacity.

Author details

Riccardo Amirante[1*] and Antonello Paduano[2]

*Address all correspondence to: riccardo.amirante@poliba.it

1 Department of Mechanics, Mathematics and Management (DMMM), Polytechnic University of Bari, Bari, Italy

2 Department of Agricultural and Environmental Science, University of Bari, Bari, Italy

References

[1] Jermann C, Koutchma T, Margas E, Leadley C & Ros-Polski V. Mapping trends in novel and emerging food processing technologies around the world. Innov Food Sci Emerg Technol. 2015;31,14–27. doi:10.1016/j.ifset.2015.06.007

[2] Jiménez A, Beltrán G & Uceda M. High-power ultrasound in olive paste pretreatment. Effect on process yield and virgin olive oil characteristics. Ultrason Sonochem. 2007;14,725–731. doi:10.1016/j.ultsonch.2006.12.006

[3] Clodoveo M L, Durante V, La Notte D, Punzi R & Gambacorta G. Ultrasound-assisted extraction of virgin olive oil to improve the process efficiency. Eur J Lipid Sci Technol. 2013;115,1062–1069. DOI: 10.1002/ejlt.201200426

[4] Clodoveo M L, Durante V & La Notte D. Working towards the development of innovative ultrasound equipment for the extraction of virgin olive oil. Ultrason Sonochem. 2013;20,1261–1270. doi:10.1016/j.ultsonch.2013.02.001

[5] Bejaoui M A, Beltran G, Sánchez-Ortiz A, Sanchez S & Jimenez A. Continuous high power ultrasound treatment before malaxation, a laboratory scale approach: effect on virgin olive oil quality criteria and yield. Eur J Lipid Sci Technol. 2015;118,332–336. doi: 10.1002/ejlt.201500020

[6] Bejaoui M A, Beltran G, Aguilera M P & Jimenez A. Continuous conditioning of olive paste by high power ultrasounds: response surface methodology to predict temperature and its effect on oil yield and virgin olive oil characteristics. LWT-Food Sci Technol. 2016;69,175–184. doi:10.1016/j.lwt.2016.01.048

[7] Clodoveo M L, Camposeo S, Amirante R, Dugo G, Cicero N & Boskou D Research and innovative approaches to obtain virgin olive oils with a higher level of bioactive constituents. In: Olives and Olive Oil Bioactive Constituents, Boskou D. (Ed.), Urbana, IL: AOCS Press. 2015, pp. 179–216. ISBN: 978-1-630670-41-2.

[8] Clodoveo M L. An overview of emerging techniques in virgin olive oil extraction process: strategies in the development of innovative plants. J Agric Engine. 2013;44,297–305

[9] Amirante R, Distaso E & Tamburrano P. Experimental and numerical analysis of cavitation in hydraulic proportional directional valves. Energ Convers Manage. 2014;87,208–219. doi:10.1016/j.enconman.2014.07.031

[10] Clodoveo M L. Malaxation: influence on virgin olive oil quality. Past, present and future-an overview. Trends Food Sci Tech. 2012;25,13–23. DOI: 10.1016/j.tifs.2011.11.004

[11] Clodoveo M L, Hbaieb R H, Kotti F, Mugnozza G S & Gargouri M. Mechanical strategies to increase nutritional and sensory quality of virgin olive oil by modulating the endogenous enzyme activities. Compr Rev Food Sci F. 2014;13,135–154. doi: 10.1111/1541-4337.12054

[12] Jordan C E. Ultrasonic flotation system. Patent US 5059309A, 21.06.1990

[13] Filippov L O, Matinin A S, Samiguin V D & Filippova I V. Effect of ultrasound on flotation kinetics in the reactor-separator. In: Journal of Physics, Conference Series. IOP Publishing. 2013;416,012016

[14] Yang X G, Tan W & Tan X F. Demulsification of crude oil emulsion via ultrasonic chemical method. Pet Sci Technol. 2009;27,2010–2020. doi:10.1080/10916460802637577

[15] Clodoveo ML, Dipalmo T, Crupi P, Durante V, Pesce V, Maiellaro I, Lovece A, Mercurio A, Laghezza A, Corbo F & Franchini C. Comparison between different flavored olive oil production techniques: healthy value and process efficiency. Plant Foods Hum Nutr. 2016;71,81–87. doi:10.1007/s11130-016-0528-7

[16] Jimenez M A, Beltran M G & Aguilera Herrera M P. Apparatus and method for the continuous heating and uniform ultrasonic treatment of olive paste. ES2327308 B1, 26.12.2007

[17] Pieralisi G. Installation for extraction of oil from olive paste. EP2535399 B1, 15.06.2011

[18] Femenia M A, Adrover O S, Simal F S, & Rosselló M M C. Method for preventing total or partial crystallization of olive oil during storage at low temperature. WO2012160226 A1, 16.05.2012

[19] Bates D M & Bagnall W. Viscosity reduction. Patent EP2334424 A4, 29.07. 2009

[20] Bates D M & Bagnall W. Methods for isolating oil from plant material and for improving separation efficiency. Patent WO2012106768 A1, 09.02.2012

[21] Adnan A, Bates D M, Kennedy M P, Patist A & Purtle I C. Improving oil recovery and reducing the oxygen demand of palm oil mill effluent. Patent WO2010138254 A1, 16.04.2010

Chapter 4

Stabilization of Extra-Virgin Olive Oil

Lorenzo Guerrini and Alessandro Parenti

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64401

Abstract

The conservation of virgin olive oil quality during its shelf life could be considered a key issue for olive oil industry. To improve the product stability, virgin olive oils should not be stored with considerable amounts of suspended solids and water. The latter have to be removed from oil musts. The chapter reviews the main spread technologies and those recently proposed for the removal of suspended solids and the water from extra-virgin olive oils. These technologies are described from an engineering perspective, and their effects on product quality during storage are discussed.

Keywords: shelf life, biophenols, quality, vertical centrifuge, filtration

1. Introduction

Virgin olive oil (VOO) is a product with a relatively long shelf life. Producers consider 12–18 months as the optimal period from production to consumption. However, the olive oil produced in a harvest season is usually consumed before the following season [1]. Furthermore, at the beginning of a harvest season, the VOO from the previous season undergoes a serious price reduction. During the storage, VOO undergoes a large number of changes in its chemical and sensory characteristics. For this reason, many solutions have been proposed to retain VOO quality and extend its shelf life.

2. Oil must characterization

The VOO coming from the decanter centrifuge, the oil must, is veiled. Decanters separate the oil from olive paste and water, and the centrifuge settings affect the VOO cloudiness and the

yield. Oil must is an intermediate product of the VOO production process, and it has to be filtered or precipitated before bottling. Otherwise, after a certain period of time, the veiled oil turns into a separate phase system and forms a brown residue that settles into the bottom of the container. It is usually considered unacceptable by consumers if it appears in bottles [2]. This deposit is made by solids as well as a certain amount of water, whose characteristics affect the settling time. The amount of material in suspension in the oil changes with the extraction system; with the traditional extraction system, it is about 8% [2], while in a continuous extraction plant (after the vertical centrifuge), the suspended solids were, are on average, equal to 0.01% [3]. The VOO turbidity affects color and appearance of olive oil [4].

In oil musts, the water content is highly variable with an average value of roughly 0.5% by weight [5], while the International Olive Oil Council suggests an upper limit of 0.2% [6]. The presence of water could reduce the rate of oxidation and improve the stability of VOO [7] because water is rich in polar compounds, especially phenols with a strong antioxidant effect [8].

Suspended solids are usually small olive fragments. They remain in the VOO after the extraction, and they consist of proteins, sugar glycosides, sugar bounded with proteins, phospholipids, and phenolic compounds [2, 3].

Some of the above-mentioned compounds have amphiphilic behavior showing both lipophilic and hydrophilic properties. They organize themselves in colloidal forms (mainly in reverse micelles and in lamellae because these forms are energetically preferred) and aggregate at the interphase between water and oil [9]. Colloidal suspended solids have a large reactive surface and, containing both water and oil, allow the presence of both lipophilic and hydrophilic compounds. The size of such colloidal formations has been studied by Papadimitriou et al. [5], who relate the average size of these suspensions with the extraction process. Suspensions give to the VOO a cloudy appearance and cause hydrolytic reactions, which could result in sensorial defects [5].

Furthermore, solids and water in cloudy VOO allow the development of a microflora, mainly represented by yeasts [10]. Before olive processing, microflora lives on the olive carposphere and migrates into the oil together with the olive fragments and water. The substances on the olive fragments and the water allow these micro-organisms to survive. In just a few hours after the olive oil extraction, some yeasts such as *Candida* and *Saccharomyces* are able to colonize the environment [11]. The selected microflora could survive during the entire storage period and could damage the sensory characteristics of VOO. It has been demonstrated that some lipase-producing yeasts can hydrolyze triglycerides triacylglycerols in different manners according to the size of suspended micro-drops of vegetation water [11, 12]. The presence of water and solids together with some micro-organisms could lead to the formation of the "muddy-sediments" and "rancid" defects after a short storage time [13].

Changes in chemical and sensorial parameters in oil musts have been recently described in the literature [14], while different strategies have been developed to eliminate part or the whole suspended solids fraction.

http://dx.doi.org/10.5772/64401

3. Vertical centrifugation

To produce an olive oil clearer and stable, suspended solids are removed with static or dynamic filtration. The vertical centrifuge represents the most widespread technology for the clarification of the olive oil must coming from the decanter. Almost every olive oil extraction plant, working with the continuous system is equipped with this device. The vertical centrifuge removes many of the present impurities due to the difference in specific weight between water and oil. The physical principle of operation is the reduction in the settling time obtained by replacing the gravitational acceleration with a centrifugal acceleration provided by the machine. The sedimentation rate with the gravitational acceleration is governed by Stokes' law:

$$V_c = d^2\left(sw_1 - sw_2\right) g / 18\eta \tag{1}$$

where V_c is the sedimentation rate, d is the equivalent diameter of the particles to be removed, sw_1–sw_2 is the difference in specific weight of the substances to be separated, g is the gravitational acceleration (9.81 m/s^2), and η is the viscosity of the main phase. In vertical centrifuge, the term g is replaced by the squared angular speed of rotation of the centrifuge (at n rpm), multiplied by the radius of the centrifuge. The Stokes equation for the calculation of the rate of sedimentation in the vertical centrifuge then becomes:

$$V_c = d^2\left(sw_1 - sw_2\right)\left(2\pi / 60\right)2r / 18\eta \tag{2}$$

The vertical centrifuge usually works at rotational speed of approximately 6000–6500 rpm on its axis. The particles to be separated move perpendicularly to the plane of decantation and quickly settle on the wall of the vessel. Thus, the high rotational speed allows sedimentation up to 20,000 times more quickly than those achievable with the gravitational acceleration, providing obvious benefits in terms of operational capability. The centrifuge, after a short lag phase due to the filling of the working chamber with the liquid to be clarified, works continuously, and it is usually able, by means of the lowering of a shutter with hydraulic control, to "shoot" outside the separated sediment and water. The central body has a vertical axis rotor, which allows centrifugation of the process liquid. The rotor is provided with a central feed tube, which carries a series of vertical disks superimposed and fixed to one another at a distance of about 1 mm. Near to the axis of rotation, these disks are perforated to form vertical channels. The disks have different functions: they reduce the turbulence of the introduced process liquid in the drum, reduce the radial space that the solid particles must travel to reach the collection chamber, and keep separated the liquid from the solid particles. In this way, the sedimentation speed and clarification are improved.

The oil is continuously introduced through the axial feed tube placed on the top of the drum and accelerates up to the operating rotational speed. Then, the oil rises through the conical

spaces of the stack of disks and separates, thanks to the effect of the centrifugal force. Afterward, a centripetal pump sends it to the outlet pipe. The centripetal pump is placed at the exit of the liquid in the upper part of the drum. The kinetic energy of the liquid having an impact on the vanes is converted into pressure energy (3–6 bar) and pushes the oil in the outlet line.

The main technical parameters of vertical centrifuges are:

- nominal flow: the maximum flow capacity (L/h) that the centrifuge can reach operating water;
- volume of the sludge chamber: storage volume (L) of the solid decanted;
- hourly capacity: quantity of product obtainable from a murky clarified. It can be estimated with the following report and is a function of the nominal flow rate and the volume of the sludge chamber:

$$Q = VP_n / t\Delta s \tag{3}$$

where Q is the hourly flow rate, V is the volume of the sludge chamber, P_n is the nominal flow rate, t is the interval between the "shoots," and Δs is the percentage of suspended solids separate;

- effective range: less than or equal to the hourly capacity. Lower if the power cuts out during the shooting and the same if this does not happen; and
- flow factor: it is calculated based on the number of revolutions of the drum, the number of disks, their diameter, and their angle of inclination [15].

Despite the operative benefits provided, vertical centrifuge has several negative effects on olive oil quality.

First of all, during the centrifugation, operation water is generally added to the VOO. This water reduces the concentration of phenolic compounds, because they are generally hydrophilic molecules, and consequently decreases the stability of the VOO [16, 17]. The amount of each compound transferred from the oily phase to the water phase is regulated by the partition coefficient between water and oil. This coefficient is a function of the solubility of each compound in the two phases. The partition coefficients between oil and water for some phenolic compounds were calculated by Rodis et al. [17]. They were found to be highly variable and ranging from 0.0004 of hydroxytyrosol to 0.187 dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol at 25°C. The exception is the elenolic acid linked to hydroxytyrosol that showed a value of 11.8 [17]. These values show how the added process water can be detrimental for the quality of olive oil.

In the vertical centrifuge, oxygen is dissolved in the VOO. The latter is almost saturated in oxygen, while the oil must from the decanter shows half of the saturation value. Dissolved oxygen is quickly consumed by the VOO; this has been correlated with a considerable shelf life reduction. A linear regression between dissolved oxygen and peroxide number has been found in olive oils [18]. In the same work, a shelf life test showed the effect of high initial

http://dx.doi.org/10.5772/64401

concentration of dissolved oxygen during the storage. In both cases, there is a linear increase in peroxide value during storage. However, the slopes of the two lines are significantly different, and the centrifuged oil reaches the legal limit (20 meqO_2/kg—EC2568/91) more quickly than the non-centrifuged oil. Therefore, it is reasonable to assume that the dissolved oxygen added from the vertical centrifuge initiates the reactions of autoxidation during storage. Other negative effects of vertical centrifuge are higher values of K_{232} and lower concentration of biophenols during the olive oil storage [18].

To avoid or to reduce the negative effects of vertical centrifugation, some technology has been proposed, for example, centrifugation under inert gas [19] and nitrogen stripping [20]. The blanketing of the vertical centrifuge with an inert gas protects the VOO from oxidation and provides benefits in term of oxidative indexes (i.e., peroxide number and K_{232}). Inert vertical centrifuges are used in oenology [21] but only few in the VOO industry due to their high purchase costs.

The nitrogen stripping technique removes the dissolved oxygen from VOO. Nitrogen stripping results in lower oxidation of VOO (i.e., lower peroxide number). However, after stripping, a loss of some VOO key odorants (i.e., E-2-hexenal) is observed [20]. Stripping appears to be promising for VOO, but other studies are required to deeply understand its effect on the product. Inert centrifuges and nitrogen stripping could be useful to protect VOO from oxidation, but they cannot prevent the losses of phenolic compounds due to the use of process water.

In recent years, some strategies to avoid vertical centrifuge have been implemented. Altieri and coworkers propose an online sedimentation approach [22]. Sedimentation is an ancient procedure used to clarify vegetable oils, requiring large basins and long times. The Altieri and co-workers' [22] approach reduces the time required by sedimentation and allows a fast VOO clarification.

Some small olive mills, producing high quality olive oils, start to filter the VOO directly from the decanter, avoiding the vertical centrifuge. This approach requires a careful sizing of the filter to equal the working capacity of the decanter. If the filter is well sized, this approach seems to be able to protect the VOO quality, avoiding the introduction of oxygen and water due to the vertical centrifugation [14, 23].

4. Filtration

Filtration is one of the most debatable steps in olive oil production. Some consumers appreciate veiled olive oils recently produced, while others prefer filtered olive oil.

Filtration could be considered one of the simpler and spread solid-liquid separation. During filtration, a suspension is forced into a porous medium able to hold the solids and let the liquid pass through. The liquid will be consequently clearer. Two theoretical models explain the filter behavior: surface and depth filtration. In the former, the porous medium has holes smaller than the suspended particles. In the latter, the separation is due to the adsorption of particles

into the porous. In this case, the porosity is greater than the retained solids. The division into surface filters and depth is a simplification of what happens in real filters where, usually, both theoretical models occur simultaneously. For instance, the layer of solid particles that are deposited on a surface filter exerts increasingly greater depth of action as it increases in thickness [24, 25].

The speed of a filtration process is defined as the quantity of filtrate (V) processed per unit of time (θ). This speed is governed by the equation of Hagen-Poiseuille:

$$\frac{1}{A}\frac{dV}{d\theta} = \frac{\Delta P}{\mu\left(\alpha w V / A + r\right)} \tag{4}$$

where A is the surface of the filter, ΔP is the difference of pressures at the two sides of the filter medium, μ is the viscosity, α is a coefficient due to the filtered resistance that has accumulated in the medium, w is the content of solids in the filtrate in weight per unit of volume, and r is the resistance of the filter medium. From the equation, it can be noted that the driving force that allows the filtration of a solid-liquid mixture is the pressure difference between the two sides of the filter media (ΔP). The pressure difference is theoretically proportional to the speed with which the process takes place. Actually, the physicomechanical characteristics of the solid residue of olive oil divert from linearity, and the pressure increase is often not proportional to the increase in the rate of filtration. From the formula, it is easy to understand the importance of the correct size of the filter, hence the correct determination of the filtering surface (A). Another important parameter for VOO filtration is the temperature of the mixture during the process. Temperature does not appear directly in the equation of Hagen-Poiseuille but strongly influences the viscosity. The higher the temperature, the lower the viscosity of the oil to be filtered. For this reason, the filtration speed of VOO at 30°C is twice than a filtration at 15°C [26]. Finally, the parameter α produces strong variation during filtration. The parameter α is minimum at the beginning of the process and increases progressively until the filtration cycle ends due to the low speed. This effect is caused by the progressive increase in the amount of solids on the filter medium [27].

Many filtration technologies are adopted to remove solids and water from VOO: conventional systems such as filter tank, filter press [27], cross-flow systems [28], inert gas flow filtration systems, and filter bags, as reviewed by Frankel et al. [29].

The simplest filter used for VOO is the cotton filter (or "alla barese" filter, an old gravity filter that may be used in a small scale). The VOO is introduced into a vessel lying on the bottom of one or more layers of textile fiber. The filtered oil is collected in a second vessel placed at a lower level than the first. This type of filtration is carried out without pumps, using the gravity acceleration. The cotton filter is a cheap and easy solution. The main drawback of cotton filter is related to the very long time required for the operation. The pressure difference between the two parts of the filter is low due to the force of gravity and by the hydrostatic pressure of the column of oil. The filtering surface is limited. During this operation, the oil is exposed to oxygen and consequently to the risk of oxidation [27].

http://dx.doi.org/10.5772/64401

The most common filter used in small- and medium-sized production companies of VOO is the filter press. Filter press consists of a series of flat chambers placed vertically next to each other. The chambers are composed of a plate used for support and turbid oil distribution, and by the filter medium, composed of filter sheets. To avoid loss of oil when the pressure rises, chambers and filter sheets are adhered to each other through a central screw and two metal plates. The filter is dimensioned by choosing the appropriate number of filter media (sheets). They usually have a square shape and size between 20 and 120 cm [25]. The filter sheets for filter press are depth filters and consist mainly of cellulose [23]. To reduce the use of sheets and the VOO losses, the filter press could be coupled with stainless steel pre-filters [23].

On a larger scale, Kieselguhr filters are often used. In this kind of filters, the filtration adjuvant is continuously added to the olive oil suspension. That colloidal suspension gives rise to highly compressible deposits, and the adjuvant confers to the deposit stiffness and porosity to obtain good permeability and ensure higher flow throughout the filtration cycle. However, in the last years, the cross-flow filtration (membrane filtration) began to spread [28].

Membrane filtration is a very common practice for seed oils, which recently has also been proposed for the VOO. Membrane filters, when compared with other filtration systems, provide some benefits. For instance, they eliminate the filtration adjuvants. Usually, adjuvants retain VOO, causing some losses. Furthermore, the disposal of these adjuvants could be problematic. In the Kieselguhr filtered oils, it is common to find traces of some filter aids; this does not happen in the membrane-filtered oils. In addition to the solution of these problems, the membrane filtration allows to remove traces of heavy metals, such as copper, manganese, and iron, which sometimes contaminate the oils. The cross-flow filters are typically surface filters, characterized by a high efficiency of retention at low porosity [28].

Filtration affects chemical composition of VOO, changing the minor compound concentration and olive oil stability during storage [30]. Lower stability and greater susceptibility to alterations are attributed by some authors to cloudiness. However, water and suspended solid particles contain antioxidant compounds. Koidis and Boskou [3] explain this decrease in the stability of filtered oils on the basis of total phenol contents because some of these compounds are highly soluble in water. Filtration may cause a reduction in the stability of the oils, removing phenolic compounds [2, 30]. Furthermore, from the observations on a model system obtained by emulsifying the olive oil, Ambrosone and coworkers [7] concluded that the presence of dispersed water reduces the rate of oxidation. Hidalgo and co-workers [32, 33] pointed out that traces of peptide compounds may also play a stabilizing role. Observations related to a lower stability of filtered oils have been made by Tsimidou et al. [34] and Papadimitriou et al. [5].

Filtration affects not only the concentration of biophenols but also other minor compounds. Chlorophylls [28] and waxes [35] are reduced by this operation. On the other hand, tocopherols seem to be not affected by filtration [36, 37]. Finally, filtration can influence the aroma of the oils. Few studies focus on the effects of filtration on volatile organic compounds in olive oil. Bottino et al. [28] quantified losses ranging from 57 to 72% of carbonyl compounds (mainly aldehydes) with 5–13 carbon atoms. Bubola et al. [38] report similar changes and point out that ketones and compounds with five carbon atoms are more easily removed.

Brenes et al. [36] indicated that unfiltered oils have a higher peroxide value, K_{232}, and are prone to more rapid oxidation. These changes could be explained with the presence in the suspended solids of oxidative enzymes [39, 40], as well as esterases and glycosidases [36]. With water and solids removal, filtration could reduce the rate of enzyme activity, preserve the biophenol fraction, and retard the degradation. Filtration removes these enzymes and reduces the hydrolysis of secoiridoids [14]. Hence, in cloudy oils, phenolic alcohols, namely tyrosol and hydroxytyrosol, are quickly produced, while in filtered oil, the increase in phenolic alcohol concentrations is slower. This increase is due to the degradation of the secoiridoid fraction (oleuropein and ligstroside derivatives). These compounds remain quite stable in filtered oils over time. Regarding the volatile fraction, the same authors [14] found that compounds of LOX pathways such as E-2-hexenal and other C6 are constant in filtered oils. Molecules related to rancidity (i.e., 2,4-decadienal, 2,4-heptadienal, E-2-decenal, octane) and to a winey attribute (ethyl acetate) have been found to increase more quickly in cloudy than in filtered oils.

In conclusion, filtration provides greater flavor stability by reducing the formation rate of off-flavor compounds and the appearance of sensory defects. The absence of these defects is required by the European law to label olive oils as extra-virgin.

Author details

Lorenzo Guerrini and Alessandro Parenti*

*Address all correspondence to: alessandro.parenti@unifi.it

Department of Agricultural, Food Forestry Systems—Biosystems Engineering Section, Florence, Italy

References

[1] Morelló, J. R., Motilva, M. J., Tovar, M. J., & Romero, M. P. Changes in commercial virgin olive oil (cv Arbequina) during storage, with special emphasis on the phenolic fraction. Food Chemistry. 2004; 85(3):357–364.

[2] Lerker, G., Frega, N., Bocci, F., & Servidio, G. "Veiled" extra-virgin olive oils: dispersion response related to oil quality. Journal of American Oil Chemists' Society. 1994; 71(6): 657–658.

[3] Koidis, A., & Boskou, D. The contents of proteins and phospholipids in cloudy (veiled) virgin olive oils. European Journal of Lipid Science and Technology. 2006; 108(4):323–328.

[4] Gordillo, B., Ciaccheri, L., Mignani, A. G., Gonzalez-Miret, M. L., & Heredia, F. J. Influence of turbidity grade on color and appearance of virgin olive oil. Journal of the American Oil Chemists' Society. 2011; 88(9):1317–1327.

[5] Papadimitriou, V., Dulle, M., Wachter, W., Sotiroudis, T. G., Glatter, O., & Xenakis, A. Structure and dynamics of veiled virgin olive oil: influence of production conditions and relation to its antioxidant capacity. Food Biophysics. 2013; 8(2):112–121.

[6] International Olive Oil Council. Trade Standard Applying to Olive Oil and Olive Pomace Oils. 2009, COI/T.15/NC No3. Rev 8, 2015.

[7] Ambrosone, L., Angelico, R., Cinelli, G., Di Lorenzo, V., & Ceglie, A. The role of water in the oxidation process of extra virgin olive oils. Journal of the American Oil Chemists' Society. 2002; 79(6):577–582.

[8] Bakhouche, A., Lozano-Sánchez, J., Ballus, C. A., Martínez-García, M., Velasco, M. G., Govantes, Á. O., Gallina-Toschi, T., Fernández-Gutiérrez, A., & Segura-Carretero, A. Monitoring the moisture reduction and status of bioactive compounds in extra-virgin olive oil over the industrial filtration process. Food Control. 2014; 40:292–299.

[9] Xenakis, A., Papadimitriou, V., & Sotiroudis, T. G. Colloidal structures in natural oils. Current Opinion in Colloid & Interface Science. 2010; 15(1):55–60.

[10] Ciafardini, G., & Zullo, B. A. Microbiological activity in stored olive oil. International Journal of Food Microbiology. 2002; 75(1):111–118.

[11] Ciafardini, G., Zullo, B. A., Cioccia, G., & Iride, A. Lipolytic activity of Williopsis californica and Saccharomyces cerevisiae in extra virgin olive oil. International Journal of Food Microbiology. 2006; 107(1):27–32.

[12] Ciafardini, G., Zullo, B. A., & Iride, A. Lipase production by yeasts from extra virgin olive oil. Food Microbiology. 2006; 23(1):60–67.

[13] Zullo, B. A., Cioccia, G., & Ciafardini, G. Effects of some oil-born yeasts on the sensory characteristics of Italian virgin olive oil during its storage. Food Microbiology. 2013; 36(1):70–78.

[14] Fortini, M., Migliorini, M., Cherubini, C., Cecchi, L., Guerrini, L., Masella, P., & Parenti, A. Shelf life and quality of olive oil filtered without vertical centrifugation. European Journal of Lipid Science and Technology. 2015; 117. In press DOI: 10.1002/ejlt.201500229.

[15] De Vita, P. Corso di meccanica enologica. Hoepli, Milano, Italy. 1997. 532 p.

[16] Di Giovacchino, L., Solinas, M., & Miccoli, M. Effect of extraction systems on the quality of virgin olive oil. Journal of American Oil Chemists' Society. 1994; 71(11):1189–1194.

[17] Rodis, P. S., Karathanos, V. T., & Mantzavinou, A. Partitioning of olive oil antioxidants between oil and water phases. Journal of Agricultural and Food Chemistry. 2002; 50(3): 596–601.

[18] Masella, P., Parenti, A., Spugnoli, P., & Calamai, L. Influence of vertical centrifugation on extra virgin olive oil quality. Journal of the American Oil Chemists' Society. 2009; 86(11):1137–1140.

[19] Masella, P., Parenti, A., Spugnoli, P., & Calamai, L. Vertical centrifugation of virgin olive oil under inert gas. European Journal of Lipid Science and Technology. 2012; 114(9): 1094–1096.

[20] Masella, P., Parenti, A., Spugnoli, P., & Calamai, L. Nitrogen stripping to remove dissolved oxygen from extra virgin olive oil. European Journal of Lipid Science and Technology. 2010; 112(12):1389–1392.

[21] Jackson, R. S. Wine science: principles and applications. Academic Press, San Diego. 2008.

[22] Altieri, G., Di Renzo, G. C., Genovese, F., Tauriello, A., D'Auria, M., Racioppi, R., & Viggiani, L. Olive oil quality improvement using a natural sedimentation plant at industrial scale. Biosystems Engineering. 2014;122:99–114.

[23] Guerrini, L., Masella, P., Migliorini, M., Cherubini, C., & Parenti, A. Addition of a steel pre-filter to improve plate filter-press performance in olive oil filtration. Journal of Food Engineering. 2015; 157:84–87.

[24] Peri, C. La filtrazione nelle industrie alimentari. Edizioni AEB, Brescia, Italia. 1983. 478 p.

[25] Sutherland, K. Filters and filtration handbook. 5th ed. Elsevier, UK. 2008. 523 p.

[26] Bonnet, J. P., Devesvre, L., Artaud, J., & Moulin, P. Dynamic viscosity of olive oil as a function of composition and temperature: a first approach. European Journal of Lipid Science and Technology. 2011; 113(8):1019–1025.

[27] Peri, C. The extra-virgin olive oil handbook. Wiley Blackwell, United Kingdom. 2014. 280 p.

[28] Bottino, A., Capannelli, G., Comite, A., Ferrari, F., Marotta, F., Mattei, A., & Turchini, A. Application of membrane processes for the filtration of extra virgin olive oil. Journal of Food Engineering. 2004; 65(2):303–309.

[29] Frankel, E., Bakhouche, A., Lozano-Sánchez, J., Segura-Carretero, A., & Fernández-Gutiérrez, A. Literature review on production process to obtain extra virgin olive oil enriched in bioactive compounds. Potential use of byproducts as alternative sources of polyphenols. Journal of Agricultural and Food Chemistry. 2013; 61(22):5179–5188.

[30] Lozano-Sánchez, J., Cerretani, L., Bendini, A., Segura-Carretero, A., & Fernández-Gutiérrez, A. Filtration process of extra virgin olive oil: effect on minor components, oxidative stability and sensorial and physicochemical characteristics. Trends in Food Science & Technology. 2010; 21(4):201–211.

[31] Frega, N., Mozzon, M., & Lercker, G. Effects of free fatty acids on oxidative stability of vegetable oil. Journal of the American Oil Chemists' Society. 1999; 76(3):325–329.

[32] Hidalgo, F. J., Alaiz, M., & Zamora, R. Determination of peptides and proteins in fats and oils. Analytical Chemistry. 2001: 73(3):698–702.

[33] Hidalgo, F. J., Alaiz, M., & Zamora, R. Low molecular weight polypeptides in virgin and refined olive oils. Journal of the American Oil Chemists' Society. 2002; 79(7):685–689.

[34] Tsimidou, M. Z., Georgiou, A., Koidis, A., & Boskou, D. Loss of stability of "veiled" (cloudy) virgin olive oils in storage. Food Chemistry. 2005; 93(3):377–383.

[35] Parenti, A., Spugnoli, P., Baldi, F., Masella, P., Calamai, L., & Mattei, A. Preliminary observations on veiled olive oil turbidity with regards to wax content. La Rivista Italiana Delle Sostanze Grasse. 2008; 85:221–228.

[36] Brenes, M., García, A., García, P., & Garrido, A. Acid hydrolysis of secoiridoid aglycons during storage of virgin olive oil. Journal of Agricultural and Food Chemistry. 2001; 49(11):5609–5614.

[37] Fregapane, G., Lavelli, V., León, S., Kapuralin, J., & Desamparados Salvador, M. Effect of filtration on virgin olive oil stability during storage. European Journal of Lipid Science and Technology. 2006; 108(2):134–142.

[38] Brkić Bubola, K., Koprivnjak, O., & Sladonja, B. Influence of filtration on volatile compounds and sensory profile of virgin olive oils. Food Chemistry. 2012; 132(1):98–103.

[39] Georgalaki, M. D., Sotiroudis, T. G., & Xenakis, A. The presence of oxidizing enzyme activities in virgin olive oil. Journal of the American Oil Chemists' Society. 1998; 75(2): 155–159.

[40] Valgimigli, L., Sanjust, E., Curreli, N., Rinaldi, A., Pedulli, G. F., & Rescigno, A. Photometric assay for polyphenol oxidase activity in olives, olive pastes, and virgin olive oils. Journal of the American Oil Chemists' Society. 2001; 78(12):1245–1248.

Chapter 5

Chlorophylls and Carotenoids in Food Products from Olive Tree

Beatriz Gandul-Rojas, María Roca and
Lourdes Gallardo-Guerrero

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64688

Abstract

This chapter provides an updated overview about the chlorophyll and carotenoid pigments present in olive fruits and their products, table olive, and olive oil. The metabolism of these pigments during growth and ripening of the olive fruit is described. General aspects related to photosynthetic tissues and non-carotenogenic fruits, varieties and the presence of exclusive pigments, the total pigment content, and their relative proportions are highlighted. Chlorophyll and carotenoid changes during the processing of green table olives according to the main styles of preparation are described. Different reaction mechanisms depending on the removal of the bitter components by alkaline hydrolysis or by slow diffusion in brine, as well as the development of the fermentation process, are discussed. The chlorophyll degradation associated with the green staining alteration is specifically mentioned. Changes in the pigment profiles and in their concentrations associated with the virgin olive oil (VOO) elaboration are also described. Recent research works related to thermal degradation kinetics and prediction mathematical model for VOO storage are summarized. The role of the chlorophylls in the photo-oxidation of VOO is also pointed out. Finally, the pigment profiles as authenticity and freshness indices for VOO quality are emphasized.

Keywords: chlorophylls, carotenoids, metabolism, table olive, olive oil

1. Introduction

The olive fruit (*Olea europaea* L.) is a small drupe that has a long ripening period involving important color changes. There are numerous varieties of olives, which are classified into three categories according to their use: table olives, oil extraction, or both purposes. Olive fruit contains

water (60–70%), lipids (10–25%), sugars (3–6%), fiber (1–4%), proteins (1–3%), and other minor compounds, such as hydrocarbons, biophenols, terpenes, sterols, alcohols, chlorophyll and carotenoid pigments, and volatile compounds, which are responsible for the unique characteristics of its products [1]. The quality of table olives and olive oil depend on the chemical composition and physical properties of the fruit, and this in turn is principally related to the olive variety, degree of fruit ripeness, environmental conditions, growing region, and processing and storage techniques. These factors influence the color of the table olive and olive oil, which is one of the most important quality attributes for products from the olive tree. In the case of the olive oil, the importance of the color, and the applicable legislation and regulation, has been discussed, and the different approaches (visual and instrumental methods) used for color measurements have been reviewed in depth [2].

Chlorophyll and carotenoid pigments are the compounds mainly responsible for the color of green table olives and virgin olive oil. Information about the influence of olive varieties on the chlorophyll composition in olives, as well as about the influence of agronomic and technological factors, and of storage on the chlorophylls in olive oil, can be found in a review article [3]. The study of chlorophylls and their structural transformations in olive is very complex. The high lipid content of the olive (15–30%) is a great obstacle in isolating chlorophylls and their derivatives and interferes with any analysis of these liposoluble pigments. Saponification is the technique mainly used for the removal of fatty matter but it cannot be used when analyzing chlorophylls, which are destroyed by alkali. The first detailed studies on pigment composition in olives and their food products started at the end of the 1980s. At that time, a liquid-phase extraction method was developed for obtaining a pigment extract from olive fruit, free of fatty matter [4]. Nowadays, different methodologies for the simultaneous analysis of chlorophyllic and carotenoid compounds in olive oil have been described. The growing interest in this subject initiated the inclusion of a new chapter [5] in the second edition of a recently published handbook of olive oil. It provides essential information about different chromatographic methodologies for the analysis of chlorophyllic and carotenoid pigments in olive oil and includes general aspects about these pigments. Information concerning the presence of chlorophyllic and carotenoid pigments in fruit and olive oil and their possible use for determining the genuineness and correct processing of VOO can be found in broad outline, together with some researches related to kinetic studies of thermodegradation of pigments [5].

A great number of studies that include the chlorophyll and carotenoid contents of olive oil can be found in the bibliography. However, many of them provide only total data of each fraction of pigments obtained by a direct determination from the absorption spectra of the olive oil dissolved in cyclohexane [6]. In the case of green table olive, there are numerous works that determine the surface color of the olive by instrumental methods, but the pigments responsible for that color are not studied in most of them. This chapter is intended to provide detailed information about the chlorophyllic and carotenoid pigments present in olives, table olive, and olive oil. Essential, specific, and updated information related to the pigments composition responsible for the color of green table olive and olive oil and their application as indices of authenticity, freshness, and quality for their different commercial products is gathered in one manuscript.

http://dx.doi.org/10.5772/64688

2. Metabolism of chlorophylls and carotenoids in olive fruits

2.1. Growth

The chlorophyllic and carotenoid profiles of olives are similar in general terms for all varieties (with the exception of the Arbequina variety). The chlorophyllic fraction is mainly composed of chlorophyll *a* and chlorophyll *b* forms, although allomerized chlorophylls, such as 13^2-hydroxy and 15^1-hydroxy-lactone derivatives, are also detected in smaller quantities (**Figure 1**) [7–9]. In the carotenoid fraction, lutein represents the major carotenoid present in olives and is the only representative of the β, ε series of carotenoids. The other carotenoids present in olives belong to the β, β series and include β-carotene, violaxanthin, neoxanthin, antheraxanthin, and β-cryptoxanthin (**Figure 2**). Lutein, β-carotene, violaxanthin, and neoxanthin constitute more than 95% of the carotenoids present in olives and are the characteristic carotenoids of the green fruit [10]. When light intensity is high, violaxanthin is transformed via antheraxanthin to zeaxanthin [11]. However, zeaxanthin has not been identified at any stage of the growth or maturation of olives [12–16]. In parallel, and independently of the high fat content of the fruit, the xanthophylls of the olive fruit remain unesterified [4], indicating that the chloroplast remains intact [17].

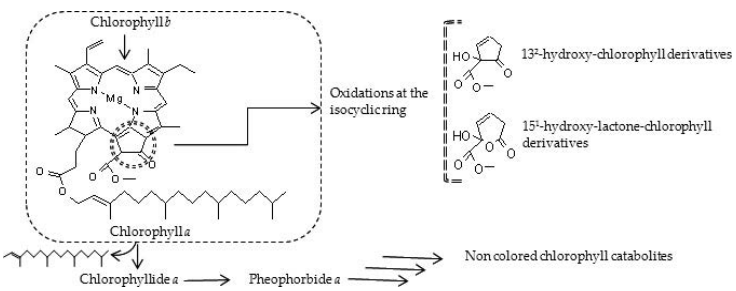


Figure 1. Chlorophyll derivatives present in the olive fruit. Dashed frame indicates the major pigments.

From a quantitative point of view, olive varieties can be divided into three groups: those categorized as high pigmentation, such as the Hojiblanca or Picual varieties, with more than 350 mg pigment per kg dry weight in the green fruit; varieties with an intermediate content, such as Cornicabra, with more than 250 mg pigment per kg dry weight; and varieties characterized as low pigmentation, such as Arbequina and Blanqueta types, with a pigment content lower than 100 mg/kg dry weight in the green fruit [18]. Commonly, the ratio of total chlorophylls to total carotenoids in thylakoids is maintained between 3 and 4 for the majority of the varieties analyzed [19], although varieties have been described that exhibit a higher chlorophyllic content: Gordal [12], Frantoio, Koroneiki, and Coratina [15]. In any case, the maturation period of the fruit implicates higher rates of degradation for the chlorophyllic fraction than for the carotenoid fraction [19]. As a consequence, the ratio of chlorophyll/carotenoid decreases as maturation advances [12, 15, 16, 20]. The chlorophyll *a*/chlorophyll *b* ratio is an indirect measure of the packing density of the thylakoids in the chloroplast. In the olive fruit, this ratio

is usually in the range of 3–4 [12, 18] for the majority of the varieties analyzed. Fruits of the Arbequina [18] and Koroneiki [15] varieties are exceptional in that they have values close to 5, implying that they show relatively fewer antenna complexes.

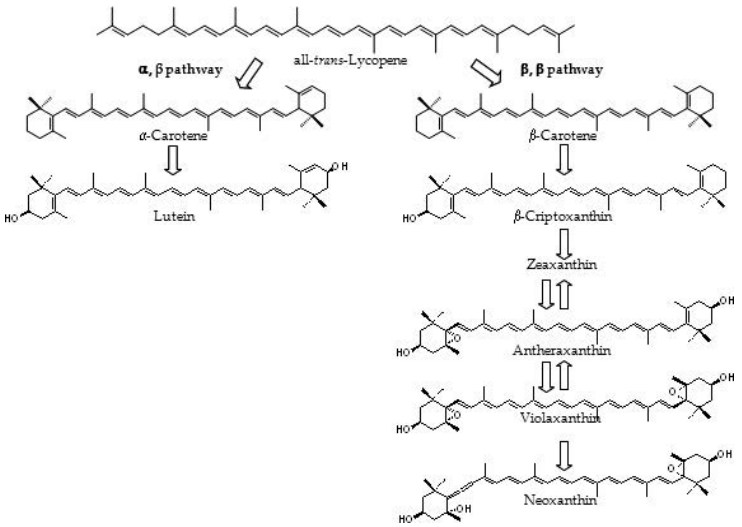


Figure 2. Biosynthetic pathway of carotenoids presents in the olive fruit.

Few studies have investigated the biosynthesis of chlorophylls and carotenoids during the olive's growth period. Roca and Mínguez-Mosquera [8, 20] specifically studied the evolution of the content of the major chlorophylls and carotenoids in the Hojiblanca, Picual, and Arbequina varieties during the growth period, which lasts for 12–16 weeks. The biosynthesis curve of both fractions exhibited a continuous increase in the first phases of the period, and later remained constant—at different levels depending on the variety—until the fruit had completely developed. Studies of the individual carotenoids show that the concentration of lutein remains constant during the growth period of the three varieties of fruits. Lutein has the highest percentage of the four basic carotenoids that constitute the "universal chloroplast," confirming it is vital in photosynthesis. In terms of the β, β series carotenoids, β-carotene is the precursor. The fluctuations of β-carotene's line of evolution [20] correspond to those of the carotenoids whose formation depends directly or indirectly on the synthesis of this molecule. As a consequence of the biosynthetic process, the composition of carotenoids in green olives is 50–55% lutein, 20–23% β-carotene, 12% violaxanthin, 8% neoxanthin, and 2% antheraxanthin.

2.2. Maturation

Carotenoids are associated with the chlorophylls of photosynthetic tissues, and for that reason, the majority of olive fruits are green color when unripe. As the stage of ripening advance, the photosynthetic activity decreases and the chlorophylls are degraded. The carotenoids associated with these compounds are usually catabolized at the same time (non-carotenogenic fruit)

or may remain constant. Alternatively, as a result of the new synthesis of carotenoids, the concentration of carotenoids may even increase (carotenogenic fruit). The olive, like other fruits whose maturation is associated with the synthesis of anthocyanins or betalains [10], is classified as a non-carotenogenic fruit. In such cases, the typical pattern of carotenoids in the chloroplast does not change during maturation. Nevertheless, the rate of degradation of each of the chloroplast carotenoids can vary significantly, and so the relative proportions of the carotenoids in a mature chloroplast may be modified.

The maturation period of the olive usually begins in November or December and has a duration of 4–6 weeks, depending on the variety. The degradation process of chlorophylls and carotenoids during the maturation phase of the olive fruit has been studied in many varieties: Gordal [12], Hojiblanca, Picual, Blanqueta, Cornicabra [8, 20], Farga [14], Coratina, Frantoio, Koroneiki [15], and Sikitita [16]. For all of the varieties studied, the profile of chlorophylls and carotenoids of olives do not change qualitatively during the maturation process. The first enzyme implicated in the degradation of chlorophylls in fruits is chlorophyllase, which is responsible for elimination of the phytol chain, generating chlorophyllides [21]. Dephytylated derivatives are subsequently degraded to colorless compounds (**Figure 1**), and therefore, in most fruits, dephytylated chlorophylls do not usually accumulate [22]. However, in the profile of certain olive varieties—Arbequina and Blanqueta—an accumulation of dephytylated chlorophyllic derivatives (chlorophyllides and pheophorbides) has been detected during the maturation period exclusively, as a consequence of high chlorophyllase activity [8, 9]. Likewise, an accumulation of certain oxidized chlorophyll derivatives (13^2-hydroxy and 15^1-hydroxy-lactone chlorophylls, **Figure 1**) has been detected in the transition period from growth to maturation in the chlorophyllic profile of the Arbequina variety of olives only [8]. Such an accumulation is due to the high peroxidase activity unique for the thylakoids of this variety [23]. These chlorophyll derivatives during the maturation of specific olive varieties are usually transferred to the corresponding single-variety oils and can be used as a parameter of authenticity.

During the period of maturation, olive fruits undergo a gradual and progressive degradation of all the carotenoids, of both the β, β and β, ε series. The degradation kinetics for the main carotenoids have been analyzed during the maturation of the Hojiblanca and Picual olive varieties [20]. In both varieties, lutein was degraded the slowest, followed by antheraxanthin, and then β-carotene and neoxanthin, with similar values. Violaxanthin was the most rapidly degraded carotenoid. As a clear exception, olive fruits of the Arbequina and Sikitita varieties have a carotenogenic profile. For these varieties, as the chlorophylls are catabolized, the synthesis of preexisting or new carotenoids is underway during ripening. In carotenogenic fruits—in which β-carotene, lutein, violaxanthin, and neoxanthin are the major types of chloroplast carotenoids—the carotenoid pattern is gradually transformed, and in some cases it becomes more complex, to a pattern more typical of chromoplasts. In the fruits of the Arbequina [24] and Sikitita [16] varieties exclusively, in addition to the typical carotenoids of green chloroplasts, esterified xanthophylls, particularly neoxanthin and violaxanthin, accumulate during the maturation phase. In the first stage of maturation, net increases of the concentrations of lutein, β-carotene, violaxanthin, and antheraxanthin are observed in the

Arbequina fruits, while the content of esterified xanthophylls gradually increases in a continuous manner during the entire period [13].

Investigations on the carotenogenic processes that take place during the ripening of certain fruits have generally been developed using species in which the biosynthetic process is very intense, like tomatoes [25] or peppers [26]. In contrast to the chloroplasts, where a similar set of carotenoids are found in distinct plant species, the chromoplasts have been found to accumulate an enormous diversity of new carotenoids in some cases [27]. The carotenogenic process is expressed at a low level in the olive fruits of the Arbequina variety, with significant punctual, albeit slight, increments of carotenoids. The identification of a progressive accumulation of esterified xanthophylls in the olive fruits during the maturation stages confirms the carotenogenic nature of this variety. The esterification of xanthophylls takes place exclusively for *de novo* carotenoids synthesized in chromoplasts but not in chloroplasts. This esterification increases the lipophilic nature of the carotenoids, contributing to their accumulation in the plastoglobuli. In this sense, it's plausible to hypothesize that a certain fraction of chloroplasts of the olive fruits of the Arbequina variety will evolve into chromoplasts rather than be transformed into gerontoplasts during maturation.

As a consequence of the specific metabolism of each olive variety, the percentage contribution of each carotenoid in the ripe olive fruit is highly dependent on the variety. In a model variety such as Hojiblanca, due to the relative differences in their degradation, the carotenoid profile of the mature fruit is enriched in lutein (reaching some 70% of the total carotenoid content). This occurs at the expense of a lower representation of the other carotenoids, namely β-carotene, violaxanthin, neoxanthin, and antheraxanthin, whose percentages in the ripe fruit are reduced with respect to the immature fruit. In the fruits of the Arbequina variety exclusively, maturation does not involve an increase in the proportion of lutein (as a consequence of the carotenogenic process), and the same relative proportion of lutein is maintained in the green fruit (around 50%); whereas β-carotene and neoxanthin are found in lower levels and higher proportions of violaxanthin and antheraxanthin are found in the mature fruit of Arbequina. The differences in the carotenoid metabolism of Arbequina olives with respect to other varieties are seen in the significant differences in the carotenoid content of the respective oils. In fact, carotenoid profiles have become useful parameters to authenticate monovarietal virgin olive oils (Section 4.4) [28].

3. Chlorophylls and carotenoids in table olives

Table olives are, together with olive oil, one of the most traditional foods of the Mediterranean diet. There are many different treatments for the preparation of table olives, with a common characteristic being the removal of the glucoside oleuropein, responsible for the extreme bitterness of the olive fruit [29]. According to the degree of ripeness of the fresh fruit, table olives are classified as green olives, turning color, or black olives [30]. The green color of olives is due to the chloroplastic pigments: chlorophylls and carotenoids [4], whereas for black ripe olives, the color is mainly due to anthocyanins that are synthesized during the ripening of the

fruit [31]. The color of table olives is one of the most important sensory attributes assessed by consumers and is considered as an important quality index. This section will examine the different aspects of the pigments responsible for the color of green table olives.

3.1. Pigment changes during the processing of table olives

Green table olives are prepared with fruits harvested during the ripening period, prior to coloring and when they have reached normal size [30]. At this time, the color of the fruits varies between green and yellowish-green, and once processed can vary from green to straw-yellow. As stated above, the color of green olives is due to the presence of chlorophylls *a* and *b*, and the typical chloroplastic carotenoids, namely lutein, β-carotene, violaxanthin, neoxanthin, and antheraxanthin [4, 12]. In addition, small amounts of the chlorophyll derivatives 13^2-OH-chlorophylls *a* and *b*, and the carotenoid β-cryptoxanthin are frequently present [7, 8, 18, 32, 33].

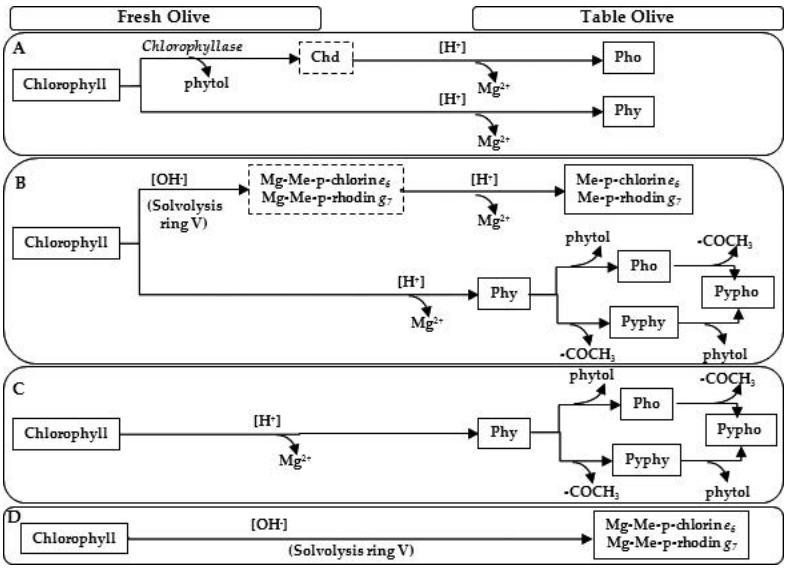


Figure 3. Main transformations of chlorophylls (*a* and *b*) and their derivatives during processing of table olives according to: (A) traditional Spanish style; (B) actual Spanish style; (C) natural green olive; (D) Castelvetrano-style. Abbreviations: Chd, chlorophyllide; Phy, pheophytin; Pho, pheophorbide; Pyphy, pyropheophytin; Pypho, pyropheophorbide; Me-p-chlorin e_6,15^2-Me-phytol-chlorin e_6 ester; Me-p-rhodin g_7,15^2-Me-phytol-rhodin g_7 ester.

The main green table olive preparation is the so-called Spanish or Seville style. The processing consists of treating the fruits with a dilute solution of sodium hydroxide to increase the skin permeability and remove the bitter glucoside oleuropein. Olives are then washed with tap water and placed in brine, where spontaneous lactic acid fermentation takes place [29, 34]. These processing conditions provoke several transformations of the chloroplastic pigments present in the fresh fruit that are desirable to obtain the characteristic and appreciated golden-yellow coloration of Spanish-style green table olives. Traditionally, the olive fermentation was done in small containers with capacities about 150–300 kg. During the processing of table olives

according to the traditional Spanish style, chlorophylls (*a* and *b*) are totally transformed to pheophytins and pheophorbides, both with grey-brownish colors, by two different and coexisting mechanisms: one enzymatic and the other chemical (**Figure 3A**) [35–37]. Firstly, a proportion of the chlorophylls are transformed into chlorophyllides by the action of chlorophyllase during the period prior to the start of the fermentation process. Afterwards, the acidic pH resulting from the lactic fermentation leads to the replacement of the Mg^{2+} ion by 2 H^+ in the remaining chlorophylls (which have not been dephytylated by the action of chlorophyllase) and chlorophyllides, causing the formation of pheophytins and pheophorbides, respectively (**Figure 4**). The degradation of chlorophylls to pheophytins and chlorophyllides to pheophorbides follows first-order kinetics with respect to the pigment concentration [37].

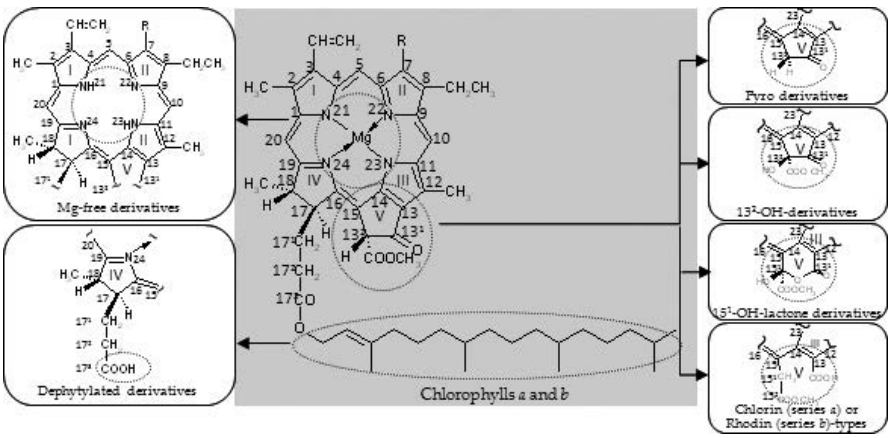


Figure 4. Structural comparison between chlorophylls (*a* and *b*) and their main derivatives found in table olives or virgin olive oil.

The alkaline treatment of the olive fruits does not produce any change in the carotenoid pigments since they are alkali-stable compounds [38]. However, the subsequent decrease of the pH during the fermentation process affects some carotenoids whose molecular structures are sensitive to the acid medium (**Figure 5**). This is the case for carotenoids with 5,6-epoxy groups, which are transformed to 5,8-furanoid groups in acidic conditions (**Figure 6**). Therefore, during the fermentation phase of olives, violaxanthin, with two 5,6-epoxy groups, is first transformed into luteoxanthin, with one 5,6-epoxy group and one 5,8-furanoid group; finally, both pigments give rise to auroxanthin with two 5,8-furanoid groups. In a similar reaction, neoxanthin and antheraxanthin, both with one 5,6-epoxy group in their structure, are transformed to their 5,8-furanoid derivatives, neochrome and mutatoxanthin, respectively (**Figure 5**). The transformation of violaxanthin and neoxanthin to auroxanthin and neochrome, respectively, follows first-order kinetics with respect to pigment concentration [39]. Thus, during the processing of Spanish-style table olives, the concentrations of violaxanthin, neoxanthin, and antheraxanthin diminish progressively while those of auroxanthin, neochrome, and mutatoxanthin increase. In the case of lutein and β-carotene, their concentrations remain constant during the complete process. The total content of the chlorophyll and

http://dx.doi.org/10.5772/64688

carotenoid pigments also remains unchanged, indicating the absence of oxidative reactions that would degrade these compounds to uncolored products [35, 37, 39].

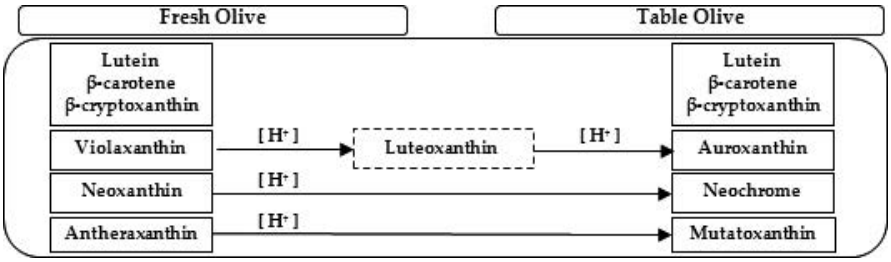


Figure 5. Transformation of carotenoids during the table olive fermentation process or the virgin olive oil elaboration.

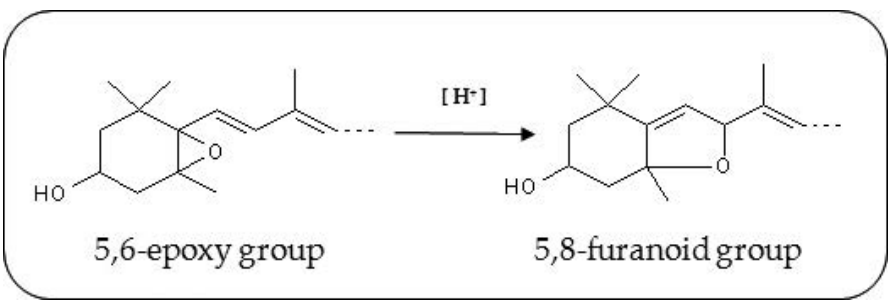


Figure 6. Transformation of carotenoids with 5,6-epoxy group to carotenoids with 5,8-furanoid group.

Actually, the use of large fermenters, with 10,000–12,000 kg capacity, and some innovations introduced in the traditional system of Spanish-style processing, such as the reuse of sodium hydroxide solution and brines, decreased fruit washes to reduce the volume of wastewaters, addition of culture initiators with recirculation, etc. [34], have partially modified the mechanism of chlorophyll degradation (**Figure 3B**). In these cases, the chlorophyllase activity is not promoted and the alkaline treatment of the olives provokes oxidative reactions that affect the chlorophyll isocyclic ring, producing allomerized chlorophylls with chlorin (series *a*)- and rhodin (series *b*)- type structures (**Figure 4**) [33, 40]. During the fermentation process, those allomerized compounds (Mg-15^2-Me-phytol-chlorin e_6 ester and Mg-15^2-Me-phytol-rhodin g_7 ester) are transformed to their corresponding Mg-free derivatives; meanwhile, the minor amounts of pheophorbides (*a* and *b*), pyropheophytins (*a* and *b*), and pyropheophorbide *a* are also formed. Moreover, a slow but progressive decrease in the concentration of the chlorophyll and carotenoid pigments takes place at the end of the process, indicating that a certain proportion of these pigments are degraded to colorless products.

In addition to the Spanish-style table olives, there are other trade preparations of green olives, which are also highly appreciated by consumers. Among them, natural green olives, which are directly fermented in brine without any alkaline treatment, are popular. In this type of table

olive elaboration, the main pigment transformations that take place are those due to the acid pH originated by the fermentation process, and no chlorophyll derivatives with chlorin- or rhodin-type structure are formed (**Figure 3C**) [33]. A particular type of Natural green olive is the Protected Designation of Origin *Aloreña de Málaga*. This is a seasoned table olive specialty that includes an initial cracking of the fruits. During the cracking process, free organic acids are released, promoting a slight transformation of chlorophylls to pheophytins and some isomerization of violaxanthin and antheraxanthin to their respective 5,8-furanoid isomers [32]. Moreover, some cellular rupture is also provoked, favoring the contact between endogenous chlorophyllase enzyme and chlorophylls, and giving rise to small amounts of pheophorbide *a*. Subsequently, the reactions of chlorophylls and carotenoids catalyzed by acids continue as the fruits remain in brine.

There are other green table olive specialties whose elaboration includes an alkaline treatment but no lactic fermentation phase, and to which are given a particular name in each producing country. Such is the case of the Castelvetrano-style table olive from Sicily, which has been used as a model to study the changes undergone by the chloroplastic pigments in the preparations of these specialties. In the processing of Castelvetrano-style table olives, the fruits are subjected to a high alkaline pH (pH 10–11) during 10–15 days. As a consequence, the chlorophyll pigments are transformed by solvolysis reactions that affect the isocyclic ring of the chlorophyll structures (**Figures 3D** and **4**), such as those that take place during the alkaline treatment of Spanish-style table olives. The absence of a fermentation process for these specialties of table olive does not lead to any acid-catalyzed reactions, and neither Mg-chlorophyll derivatives nor carotenoid isomers with 5,8-furanoid groups is formed [41]. As a result, this type of table olive is bright green in color, which is one of its most highly valued features. Unfortunately, this appreciated color is highly unstable and easily degraded to yellowish colorations with time, or due to the thermal treatments that are frequently used to prolong the shelf life of the product. To maintain a permanent green color, the food colorant E-141ii is sometimes added, although this practice is not permitted in the European Union or the United States of America [42, 43]. The E-141ii colorant is a mixture of various compounds, most of them with Cu-chlorin-type structures, which are formed by alkaline hydrolysis of natural chlorophyll with the subsequent addition of copper salt. An analytical procedure for the detection of the color adulteration of green table olives with the E-141ii colorant has been developed [44]. In commercial green table olives with striking bright green color, and labeled both as "green olives in soda" and Castelvetrano-style, great presence of metallochlorophyll complexes of Cu has been also detected (private reports requested by table olive industries, 2005–2010), and amounts up to 90% of the total chlorophyll pigments have been estimated [45]. These Cu–chlorophyll complexes are structurally different from those that make up the E-141ii colorant, and they can be generated during the industrial processing of food by adding Cu salts, such as $CuCl_2$ or $CuSO_4$ [46]. The formation of the same Cu–chlorophyll complexes, but with endogenous copper of the fruits, has been demonstrated in Spanish-style table olives of the Gordal variety affected by the alteration known as green staining (Section 3.2). In the case of Castelvetrano-style table olives, the processing method does not cause the formation of Cu–chlorophyll complexes by itself [41]. The intense alkaline treatment of the process provokes a high degree of cell damage in the olive that allows certain level of complexation between

chlorophyll derivatives and endogenous copper of the fruit during its shelf life under different systems of conservation (acid brine, sterilization, and pasteurization), but the amounts formed are not enough to re-green the product [47]. This result leads to the suspicion that the high proportion of Cu–chlorophyll complexes detected in samples of commercial bright green table olives, which reaches up to 90% of the total chlorophylls, is due to a fraudulent addition of Cu salts, which are only permitted as minerals to fortify foods [48] and not as food additives [49].

3.2. Green staining alteration

The Gordal olive is one of the most important table olive varieties at the international trade level [29]. For a long time, this olive variety has been affected by the occasional appearance of green spots on the surface of the table olives processed according to the Spanish style, with this problem known as green staining alteration. This alteration is seen as brilliant green spots of different sizes distributed over the olive surface, which contrast with the natural olive-green color of the fermented fruit. Several studies carried out to investigate this color alteration have shown that various copper–chlorophyll derivatives are the pigments responsible for the appearance of green spots. The main metallochlorophyll complexes that have been identified in the green staining alteration are copper complexes of chlorophyll derivatives with chlorin- and rhodin-type structures, which are formed during Spanish-style table olive processing, as well as copper complexes of pheophytin *a* and pyropheophytin *a* [50, 51]. These copper complexes are formed stepwise, in such a way that new metallochlorophyll compounds are detected as the fruits become more affected. In addition, the concentrations of the copper complexes increase progressively as the alteration spreads over the surface of the processed olive [52]. In relation to the copper involved in the green staining alteration, it has been shown that it comes from the fruit itself, rather than from exogenous origin [50] and that the pectin chains of the fruit might be the source of the copper [53]. The existence of a great loss of cell integrity in the zone of the olive with green spots is related to the contact of the copper with the chlorophyll derivatives [54].

4. Chlorophylls and carotenoids in virgin olive oil

According to the International Olive Council [55], 90% of the total annual production of olives is used for producing olive oil. Virgin olive oil (VOO) is a natural food product that is obtained solely from the olive fruit (*Olea europaea* L.). Its production is more than just a simple process of extraction and physical separation; during the crushing and malaxation stages, a complex biochemical changes takes place, which are important for both the quality and composition of the final product (**Figure 7**) [56]. Chlorophylls and carotenoids in VOO are determined by the initial pigment composition of the olive fruit, its chemical or enzymatic transformations at the different stages of their elaboration and its transfer to the oil phase. Malaxation and coalescence processes generate an oil emulsion containing water from the olive fruit as well as the water added during the extraction procedure, leaving a certain amount of dispersed oil that is not recoverable and will be lost with the extraction byproducts ("alperujo" in **Figure 7**). The qualitative and quantitative composition of the minor components of VOO are fundamentally

related with the efficiency of the malaxation process and are determined by the rheological properties of the olive paste as well as the variables of the operation. A range of parameters, including the time and temperature of malaxation, atmosphere in contact with the olive paste, and the addition of warm water and/or technological co-adjuvants, determine the equilibrium between the quality and quantity of the VOO extracted [57, 58].

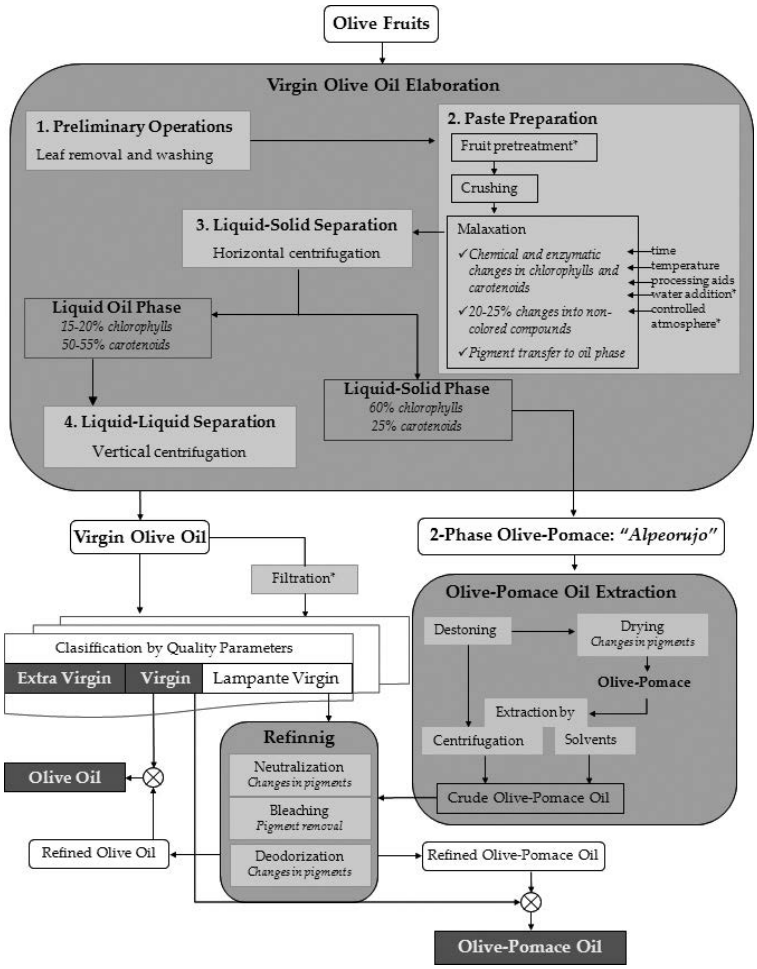


Figure 7. Changes and transfer of pigments in the processing of olive fruits to obtain olive oils and olive pomace oil. *Indicates optional activities. Fruit pretreatments could include destoning or some emerging technologies such as electric pulses, microwaves, ultrasonic, or thermal conditioning. Darker rectangle indicates commercial categories.

4.1. Transfer and changes of pigments from the olive fruit to olive oil

The composition of pigments in VOO is influenced by factors that affect the fruit, such as the olive variety [14, 15, 18, 24, 59–63], the ripening degree [20, 64–66], or the growth conditions, like irrigation [67], as well as the specific conditions employed in each industrial oil extraction

process [28, 60, 67–71]. For the pigments, the key stage of the oil extraction process is malaxation of the olive paste, during which the pigments are transferred from the crushed plant tissue to the oil. To obtain olive oil, ripe fruits—purple-black in color—are used due to their higher fat content. The chlorophyllic and carotenoid compounds are the only pigments transferred from the thylakoid membranes to the oil phase because of their lipophilic nature, and they are responsible for the characteristic yellowish-green color of the oil. The anthocyanins, which are responsible for the dark coloration of the ripe fruit, are retained in the aqueous phase (alperujo) due to their hydrophilic nature. As the chlorophyllic and carotenoid pigments are transferred to the oil phase, they undergo a series of structural transformations that are inherent to the oil extraction process. These changes are influenced by the liberation of acids to the medium, oxygenation, and the greater accessibility of the substrates and enzymes, including those of the seed—since the fruit is ground with the pit. In the chlorophyllic fraction, the main reaction that occurs is the pheophytinization of chlorophylls, in which the chelated Mg ion is substituted for two hydrogen ions. A certain amount of allomerization also occurs, albeit to a lesser extent, generating 13^2-hydroxy and 15^1- hydroxy-lactone chlorophyll derivatives and pheophytins (**Figure 1**), in addition to the oxidative ring-opening of porphyrin macrocyles with the formation of uncolored derivatives. Accessibility of the chlorophyllase enzyme to chlorophyllic substrates during the grinding and mixing of the olive paste can, depending on the variables of the operation, provoke the enzymatic de-esterification of the phytol chains and gives rise to chlorophyllides, which in acidic conditions substitute the Mg ion and generate pheophorbides (**Figure 1**). The presence of de-esterified chlorophyll derivatives in VOO is exclusive for the olive varieties with high chlorophyllase activity, such as Arbequina, Blanqueta, and Koroneiki [15, 59, 72], and therefore, they can be used as chemical markers for the determination of the varietal origin of the VOO. In the carotenoid fraction, the most common reaction is the formation of 5,8-furanoid isomers, although the formation of *Z*/*E* (*cis-trans*) isomers and the degradation to uncolored products are also frequent [28, 59, 64, 72].

The pigment profile inherent to VOO consists of chlorophyll *a* and *b*, lutein, β-carotene, and the minor xanthophylls violaxanthin, neoxanthin, antheraxanthin, and β-cryptoxanthin that originate from the fresh olive fruit, together with pheophytin *a* and *b* and the 5,8-furanoid xanthophylls (luteoxanthin, auroxanthin, neochrome, and mutatoxanthin), which are formed during the oil extraction process [59, 64]. Traces of allomerized chlorophyll derivatives, and in certain varieties, some exclusive pigments such as de-esterified chlorophyll derivatives, α-carotene, or esterified xanthophylls may also be found (**Figure 8**) [59]. The olive variety and the ripening degree of the olive fruits are factors that significantly affect the content and percentage composition of pigments in VOO. The specific metabolism of pigments for each olive variety (Section 2.2) allows a differentiation according to the total pigment content, the percentage of violaxanthin, and the percentage of lutein. These parameters have been successfully used as model indicators to classify VOO varieties of Spanish origin [28]. Likewise, the generalized reduction of pigment concentrations as maturation progresses and the higher rate of degradation of the chlorophyllic fraction are directly reflected by the tone and intensity of color of the VOO, which can vary across the production campaign from intense green to light yellow [6].

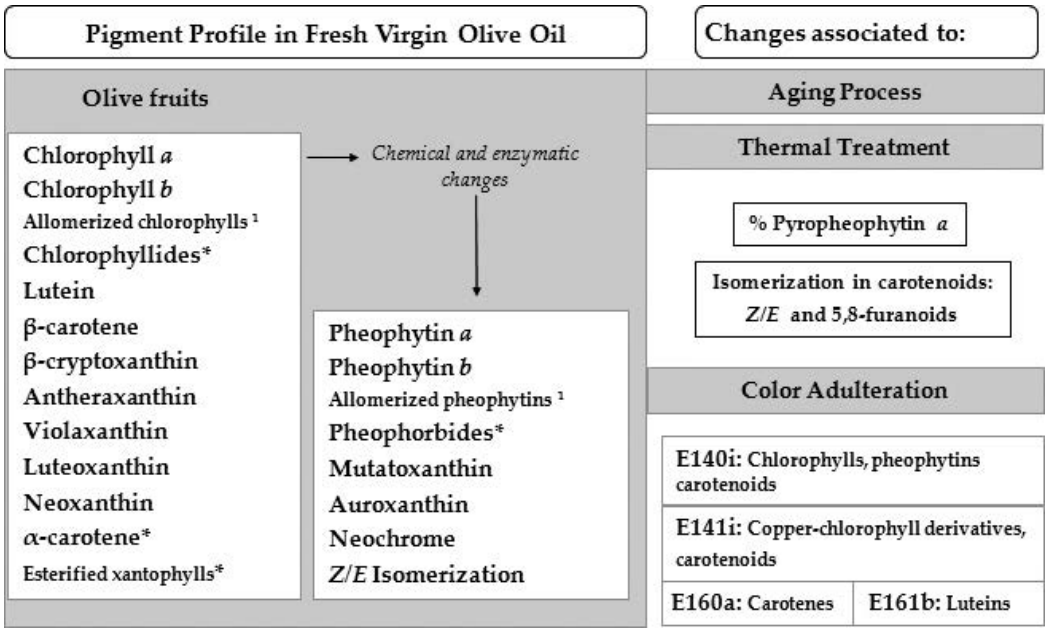


Figure 8. Pigment profile in fresh virgin olive oil and changes associated to aging process, thermal treatment, or colorant addition. [1]Minor compounds. *Exclusive pigments for certain olive varieties as Arbequina.

From a quantitative point of view, the main modification that occurs during oil extraction is the considerable reduction in the ratio of total chlorophylls to total carotenoids as the pigments pass from the fruit to the oil. A mass balance of the extraction process reveals that despite the lipophilic nature of these pigments, their transfer to the oil phase is only partial; a part of these pigments is retained in the alperujo while another part is oxidized to colorless products (**Figure 7**). A higher percentage of carotenoids are transferred to the oil phase than chlorophyllic pigments; this explains why the ratio between the two fractions is lower in the oil than in the fruit. These differences mainly lie in the lower retention of carotenoids in the alperujo, with around 25% of total carotenoids retained, whereas the retention of chlorophylls can reach a level up to 60%. The percentage of pigments that are oxidized to colorless products has been estimated to be between 20 and 25% for both pigment fractions [64, 65].

Each portion of pigments that is transferred to the oil, remains in the alperujo, or is degraded to colorless compounds, is different and this variability depends on the technological innovations introduced at each stage of the extraction process. Some techniques permit the processing of less mature olive fruits, such as thermal treatments with warm air [73], or a water bath [74–76], and their use allows the production campaign to start earlier in the season. These techniques can also decrease the stability to some extent, modulate the intensity of bitterness and lead to an increase in pigmentation of the VOO. Pitted olives produce oils with optimal qualitative characteristics, with increased content of volatile compounds and phenols; yet pitting has a negative effect on the transfer of chlorophylls and carotenoids, leading to the production of oil with a less intense color [77, 78]. During malaxation, it has been observed

that as the mixing time and temperature are increased in an optimal range between 20 and 30°C, the pigment concentration in the oil is greater [79–81] due to a larger release from the plant tissue [79]. For temperatures higher than 30°C, further increase in the pigment concentration is not observed, due to the thermal destruction of the pigments [69, 79]. The use of extraction co-adjuvants that generally improve the yield of oil, like plant enzymes with pectinolytic, cellulolytic, and hemicellulolytic activities [69], micronized talc [67], or common salt (NaCl) [82], also produce oils that are richer in pigments, although the only additives authorized for VOO extraction by the EU are those of physical action such as micronized natural talc and kaolinite clay [83].

4.2. Thermal degradation kinetics

Olive oil will deteriorate over time even if suitable conditions for storage are used, protected from light and heat [84]. This loss of freshness or aging in VOO can be monitored by measuring any parameter that is sensitive to some degradation that inevitably occurs during storage. The values of parameters and their kinetic variations will depend on both the operative conditions, essentially the temperature, and on the compositional characteristics of the oil matrix. Some studies on actual modification of these parameters have been reported, which have led to develop some empirical models able to perform predictions under specific conditions. One more advanced step in this area is the development of kinetic models, capable of predicting the evolution of the selected parameter, not only under specific conditions, but in terms of the different variables that affect storage.

The chlorophyll and carotenoid pigments can be good tracers of storage conditions and preservation of VOO, as they are quite sensitive to operational factors such as temperature, light, and oxygen. The kinetic and thermodynamic parameters related to the oxidation of the main chlorophyll and carotenoid pigments have been characterized by thermodegradation studies of VOO at different temperatures and in the absence of light and oxygen. First-order kinetics of an irreversible reaction mechanism was determined appropriate to describe the thermal degradation of pheophytin *a* [85], lutein, β-carotene, and β-cryptoxanthin [86], as well as the epoxidized xanthophylls, neoxanthin, violaxanthin, and mutatoxanthin [87]. Details have been reviewed by Gandul-Rojas et al. [5].

A complex kinetic mechanism of parallel and consecutive reactions has been proposed for pheophytin *a* (phy a) degradation (**Figure 9**) [85]. Of these competitive reactions, the formation of pyropheophytin *a* (pyphy *a*) shows significantly higher kinetic constants in dark conditions and in the absence of air. This result confirms that pyphy *a* could be a good chemical marker for monitoring the aging of VOO during storage in dark conditions. Oxygen availability is a critical factor in the allomerization reactions that give rise to 13^2-OH-pheophytin *a* and 15^1-OH-lactone-pheophytin *a*. Allomerization takes place via mechanisms involving free radicals [88] and are, therefore, favored when VOO is exposed to the air [89]. The final reactions that produce colorless compounds are prevalent when VOO is exposed to the light and the chlorophyllic structure is photo-oxygenated by a singlet oxygen [90] due to its photosensitizing capability [88] through a self-destruction mechanism of porphyrins.

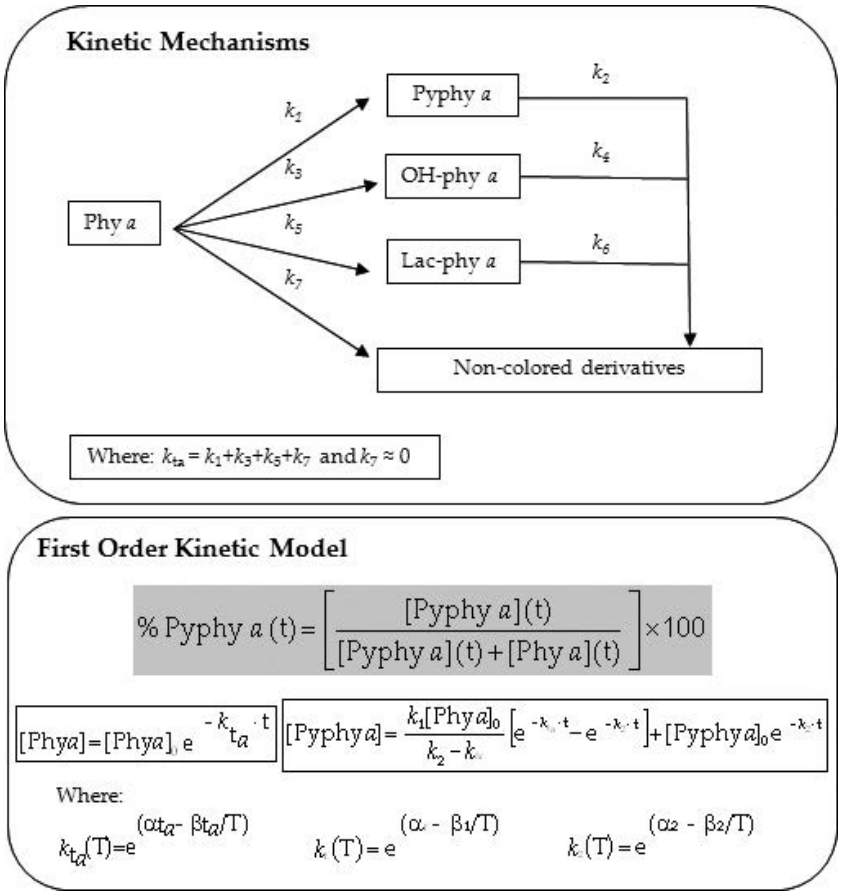


Figure 9. Kinetic mechanisms for thermodegradation of pheophytin *a* in virgin olive oil and expression of a first-order kinetic model for prediction of the percentage of pyropheophytin *a* [85, 102]. Abbreviations as in **Figure 3**.

An isokinetic study compared the kinetic and thermodynamic parameters of three VOO matrices obtained from olive fruits of distinct levels of maturity and with a different pigment content (high, medium, or low); it demonstrated that the oil matrix does not significantly affect the reaction mechanisms that predominate in dark conditions and in conditions of limited oxygen availability [85]. Consequently, the kinetic parameters obtained as a function of the temperature (according to the Arrhenius equation) could be used to develop mathematical models to predict the formation of pyphy *a* (**Figure 9**), the *Z* isomers of lutein, and 5,8-furanoid xanthophylls, in addition to the global degradation of carotenoids to colorless products [86, 87, 91]. In contrast, the mechanism for the formation of 13^2-OH-pheophytin is affected by the composition of VOO, which is logical due to the presence of compounds that act as radical scavengers in VOO that can partially inhibit the mechanism of degradation by free radicals. A clear relationship between the content of radical scavengers and the formation of OH-phy in VOO stored in darkness has not been found [92]. A marked effect of temperature has been discovered for the thermodegradation reactions of pigments in VOO, and perhaps contrary to

what one might expect, the kinetic constants for the degradation of the carotenoid fraction were about 3.6-fold higher than for the chlorophyll fraction, demonstrating a structure that is more stable against discoloration. Likewise, higher activation energy in chlorophylls indicates that a lower increase of temperature is needed in order to increase the kinetic constants for the carotenoid fraction [91].

4.3. Role of the chlorophylls in the photo-oxidation of virgin olive oil

VOO is one of the vegetable oils most resistant to oxidation due to its composition of triacylglycerols low in polyunsaturated fatty acids and the presence of natural antioxidants [93]. However, it also contains other minor compounds such as chlorophyllic pigments, which can have a catalytic effect on the oxidation of VOO in the presence of light [94]. The possible participation of the chlorophylls and their derivatives in the photo-oxidation of VOO has been studied by several groups. Initial investigations were carried out with bleached olive oil, to which compounds including chlorophyll *a*, pheophytin *a* and *b*, and others were added, demonstrating the capacity of these pigments to act as photosensitizers and promote the oxidation of the oil in the presence of light [95]. In a later study, Gutiérrez et al. [96] did not observe any pro-oxidant effects when chlorophylls *a* and *b* were added to a real VOO system that was maintained for three months under artificial light. The authors concluded that the effect of the light itself caused a greater oxidation of the oil than the added chlorophylls, with the chlorophyllic pigments totally destroyed after one week in these study conditions. Nevertheless, the authors did find a slight antioxidant effect when the same VOO system was maintained in dark conditions, with a more pronounced effect for chlorophyll *a* than for chlorophyll *b*. Other later studies carried out with VOOs have found a positive influence of the chlorophyllic pigments on the photo-oxidation of oils subjected to different conditions of light and storage times [90, 97, 98]. In relation to this result, Psomiadou and Tsimidou [90] suggested a concentration-dependent photosensitizing activity for pheophytin *a* that is favored by the availability of oxygen.

4.4. Pigment profiles as authenticity and freshness indices of virgin olive oil

The qualitative changes in the profiles of chlorophyllic and carotenoid pigments, namely their structural transformations to other detectable colored compounds during the oil extraction process, were described in Section 4.1. Such modifications leave a signature specific for a product and they can be used as an index of its authenticity and quality (**Figure 8**) [28, 99]. Beside the basic profile of pigments common to all VOOs, the presence of specific pigments, such as de-esterified chlorophyllic derivatives, α-carotene, or esterified xanthophylls, is a good marker of the varietal origin of the oil [28, 59]. Moreover, three ratios have been proposed to determine the authenticity and correct processing of Spanish VOOs [28, 99]. The genuine pigment profile of the fresh VOO is modified also during its storage, and it is therefore a good indicator of the suitability of the conditions employed in the preservation of the product [100].

For commercial olive oil products (a mix of VOO and refined olive oil), different intensities of the sensory characteristics can be obtained depending on the proportion of VOO. As such, we can find olive oils labeled as intense flavor or mild flavor, which should correspond to the

percentage of VOO used in the mix, although this concept is not yet regulated. The color of the VOO can range from dark green to pale yellow depending on the composition of the pigments of the olive fruit used as the raw material. As described in Section 4.1, this content is subject to wide variability—for chlorophylls between 1 and 40 mg/kg, and for carotenoids between 2 and 20 mg/kg—depending on the olive variety (of high or low pigmentation) and the stage of fruit maturity [28, 64]. Therefore, the color of bottled oil is indicative but it cannot be used to identify exactly the quantity of VOO present in the mixture. Some methods exist to determine a global quality index of olive oil color via the measurement of absorption at the wavelengths of maximum absorption of the oil against an air blank. The use of such methods allows for a measurement of the oil's color to be made, but in no instance are valid to scientifically discriminate taste or the proportion of VOO in the mix, nor can they substitute the analysis of individual pigments as a method for the detection of the adulteration of color. In this case, only the detection of pigments outside the common profile as defined for the VOO, or changes in the aforementioned quantitative ratios of certain pigments, would indicate the color adulteration of commercialized olive oil [55,84,101].

In the list of colorants permitted by the EU for use as food additives are two natural colorants named "chlorophylls" or "natural green" (E-140i) and "copper complexes of chlorophyll" (E-141i), which are obtained by solvent extraction from different edible plant sources. The colorant compounds of E-140i—chlorophylls, pheophytins, and carotenoids—undergo the same transformations as the natural pigments of VOO. The additive E-141i, produced by the addition of copper (II) salts to the pigments causing the formation of copper–chlorophyll derivatives primarily, is preferentially used for the adulteration of olive oil because of its highly stable green color. It is not a pure compound but rather a heterogeneous mixture of chlorophyllic derivatives, whose variability depends on the starting material, the process used for its synthesis, and even the particular commercial batch. In any case, analysis of different commercial samples of the colorant E-141i [101] showed that more than 76% of the compounds were copper–chlorophyll derivatives, with the major component being Cu–pyropheophytin *a*. Of the chlorophyllic pigments present in the colorant E-141i, 99.6% are not found in olive oil; therefore, a simple detection of one of these compounds in olive oil would reveal the oil's adulteration.

A maximum allowable level of copper-chlorophyll derivatives cannot be set for edible oils in general. This is because the detection of these copper–chlorophyll derivatives in VOO is indicative of adulteration by E-141i addition [101], yet this claim cannot be made for olive pomace oil. In this case, it is possible for copper–chlorophyll derivatives to form "natural" complexes via reactions between the chlorophyll pigments and copper from the olive pulp under acidic conditions during the storage and/or drying of the raw material (olive pomaces) (**Figure 7**). These fat-soluble compounds could be transferred to the crude oil during the extraction process and are not completely removed during the refining step (industrial sector private reports). At present, there is no regulation for these refined oils and the detection of copper–chlorophyll in refined olive pomace oil is necessary but not sufficient to certify that the oil has been adulterated by the addition of colorant E-141i.

http://dx.doi.org/10.5772/64688

The fraudulent use of yellow colorants has also been reported. The most likely additives to be used are the carotenes (E-160a) and lutein (E-161b) due to their structural similarity to the carotenoids naturally present in VOO. Cases have been reported of commercialized olive oils where minor xanthophylls were not detected and β-carotene or lutein was found exclusively (or at percentages greater than 90%). This quantitative profile of carotenoids is not within the margins defined for VOO [15, 28] and indicates adulteration by the addition of the colorants E-160a or E-161b, respectively. In other cases, esterified lutein has been detected with spectroscopic characteristics identical to those of free lutein but with different chromatographic properties due to the less polar nature. Lutein is susceptible to esterification with fatty acids on the two hydroxyl groups of its structure; this process, however, does not occur in the olive fruit (see Section 2.2)—despite its high content in fatty acids—as the fruit has a typical non-carotenogenic metabolism, and lutein and xanthophylls remain unesterified. Thus, the presence of esterified lutein in VOO is indicative of the addition of the colorant E-161b.

The qualitative pigment profile of genuine VOO is affected by the small degree of degradation of the oil that can take place during its storage, even under appropriate storage conditions—in darkness and at a controlled temperature [100, 102]. In general, all the degradation reactions that began during the oil extraction progress during its storage; the carotenoid pigment fraction is affected by geometric isomerization (*Z*/*E* isomerizations); 5,6-epoxide is transformed to 5,8-furanoid xanthophylls; degradation of pigments gives colorless products. Mainly in the chlorophyllic pigments fraction, pheophytinization is completed and a certain grade of allomerization continues. Beside, a new reaction commences: pyropheophytinization (**Figure 8**), which has been found to be strongly affected by temperature (Section 4.2). Pyphy *a* (PP) content is a highly variable parameter that depends not only on the operating conditions (time and temperature) but also on the initial amount of pheophytin *a* (P) in the starting oil. This compound is the precursor pigment for PP and is associated with variety and ripening degree [100]. However, when the PP content is expressed as percentage (PPP), with respect to the sum of PP+P, the parameter shows a considerably lower variation margin. In oils stored at controlled temperatures of 15°C, less than 3% of PPP are formed after a year [100], whereas at an average annual temperature of 22°C, the compound can reach values of 7–14% [102]. Therefore, the PPP parameter has been suggested as a chemical marker for monitoring the degradation of VOO [89, 100, 103].

Traditionally, the presence of pyropheophytins in foods of plant origin has been associated with heat treatments of cooking or conservation [104], and in this sense, their presence in VOOs is related with "deodorato" or deodorized olive oils [105, 106]. PP becomes the major chlorophyllic derivative in olive oil that has been thermally treated by deodorization to eliminate organoleptic defects, and its percentage content can reach values of 60% and above. Milder treatments of physical distillation to eliminate slight sensory defects—in conditions of low temperature (<100°C), and under a current of nitrogen and vacuum (2–6 mbar)—to produce what is known as "deodorato" oil, reduce this pyropheophytinization reaction while the other chemical parameters remain unaltered [107]. Therefore, the PPP has also been suggested as a chemical marker of VOO thermal treatments [108]. These results have prompted researchers to study the kinetic behavior of chlorophyll pigments [85] to establish a prediction model of

PPP evolution over time as a function of temperature (**Figure 9**) [102]. The model obtained can be applied to establish a best-before date or shelf life of the VOO, a statement obligatory in the label accordance with the latest regulations [109]. Although this initiative is positive, one must not forget the complexity of putting the model into practice, given that the best-before date requires studies of the shelf life so that the legislator has elements of technical and scientific judgment to support the established timescales. Therefore, the availability of a scientific basis to determine the best-before date for the product is important for the olive oil sector.

In the aforementioned regulations, the inclusion of any special storage conditions for the VOO, such as "must be stored away from light and heat" is also obligatory on the label [84]. The chlorophyllic pigments are quite sensitive to both factors, and therefore, the pigments are good markers of the storage/conservation conditions of VOO. The best-before for VOO is the period in which the oil can be maintained—in the specified storage conditions—without losing optimal quality. Although PPP is not a parameter that is directly related to the sensory quality of VOO, it has been demonstrated to be a good chemical marker to trace the oil's storage conditions. As such, and in the absence of scientific studies that describe the kinetic behavior of the chemical components responsible for the positive and negative attributes used in the evaluation of the organoleptic quality of VOO, PPP could be a very useful tool for establishing a best-before date. Although the freshness of oil does not necessarily mean that the oil has a high quality, it is indisputable that a high-quality VOO (ExtraVOO) loses freshness during storage and, in parallel, it loses their quality because their positive or desirable sensory descriptors will also decrease in intensity over time [110, 111]. The oil can even develop a sensory defect in which case, it would lower the commercial category "extra virgin" (EVOO) to "virgin" (VOO). These sensory changes are strongly influenced by temperature and illumination degree, and PPP may be an indirect marker of such changes.

An analysis of PPP in the initial EVOO (before it is bottled) permits, in accordance with the predicted model established by Aparicio-Ruiz et al. [102] (**Figure 9**), the calculation of the storage time remaining for the oil (protected from the heat and light), to exceed a PPP limit established by the regulations, and gives a specific best-before date for each oil. What limit will be set as the standard? It could be the level that an EVOO reaches a year after its production —if it is stipulated that this is the time in which the EVOO loses its sensory quality. In accordance with this proposition, the maximum limit of PPP can be established at around 14%, in accordance with the study of storage carried out for monovarietal EVOOs obtained from different olive varieties and at distinct ripening degrees [102], and which served to validate the mathematical prediction model of PPP. In Australia, a limit for PPP ≤17 has been included in the olive oil standards [112]. A study from the Davis University aimed to correlate sensory and chemistry results [113] revealed that of 141 samples of commercial EVOO that failed the sensory standard, few samples (at most 29) failed some IOC chemical standards, while more samples (67 and 68) failed the additional chemical tests (diacylglycerols and PPP, respectively) adopted by Australia [112]. If the PPP standard ≤17 is decreased to ≤15 according to the German Society for Fat Science (DGF) [114], the number of failed samples increases to 83.

The mathematical model for PPP provides the producer and/or wholesaler with a tool to determine the speed of the pyropheophytinization reaction as a function of temperature and

the storage conditions that can delay it, facilitating the distinction between aged oil and oil that has been heat-treated. If the storage location of an EVOO, and therefore the temperatures reached during the storage period, is known, it is possible to calculate the estimated PPP and compare it to the experimental value obtained by chemical analysis. An experimental value higher than the theoretical value could indicate that the VOO has not been sheltered from heat and light or has been submitted to a mild deodorization treatment.

The quantitative pigment analysis in the VOO is usually carried out by HPLC with visible detection. This subject has been reviewed by Gandul-Rojas et al [5]. PP and P show the same response signal with a UV–vis detector because both compounds have identical electronic absorption spectrum. Since PPP is a relative parameter, independent of the absolute PP amounts, it can be directly obtained from the ratio between the peak areas that correspond to P and PP in the chromatogram, and the calibration process is not essential. However, there are wide differences between the limit of quantification (LOQ) of the different methods proposed for the analysis of PPP which depend mainly on the system used for purification and concentration of the sample prior to the chromatographic analysis.

The LOQ is an important aspect when the oil is stored in adverse conditions such as unprotected from light. These conditions cause a pigment photo-oxidation to colorless products but surprisingly a rapid increase in PPP is also observed [111]. This result is not due to an increase of PP content but to a faster degradation of P than PP. It is important to note that the oil storage during long time under light conditions can decrease the PP content so much that its analytical detection is impossible, and PPP may result in an erroneous value of 0 (industrial sector private reports).

A marked effect of temperature on the rate of degradation reactions is also observed for the carotenoid pigments fraction, and some ratios, such as the percentage of lutein *Z* isomers, or the percentage of neochrome, could be suggested as chemical markers to monitor the degradation of VOO, as "freshness indicators" [86, 87]. The presence of certain geometric isomers of carotenoids in foods is also related with heat processing. Reaction conditions similar to those utilized in the mild deodorization of VOO are sufficient to significantly increase the percentage of lutein *Z* isomers [86] or the 5,8-furanoid isomer of neoxanthin, namely neochrome [87]. This has indicated the need for some criteria as markers of VOO heat treatment, in addition to the marker established by the chlorophyllic pigments fraction (PPP) [102]. The kinetic behavior of the isomerization of the carotenoid pigments in olive oil has been studied and mathematical models for the prediction of their evolution over time as a function of temperature has been established [86, 87].

Acknowledgements

We acknowledge the financial support of the Spanish Government (Projects AGL 2012-39714/ALI and AGL2015-63890-R) and Junta de Andalucía (Project AGR2010-6271).

Author details

Beatriz Gandul-Rojas, María Roca and Lourdes Gallardo-Guerrero*

*Address all correspondence to: lgallardo@ig.csic.es

Food Phytochemistry Department, Instituto de la Grasa (CSIC), Pablo de Olavide University Campus, Sevilla, Spain

References

[1] Ghanbari R, Anwar F, Alkharfy KM, Gilani AH, Saari N. Valuable nutrients and functional bioactives in different parts of olive (Olea europaea L.)—A Review. Int. J. Mol. Sci. 2012;13:3291–3340. doi:10.3390/ijms13033291.

[2] Moyano MJ, Heredia FJ, Meléndez-MartínezAJ. The color of olive oils: The pigments and their likely health benefits and visual and instrumental methods of analysis. Compr. Rev. Food Sci. Food Saf. 2010;9:278–291. doi:10.1111/j.1541-4337.2010.00109.x.

[3] Giuliani A, Cerretani L, Cichelli A. Chlorophylls in olive and in olive oil: Chemistry and occurrences. Crit. Rev. Food Sci. Nutr. 2011;51:678–690. doi: 10.1080/10408391003768199.

[4] Mínguez-Mosquera MI, Garrido-Fernández J. Chorophyll and carotenoid presence in olive fruit (Olea europaea). J. Agric. Food Chem. 1989;37:1–7. doi:10.1021/jf00085a001

[5] Gandul-Rojas B, Gallardo-Guerrero L, Roca M, Aparicio-Ruiz R. Chromatographic methodologies: Compounds for olive oil color issues. In: Aparicio R, Harwood J, editors. Handbook of Olive Oil: Analysis and Properties. 2nd ed. New York: Springer Science+Business Media; 2013. p. 219–259. doi:10.1007/978-1-4614-7777-8

[6] Mínguez-Mosquera MI, Rejano-Navarro L, Gandul-Rojas B, Sánchez-Gómez AH,Garrido-Fernández J. Color-pigment correlation in virgin olive oil. J. Am. Oil Chem. Soc. 1991;69:332–336. doi:10.1007/BF02657688

[7] Gallardo-Guerrero L, Jaren-Galán M, Hornero-Méndez D, Mínguez-Mosquera MI. Evidence for the involvement of lipoxygenase in the oxidative processes associated with the appearance of green staining alteration in the Gordal olive. J. Sci. Food Agric. 2003;83:1487–1492. doi:10.1002/jsfa.1564.

[8] Roca M, Mínguez-Mosquera MI. Involvement of chlorophyllase in chlorophyll metabolism in olive varieties with high and low chlorophyll content. Physiol. Plantarum. 2003;117:459–466. doi:10.1034/j.1399-3054.2003.00073.x.

[9] Roca M, Gandul-Rojas B, Minguez-Mosquera MI. Varietal differences in catabolic intermediates of chlorophylls in Olea europaea (L.) fruits cvs. Arbequina and Blanqueta. Postharvest Biol. Tec. 2007;44:150–156. doi:10.1016/j.postharvbio.2006.12.001.

[10] Gross J. Pigment in Fruits. Orlando: Academic Press; 1987, 303p.

[11] Schindler C, Lichenthaler HK. Photosynthetic CO_2 assimilation, chlorophyll fluorescence and zeaxanthin accumulation in field-grown maple trees in the course of a sunny and a cloudy day. J. Plant Physiol. 1996;148:399–412. doi:10.1016/S0176-1617(96)80272-0.

[12] Mínguez-Mosquera MI, Gallardo-Guerrero L. Disappearance of chlorophylls and carotenoids during the ripening of the olive. J. Sci. Food Agric. 1995;69:1–6. doi:10.1002/jsfa.2740690102.

[13] Roca M, Mínguez-Mosquera MI. Unusual carotenogenesis in fruits with pronounced anthocyanic ripening (Olea europaea var. Arbequina). J. Agric. Food Chem. 2001;49:4414–4419. doi:10.1021/jf0100200.

[14] Criado NN, Motilva MJ, Goñi M, Romero MP. Comparative study of the effect of the maturation process of the olive fruit on the chlorophyll and carotenoid fractions of drupes and virgin oils from Arbequina and Farga cultivars. Food Chem. 2007;100:748–755. doi:10.1016/j.foodchem.2005.10.

[15] Aparicio-Ruiz R, Gandul-Rojas B, Roca M. Pigmentprofileinnon-Spanisholivevarieties (OleaeuropaeaL. Var. Coratina, Frantoio, andKoroneiki). J. Agric. Food Chem. 2009;57:10831–10836. doi:10.1021/jf9027393.

[16] Roca M, León L, de la Rosa R. Pigment metabolism of 'Sikitita' olive (Olea europaea L.): A new cultivar obtained by cross-breeding. J. Agric. Food Chem. 2011;59:2049–2055. doi:10.1021/jf104374t

[17] Goodwin TW, editor. Chemistry and Biochemistry of Plant Pigments. Vol. 2. London: Academic Press; 1976, 373 p.

[18] Roca M. Mínguez-Mosquera MI. Changes in chloroplast pigments of olive varieties during fruit ripening. J. Agric. Food Chem. 2001;49:832–839. doi:10.1021/jf001000l.

[19] Roca M, Mínguez-Mosquera MI. Change in the natural ratio between chlorophylls and carotenoids in olive fruit during processing for virgin olive oil. J. Am. Oil Chem. Soc. 2001;78:133–138. doi:10.1007/s11746-001-0233-z.

[20] Roca M, Mínguez-Mosquera MI. Carotenoid levels during the period of growth and ripening in fruits of different olive varieties (Hojiblanca, Picual and Arbequina). J. Plant Physiol. 2003;160:451–459. doi:10.1078/0176-1617-00759.

[21] Shemer TA, Harpaz-Saad S, Belausov E, Lovat N, Krokhin O, Spicer V, Standing KG, Goldschmidt EE, Eyal Y. Citruschlorophyllase dynamics at ethylene-induced fruit color-break: A study of chlorophyllase expression, posttranslational processing

kinetics, and in situ intracellular localization. Plant Physiol. 2008;148:108–118. doi:http://dx.doi.org/10.1104/pp.108.124933.

[22] Kräutler B, Hörtensteiner S. Chlorophyll breakdown: chemistry, biochemistry and biology. In: Ferreira GC, Kadish KM, Smith KM, Guilard R, editors. Handbook of Porphyrin Science. Vol. 28. USA: World Scientific Publishing; 2013. p. 117–185.

[23] Vergara-Domínguez H, Roca M, Gandul-Rojas B. Thylakoid peroxidase activity responsible for oxidized chlorophyll accumulation during ripening of olive fruits (Olea europaea L.). Food Res. Int. 2014;65:247–254. doi:10.1016/j.foodres.2014.04.030.

[24] Gandul-Rojas B, Roca-L. Cepero M, Mínguez-Mosquera MI. Chlorophyll and carotenoid patterns in olive fruits, Olea europaea Cv. 'Arbequina'. J. Agric. Food Chem. 1999;47:2207–2212. doi:10.1021/jf981158u.

[25] Bramley PM. Regulation of carotenoid formation during tomato fruit ripening and development. J. Exp. Botany. 2002;53:2107–2113. doi:10.1093/jxb/erf059.

[26] Rodriguez-Uribe L, Guzman I, Rajapakse W, Richins RD, O'Connell MA. Carotenoid accumulation in orange-pigmented Capsicum annuum fruit, regulated at multiple levels. J. Exp. Botany. 2011;63:517–526. doi:10.1093/jxb/err302.

[27] Price CA, Hadjeb N, Newman LA, Reardon EM. Chromoplast. Methods Cell Biol. 1995;50:189–207. doi:10.1016/S0091-679X(08)61031-6.

[28] Gandul-Rojas B, Roca-L. Cepero M, Mínguez-Mosquera MI. Use of chlorophyll and carotenoid pigment composition to determine authenticity of virgin olive oil. J. Am. Oil Chem. Soc. 2000;77:853–858. doi:10.1007/s11746-000-0136-z.

[29] Rejano L, Montaño A, Casado FJ, Sánchez AH, de Castro A. Table olives: Varieties and variations. In: Preedy VR, Watson RR, editors. Olives and Olive Oil in Health and Disease Prevention. Oxford: Academic Press; 2010. p. 5–15.

[30] International Olive Council. Trade Standard Applying to Table Olives. COI/OT/NC no. 1 [Internet]. 2004. Available from: http://www.internationaloliveoil.org/estaticos/view/222-standards [accessed: 2016-03-16].

[31] Vázquez-Roncero A, Maestro-Durán R. Los colorantes antociánicos de la aceituna madura. I. Estudio cualitativo. Grasas y Aceites. 1970; 21:208–214.

[32] Gallardo-Guerrero L, Gandul-Rojas B, Moreno-Baquero JM, López-López A, Bautista-Gallego J, Garrido-Fernández A. Pigment, physicochemical, and microbiological changes related to the freshness of cracked table olives. J. Agric. Food Chem. 2013;61:3737–3747. doi:10.1021/jf400240e.

[33] Ramírez E, Gandul-Rojas B, Romero C, Brenes M, Gallardo-Guerrero L. Composition of pigments and colour changes in green table olives related to processing type. Food Chem. 2015;166:115–124. doi:10.1016/j.foodchem.2014.05.154.

[34] Garrido-Fernández A, Fernández-Díez MJ, Adams MR. Table olives: Production and processing. London: Chapman and Hall; 1997. 496 p.

[35] Mínguez-Mosquera MI, Garrido-Fernández J, Gandul-Rojas B. Pigment changes in olives during fermentation and brine storage. J. Agric. Food Chem. 1989;37:8–11. doi: 10.1021/jf00085a002.

[36] Mínguez-Mosquera MI, Garrido-Fernández J, Gandul-Rojas B. Quantification of pigments in fermented Manzanilla and Hojiblanca olives. J. Agric. Food Chem. 1990;38:1662–1666. doi:10.1021/jf00098a008.

[37] Mínguez-Mosquera MI, Gandul-Rojas B, Mínguez-Mosquera J. Mechanism and kinetics of the degradation of chlorophylls during the processing of green table olives. J. Agric. Food Chem. 1994;42:1089–1095. doi:10.1021/jf00041a008.

[38] Schiedt H, Liaaen-Jensen S. Isolation and analysis. In: Britton G, Liaaen-Jensen S, Pfander H. Carotenoids. Vol 1A: Isolation and Analysis. Basel: BirkhäuserVerlag: 1995. p. 81–108.

[39] Mínguez-Mosquera MI, Gandul-Rojas B. Mechanism and kinetics of carotenoid degradation during the processing of green table olives. J. Agric. Food Chem. 1994;42:1551–1554. doi:10.1021/jf00043a030.

[40] Mínguez-Mosquera MI, Gallardo-Guerrero L. Anomalous transformation of chloroplastic pigments in Gordal variety olives during processing for table olives. J. Food Prot. 1995;58:1241–1248.

[41] Gandul-Rojas B, Gallardo-Guerrero L. Pigment changes during processing of green table olive specialities treated with alkali and without fermentation. Food Res. Int. 2014;65:224–230. doi: 10.1016/j.foodres.2014.05.007.

[42] Official Journal of the European Communities. European Parliament and Council Directive 94/36/CE, L237 [Internet]. 1994. Available from: http://ec.europa.eu/food/fs/sfp/addit_flavor/flav08_en.pdf [accessed: 2016-03-16].

[43] US Food and Drug Administration. Color Additives Approved for Use in Human Food [Internet]. 2006. Available from: http://www.fda.gov/ForIndustry/ColorAdditives/ColorAdditiveInventories/ucm115641.htm#table1A [accessed: 2016-03-17].

[44] Gandul-Rojas B, Roca M, Gallardo-Guerrero L. Detection of the color adulteration of green table olives with copper chlorophyllin complexes (E-141ii colorant). LWT Food Sci. Technol. 2012;46:311–318. doi:10.1016/j.lwt.2011.09.012.

[45] Aparicio-Ruiz R, Riedl KM, Schwartz SJ. Identification and quantification of metallo–chlorophyll complexes in bright green table olives by high-performance liquid chromatography–mass spectrometry quadrupole/time-of-flight. J. Agric. Food Chem. 2011;59:11100–11108. doi:10.1021/jf201643s.

[46] LaBorde LF, von Elbe JH. Method for improving the color of containerized green vegetables. U. S. patent 5,482,727, 1996.

[47] Gandul-Rojas B, Gallardo-Guerrero L. Pigment changes during preservation of green table olive specialities treated with alkali and without fermentation: effect of thermal treatments and storage conditions. In: Socaciu C, Pintea A, editors. Abstract of the 8th International Congress on Pigments in Food (PIF 2016); 28 June-1 July 2016. Cluj-Napoca, Romania; 2016.

[48] Official Journal of the European Union. Commission Implementing Regulation (EU) No. 1170/2009, L314 [Internet]. 2009. Available from: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R1170&from=EN [accessed: 2016-05-09].

[49] Codex Alimentarius Commission (FAO/WHO). Codex standard for table olives (Codex Stan 66-1981). 1981. Revision 1987, 2013. Available from: http://www.fao.org/input/download/standards/243/CXS_066e.pdf [accessed 2016-05-09].

[50] Mínguez-Mosquera MI, Gallardo-Guerrero L, Hornero-Méndez D, Garrido-Fernández J. Involvement of copper and zinc ions in green staining of table olives of the variety Gordal. J. Food Prot. 1995;58:564–569.

[51] Gandul-Rojas B, Gallardo-Guerrero L, Mínguez-Mosquera MI. Identification of oxidized chlorophylls and metallochlorophyllic complexes of copper in table olives (cv. Gordal) with green staining alteration. J. Food Prot. 1999;62:1172–1177.

[52] Gallardo-Guerrero L, Gandul-Rojas B, Mínguez-Mosquera MI. Chlorophyll pigment composition:1172–1177. in table olives (cv. Gordal) with green staining alteration. J. Food Prot. 1999;62:1167–1171.

[53] Gallardo-Guerrero L, Hornero-Méndez D, Mínguez-Mosquera MI. Pectins as possible source of the copper involved in the green staining alteration of cv. Gordal table olives. J. Agric. Food Chem. 2002;50:6746–6751. doi:10.1021/jf025682j.

[54] Gallardo-Guerrero L, Milicua JCG, Salvador AM, Jarén-Galán M, Mínguez-Mosquera MI. Pigment-lipoprotein complexes in table olives (cv. Gordal) with green staining alteration. J. Agric. Food Chem. 2003;51:1724–1727. doi:10.1021/jf025965b.

[55] International Olive Council. Trade Standard Applying to Olive Oils and Olive-Pomace Oils. COI/T.15/NC No 3/Rev. 10 [Internet] 2015. Available from: http://www.internationaloliveoil.org/estaticos/view/222-standards [accessed: 2016-03-16].

[56] Gómez-Herrera C, Matter transfer during virgin olive oil elaboration. Grasas y Aceites. 2007;58(2):194–205. doi:10.3989/gya.2007.v58.i2.85.

[57] Fregapane G, Salvador MD. Production of superior quality extra virgin olive oil modulating the content and profile of its minor components. Food Res. Int. 2013;54:1907–1914. doi:10.1016/j.foodres.2013.04.022.

[58] Clodoveo ML. Malaxation: Influence on virgin olive oil quality. Past, present and future-An overview. Trends in Food Sci. Tech. 2012;25:13–23. doi:10.1016/j.tifs.2011.11.004.

[59] Gandul-Rojas B, Mínguez-Mosquera MI. Chlorophyll and carotenoid composition in virgin olive oils from various Spanish olive varieties. J. Sci. Food Agric. 1996;72:31–39. doi:10.1002/(SICI)1097-0010(199609)72:1<31::AID-JSFA619>3.0.CO;2-5.

[60] Psomiadou E, Tsimidou M. Pigments in Greek virgin olive oils: Occurrence and levels. J. Sci. Food Agric. 2001;81:640–647. doi:10.1002/jsfa.859.

[61] Giuffrida D, Salvo F, Salvo A, La Pera L, Dugo G. Pigments composition in monovarietal virgin olive oils from various sicilian olive varieties. Food Chem. 2007;101:833–837. doi: 10.1016/j.foodchem.2005.12.030.

[62] Oueslati I, Anniva C, Daoud D, Tsimidou M, Zarrouk M. Virgin olive oil (VOO) production in Tunisia: The commercial potential of the major olive varieties from the arid Tataouine zone. Food Chem. 2009;112:733–741. doi:10.1016/j.foodchem. 2008.06.041.

[63] Giuffrida D, Salvo F, Salvo A, Cossignani L, Dugo G. Pigments profile in monovarietal virgin olive oils from various Italian olive varieties. Food Chem. 2011;124:1119–1123. doi:10.1016/j.foodchem.2010.07.012.

[64] Mínguez-Mosquera MI, Gandul-Rojas B, Garrido Fernández J, Gallardo-Guerrero L. Pigments present in virgin olive oil. J. Am. Oil Chem. Soc. 1990;67:192–196. doi:10.1007/ BF02539624.

[65] Gallardo-Guerrero L, Roca M, Mínguez-Mosquera MI. Distribution of chlorophylls and carotenoids in ripening olives and between oil and alperujo when processed using a two-phase extraction system. J. Am. Oil Chem. Soc. 2002;79:105–109. doi:10.1007/ s11746-002-0442-5.

[66] Criado MN, Romero MP, Casanovas M, Motilva MJ. Pigment profile and colour of monovarietal virgin olive oils from Arbequina cultivar obtained during two consecutive crop seasons. Food Chem. 2008;110:873–880. doi:10.1016/j.foodchem.2008.02.075.

[67] Criado MN, Romero MP, Motilva MJ. Effect of the technological and agronomical factors on pigment transfer during olive oil extraction. J. Agric. Food Chem. 2007;55:5681–5688. doi:10.1021/jf070303d.

[68] Morelló JR, Motilva MJ, Ramo T. Romero MP. Effect of freeze injuries in olive fruit on virgin olive oil composition. Food Chem. 2003;81:547–553. doi:10.1016/ S0308-8146(02)00488-0.

[69] Ranalli A, Malfatti A, Lucera L, Contento S, Sotiriou E. Effects of processing techniques on the natural colourings and the other functional constituents in virgin olive oil. Food Res. Int. 2005;38:873–878. doi:10.1016/j.foodres.2005.02.011.

[70] Salvador MD, Aranda F, Gómez-Alonso S, Fregapane G. Influence of extraction system, production year and area on Cornicabra virgin olive oil: A study of five crop seasons. Food Chem. 2003;80:359–366. doi:10.1016/S0308-8146(02)00273-X.

[71] Altieri G, Genovese F, Tauriello A, Di Renzo GC. Innovative plant for the separation of high quality virgin olive oil (VOO) at industrial scale. J. Food Eng. 2015;166:325–334. doi:10.1016/j.jfoodeng.2015.06.033.

[72] Gandul-Rojas B, Mínguez-Mosquera MI. Chlorophyllase activity in olive fruits and its relationship with the loss of chlrorophyll pigments in the fruits and oils. J. Sci. Food Agric. 1996;72:291–294. doi:10.1002/(SICI)1097-0010(199611)72:3<291::AID-JSFA654>3.0.CO;2-O.

[73] García JM, Yousfi K, Mateos R, Olmo M, Cert A. Reduction of oil bitterness by heating of olive (Oleaeuropaea) fruits. J. Agric. Food Chem. 2001;49:4231–4235. doi:10.1021/jf001302n.

[74] García JM, Yousfi K, Oliva JS, García-Diaz MT, Pérez-Camino MC. Hot water dipping of olives (Olea europaea) for virgin oil debittering. J. Agric. Food Chem. 2005;53:8248–8252. doi:10.1021/jf050616d.

[75] Luaces P, Pérez AG, García JM, Sanz C. Effects of heat-treatments of olive fruit on pigment composition of virgin olive oil. Food Chem. 2005;90:169–174. doi:10.1016/j.foodchem.2004.03.035.

[76] Yousfi K, Moyano MJ, Martínez F, Cayuela JA, García JM. Postharvest heat treatment for olive oil debittering at the industrial scale. J. Am. Oil Chem. Soc. 2010;87:1053–1061. doi:10.1007/s11746-010-1588-8.

[77] Ranalli A, Benzi M, Gomes T, Delcuratolo D, Marchegiani D, Lucera L. Concentration of natural pigments and other bioactive components in pulp oils from de-stoned olives. Inn. Food Sci. Em. Tech. 2007;8:437–442. doi:10.1016/j.ifset.2007.03.020.

[78] Ranalli A, Marchegiani D, Pardi D, Contento S, Pardi D, Girardi F, Kotti F. Evaluation of functional phytochemicals in destoned virgin olive oil. Food Bioprocess Tech. 2009;2:322–327. doi:10.1007/s11947-008-0128-0.

[79] Ranalli A, Contento S, Schiavone C, Simone N. Malaxing temperature affects volatile and phenol composition as well as other analytical features of virgin olive oil. Eur. J. Lip. Sci. Tech. 2001;103:228–238. doi:10.1002/1438-9312(200104)103:4<228::AID-EJLT228>3.0.CO;2-7.

[80] Ranalli A, Pollastri L, Contento S, Iannucci E, Lucera L. Effect of olive paste kneading process time on the overall quality of virgin olive oil. Eur. J. Lip. Sci. Tech. 2003;105:57–67. doi:10.1002/ejlt.200390018.

[81] Stefanoudaki E, Koutsaftakis A, Harwood JL. Influence of malaxation conditions on characteristic qualities of olive oil. Food Chem. 2011;127:1481–1486. doi:10.1016/j.foodchem.2011.01.120.

[82] Pérez AG, Romero C, YousfiK, García JM. Modulation of olive oil quality using NaCl as extraction coadjuvant. J. Am. Oil Chem. Soc. 2008;85:685–691. doi:10.1007/s11746-008-1252-8.

[83] Spain. Real Decreto 640/2015, of July 10. List of approved processing aids for the production of edible vegetable oils and their identity and purity criteria. BOE, 28 July 2015, no. 179, Sec.1, p. 64243-64249. [Internet]. 2015. Available from: https://www.boe.es/boe/dias/2015/07/28/pdfs/BOE-A-2015-8443.pdf [accessed: 2016-03-16].

[84] Official Journal of the European Union. Commission Implementing Regulation (EU) No. 1335/2013, L335 [Internet]. 2013. Available from: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013R1335&rid=1 [accessed: 2016-03-16].

[85] Aparicio-Ruiz R, Mínguez-Mosquera MI, Gandul-Rojas B. Thermal degradation kinetics of chlorophyll pigments in virgin olive oils. 1. Compounds of series "a". J. Agric. Food Chem. 2010;58:6200–6208. doi:10.1021/jf9043937.

[86] Aparicio-Ruiz R, Minguez-Mosquera MI, Gandul-Rojas B. Thermal degradation kinetics of lutein, β-carotene and β-cryptoxanthin pigments in virgin olive oils. J. Food Comp. Anal. 2011;24:811–820. doi:10.1016/j.jfca.2011.04.009.

[87] Aparicio-Ruiz R, Gandul-Rojas B. Thermal degradation kinetics of neoxanthin, violaxanthin, and antheraxanthin in virgin olive oils. J. Agric. Food Chem. 2012;60:5180–5195. doi:10.1021/jf300332m.

[88] Hynninen PH. Chemistry of chlorophyll modifications. In: Scheer H, editor. Chlorophylls. Boca Raton, FL: CRC Press; 1991. p. 145–210.

[89] Anniva C, Grigoriadou D,Psomiadou E, Tsimidou M Z. Pheophytin adegradation products as useful indices in the quality control of virgin olive oil. J. Am. Oil Chem. Soc. 2006;83: 371–375. doi:10.1007/s11746-006-1215-x.

[90] Psomiadou E, Tsimidou M. Stability of virgin olive oil. 2. Photo-oxidation studies. J. Agric. Food Chem. 2002;50:722–727. doi:10.1021/jf010847u.

[91] Aparicio-Ruiz R, Gandul-Rojas B. Decolouration kinetics of chlorophylls and carotenoids in virgin olive oil by autoxidation. Food Res. Int. 2014;65:199–206. doi:10.1016/j.foodres.2014.05.046.

[92] Psomiadou E, Tsimidou M. Stability of virgin olive oil. 1. Autoxidation studies. J. Agric. Food Chem. 2002;50: 716–721. doi:10.1021/jf0108462.

[93] Velasco J, Dobarganes C. Oxidative stability of virgin olive oil. Eur. J. Lipid Sci. Technol. 2002;104:661–676. doi:10.1002/1438-9312(200210)104:9/10<661::AID-EJLT661>3.0.CO;2-D.

[94] Frankel EN. Photooxidation of unsaturated fats. In: Frankel EN, editor. Lipid Oxidation. 2nd ed. Cambridge: Woodhead Publishing Limited. 2005. p. 51–64.

[95] Kiritsakis A, Dugan LR. Studies in photooxidation of olive oil. J. Am. Oil Chem. Soc. 1985;62:892–896. doi:10.1007/BF02541753.

[96] Gutiérrez-Rosales F, Garrido-Fernández J, Gallardo-Guerrero L, Gandul-Rojas B, Mínguez-Mosquera MI. Action of chlorophylls on the stability of virgin olive oil. J. Am. Oil Chem. Soc. 1992;69:866–871. doi:10.1007/BF02636334.

[97] Rahmani M, Csallany AS. Role of minor constituents in the photo-oxidation of virgin olive oil. J. Am. Oil Chem. Soc.1998;75:837–843. doi:10.1007/s11746-998-0234-1.

[98] Khan MA, Shahidi F. Rapid oxidation of commercial extra virgin olive oil stored under fluorescent light. J. Food Lipids. 1999;6:331–339. doi:10.1111/j.1745-4522.1999.tb00154.x.

[99] Roca M, Gandul-Rojas B, Gallardo-Guerrero L, Mínguez-Mosquera MI. Pigment parameters determining Spanish virgin olive oil authenticity: stability during storage. J. Am. Oil Chem. Soc. 2003;80:1237–1240. doi:10.1007/s11746-003-0848-0.

[100] Gallardo-Guerrero L, Gandul-Rojas B, Roca M,Mínguez-Mosquera MI. Effect of storage on the original pigment profile of Spanish virgin olive oil. J. Am. Oil Chem. Soc. 2005;82:33–39. doi:10.1007/s11746-005-1039-8

[101] Roca M, Gallardo-Guerrero L, Mínguez-Mosquera MI, Gandul-Rojas B. Control of olive oil adulteration with copper-chlorophyll derivatives. J. Agric. Food Chem.2010;58:51–56. doi:10.1021/jf902084d.

[102] Aparicio-Ruiz R, Roca M, Gandul-Rojas B. Mathematical model to predict formation of pyropheophytin a in virgin olive oil during the storage. J. Agric. Food Chem. 2012;60:7040–7049. doi:10.1021/jf3010965.

[103] Gandul-Rojas B, Roca-López-Cepero C, Carmona-Ramón C, Mínguez-Mosquera MI. Stability of chlorophyll pigments during storage of virgin olive oil. In: Mínguez-Mosquera MI, Jarén-Galán M, Hornero-Méndez D, editors. Proceeding of the 1st International Congress on Pigments in Food Technology (PIF'99); 24–26 March 1999. Sevilla, Spain; 1999. p. 271–275.

[104] Schwartz SJ, Woo SL, von Elbe JH. High performance liquid chromatography of chlorophylls and their derivatives in fresh and processed spinach. J. Agric. Food Chem. 1981;29:533–535. doi:10.1021/jf00105a025.

[105] Gandul-Rojas B, Roca-López-Cepero C, Mínguez-Mosquera MI. Chlorophyll and carotenoid pattern in virgin olive oil. Adulteration control. In: Mínguez-Mosquera MI, Jarén-Galán M, Hornero-Méndez D, editors. Proceeding of the 1th International Congress on Pigments in Food Technology (PIF'99); 24–26 March 1999. Sevilla, Spain; 1999. p. 381–386.

[106] Serani A, Piacenti D. Identification of deodorized oils in virgin olive oils. I. Analysis of chlorophyllian pigments in virgin olive oils. Riv. Ital. Sostaze Grasse 2001;78:459-463.

[107] Gandul-Rojas B, Hornero-Méndez D, Roca M, Aparicio-Ruiz R, Mínguez Mosquera MI. Determination of pyropheophytin to pheophytin ratio as quality index in extra virgin

olive oils. In Abstract of the 26th World Congress and Exibition of the ISF. Modern Aspect of Fats and Oils. 25–28 September 2005. Praga (Rep. Checa), 2005. p. 99. Available from: http://digital.csic.es/browse?type=author&sort_by=1&order=ASC&rpp=20&etal=10&value=Gandul-Rojas%2C+Beatriz&offset=20.

[108] Gertz C, Fiebig HJ. Pyropheophytin a –determination of thermal degradation products of chlorophyll a in virgin olive oil. Eur. J. Lipid Sci. Technol. 2006;108:1062−1065. doi: 10.1002/ejlt.200600164.

[109] Official Journal of the European Union. Regulation (EU) No. 1169/2011 of the European Parliament and of the Council, L304, [Internet]. 2011. Available from: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R1169&rid=1 [accessed: 2016-03-16].

[110] Ayton J, Mailer RJ, Graham K. The effect of storage conditions on extra virgin olive oil quality. Australian Government, Rural Industries Research and Development Corporation. ISBN 978-1-74254-379-6. [Internet]. 2012. Available from: http://cdn.oliveoil-times.com/library/Olive-Oil-Storage-Conditions.pdf [accessed: 2016-04-18].

[111] Guillaume C, Gertz Ch, Ravetti L. Pyropheophytin a and 1,2 di-acyl-glycerols over time under different storage conditions in natural olive oils. J. Am. Oil. Chem Soc. 2014;91:697–709. doi:10.1007/s11746-014-2415-4.

[112] Australian Standards for Olive oil and olive-pomace oils. AS 5264–2011. Available from: http://www.aph.gov.au/DocumentStore.ashx?id=ca0c9dd8-b5ee-45fa-b7e3-3078219d7be9 [accessed: 2016-04-18].

[113] Flynn D, Li X, Wang S. Correlating olive oil sensory and chemistry results. Report of the UC Davis Olive Center, [Internet]. 2014. Available from: http://olivecenter.ucdavis.edu/research/files/correlating-olive-oil-sensory-and-chemistry-results [accessed: 2016-04-18].

[114] German Society for Fat Science (DGF). Statement on the Applicability of Methods for the Determination of Pyropheophytin a and Isomeric Diacylglycerols in Virgin Olive Oils. [Internet].2010. Available from: http://www.dgfett.de/meetings/archiv/hageno-live/Conclusions/Statement_Methods.pdf [accessed: 2016-04-18].

Chapter 6

Pigments in Extra-Virgin Olive Oil: Authenticity and Quality

Cristina Lazzerini, Mario Cifelli and
Valentina Domenici

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64736

Abstract

Pigments, divided into carotenoids and chlorophyll derivatives, are responsible for the colour of extra-virgin olive oil (EVOO). The concentration of pigments in EVOO depends on several factors, such as the maturity of olives before oil production, the cultivar and the geographic origin of olives. Pigments naturally degrade in olive oil (OO) during storage, and they may decompose due to light, temperature and oxygen exposure. The nature and concentration of pigments in EVOOs are different from seed oils, and this is a base of their use to reveal oil treatments and sophistication. In this chapter, the analytical methods, mainly chromatographic and spectroscopic, applied to identify and quantify pigments are overviewed. In particular, the applications of these methods to check the authenticity and the quality of extra-virgin olive oil are discussed.

Keywords: carotenoids, chlorophylls, quantitative methods, adulterations, EVOOs

1. Introduction

The authenticity of edible olive oils is a subject of intense research all over the world. The aim is to guarantee the protection of consumers and to avoid frauds damaging not only the consumers but also the producers. Olive oil (OO), and in particular extra-virgin olive oil (EVOO), is one of the most susceptible to fraud foods. There are many different frauds which can be roughly divided into [1]:

- *Adulteration*: at least a component is substituted with one of a lower value;

- *Tampering*: legitimate products are used in a fraudulent way;
- *Overrun*: a legitimate product is produced with a certain amount in excess with respect to product agreement;
- *Diversion*: the sale or distribution of legitimate products out of the intended market;
- *Simulation*: illegitimate products are designed to look like but not exactly copy the original product;
- *Counterfeit*: all aspects of the fraudulent product are fully replicated.

All the above frauds are usually intentional, and the main motivation is economical.

Most of the known frauds in the field of EVOOs concern their saponifiable fraction, which represent the 98–99% of olive oil. It is composed of saturated and unsaturated fatty acids, esterified almost entirely to glycerol to form triacylglycerols. Diacylglycerols, monoacylglycerols and free fatty acids are also components of the saponifiable fraction. The unsaponifiable fraction is composed of a very large number of minor compounds, very important for the flavour and the nutritional properties of EVOOs [2]. Minor components of olive oil, which include phenols, aliphatic and other alcohols, hydrocarbons, tocopherols, sterols and those responsible of the colour [3], namely the pigments, can be the object of alterations and frauds, too. An example is the addition of an artificial pigment, called E141, which is similar to chlorophyll, where the inner metal ion, Mg^{++}, is substituted with the more stable Cu^{++}. This fraud can be unmasked by a simple spectroscopic analysis of the sophisticated olive oil [4]. Since the amount of pigments is a distinct feature of EVOOs, with respect to other oils, the identification and quantification of pigments have become the subject of intense research, and several methodologies are now available [5–15].

2. Carotenoids and chlorophyll derivatives

The unique colour of olive oil related to its pigment content [16] varies from a light gold to a rich green. Green olives produce a green oil because of the high chlorophyll content, while ripe olives yield a yellow oil because of the carotenoids (yellow red). The exact combination and proportions of pigments determine the final colour of the oil. Their presence in olive oil depends on olive fruits (*Olea europa, L.*), but also on genetic factors (olive cultivar), the stage of fruit ripeness, environmental conditions, the extraction processing [8, 17–19] and storage conditions. The role of chlorophylls as natural pigments accounting for greenish colours and in photosynthesis is well known. There are also some reports about the benefits of dietary chlorophylls for human health [20].

The structure of chlorophyll pigments, consisting of one tetrapyrrole macrocycle, coordinated to a Mg^{++} ion to form a planar complex, is responsible for the absorption in the visible region of the spectrum of olive oils. Here, both the bluish-green chlorophyll-a and the yellowish-green chlorophyll-b can be found. Chlorophylls in olive oils are mostly converted to pheophytins, due to the exchange of the central Mg^{++} ion with acid protons. Pheophytin-a (see **Figure 1a**) is

http://dx.doi.org/10.5772/64736

predominant with respect to pheophytin-b. In the case of bad storage conditions, pheophytins are further degraded to pyropheophytins [21, 22]. The main carotenoids present in olive oils are lutein and β-carotene (see **Figures 1b**, **c**).

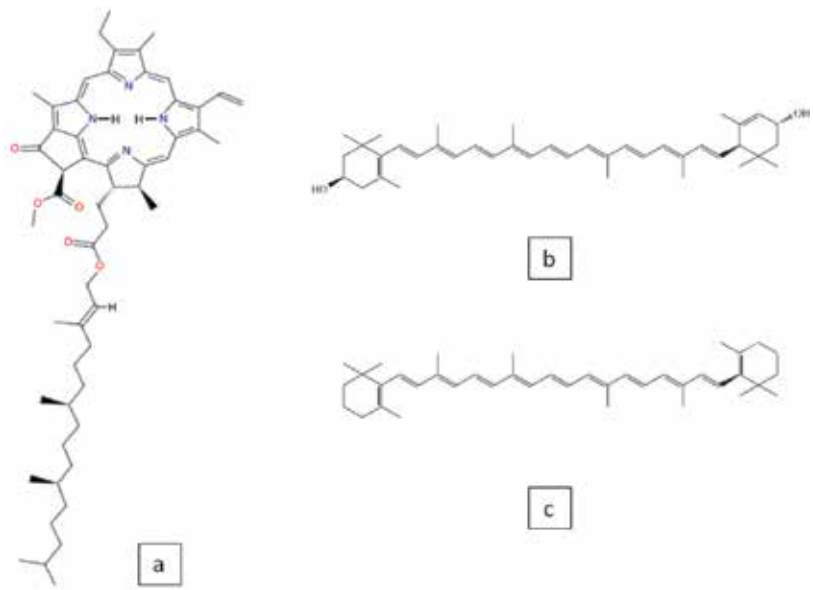


Figure 1. Molecular structure of (a) pheophytin-a, (b) lutein and (c) β-carotene.

The level of pigments ranges from a few ppm to almost 100 ppm. Fresh olive oils usually have a higher pigments content. The major components are pheophytin-a (from few ppm up to 25 ppm), followed by β-carotene (from few ppm up to 15 ppm) and lutein (from few ppm up to 10 ppm).

Carotenoids are isoprenoid compounds having a hydrocarbon structure with carbon double bonds that are responsible for many of their properties [23, 24]. Carotenoids can be divided into carotenes (carotenoids containing only carbon and hydrogen) and xanthophylls (carotenoids that also contain oxygenated functions, such as epoxide, hydroxyl, acetate, carbonyl and carboxylic groups, among others). In EVOOs, the main carotenoids are β-carotene and lutein [25]. The carotenoid fraction of olive oil also includes other xantophylls [15, 16].

See also Chapter entitled "Chlorophylls and Carotenoids in Food Products from Olive Tree" by Beatriz Gandul-Rojas, María Roca and Lourdes Gallardo-Guerrero.

3. Methods to identify and quantify pigments in olive oils

In the literature, several works have been published about analytical methods able to identify and quantify pigments in oil matrices. These methods can be divided into two main categories

based on the physical principle: (1) chromatographic techniques (characterized by pretreatment of the samples, such as extraction and/or saponification) and (2) spectroscopic techniques (without pretreatment of the samples). In the following, these two main classes of methods are overviewed.

3.1. Chromatographic techniques

The most successful chromatographic technique for pigments quantification is the *high-performance liquid chromatography,* HPLC, coupled with mass spectroscopy or UV-vis absorp-

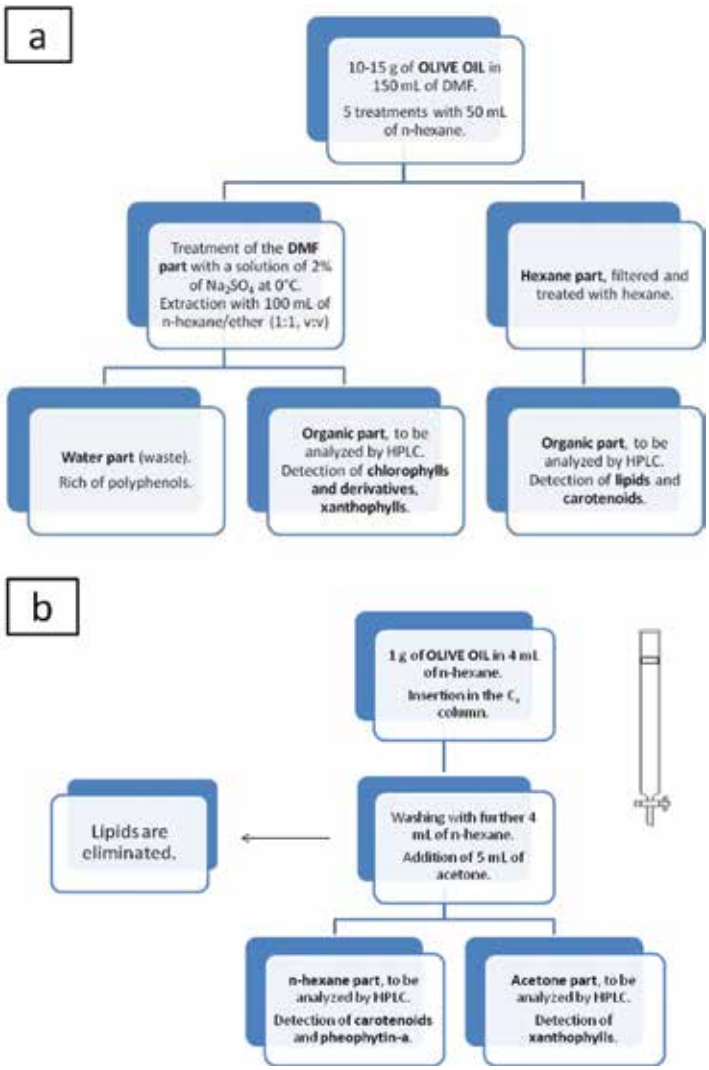


Figure 2. Basic treatment schemes to analyse the pigments' content in EVOOs according to (a) the (LPD) [26] and (b) the SPE [27] chromatographic methods.

http://dx.doi.org/10.5772/64736

tion through the diode array detection (DAD). These methods consist of three steps: (a) extraction/purification of the pigments from olive oil sample; (b) chromatographic separation of pigments; and (c) fraction analysis. The extraction/purification step can be done by *liquid phase distribution* (LPD) [26] or by *solid phase extraction* (SPE) [27]. The LPD method includes the separation of pigments between two phases, one in hexane and the other one in N,N-dimethyl formaldehyde (DMF), as shown in **Figure 2a**. On the contrary, the SPE method is based on the use of C_{18} columns, which are previously conditioned with a mixture of methanol and hexane. Details of the procedure are reported in **Figure 2b**. The comparison between the two procedures shows that the recoveries of lutein, β-carotene and pheophytin-a are much better in the SPE than LPD method [28]. The chromatographic separation of pigments is highly dependent on the column: direct columns are normally very sensitive to the eventual presence of water and temperature gradients, and they require longer retention times [29]. Inverse column gives better results. The use of C_{18} inverse column allows optimal separation of chlorophylls and their derivatives, as well as separation of short chain and low molecular weight carotenoids [30].

The recent introduction of C_{30} inverse columns allowed a good separation for long chain carotenoids and their isomers with lower polarity, such as lycopene and β-carotene [15, 31]. A disadvantage of C_{30}, with respect to C_{18}, is the longer retention times. Normally, the better choice of the column depends on the starting matrix [32-34].

Solvent mixtures to analyse an oil matrix have been optimized [33], and they consist of eluent A, based on water/reagent ion pair/methanol (1:1:8, v/v), and eluent B, based on acetone/methanol (1:1, v/v). The reagent *ion pair* is represented by a solution of tetrabutylammonium (0.05 M) and ammonium acetate (1 M).

	Mobile phase	
Time (min)	**A (%)**	**B (%)**
0	75	25
8	25	75
10	25	75
18	10	90
23	0	100
30	75	25

Table 1. Elution gradient for pigments separation commonly used in HPLC methods.

The gradient scheme used for pigments separation is reported in **Table 1**.

Mass spectroscopy (MS) has been used less frequently to analyse pigments in olive oils. This technique is very useful to understand the structural features and degradation of chlorophylls

due to oxidative effects. Recently, a new methodology based on HPLC coupled with high-resolution time-of-flight (hrTOF) mass spectrometry has been developed, and thanks to the computer-assisted analysis, the completion of MS fragmentation of chlorophylls and their derivatives has been reached [35]. No applications of this technique in terms of authentication and quality aspects of olive oils have been reported so far.

Pigments	k	Peak positions (nm)			Ratio between peaks	
		I	II	III	100 III/II	I/II
Neoxanthin	2.4	415	438	467	88	–
Neoxanthin	2.8	415	438	467	88	–
Violaxanthin	3.1	413	434	464	93	–
Luteoxanthin	3.4	398	420	447	100	–
Auroxanthin	3.6	379	400	425	94	–
Antheraxanthin	4.1	421	445	470	45	–
Mutatoxanthin	4.4	414	438	464	60	–
Auroxanthin isomer	4.7	379	400	425	92	–
Chlorophyll-b	4.8	465	602	650	–	3.3
Lutein	5.1	423	444	472	63	–
b-Cryptoxanthin	5.8	431	450	477	28	–
cis-Lutein	6.3	416	438	466	38	–
Chlorophyll-a	6.7	430	620	666	–	1.1
Neoxanthin	7.3	415	438	467	88	–
Pyropheophytin-a	9.7	406	506	666	–	2.2
Pheophytin-a′	12.1	406	506	665	–	2.1
Pheophytin-a	12.4	406	506	665	–	2.1
Pheophytin-a isomer	12.7	406	506	665	–	2.1

This table is modified from **Table 2** in Ref. [33] (reproduced with permission from Wiley Company).

Table 2. Parameters commonly used for the HPLC-DAD detection of pigments in EVOOs. The retention factor, k, the peak positions of the UV-vis spectra for DAD detection and the ratios between relevant peaks are reported (see reference 33).

3.2. Spectroscopic techniques

Spectroscopic techniques, such as UV-vis absorption, nuclear magnetic resonance (NMR), Fourier transform infrared (FT-IR) spectroscopy and fluorescence, are used to investigate olive oil chemical composition and to differentiate oils according to cultivar, variety and storage conditions. The main feature of these techniques is the possibility to use them directly on olive oil samples, without any pretreatment. In most of the cases, these techniques are suitable for

http://dx.doi.org/10.5772/64736

investigating the saponifiable fraction of olive oil, as is the case of FT-IR [36], and NMR spectroscopy [37, 38].

The broadness of the electromagnetic spectrum allows to get information from different spectral regions; those ones mostly used to study food and relative frauds are three: (1) the ultraviolet (UV) region (200–400 nm), (2) the visible (Vis) region (400–779 nm) and (3) the near-infrared (NIR) region (780–2500 nm).

In particular, the UV region is characterized by high signal to noise ratio and high sensitivity, due to the presence of many signals from tocopherols, anthocyanins, phenols and diene/triene compounds [39].

As it is shown in **Figure 3**, the UV region is characterized by very intense signals in extra-virgin olive oils, due to the variety of minor components.

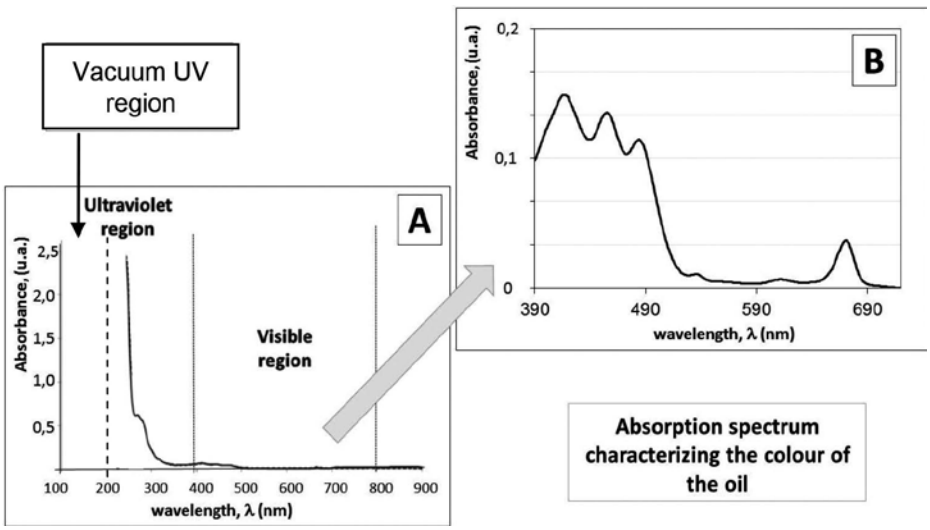


Figure 3. (A) Full experimental spectrum in the UV-vis region of an extra-virgin olive oil, showing the two separated regions: UV and Vis one. (B) Enlargement of the spectrum in the sole Vis region showing the typical shape of the Vis absorption spectrum of extra-virgin olive oils, due to the pigments content and responsible of the colour.

Concerning the NIR absorption region, most of the applications on olive oils concern the acidic part, allowing a differentiation among different botanic origin of seeds oils [40], but with this technique, pigments cannot be investigated.

A useful technique to study chlorophylls and carotenoids is fluorescence spectroscopy [41], which is a photoluminescence process. Fluorescence spectroscopy can be performed either by right-angle and front-face techniques, with several possibilities of investigation. Different types of signals can be indeed acquired: emission spectra, excitation spectra and synchronous spectra [42]. Several works have been published based on fluorescence spectroscopy applied to extra-virgin olive oils [43–49], since several chemical constituents, including pigments, give fluorescence in specific conditions and they can be identified. The quantification of fluoro-

phores is less straightforward than in absorption spectroscopies, as deeply addressed in Ref. [49]. In particular, this work shows that the right-angle method gives several artefacts, while front face technique is more appropriate for quantification of fluorophores. In emission spectra of olive oils, the band centred at 670–680 nm is clearly due to chlorophyll-like chromophores. In particular, the pheophytins' signals are expected around 670 nm, but the maximum of the emission band can be slightly shifted depending on the used technique (right-angle or front-face). Interestingly, a weak emission band, centred at about 666 nm in the spectrum of sunflower oil, reveals the presence of fluorescent chlorophyll derivatives. Such a band is undetectable in peanut oil [49]. Carotenoids, present in olive oil with a relatively high concentration, are characterized by a less intense emission. In fact, carotenoids strongly compete for incident light with other chromophores present in olive oil due to their concentration and high extinction coefficient. Carotenoids signals can be observed in the 430–480 nm region, but their quantification remains very difficult by fluorescence techniques.

The synchronous fluorescence technique has been developed to analyse large sets of olive oil samples with the aim of differentiate them on the basis of botanic origin. This technique consists in acquiring emission and excitation spectra simultaneously, by fixing a constant distance between the emission and excitation wavelengths [45–48]. This spectroscopic method is coupled with a multivariate statistical analysis, such as PLS, *partial least squares*, PCA, *principal components analysis*, or PARAFAC, *PARAllel FACtor analysis*. These methods have been proved to be satisfactory for discriminating oils of different botanic origin based on chlorophylls and their derivatives quantification, but not for carotenoids. Nevertheless, despite of good potentialities, no works are known about applications in the field of frauds and authentication of EVOOs.

3.2.1. Methods based on Vis absorption for pigments identification and quantification

As previously stated, Vis absorption spectra of extra-virgin olive oils have characteristic features [50, 51]: a three-peak band in the range 390–520 nm and a sharper band around 660–675 nm. This last absorption band is due to the electronic transition of chlorophylls and their derivatives, while the first band is more complex, since it is due to the overlap among carotenoids and chlorophylls absorption signals. As it can been seen in **Figure 4**, the visible absorption spectrum of extra-virgin olive oils is very different from the absorption spectra of other seeds and fruits oils. This evidence is at the basis of several works [5, 11, 52–54] devoted to the authentication of EVOOs and the identification of specific frauds, such as the mixing between olive oils and oils obtained from other seeds.

Visible (vis) light absorption of EVOOs is associated with the pigments' content, and this specificity is at the origin of several research works aiming to substitute the chromatographic methods with much faster and direct spectrophotometric methods [5, 11, 12, 39].

A recent methodology proposed by Cayuela et al. [12] associates the absorbance measured at specific wavelengths in the visible region, namely the K470 and K670 indexes, to the amount of carotenoids and chlorophyll derivatives, respectively. This method has the advantages to be fast, non-destructive and inexpensive. However, the single absorbance values at specific wavelengths in the Vis spectrum of EVOOs do not allow a reliable and

http://dx.doi.org/10.5772/64736

unambiguous quantification of the pigments' content, in particular for the superposition of carotenoids and pheophytins signals in the 390–520 nm region. Moreover, this method is able to quantify only the total amount of carotenoids and chlorophyll derivatives, and not the single compounds.

In order to get a more robust and accurate method for pigments quantification, fast and rapid at the same time, a mathematical approach based on the spectral deconvolution of the Vis absorption of EVOOs in terms of its main pigment components can be performed. A first mathematical approach was proposed by Ayuso et al. [50]: the experimental Vis spectrum is reproduced by a combination of two signals, from pure β-carotene and chlorophyll-a spectra. More recently, a new mathematical approach has been developed by using four orthogonal functions derived from the experimental spectra of two carotenoids (β-carotene and lutein) and two chlorophyll derivatives (pheophytin-a and pheophytin-b). This procedure, shown in **Figure 5**, consists of the following steps: (1) the acquisition of the experimental UV-vis spectrum of the sample; (2) the fitting of the experimental spectrum as a linear combination of the four orthogonal functions; and (3) the calculation of pigments' concentrations and relevant statistical parameters. Steps 2 and 3 are done automatically by a home-made program compatible with Excel software [5].

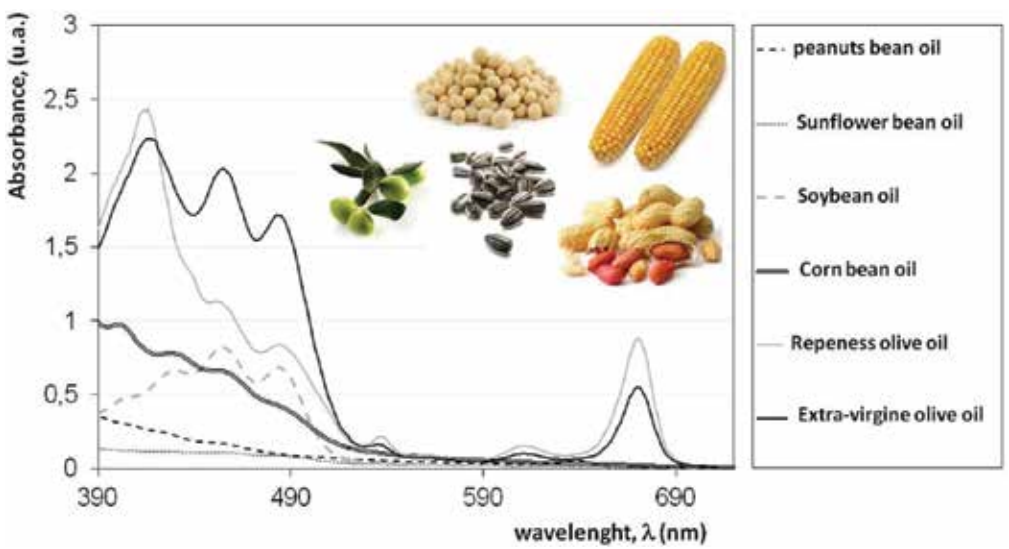


Figure 4. Visible absorption spectra of several vegetable oils obtained from olives, peanuts, sunflower beans, soya beans and corn beans.

This method has been applied to more than 150 EVOOs having different cultivars and with different geographic origin [5, 51, 52, 54], showing a very high quality of the fitting in all cases (R^2 ranges from 0.996 to 0.998) and good possibilities of discriminating EVOOs from not EVOOs.

Additional methods to analyse olive oils from UV-vis absorption spectra base on statistical multivariate approaches. In such cases, the discriminating factors are derived from the absorption spectra, but they are not directly related to the pigments concentration. An example is the method based on the calculation of chaotic parameters (Lyapunov exponent, autocorrelation coefficients and fractal dimensions), further treated to find correlations index [10, 11, 55] and the application of PCA on large sets of data, including UV-vis spectral ones, further treated with class-modelling methods [56].

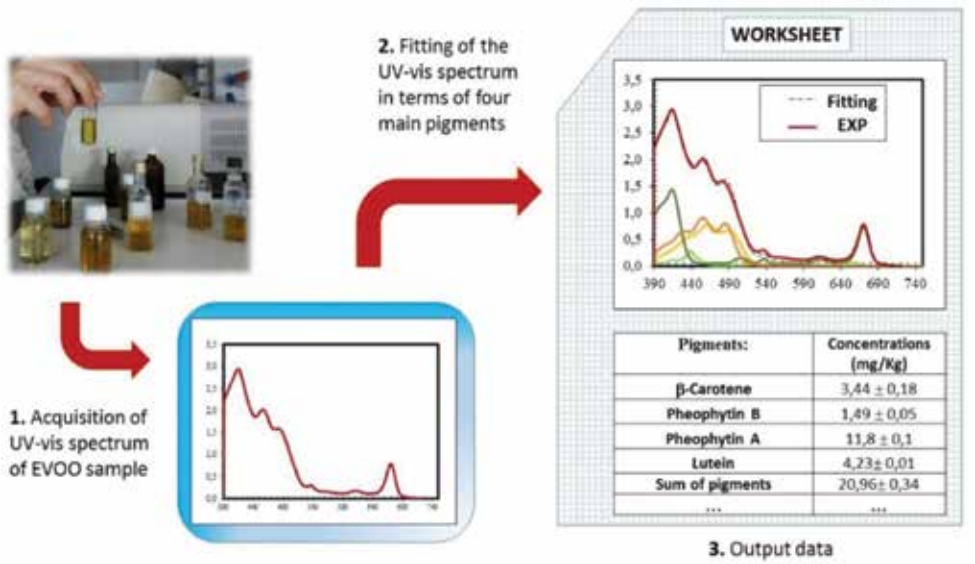


Figure 5. Scheme of the mathematical method develop [5] to obtain the concentration of four main pigments by analysing the Near UV-vis absorption spectrum of an EVOO.

4. Authenticity and quality studies based on pigments quantification

As seen in the previous sections, there are several analytical methods, both chromatographic and spectroscopic ones, able to identify and quantify pigments in EVOOs. Since the concentration of pigments strongly differs depending on several variables, their use was discouraged. It is well known that chlorophylls completely degrade into pheophytins after few months after the bottling and that the pheophytins themselves evolve into pyropheophytins depending on storage conditions. However, as reported in several works [5, 51–60], typical range of concentrations of various pigments can be associated with EVOOs and these quantities can be used to discriminate them from non-EVOOs. Most of the applications of the chromatographic methods refer to Spanish EVOOs [56–58], while the recently developed UV-vis based mathematical approach [5] has been mainly applied to Italian [5, 51, 52, 54] and Spanish EVOOs [5, 53] as well as Greek and Tunisian EVOOs [53, 54].

http://dx.doi.org/10.5772/64736

The analytical methodologies described in the previous section can be roughly divided into two types: methods able to characterize the olive varieties and cultivars and methods developed to identify the geographic origin of the olives. Some parameters used for EVOOs authentication are the ratio between the total amount of chlorophylls' derivatives and the total amount of carotenoids, the ratio between the carotenoids and lutein contents, the percentage of violaxhantin and the percentage of lutein [57–59].

For instance, according to Ref. [56], Spanish EVOOs typically have a ratio between chlorophylls' derivatives and carotenoids close to 1, in case of Italian EVOOs, a higher variability of this ratio has been found [15, 49–52]. According to Ref. [57], the ratio between carotenoids and lutein varies in a range between 0.4 and 1.5, depending on the variety and cultivar and this factor could be used as authentication parameter. Another parameter to be taken into account for the authenticity of EVOOs is the ratio between the concentration of lutein and β-carotene, which typically varies between 0.15 and 5 [15, 53, 54, 60]. However, as also reported in Refs. [19, 54], the amount of pigments in EVOOs depend strongly by olives variety, by the moment when the olives are picked and by the storage conditions [13, 57, 59].

5. Conclusions

In this chapter, the main analytical methods developed and applied to the identification and quantification of pigments in edible oils, and in particular, extra-virgin olive oils are described. New methodologies, divided between chromatographic and spectroscopic ones, are discussed putting in evidence advantages and disadvantages.

Pigments' concentration and their relative ratios may indicate the age and storage conditions (i.e. temperature, light and oxygen exposure), but they are also a good parameter to check authenticity and quality and to reveal mixtures with other seed oils, as deeply addressed in this chapter.

Acknowledgements

We would like to thank Dr. Andrea Serani and Prof. Maurizio Zandomeneghi for helpful discussions during our researches on olive oils.

Author details

Cristina Lazzerini, Mario Cifelli and Valentina Domenici*

*Address all correspondence to: valentina.domenici@unipi.it

University of Pisa, Pisa, Italy

References

[1] Spink J., Moyer D.C. Understanding and combating food fraud. Food Technology Magazine. 2013;67:30–35.

[2] Boskou D., Blekas G., Tsimidou M. Olive oil composition. In: Boskou D., editor. Olive Oil Chemistry and Technology. 2nd ed. Champain: AOCS Press; 2006. p. 40–72.

[3] Clydesdale F.M. Color as a factor in food choice. Critical Reviews in Food Science and Nutrition. 1993;33:83–101.

[4] Del Giovine L., Fabietti F. Copper chlorophyll in olive oils: identifications and determination by LIF capillary electrophoresis. Food Control. 2005;16:267–272.

[5] Domenici V., Ancora D., Cifelli M., Serani A., Veracini C.A., Zandomeneghi M. Extraction of pigment information from near UV-Vis absorption spectra of extra virgin olive oils. Journal of Agricultural and Food Chemistry. 2014;62:9317–9325.

[6] Serani A., Piacenti D. Analytical technique for the identification of deodorized oils in virgin olive oils. Note 1 – Analysis of chlorophylls' derivatives in virgin olive oils. The Italian Journal of Fatty Compounds. 2001;LXXVIII:459–463.

[7] Angerosa F., Campestre C., Giansante L. Analysis and authentication. In: Boskou D., editor. Olive Oil Chemistry and Technology. 2nd ed. Champain: AOCS Press; 2006. p. 113–172.

[8] Gandul-Rojas B., Roca-L., Cepero M., Mínguez-Mosquera M.I. Use of chlorophyll and carotenoid pigment composition to determine authenticity of virgin olive oil. Journal of American Oil Chemists's Society. 2000;77:8.

[9] Moyano M.J., Melendez-Martinez A.J., Alba J., Heredia F.J. A comprehensive study of the colour of the virgin olive oils and its relationship with the chlorophylls and carotenoids indexes (I): CIEXYZ non-uniform colour space. Food Research International. 2008;41:505–512.

[10] Torrecilla J.S., Rojo E., Domínguez J.C., Rodríguez F. Linear and non linear chemometric models to quantify the adulteration of extra virgin olive oil. Talanta. 2010;83:404–409.

[11] Torrecilla J.S., Rojo E., Dominguez J.C., Rodriguez F. A novel method to quantify the adulteration of extra-virgin olive oil with low grade olive oils by UV-Vis. Journal of Agricultural Food Chemistry. 2010;58:1679–1684.

[12] Cayuela J.A., Yousfi K., Martinez M.C., Garcia J.M. Rapid determination of olive oil chlorophylls and carotenoids by using UV-Vis spectroscopy. Journal of American Oil Chemists's Society. 2014;91:1677–1684.

[13] Psomiadou E., Tsimidou M. Simultaneous HPLC determination of tocopherols, carotenoids and chlorophylls for monitoring their effect on virgin olive oil oxidation. Journal of Agricultural and Food Chemistry. 1998;46:5132–5138.

[14] Seppanem C.M., Rahmani M., Csallany A. Simultaneous determination of chlorophylls, pheophytins, b-carotene, tocopherols and tocotrienols in olive and soybean oils by HPLC. Journal of Food Science. 2003;68:1644–1647.

[15] Giuffrida D., Salvo F., Salvo A., Cossignani L., Dugo G. Pigments profile in monovarietal virgin olive oils from various Italian olive varieties. Food Chemistry. 2011;124:1119–1123.

[16] Minguez-Mosquera I., Gandul B., Garrido J., Gallardo L. Pigments present in virgin olive oils. Journal of American Oil Chemists's Society. 1990;67:192–196.

[17] Giuliani A. Chlorophylls in olive and in olive oil: chemistry and occurrences. Critical Reviews in Food Science and Nutrition. 2011;51:678–690.

[18] Ramirez E., Gandul-Rojas B., Romero C., Brenes M., Guerrero L.G. Composition of pigments and colour changes in green table olives related to processing type. Food Chemistry. 2015;66:115–124.

[19] Criado M.N., Romero M.P., Motilva M.I. Effect of the technological and agronomical factors on pigment transfer during olive oil extraction. Journal of Agricultural and Food Chemistry. 2007;55:5681–5688.

[20] Ferruzzi M.G., Blakeslee J. Digestion, absorption and cancer preventative activity of dietary chlorophyll derivatives. Nutrition Research. 2007;27:1–12.

[21] Gertz C., Fiebing H.J. Pyropheophytin a determination of thermal degradation products of chlorophyll a in virgin olive oil. European Journal of Lipid Science and Technology. 2006;108:1062–1065.

[22] Aparicio R. Thermal degradation kinetics of chlorophyll pigments in virgin olive oils, A. Compounds of series A. Journal of Agricultural and Food Chemistry. 2010;58:6200–6208.

[23] Britton G. Structure and properties of carotenoids in relation to functions. The Federation of American Societies for Experimental Biology Journal. 1995;9:1551–1558.

[24] Mendez-Martinez A.J., Vicario I.M., Heredia F.J. Carotenoid pigments: structural and physicochemical considerations. Latin-American Archive of Nutrition. 2007;57:109–117.

[25] Jaswir I., Noviendri D., Rewo F.H., Octavianti F. Carotenoids: sources, medical properties and their application in food and nutraceutical industry. Journal of Medical Plants Research. 2011;71:7119–7131.

[26] Minguez-Mosquera M.I., Garrido-Fernandez J. Chlorophyll and carotenoid presence in olive fruit (Olea europeae). Journal of Agricultural and Food Chemistry. 1989;37:1–7.

[27] Gutierrez F., Albi M.A., Palma R., Rios J.L., Olias J.M. Bitter taste of virgin oil: correlation of sensory evaluation and instrumental HPLC. Journal of Food Science. 1989;54:68–73.

[28] Minguez-Mosquera M.I., Gandul-Rojas B., Gallardo-Guerrero M.L. Rapid method of quantification of chlorophylls and carotenoids in virgin olive oils by HPLC. Journal of Agricultural and Food Chemistry. 1992;40:60–63.

[29] Schmitz H.H., Emenhiser C., Schartz J.J. HPLC separation of geometric carotene isomers using a calcium hydroxide stationary phase. Journal of Agricultural and Food Chemistry. 1995;43:1212–1218.

[30] Kamal-Eldin A. Minor components of fats and oils. Bailey's Industrial Oil and Fat Products. 2005;3:12.

[31] Giuffrida D., Salvo F., Salvo A., La Pera L., Dugo G. Pigments composition in monovarietal virgin olive oils from various sicilian varieties. Food Chemistry. 2007;101:833–837.

[32] Crupi P., Milella R.A., Antonacci D. Simultaneous HPLC-DAD-MS (ESI^+) determination of structural and geometrical isomers of carotenoids in mature grapes. Journal of Mass Spectrometry. 2010;45:971–980.

[33] Gandul-Rojas B., Mìnguez-Mosquera M.I. Chlorophyll and carotenoid composition in virgin olive oils from various Spanish olive varieties. Journal of the Science of Food and Agriculture. 1996;72:31–39.

[34] Criado M.N., Motilva M.J., Gòni M., Romero M.P. Comparative study of the effect of the maturation process of the olive fruit on the chlorophyll and carotenoid fractions of drupes and virgin oils from Aberquina and Farga cultivars. Food Chemistry. 2007;100:748–755.

[35] Chen K., Rìos J.J., Pèrez-Gàlvez A., Roca M. Development of an accurate and high-throughput methodology for structural comprehension of chlorophylls derivatives (I). Dephytylated derivatives. Journal of Chromatography A. 2015;14:99–108.

[36] Baeten V., Dardenne P., Aparicio R. Interpretation of Fourier transform Raman spectra of the unsaponifiable matter in a selection of edible oils. Journal of Agricultural and Food Chemistry. 2001;49:5098–5107.

[37] Dais P., Hatzakis E. Quality assessment and authentication of virgin olive oil by NMR spectroscopy: a critical review. Analytica Chimica Acta. 2013;765:1–27.

[38] Del Coco L., Schena F.P., Fanizzi F.P. ^1H nuclear magnetic resonance study of olive oils commercially available as Italian products in the United States of America. Nutrients. 2012;4:343–355.

[39] Pizarro C., Rodrìguez-Tecedor S., Pèrez-del Notario N., Esteban-Dìez I., Gonzàlez-Sàiz J.M. Classification of Spanish extra virgin olive oils by data fusion of visible spectroscopic fingerprints and chemical descriptors. Food Chemistry. 2013;138:915–922.

[40] Mignani A.G., Garcìa-Allende P.B., Ciaccheri L., Conde M.O., Cimato A., Attilio C., Tura D. Comparative analysis of quality parameters of Italian extra virgine olive oils according to their region of origin. Optical Sensor. 2008;70:1–6.

[41] Sikorska E., Khmelinskii I., Sikorski M. Analysis of olive oils by fluorescence spectroscopy: methods and applications. In: Boskou D., editor. Olive Oil-Constituents, Quality, Health Properties and Bioconversions. Rijeka; InTech; 2012. p. 63–89.

[42] Lakowicz J.R. Principles of Fluorescence Spectroscopy. 3rd ed. New York: KluwerAcademic/Plenum Publishers; 2006.

[43] Young A.J., Frank H.A. Energy transfer reactions involving carotenoids: quenching of chlorophyll fluorescence. Journal of Photochemistry and Photobiology. 1996;36:3–15.

[44] Choe E., Min D.B. Mechanisms and factors for edible oils oxidation. Comprehensive Reviews in Food Science and Food Safety. 2006;5:169–186.

[45] Guimet F., Boque R., Ferre J. Cluster analysis applied to the exploratory analysis of commercial Spanish olive oils by means of excitation-emission fluorescence spectroscopy. Journal of Agricultural and Food Chemistry. 2004;52:6673–6679.

[46] Poulli K.I., Mousdis G.A., Georgiou C.A. Classification of edible and lampante virgin olive oil based on synchronous fluorescence and total luminescence spectroscopy. Analytica Chimica Acta. 2005;542:151–156.

[47] Dìaz G.T., Meràs I.D., Correa C.A., Roldan B. Simultaneous fluorometric determination of chlorophylls a and b and Pheophytins a and b in olive oil by partial least-squares calibration. Journal of Agricultural and Food Chemistry. 2003;51:6934–6940.

[48] Moberg L., Robertsson G., Karlberg B. Spectrofluorimetric determination of chlorophylls and pheopigments using parallel factor analysis. Talanta. 2001;54:161–170.

[49] Zandomeneghi M., Carbonaro L., Caffarata C. Fluorescence of vegetable oils: olive oils. Journal of Agricultural and Food Chemistry. 2005;53:759–766.

[50] Ayuso J., Haro M.R., Escolar D. Simulation of the visible spectra for edible virgin olive oils: potential uses. Applied Spectroscopy. 2004;58:474–480.

[51] Ancora D., Domenici V. (tutor). UV-Vis and ^{1}H-NMR spectroscopic methods applied to the study of extra-virgin olive oil produced in Tuscany and Apulia. Master Thesis, University of Pisa; 2014.

[52] Domenici V., Lazzerini C., Cifelli M. A rapid method to evaluate extra virgin olive oils quality from near-UV Vis absorption spectral analysis. Presented at 106th AOCS annual meeting, May 3, 2015 Orlando, USA.

[53] Lazzerini C., Cifelli M., Domenici V. Italian Journal of Food Science, under review.

[54] Lazzerini C., Buti F., Cifelli M., Zandomeneghi M., Domenici V. Tuscan Extra-virgin Olive Oil: a study of the pigments' content and perspectives for a new quality index. In: Lenzi A., editor. Codice Armonico 2016, Pisa: ETS; 2016.

[55] Torrecilla J.S., Vidal S., Aroca-Santos R., Wang S.C., Cancilla J. Spectroscopic determination of the photodegradation of monovarietal extravirgin olive oils and their binary mixtures through intelligent systems. Talanta. 2015;144:363–368.

[56] Oliveri P., Casale M., Casolino M.C., Baldo M.A., Nizzi F., Forina G.F. Comparison between classical and innovative class-modelling techniques for the characterisation of a PDO olive oil. Anal Bioanal Chem. 2011;399:2105–2113.

[57] Guerrero L.G., Gandul-Rojas B., Roca M., Minguez-Mosquera I. Effect of storage on the original pigment profile of Spanish virgin olive oil. Journal of the American Oil Chemists' Society. 2005;82:33–39.

[58] Gandul-Rojas B., Roca B.M., Cepero L., Minguez-Mosquera M.I. Chlorophylls and carotenoids pattern in olive fruits: Olea europea cv. Aberquina. Journal of Agricultural and Food Chemistry. 1999;47:2207–2212.

[59] Roca M., Gandul-Rojas B., Guerrero L.G., Minguez-Mosquera M.I. Pigments parameters determining Spanish virgin olive oil authenticity: stability during storage. Journal American Oil Chemists's Society. 2003;80:1237–1240.

[60] Roca M., Minguez-Mosquera M.I. Change in the natural ratio between chlorophylls and carotenoids in olive fruit during processing for virgine olive oil. Journal of the American Oil Chemists Society. 2001;78:133–138.

Chapter 7

DNA-Based Approaches for Traceability and Authentication of Olive Oil

Christos Bazakos, Stelios Spaniolas and Panagiotis Kalaitzis

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64494

Abstract

Authentication and traceability of extra virgin olive oil is a challenging research task due to the complexity of fraudulent practices. Various chemical and biochemical techniques have been developed for determining the authenticity of olive oil and in recent years non-conventional methods based on DNA analysis have gained attention, due to high specificity, sensitivity and reliability. DNA analyses have very high discriminating power because ultimately the unique identity of a variety or species is to a great extent genetically dependent. Polymorphisms are genetic variations which refer to the variation in populations or species. Molecular markers provide information on genetic variations and are valuable tools to determine olive oil authenticity. Recently several DNA-based methods have been developed to authenticate olive oil, since analysis of the residual oil DNA with the use of molecular markers can lead to the identification of the variety or the plant species from which it was extracted. The aim of this chapter is to provide an overview of the current trends and critical issues on DNA-targeted approaches used for traceability and authenticity of olive oil. This is considered a rapidly expanding field with significant challenges and prospects which shall be discussed thoroughly.

Keywords: olive oil and plant oils authentication, traceability, DNA, molecular markers, identification of varietal origin, SNPs, SSRs, biotechnological approaches

1. Introduction

Olive oil is the main component of the Mediterranean diet and one of the most valuable food products of the agro-food industry, not only due to taste, but also due to high nutritional value

[1]. Authentication and traceability of extra-virgin olive oil is a challenging endeavour that requires persistent efforts and continuous progress due to the complexity and advancement of novel fraudulent practices. Authentication uses methodologies, tools and technological platforms aiming at detection, prevention and exposure of adulteration and mislabelling of food commodities. Traceability is the ability to trace a food commodity from the production until the distribution stage (European Council regulation-EC 178/2002).

The various ways of olive oil adulteration mainly comprise (i) economic adulteration that refers to either the mixing of lower-grade vegetable oils with extra-virgin olive oil or with minimally processed olive oils such as non-refined or cold-pressed olive oils and (ii) mislabelling and misleading origin of protected designation of origin (PDO) and protected geographical indication (PGI) olive oils that are legally protected. One of the aims of PDO and PGI is to provide added market value high-quality olive oils that are derived from well-defined geographical regions. In most of the cases, the higher quality might be attributed to either the cultivar from which the olive oil was extracted or the edaphoclimatic conditions of the area, which is known as the 'terroir' in wine industry.

Therefore, adulteration of extra-virgin olive oil occurs not only by accidental contamination during the stages of oil processing, but is an act of deliberate addition of less expensive olive oils by fraudsters for financial profit.

Plethora of chemical and biochemical techniques have been developed for detecting the adulteration while ensuring authenticity and traceability of extra-virgin olive oil [2–6]. However, neither conventional analytical chemistry methods nor the analysis of biomorphological traits is always able to accurately detect the region of origin and/or the olive oil cultivar due to the diverse climatic conditions in which olive trees are exposed every year and their impact on the chemical composition of olive oil [7, 8].

The authenticity of olive oil, especially the extra-virgin, has been extensively studied by using several analytical approaches such as chromatography, stable isotope analysis, spectroscopy and nuclear magnetic resonance [9, 10]. In an interesting review [11], an extensive analysis of the most relevant compounds used as target analytes for olive oil characterisation and authentication was presented. Among others, the division of all analytical methods for olive oil authentication into 'targeted analysis' and 'profiling or non-targeted analysis' was suggested. The targeted analysis is based on the analysis of chemical compounds that appear only in the adulterant oil species (i.e. seed oils) and not in olive oil samples. Whereas the non-targeted analysis refers to the simultaneous detection of many known or unknown analytes belonging to a pre-defined metabolic pathway; usually, there is no differentiation among them. Although the aim is to rapidly determine the genuineness of olive oils, complicated multivariate statistical procedures are needed.

Recently, non-conventional methods based on DNA analysis have gained attention due to their high specificity, sensitivity and accuracy to detect the varietal origin of olive oil as well as the botanical origin of plant oils [12–15]. Moreover, DNA-based methods overcome deficiencies of conventional methods such as denaturation of proteins due to the heating and processing of food commodities [16]. Therefore, non-conventional methods offer an alternative, comple-

http://dx.doi.org/10.5772/64494

mentary approach since they rely only on the analysis of the DNA. Plenty of biomolecular methodologies have been developed for the authentication of olive oil using DNA markers. Molecular markers provide information on polymorphisms within DNA regions and are considered valuable tools to determine olive oil authenticity. Polymorphisms are genetic variations that can be detected either in nuclear, ribosomal or mitochondrial genomes as well as in the genomes of other organelles, such as chloroplasts.

Several DNA-based methods have been developed to authenticate olive oil, since analysis of the residual oil DNA with the use of molecular markers can lead to the identification of the variety or the species from which it was extracted regardless of environmental conditions during olive fruit's growth [13]. Plethora of studies took advantage of molecular markers for the identification of the varietal origin of olive oil such as SNPs [14, 17], microsatellites [18–20], SCARs [21] and AFLPs [22, 23]. Recent advances in olive genome and transcriptome sequencing increased the analytical targets and enriched the olive molecular markers database.

However, the reliability and reproducibility of these techniques are strictly dependent on the quality of the DNA extracted from oil samples [12, 19, 24, 25]. Trace amount of DNA is found in olive oil, which is highly degradable. Therefore, a high number of DNA isolation protocols were published while several commercial kits dedicated to olive oil DNA extraction are available [26].

DNA-based reaction chemistry was combined with existing detection methods such as capillary electrophoresis, high-resolution melting (HRM), TaqMan probes, qRT-PCR and single-base extension (SNaPshot) resulting in numerous analytical approaches with particular advantages and disadvantages.

The chapter provides an overview of the current trends and critical issues on DNA-targeted approaches used for traceability and authenticity of olive oil. This is a rapidly expanding field with significant challenges and prospects that shall be discussed thoroughly. Moreover, issues of adulteration with oils of plant origin will be thoroughly discussed while recent advances in authentication and traceability of herbs and medicinal plants will be taken into consideration and compared with oil matrices.

2. Recent advances in DNA extraction from olive and other plant oil matrices

A pre-requisite for the development and improvement of polymerase chain reaction (PCR) based DNA fingerprinting methods is the isolation of adequate quality and quantity DNA. Although the quantity of recoverable DNA from plant oils is hardly detectable by any means, either nano-spectrophotometer or agarose gel electrophoresis, most of the times it is sufficient for molecular markers analysis. However, nuclease activity and the presence of PCR inhibitors such as fats, residual polysaccharides and polyphenols might inhibit PCR amplification [19, 21, 24, 27, 28].

The purity of extracted DNA is the most crucial and significant step in DNA fingerprinting methods ensuring the validity and the reproducibility of plant oil forensic methodologies. Therefore, the need for a reliable and reproducible protocol for DNA isolation from plant oils is mandatory for either research or industrial applications. The ideal plant oil DNA extraction method should be reproducible, simple, relatively cheap and capable to recover stable, free of PCR inhibitors DNA either from filtered and/or heavily processed plant oils or from unfiltered olive oils.

In the last 15 years, many extraction methods and commercial kits were used and further improved valuable tools were provided. Commercial kits were used in several reports with positive results in most cases. Testolin and Lain compared the performance of a variety of commercial kits on DNA isolation from filtered and non-filtered olive oil and concluded that most consistent results were obtained using the QIAamp DNA Stool Mini Kit [24]. The potential of this commercial kit to isolate DNA of acceptable quality from oils of plant origin such as sesame oil was validated by Spaniolas et al. [29]. However, the high cost of this commercial kit motivated researchers to develop or modify CTAB (Cetyltrimethylammonium bromide)-based protocols. Muzzalupo and Perri [28] introduced the use of Proteinase K during malaxation to prevent DNA degradation. Intact DNA was then isolated from non-filtered olive oil sediments using the CTAB method of Doyle and Doyle [30]. The main limitation of this method was the use of Proteinase K during malaxation that is not applicable commercially.

The addition of this enzyme prior to the storage of olive oil improved the amount of recovered DNA [24], although this is not recommended for commercial application. Therefore, Consolandi et al. tested the efficiency of Proteinase K by adding it to the pellet and the oily phase after hexane treatment and centrifugation of oil sample, with positive results [31]. However, the innovation of this method relies on the disruption of neutral micelles by adding the surfactant Tween-20 after hexane extraction. Busconi et al. isolated intact DNA from unfiltered laboratory and commercial olive oils, using a CTAB method on 0.5 g of pellet [32]. They were able to amplify up to 1942 bp fragments. Martins-Lopes et al. used the same method, but their starting material was 6 ml unfiltered olive oil and not 0.5 g oil sediment [33]. The various CTAB-based methodologies indicated that the strategy of using common plant DNA extraction protocols can lead to oil matrix DNA isolates of adequate quality.

Alternatively, Breton et al. extracted DNA from variable amounts of olive oil using several techniques such as magnetic beads, silica extraction, spun column and hydroxyapatite [25]. The most efficient approach which that provided free of PCR inhibitors DNA was the magnetic beads. This technique was further applied in many commercial virgin and crude olive oils leading to the claim by the authors that although it needs further improvement, it could be routinely applied in any olive oil sample. Doveri et al. [34] used the official Swiss method for lecithin extraction [35] from 15 ml unfiltered olive oils, resulting to less than 50% successful amplifications due to the low quantity and quality of extracted DNA.

Giménez et al. compared four DNA isolation methods for commercial mono-varietal olive oils [26]. The study included a CTAB-based [36] and a hexane method [31] as well as two modified CTAB-methods, CTAB-hexane and CTAB-hexane-chloroform. The CTAB-hexane-chloroform methodology comprising a washing step with chloroform/isoamyl alcohol in combination

with the addition of linear acrylamide prior to the precipitation proved to have a beneficial effect on recovering DNA of adequate quality and quantity. It is worth mentioning that the starting olive oil volume was reduced to less than 1 ml. This protocol was successfully used to extract DNA from several plant oils indicating the potential of this method for oil DNA forensic applications [37, 38]. Ramos-Gomes et al. improved this protocol by increasing the concentration of Tween-20 in the lysis buffer and reducing the lysis time to 15 minutes while the precipitation time was limited to 1 h without the addition of linear acrylamide [38]. This improved protocol increased the yield and the PCR amplification efficiency of olive and seed oils DNA [38]. Raieta et al. [39] further modified the CTAB-hexane-chloroform protocol [26] by adding more purification steps with more critical excision of major DNA band from 1% agarose gel aiming to remove most of the PCR inhibitors such as residues of organic components or other contaminants. This protocol was successfully applied on various mono- and multi-varietal commercial filtered olive oils.

Recently, Muzzalupo et al. developed an innovative and simple method that bypasses the DNA extraction/purification steps by using KAPA3G Plant DNA polymerase that enables the direct DNA amplification from virgin filtered and unfiltered olive oils [40]. Definitely, this method seems more efficient and faster than the traditionally DNA isolation protocols. However, the success of this approach remains to be tested in lab and industrial applications.

Considering that in the 1990s only a couple of olive oil DNA extraction protocols were reported, the current availability of numerous protocols ensures the positive prospects of DNA-based olive oil authentication and traceability.

3. Olive oil and varietal origin

The olive cultivar together with the region of production directly affects the quality traits of olive oil. Therefore, mono-varietal extra-virgin olive oils or blends of specific cultivars grown in certain regions are considered as premium olive oils of higher value due to specific quality characteristics. These premium olive oils are protected by the European Commission through certification labels of PDO and PGI in order to ensure authenticity and protection of consumers. Therefore, there is a pressing need for reliable identification of the genetic identity of extra-virgin olive oils.

Several attempts were carried out to relate chemical composition of olive oils with the cultivar of origin such as the mono-saturated fatty acids that are major constituents of olive oils and confer high nutritional value. In this effort, many reports used the fatty acids content to discriminate olive cultivars [41–43] while Mannina et al. performed a study in a well-defined and limited geographical region achieving a relationship between the fatty acid composition and some Sicilian cultivars [42]. These studies revealed that although the varietal effect was important for olive oil discrimination purposes based on fatty acid composition, the geographical and environmental effects were strongly affecting this approach [13]. Recently, Laroussi-Mezghani et al. were able to predict the varietal origin of six Tunisian cultivars by

their fatty acid composition and near-infrared spectra associated with chemometric treatment. Other olive oil constituents such as the triacylglycerols have also been widely studied for their discriminatory efficiency on cultivar origin [41, 44–46]. In addition, several attempts were also made to correlate a long list of minor components such as sterols, pigments, phenolic and volatile compounds, hydrocarbons and tocopherols of olive oils with the cultivar origin of olive oil. Although Matos et al. [47] were able to discriminate three olive cultivars based on sterol composition; sterol was mostly studied in combination with other chemical compounds such as fatty acids and triacylglycerols [48, 49].

The volatile compounds are known to be strongly related to the genotype and the geographic origin of olive fruit, giving the unique flavour and quality of olive oil [13]. The volatile fraction in virgin olive oil consists of more than 100 compounds, while the most important substances for olive variety discrimination are the products of the lipoxygenase pathway (LOX) [50].

Despite the plethora of biochemical methods and analytical tools that were developed for the identification of olive oil cultivar, the main issue is the significant effect of climatic conditions and olive oil processing on chemical composition of virgin olive oil.

DNA-based methods can be used as complementary approaches for olive oil authentication and traceability considering a number of advantages over conventional methods such as reliability, specificity and sensitivity without any influence by the environmental conditions.

Currently, there are plenty of reports on the discrimination of olive cultivars using various molecular markers such as amplified-fragment length polymorphism [51, 52], random amplified polymorphic DNA (RAPD) [53], sequence-characterised amplified regions [54], simple sequence repeats (SSRs) [55, 56], inter-simple sequence repeats (ISSRs) [57] and single-nucleotide polymorphisms [58, 59]. However, only few of them were tested on the identification of the varietal origin of olive oil. This can be attributed to the low quality of olive oil DNA due to severe fragmentation as a result of the degradation process.

A study showed that the DNA quality is affected during storage of filtered olive oil in retail store conditions [27]. The amplification of 107 bp DNA fragments was successful for olive oil samples stored up to 1 year, whereas DNA fragments longer than 415 bp were successfully amplified only after 20 days of storage and no longer [27]. Moreover, Montemurro et al. and Pafundo et al. employed AFLP molecular markers to study the effect of olive oil storage length on the use of DNA as an analyte for molecular traceability [28, 60]. They showed a significant deterioration of DNA quality within a month while the AFLP profiles of leaf and oil DNA were not similar after 9 and 12 months of storage. These reports indicate the absolute requirement for short DNA template molecular markers in order to be used for olive oil DNA forensic applications.

One of the first efforts to fingerprint four Italian olive oils was made by Pafundo et al. using AFLP markers [23]. AFLP markers are based on the detection of restriction fragments by PCR amplification using a genome as template [61]. The origin of polymorphism in AFLP is based on base substitutions within restriction sites or deletions/insertions between two adjacent restriction sites. Therefore, the high-quality DNA is a pre-requisite for such a molecular marker. The identity between AFLP profiles in leaves and oils reached a maximum of 70% due

to differences in the level of DNA degradation between leave and oil samples [23]. Three years later, Montemurro et al. reported enhanced AFLP profiles of 10 Italian cultivars by optimising the DNA extraction protocol and restriction/ligation conditions [22]. They also suggested the setting up of an olive oil reference data bank with AFLP profiles, which could be used as an identity card of mono-varietal olive oils. However, the requirements of high-quality DNA and short-storage time for reliable traceability lead to the advancement of other molecular markers such as SSRs and SNPs in combination with high-performance analytical platforms.

The efficiency of RAPD, ISSR and SSR molecular markers for varietal identification of olive oil was evaluated by Martins-Lopes et al. in 23 Portuguese olive oil samples [33]. This study demonstrated that the ISSR marker system was more informative as compared to the inadequate efficiency of RAPD primers in olive oil samples. SSR analysis was performed to compare the profile of DNA samples isolated from olive oil with that of leaves with satisfactory results.

SSRs are also called microsatellites and consist of 1–10 bp tandem repeats, with a variable number of repetitions [62]. They are highly polymorphic due to the variation in the number of repeats. Microsatellites can be detected by PCR amplification using specific primers annealed to the unique flanking sequences providing high discrimination power. So far they are the most popular molecular markers for olive oil fingerprinting purposes.

Although many studies reported identical olive oil and leaf profiles [18, 24, 34, 63–65], the validity of this approach due to occurrence of repeatability problems is mostly dependent on the SSR sequence and the olive oil DNA quality. Another significant concern on the interpretation of the results is related to differences in amplicon size and allele drop-out in olive oil DNA analysis. These issues might be probably attributed to the low quality and quantity of olive oil DNA, which affect the allele amplification.

In several studies, the appearance of additional than the expected alleles was reported. Muzzalupo et al. and Ben Ayed et al. showed the allele contribution of the pollinator cultivar that is present in seed embryo [12, 19]. This finding contradicts many studies that appeared thereafter. However, additional alleles might appear either by accidental mixing with other cultivars during harvesting or by mixing with traces of olive oils originated from other cultivars during oil-milling processes.

The initial attempts to identify olive cultivars in commercial olive oil samples using SSR markers were reported by Pasqualone et al. and Breton et al. [25, 65]. The SSR profiles of olive oil DNA were identical to those obtained from leaves and drupes, showing the potential of SSR marker to be used for forensic applications. Pasqualone et al. demonstrated the effectiveness of SSR analysis in verifying the identity of a PDO olive oil by the genotyping of a limited number of DNA microsatellites [20]. More recently, Pasqualone et al. showed that a single microsatellite marker was sufficient to discriminate Leucocarpa olive oil from six other mono-varietal olive oils providing an identification key based on the PCR amplification profiles [63]. Additionally, Alba et al. demonstrated the potential of a single, highly polymorphic SSR marker to discriminate seven Italian PDO mono-varietal olive oils [18]. Vietina et al. studied the traceability of 21 mono-varietal olive oils using microsatellites, concluding that in parallel with the improvement of olive oil DNA extraction methods, the implementation of authenti-

cation procedures might require selection of highly polymorphic SSR markers that display the same alleles in different laboratories and the robustness of the method should be assessed in an inter-laboratory ring trial [66].

Recently, Montemurro et al. demonstrated the applicability of SSR markers coupled with high-resolution melting analysis for the identification of olive varieties constituting the 'Terra di Bari' PDO extra-virgin olive oil: Cima di Bitonto, Coratina and Ogliarola among a panel of nine cultivars widespread in Apulia region [67]. This assay provided a flexible, cost-effective, and closed-tube microsatellite genotyping method for authentication analysis in olive oil.

Although genetic traceability using microsatellites is a proven, powerful technique, the identification of an unknown mono-varietal virgin olive oil cultivar is not possible without a reference database. Therefore, Ben Ayed et al. constructed the Olive Genetic Diversity Database (http://www.bioinfo-cbs.org/ogdd/) which is a genetic, morphological and chemical database of about 200 worldwide cultivars [68]. This reference database not only enables the identification of unknown olive cultivars based on their microsatellite allele size(s), but it also provides additional morphological and chemical information for each cultivar.

Single-nucleotide polymorphisms are the most abundant type of mutation. SNPs are the most abundant markers in the genome. They are stably inherited, bi-allelic in most cases, co-dominant, and they require short DNA amplicons for genotyping [69]. Moreover, no other molecular marker has such diverse and numerous methods of analysis as SNPs. Therefore, the recent development of low-cost, high-throughput sequencing technologies which reduced the cost of SNP identification in plant species will expand the use of SNP markers in various industrial applications including olive oil DNA forensics.

Reale et al. and Consolandi et al. bypassed the lack of olive genomic sequences and explored the potential of SNPs to discriminate olive cultivars from Europe and Australia [58, 59]. One year later, Consolandi et al. demonstrated the improvement of their previously developed assay for the genotyping of 49 Mediterranean olive cultivars by ligation detection reaction (LDR)/universal array (UA) [31]. In this analytical assay, a ligation detection reaction distinguishes the alleles that are subsequently detected by hybridisation onto a universal array.

Bazakos et al. developed a simple and efficient assay to identify the varietal origin of olive oils using SNPs [14]. A large number of SNPs were identified, and they used those residing in restriction sites as the basis for the development of a PCR-RFLP assay coupled with capillary electrophoresis. Capillary electrophoresis has a much lower limit of detection for DNA fragments compared to agarose gel electrophoresis and can easily discriminate fragments that differ only by few nucleotides in length [15]. Three SNPs were adequate to discriminate five mono-varietal olive oils. Neither paternal contribution of embryos was detected in olive oil samples nor did additional peaks in leaf samples as was the case of additional alleles observed in leaf samples when certain SSR markers were used [18]. This can be attributed to the single-locus nature of SNPs compared to SSR markers.

Kalogianni et al. took advantage of the discriminatory potential of these three SNPs, and they developed the first multiplex SNP genotyping assay for olive oil cultivar identification that is performed on a suspension of fluorescence-encoded microspheres [70]. The developed

http://dx.doi.org/10.5772/64494

analytical assay could be particularly useful in industrial sector and/or in laboratories involved in official control, that is, laboratories that require methods offering high sample throughput.

Uncu et al. developed an SNP-based identification key to ascertain the cultivar origin of Turkish olive oils [17]. They demonstrated a cleaved amplified polymorphic (CAP) DNA assay for SNPs that reside in restriction sites. Five CAPs were adequate to discriminate 17 olive cultivars. Furthermore, the efficiency and limit of their approach for detecting olive oil admixtures was down to a limit of 20%.

Bazakos et al. enriched the Greek SNP database with Lebanese and Tunisian varieties that resulted in the discrimination of six among 13 mono-varietal olive oils, three Greek and three Tunisian, using PCR-RFLP assay combined with capillary electrophoresis [71]. The highlight of this study was the detection of olive oil admixtures down to a limit of 10%. The authors attributed the better limit of detection to the use of different DNA extraction protocols for the olive oil samples between the two studies that might result in higher quality and quantity of isolated DNA. Although PCR-RFLP is a reliable and simple assay, the main limitation is the requirement of SNPs that reside in restriction sites.

4. Adulteration with oils of plant origin: trends and lessons learned

4.1. Introduction

Incidences of olive oil adulteration refer to the mixing of extra-virgin grade with refined olive oils or/and with cheaper oils of other than olive botanical origin. However, apart from the consumers' deception issue, the adulteration of olive oil with other plant oils could possibly introduce a health risk for consumers allergic to plant species from which the adulterant oils are originated [72–74].

The authenticity of olive oil, especially the extra-virgin, has been extensively studied by using several analytical approaches such as chromatography, stable isotope analysis, spectroscopy and nuclear magnetic resonance [9, 10]. In an interesting review, Aparicio et al. presented an extensive analysis of the most relevant compounds used as target analytes for olive oil characterisation and authentication [11]. Among others, it was suggested the division of all analytical methods for olive oil authentication into 'targeted analysis' and 'profiling or non-targeted analysis'. Targeted analysis is based on the analysis of chemical compounds that appear only in the adulterant oil species (i.e. seed oils) and not in olive oil samples. Whereas the non-targeted analysis refers to the simultaneous detection of many known or unknown analytes belonging to a pre-defined metabolic pathway and usually, there is no differentiation among them. Although the aim is to rapidly determine the genuineness of olive oils, complicated multi-variate statistical procedures are needed.

4.2. DNA target analytes

DNA-based methods target analytes that are characterised in terms of DNA length and/or nucleotide sequence that can be species specific, thus indicating whether an olive oil is

adulterated with oil of other botanical origin. A DNA-based analytical approach usually involves the analysis of species-specific DNA fragments or polymorphisms, the genetic variations between or within species. The characteristic of species-specific DNA fragments is the discrimination of rather distantly related organisms such as the case of food allergens [75, 76] and of genetically modified organisms (GMOs) [75, 76]. The characteristic of polymorphism detection is the discrimination of varieties or closely related species through molecular markers. However, when it comes to the exploitation of polymorphisms of organelle DNA, there is great potential for the discrimination of plant species in food matrices.

Chloroplastic DNA (cpDNA) targets have been widely used in the past for plant phylogenetic studies and nowadays have become valuable tools for the authentication studies of plant origin foods. One of the most extensively studied cpDNA fragment is the *rbcL* gene that encodes the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase and has been sequenced from 500 species [77]. Gielly and Taberlet indicated that *rbcL* coding region usually does not contain enough genetic variation to resolve relationships among closely related genera [78]. Therefore, the analysis of non-coding regions of cpDNA was suggested, such as the *trnL* (UAA) intron and the intergenic spacer between *trnL* (UAA) 3′-exon and *trnF* (GAA) gene, since these regions evolve more rapidly than coding regions do, and thus, are expected to be more useful for discrimination purposes.

For food authentication purposes, the cpDNA target of choice is amplified through PCR assay by using universal primers, thus resulting in an amplified cpDNA fragment ready to be analysed for its length. Due to the presence of insertions/deletions, a species-specific PCR product length exists for each plant species. For food mixtures of more than one species, the number of generated PCR products will correspond to the number of species that constitute the mixture. Consequently, the analysis of the length of the PCR product(s) by using standard gel electrophoresis would lead to the identification of the mixture comprising species. For validation purposes or/and in case that higher resolution is needed due to likelihood of almost similar length amplicons, more sophisticated analytical instruments can be used.

The *trnL* (UAA) intron has been used in the past as an analyte molecule in order to trace specific food crops through PCR amplification including sources of potential allergens, such as canola, corn, potato, soybean, rice, peanut and wheat [79] as well as rye, barley, oat, rice, wheat and maize through an oligonucleotide hybridisation array [80]. The same region was employed to develop a simple PCR-based approach to detect olive oil adulteration with other plant oils such as sunflower, sesame, hazelnut, walnut, cotton, soya, almond, avocado and corn using a lab-on-a-chip capillary electrophoresis system [29]. Preliminary results exhibited efficient discrimination potential except for sesame and avocado [29]. This approach was successfully used in previous authentication studies for the analysis of coffee [81] and while it was further improved by using a DNA capillary electrophoresis platform [82]. As a proof of concept, sesame oil could be easily discriminated from olive oil in a reliable way that was validated through a single-base primer extension assay [15]. The lack of discrimination between olive and avocado oils could be overcome by employing polymorphic SNPs within the cpDNA using a single-base primer extension approach [15].

http://dx.doi.org/10.5772/64494

In addition to insertions/deletions, the presence of single-nucleotide polymorphisms within the PCR target can lead to the validation of the plant species as a constituent of the food matrix. A large number of SNP-based analytical methodologies are available to scientists involved in genotyping approaches.

There are numerous reports on new methodologies, enzymatic tools, platforms and optimised protocols for SNP detection methods and analyses. All these SNP genotyping methods have been classified by Syvanen in several groups/assays, based on the main biochemical principle, thus facilitating their study and in-depth comprehension [83]. Recently, a very promising analytical approach appeared such as the high-resolution melting analysis that has been used for olive oil authentication and was first described by Reed and Wittwer [84].

HRM analysis was performed with PCR primers for the *rbcL* gene in order to detect the presence of maize and sunflower oils in artificial mixture of olive oils and particularly in olive-maize oil mix and olive-sunflower oil mix in ratios (v/v) 50/50, 70/30, 80/20 and 90/10. The HRM results showed that both maize oil and sunflower oil could be detected down to 10% limit of detection [85]. The same approach was applied by Ganopoulos et al. to identify the botanical origin of main vegetable oils and their quantitative detection in mixed oils [37]. The adulteration of olive (*Olea europaea*) oil with canola (*Brassica napus*) oil was selected as a case study. The results showed that the universal *rbcL* region is efficient enough to discriminate plant oil species and to detect the existence of 1% of canola oil admixed into olive oil. In another study, the PCR-CE-SSCP method was applied for the first time to detect cheap oils blended into olive oil, by using the chloroplastic *rbcL* gene as DNA target for PCR amplification. Olive along with six other commonly used oil plants was successfully discriminated [86].

In general, SNPs are advantageous molecular markers because of their high density in genomes that permit high-capacity discriminatory power. SNPs should be considered as the marker of choice to trace and authenticate highly degraded DNA extracted from complex food matrices such as oils of plant origin, since they can be genotyped within low size range amplicons [87]. This is very important since the possibility to amplify PCR products of around 80 bp is much higher than that of 200 bp considering that the template DNA was extracted from highly processed vegetable oils [26, 29]. In this way, the chance to detect a potential adulterant species is much higher.

4.3. Limitations

Nowadays, there are enough analytical platforms, instrumentation and discriminative DNA targets/markers for the analysis of olive oil authenticity and the detection of adulteration with plant oils of other botanical origin. However, there are still limitations related to the DNA extraction step. The availability and extractability of residual DNA is an issue that has to be thoroughly studied particularly from a physical chemistry point of view. Further progress on this issue will strongly impact the olive oil authenticity and traceability industry and the pertinent commercial applications.

5. Conclusions

The research field of DNA-based authentication and detection of adulteration of olive oil has been transformed into a very active area of olive oil-related research with substantial progress. This was accomplished through the established reliability of SSR markers and the continuously increasing use of SNPs markers for olive oil DNA forensic purposes. Moreover, the plethora of available technological platforms to support high-throughput SNP genotyping has significantly contributed towards this direction. This would not have been feasible without the unequivocal advancement of new methodologies for the extraction of high-quality DNA from olive oil and oils of plant origin matrices. The conditions are now mature for a significant boost towards industrial applications of DNA-based approaches for olive oil authentication and traceability.

Author details

Christos Bazakos[1], Stelios Spaniolas[2] and Panagiotis Kalaitzis[1,2*]

*Address all correspondence to: panagiot@maich.gr

1 Department of Horticultural Genetics and Biotechnology, Mediterranean Agronomic Institute of Chania, Alsyllio Agrokipiou, Chania, Crete, Greece

2 Hellenic Food Authority (EFET), Regional Division of Northern Aegean, Department of Laboratory Controls, Mytilini, Lesvos Island, Greece

References

[1] Wahrburg U, Kratz M, Cullen P. Mediterranean diet, olive oil and health. Eur. J. Lipid Sci. Technol. 2002;104:698–705.

[2] Aroca-Santos R, Cancilla J, Matute G, Torrecilla J. Identifying and quantifying adulterants in extra virgin olive oil of the picual varietal by absorption spectroscopy and nonlinear modeling. J. Agric. Food Chem. 2015;63:5646–5652.

[3] Bevilacqua M., Bucci R., Magri AD, Magri AL, Marini F. Tracing the origin of extra virgin olive oils by infrared spectroscopy and chemometrics: A case study. Anal. Chim. Acta. 2012;717:39–51.

[4] Giacalone R, Giuliano S, Gulotta E, Monfreda M, Presti G. Origin assessment of EV olive oils by esterified sterols analysis. Food Chem. 2015;188:279–285.

[5] Korifi R, Le Dr´eau E, Molinet J, Artaud J, Dupuy N. Composition and authentication of virgin olive oil from French PDO regions by chemometric treatment of Raman spectra. J. Raman Spectrosc. 2010;42:1540–1547.

[6] Portarena S, Gavrichkova O, Lauteri M, Brugnoli E. Authentication and traceability of Italian extra-virgin olive oils by means of stable isotopes techniques. Food Chem. 2014;164:12–16.

[7] Alessandri S, Cimato A, Modi G, Mattei A, Crescenzi A, Caselli S, Tracchi S. Univariate models to classify Tuscan virgin olive oils by zone. La Rivista Italiana Delle Sostanze Grasse. 1997;74:155–164.

[8] Barone E, Di Marco L, Motisi A, Caruso T. The Sicilian germplasm and its characterization by using statistical method. Acta Hortic. 1994;356:66–69.

[9] Aparicio R, Aparicio-Ruiz R. Authentication of vegetable oils by chromatographic techniques. J. Chromatogr. Anal. Chim. Acta. 2000;881:93–104.

[10] Osorio MT, Haughey SA, Elliott CT, Koidis A. Evaluation of methodologies to determine vegetable oil species present in oil mixtures: Proposition of an approach to meet the EU legislation demands for correct vegetable oils labelling. Food Res. Int. 2014;60:66–75.

[11] Aparicio R, Morales M, Aparicio-Ruiz R, Tena N, Garcıa-Gonzalez D. Authenticity of olive oil: Mapping and comparing official methods and promising alternatives. Food Res. Int. 2013;54:2025–2038.

[12] Muzzalupo I, Pellegrino M, Perri E. Detection of DNA in virgin olive oils extracted from destoned fruits. Eur. Food Res. Technol. 2007;224:469–475.

[13] Montealegre C, Alegre MLM, Garcia-Ruiz C. Traceability markers to the botanical origin in olive oils. J. Agric. Food Chem. 2010;58(1):28–38.

[14] Bazakos C, Dulger AO, Uncu AT, Spaniolas S, Spano T, Kalaitzis P. A SNP-based PCR–RFLP capillary electrophoresis analysis for the identification of the varietal origin of olive oils. Food Chem. 2012;134:2411–2418.

[15] Spaniolas S, Bazakos C, Spano T, Zoghby C, Kalaitzis P. The potential of plastid trnL (UAA) intron polymorphisms for the identification of the botanical origin of plant oils. Food Chem. 2010;122:850–856.

[16] Lockley AK, Bardsley RG. DNA-based methods for food authentication. Trends Food Sci. Technol. 2000;11:67–77.

[17] Uncu AT, Frary A, Doganlar S. Cultivar origin and admixture detection in Turkish olive oils by SNP-based CAPS assays. J. Agric. Food Chem. 2015;63:2284–2295.

[18] Alba V, Sabetta W, Blanco A, Pasqualone A, Montemurro C. Microsatellite markers to identify specific alleles in DNA extracted from monovarietal virgin olive oils. Eur. Food Res. Technol. 2009;229:375–382.

[19] Ben Ayed R, Grati-Kamoun N, Moreau F, Rebai A. Comparative study of microsatellite profiles of DNA from oil and leaves of two Tunisian olive cultivars. Eur. Food Res. Technol. 2009;229:757–762.

[20] Pasqualone A, Montemurro C, Summo C, Sabetta W, Caponio F, Blanco A. Effectiveness of microsatellite DNA markers in checking the identity of protected designation of origin extra virgin olive oil. J. Agric. Food Chem. 2007;55:3857–3862.

[21] Pafundo S, Agrimonti C, Maestri E, Marmiroli N. Applicability of SCAR markers to food genomics: Olive oil traceability. J. Agric. Food Chem. 2007;55:6052–6059.

[22] Montemurro C, Pasqualone A, Simeone R, Sabetta W, Blanco A. AFLP molecular markers to identify virgin olive oils from single Italian cultivars. Eur. Food Res. Technol. 2008;226:1439–1444.

[23] Pafundo S, Agrimonti C, Marmiroli N. Traceability of plant contribution in olive oil by amplified fragment length polymorphisms. J. Agric. Food Chem. 2005;53:6995–7002.

[24] Testolin R, Lain O. DNA extraction from olive oil and PCR amplification of microsatellite markers. J. Food Sci. 2005;70:c108−c112.

[25] Breton C, Claux D, Metton I, Skorski G, Berville A. Comparative study of methods for DNA preparation from olive oil samples to identify cultivar SSR alleles in commercial oil samples: Possible forensic applications. J. Agric. Food ChemE. 2004;52:531–537.

[26] Giménez MJ, Pistón F, Martín A, Atienza SG. Application of real-time PCR on the development of molecular markers and to evaluate critical aspects for olive oil authentication. Food Chem. 2010;118:482–487.

[27] Spaniolas S, Bazakos C, Ntourou T, Bihmidine S, Georgousakis A, Kalaitzis P. Use of lambda DNA as a marker to assess DNA stability in olive oil during storage. Eur. Food Res. Technol. 2008;227:175–179.

[28] Muzzalupo I, Perri M. Recovery and characterization of DNA from virgin olive oil. Eur. Food Res. Technol. 2002;214:528–531.

[29] Spaniolas S, Bazakos C, Awad M, Kalaitzis P. Exploitation of the chloroplast trnL (UAA) intron polymorphisms for the authentication of plant oils by means of a lab-on-a-chip capillary electrophoresis system. J. Agric. Food Chem. 2008;56:6886–6891.

[30] Doyle JJ, Doyle JL. A rapid isolation procedure for small quantities of fresh leaf tissues. Phytochem. Bull. 1987;19:11–15.

[31] Consolandi C, Palmieri L, Severgnini M, Maestri E, Marmiroli N, Agrimonti C, Baldoni L, Donini P, De Bellis G, Castiglioni B. A procedure for olive oil traceability and authenticity: DNA extraction, multiplex PCR and LDR-universal array analysis. Eur. Food Res. Technol. 2008;227:1429–1438.

[32] Busconi M, Foroni C, Corradi M, Bongiorni C, Cattapan F, Fogher C. DNA extraction from olive oil and its use in the identification of the production cultivar. Food Chem. 2003;83:127–134.

[33] Martins-Lopes P, Gomes S, Santos E, Guedes-Pinto H. DNA markers for Portuguese olive oil fingerprinting. J. Agric. Food Chem. 2008;56:11786–11791.

[34] Doveri S, Donal M, Lee D. Non concordance between genetics profiles of olive oil and fruit: A cautionary note to the use of DNA markers for provenance testing. J. Food Chem. 2006;54:9221–9226.

[35] Waiblinger HU, Pietsch K, Wurz A. DNA-Extraktion aus Sojalecithin und Kosmetika. Lebensmittelchemie. 1999;53:11.

[36] Doyle JJ. DNA protocols for plants. In: Hewitt G, Johnson AWB, Young JPW, eds. Molecular techniques in taxonomy. NATO ASI series H, Cell Biol: Springer-Verlag, Berlin, Germany. 1991;57:283–293.

[37] Ganopoulos I, Bazakos C, Madesis P, Kalaitzis P, Tsaftaris A. Barcode DNA high-resolution melting (Bar-HRM) analysis as a novel close-tubed and accurate tool for olive oil forensic use. J. Sci. Food Agric. 2013;93:2281–2286.

[38] Ramos-Gómez S, Busto MD, Perez-Mateos M, Ortega N. Development of a method to recovery and amplification DNA by real-time PCR from commercial vegetable oils. Food Chem. 2014;158:374–383.

[39] Raieta K, Muccillo L, Colantuoni V. A novel reliable method of DNA extraction from olive oil suitable for molecular traceability. Food Chem. 2015;172:596–602.

[40] Muzzalupo I, Pisani F, Greco F, Chiappetta A. Direct DNA amplification from virgin olive oil for traceability and authenticity. Eur. Food Res. Technol. 2015;241:151–155.

[41] Stefanoudaki E, Kotsifaki F, Koutsaftakis A. Sensory and chemical profiles of three European olive varieties (Olea europaea L): An approach for the characterisation and authentication of the extracted oils. Sci. Food Agric. 2000;80:381–389.

[42] Mannina L, Dugo G, Salvo F, Cicero L, Ansanelli G, Calcagni C, Segre A. Study of the cultivar-composition relationship in Sicilian olive oils by GC, NMR, and statistical methods. J. Agric. Food Chem. 2003;51:120–127.

[43] D'Imperio M, Dugo G, Alfa M, Mannina L, Segre AL. Statistical analysis on Sicilian olive oils. Food Chem. 2007;102:956–965.

[44] Aranda F, Gomez-Alonso S, Rivera del Alamo RM, Salvador MD, Fregapane G. Triglyceride, total and 2-position fatty acid composition of Cornicabra virgin olive oil: comparison with other Spanish cultivars. Food Chem. 2004;86:485–492.

[45] Cerretani L, Bendini A, Del Caro A, Piga A, Vacca V, Caboni MF, Gallina TT. Preliminary characterisation of virgin olive oils obtained from different cultivars in Sardinia. Eur. Food Res. Technol. 2006;222:354–361.

[46] Issaoui M, Dabbou S, Echbili A, Rjiba I, Gazzah N, Trigui A, Hammami M. Biochemical characterisation of some Tunisia virgin olive oils obtained from different cultivars growing in Sfax National Collection. J. Food Agric. Environ. 2007;5:17–21.

[47] Matos LC, Cunha SC, Amaral JS, Pereira JA, Andrade PB, Seabra RM, Oliveira B. Chemometric characterization of three varietal olive oils (cvs. Cobranc-osa, Madural and Verdeal Transmontana) extracted from olives with different maturation indices. Food Chem. 2007;102:406–414.

[48] Bucci R, Magri A.D, Magri AL, Marini D, Marini F. Chemical authentication of extra virgin olive oil varieties by supervised chemometric procedures. J. Agric. Food Chem. 2002;50:413–418.

[49] Diaz GT, Meras DI, Casas SJ, Franco MF. Characterization of virgin olive oils according to its triglycerides and sterols composition by chemometric methods. Food Control. 2005;16:339–347.

[50] Angerosa F, Basti C, Vito R. Virgin olive oil volatile compounds from lipoxygenase pathway and characterization of some Italian cultivars. J. Agric. Food Chem. 1999;47:836–839.

[51] Ercisli S, Barut E, Ipek A. Molecular characterization of olive cultivars using amplified fragment length polymorphism markers. Genet. Mol. Res. 2009;8:414–419.

[52] Owen CA, Bita EC, Banilas G, Hajjar SE, Sellianakis V, Aksoy U. AFLP reveals structural details of genetic diversity within cultivated olive germplasm from the Eastern Mediterranean. Theoret. Appl. Genet. 2005;110:1169–1176.

[53] Nikoloudakis N, Banilas G, Gazis F, Hatzopoulos P, Metzidakis J. Discrimination and genetic diversity among cultivated olives of Greece using RAPD markers. J. Am. Soci. Hortic. Sci. 2003;128:741–746.

[54] Bautista R, Crespillo R, Canovas FM, Claros MG. Identification of olive-tree cultivars with SCAR markers. Euphytica. 2003;129:33–41.

[55] Diaz A, De la Rosa R, Martin A, Rallo P. Development, characterization and inheritance of new microsatellites in olive (Olea europaea L.) and evaluation of their usefulness in cultivar identification and genetic relationship studies. Tree Genet. Genomes. 2006;2:165–175.

[56] Montemurro C, Simeone R, Pasqualone A, Ferrara E, Blanco A. Genetic relationships and cultivar identification among 112 olive accessions using AFLP and SSR markers. J. Hortic. Sci. Biotechnol. 2005;80:105–110.

[57] Terzopoulos PJ, Kolano B, Bebeli PJ, Kaltsikes PJ, Metzidakis I. Identification of Olea europaea L. cultivars using inter-simple sequence repeat markers. Sci. Hortic. 2005;105:45–51.

[58] Reale S, Doveri S, Diaz A, Angiolillo A, Lucentini L, Pilla F., et al. SNP based markers for discriminating olive (Oleaeuropaea L.) cultivars. Genome. 2006;49:1193–1205.

[59] Consolandi C, Palmieri L, Doveri S, Maestri E, Marmiroli N, Reale S., et al. Olive variety identification by ligation detection reaction in a universal array format. J. Biotechnol. 2007;129:565–574.

[60] Pafundo S, Busconi M, Agrimonti C, Fogher C, Marmiroli N. Storage time effects on olive oil DNA assessed by amplified fragments length polymorphisms. Food Chem. 2010;123:787–793.

[61] Vos P, Hogers R, Bleeker M, Reijans M, Van de Lee T, Hornes M, Frijtens A, Pot J, Peleman J, Kuiper M, Zabeau M. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 1995;23:4407–4414.

[62] Tautz D. Hypervariability of simple sequences as general source for polymorphic DNA markers. Nucleic Acids Res. 1989;17:6463–6471.

[63] Pasqualone A, Di Rienzo V, Blanco A, Summo C, Caponio F, Montemurro C. Characterization of virgin olive oil from Leucocarpa cultivar by chemical and DNA analysis. Food Res. Int. 2012;47:188−193.

[64] Corrado G, Imperato A, La Mura M, Perri E, Rao R. Genetic diversity among olive varieties of southern Italy and the traceability of olive oil using SSR markers. J. Hortic. Sci. Biotechnol. 2011;86:461–466.

[65] Pasqualone A, Montemurro C, Caponio F, Blanco A. Identification of virgin olive oil from different cultivars by analysis of DNA microsatellites. J. Agric. Food Chem. 2004;52:1068–1071.

[66] Vietina M, Agrimonti C, Marmiroli M, Bonas U, Marmiroli N. Applicability of SSR markers to the traceability of monovarietal olive oils. J. Sci. Food Agric. 2011;91:1381–1391.

[67] Montemurro C, Miazzi MM, Pasqualone A, Fanelli V, Sabetta W, Di Rienzo V. Traceability of PDO olive oil "Terra di Bari" using high resolution melting. J. Chem. 2015;DOI: http://dx.doi.org/10.1155/2015/496986.

[68] Ben Ayed R, Ben Hassen H, Ennouri K, Ben Marzoug R, Rebai A. OGDD (Olive Genetic Diversity Database): A microsatellite markers' genotypes database of worldwide olive trees for cultivar identification and virgin olive oil traceability. Database. 2016;DOI: 10.1093/database/bav090.

[69] Brookes AJ. The essence of SNPs. Gene. 1999;234:177–186.

[70] Kalogianni DP, Bazakos C, Boutsika LM, Ben Targem M, Christopoulos TK, Kalaitzis P, Ioannou PC. Olive oil DNA fingerprinting by multiplex SNP genotyping on fluorescent microspheres. J. Agric. Food Chem. 2015;63:3121–3128.

[71] Bazakos C, Khanfir E, Aoun M, Spano T, El Zein Z, Chalak L, El Riachy M, Abou-Sleymane G, Ben Ali S, Kammoun N, Kalaitzis P. The potential of SNP-based PCR-RFLP

capillary electrophoresis analysis to authenticate and detect admixtures of Mediterranean olive oils. Electrophoresis. 2016;DOI: 10.1002/elps.201500537.

[72] Ramazzotti M, Mulinacci N, Pazzagli L, Moriondo M, Manao G, Vincieri FF, Degl'Innocenti D. Analytic investigations on protein content in refined seed oils: Implications in food allergy. Food Chem. 2008;46:3383–3388.

[73] Arlorio M, Coisson JD, Bordiga M, Travaglia F, Garino C, Zuidmeer L. Olive oil adulterated with hazelnut oils: Simulation to identify possible risks to allergic consumers. Food Addit. Contam.: Part A. 2010;27:11–18.

[74] De Ceglie C, Calvano CD, Zambonin CG. Determination of hidden hazelnut oil proteins in extra virgin olive oil by cold acetone precipitation followed by in-solution tryptic digestion and MALDI-TOF-MS analysis. J. Agric. Food Chem. 2014;62:9401–9409.

[75] Brezna B, Hudecova L, Kuchta T. A novel real-time polymerase chain reaction (PCR) method for the detection of walnuts in food. Eur. Food Res. Technol. 2006;223:373–377.

[76] Koppel R, Velsen-Zimmerli F, Bucher T. Two quantitative hexaplex real-time PCR systems for the detection and quantification of DNA from twelve allergens in food. Eur. Food Res. Technol. 2012;235:843–852.

[77] Savolainen V, Chase MW. A decade of progress in plant molecular phylogenetics. Trends Genet. 2003;19:717–724.

[78] Gielly L, Taberlet P. The use of chloroplast DNA to resolve plant phylogenies: Noncoding versus rbcL sequences. Mol. Biol. Evol. 1994;11:769–777.

[79] James D, Schmidt AM. Use of an intron region of a chloroplast tRNA gene (trnL) as a target for PCR identification of specific food crops including sources of potential allergens. Food Res. Int. 2004;37:395–402.

[80] Rønning SB, Rudi K, Berdal KG, Holst-Jensen A. Differentiation of important and closely related cereal plant species (Poaceae) in food by hybridization to an oligonucleotide array. J. Agric. Food Chem. 2005;53:8874–8880.

[81] Spaniolas S, May ST, Bennett MJ, Tucker GA. Authentication of coffee by means of PCR-RFLP analysis and lab-on-a-chip capillaryelectrophoresis. J. Agric. Food Chem. 2006;54:7466–7470.

[82] Spaniolas S, Tsachaki M, Bennett MJ, Tucker GA. Toward the authentication of wines of Nemea denomination of origin through cleaved amplified polymorphic sequence (CAPS)-based assay. J. Agric. Food Chem. 2008;56:7667–7671.

[83] Syvanen AC. Accessing genetic variation: Genotyping single nucleotide polymorphisms. Nat. Rev. Genet. 2001;2:930–942.

[84] Reed GH, Wittwer CT. Sensitivity and specificity of single-nucleotide polymorphism scanning by high-resolution melting analysis. Clinical Chem. 2004;50:1748–1754.

[85] Vietina M, Agrimonti C, Marmiroli N. Detection of plant oil DNA using high resolution melting (HRM) post PCR analysis: A tool for disclosure of olive oil adulteration. Food Chem. 2013;141:3820–3826.

[86] Wu Y, Zhang H, Han J, Wang B, Wang W, Ju X, Chen Y. PCR-CE-SSCP applied to detect cheap oil blended in olive oil. Eur. Food Res. Technol. 2011;233:313–324.

[87] Agrimonti C, Vietina M, Pafundo S, Marmiroli N. The use of food genomics to ensure the traceability of olive oil. Trends Food Sci. Technol. 2011;22:237–244.

Section 3

Consumers Perception

Chapter 8

Evaluation of the "Harmony Value": A Sensory Method to Discriminate the Quality Range within the Category of EVOO

Annette Bongartz , Martin Popp ,
Reinhard Schneller and Dieter Oberg

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64727

Abstract

Besides a certain amount of relevant chemical parameters, objective quality of olive oil as well as consumer acceptance are depending mainly on its sensory characteristics. Referring to the EC Regulation 1833/2015, there exist different quality categories for olive oil, namely extra virgin, virgin and lampant. To belong to the category "extra virgin" olive oil (EVOO), an oil has to have a certain fruitiness (median > 0) and no defects (median = 0). This means that all olive oils without defect have the same quality level (extra virgin) no matter what kind of sensory characteristics they show. Within EVOOs, type and width of the parameter values of sensory descriptors show a broad variety. In order to mark differences between sensory characteristics in olive oil, the German and the Swiss Olive Oil Panel (DOP and SOP) further developed the panel test (according to EC regulation 1833/2015) by extending their profile sheet with additional sensory parameters, e.g. the "harmony"-value. The evaluation and interpretation of the "harmony" value of olive oils make it possible to monitor and thereby discriminate the sensory quality within the range of EVOOs on the market. This is important for all stakeholders in the olive oil business, aiming to produce, sell, provide and buy EVOOs at different price (and quality) levels.

Keywords: olive oil, harmony, sensory evaluation, aroma description, profiling, persistency

1. Introduction

Within the context of food production and food consumption, intrinsic product quality is defined as a product being free from defects, deficiencies and/or significant variations. The ISO 8402-1994 standard [1], for example, defines quality as the totality of characteristics (intrinsic and extrinsic) of a product that are able to satisfy stated or implied requirements.

In food production, usually strict and consistent commitments and specifications lead to a certain standardization and uniformity in order to satisfy various requirements from producers and consumers. These requirements are mainly defined by intrinsic product factors such as objective sensory characteristics as well as consumer's acceptance, preference and expectation for these characteristics. Extrinsic product factors are additionally able to define at least a certain portion of the overall quality of food products, such as packaging size or product price.

How about olive oil? Referring to the EC Regulation 2568/91 in its actual version [2] (that is EC Regulation 1833/2015 [3]), there do exist different classification levels for the identification of olive oil quality in Europe. In two categories, the so-called "virgin" olive oils are defined as oils that are obtained directly from olives and solely by mechanical means. Olive oil that is produced according to this standard can be branded either as "extra virgin" or as "virgin". Intrinsic product quality of olive oil depends especially on sensory characteristics perceived during an objective evaluation and a professional tasting. Apart from a certain range of chemical parameters within threshold values that have to be fulfilled to a different level in both categories (virgin and extra virgin), several sensory requirements are defined in the EC Regulation and have to be evaluated in a "panel test". All "virgin" olive oils have to show certain fruitiness (median > 0). In addition to this, olive oil in the category "extra virgin" has to show a total absence of defects (median = 0), whereas olive oil in the category "virgin" is allowed to have a certain amount of defects (median > 0 and < 3.5). Both chemical and sensory requirements have to be fulfilled in order to get classified into the described categories. If sensory characteristics fail, based on the evaluation within a panel test, an immediate downgrade of the olive oil is obligatory. That means that from a regulatory point of view sensory evaluation of relevant parameters has the same importance to clarify the intrinsic product quality compared with chemical analysis. But, chemical analysis is almost not able to detect sensory defects, except changes due to oxidation processes. Threshold values for the defined chemical requirements normally can be met quite easily. As a consequence, sensory evaluation in this context has far more "power".

The classification according to the EC Regulation 2568/91 in its actual version [2] has, from a sensory point of view, only the potential to make a separation between defective olive oils and oils that do not show any sensory defects. Defective olive oils are classified as either virgin or lampante olive oils, depending on the intensity of their organoleptic defects. Within the range of extra virgin olive oils, no further discrimination due to their different sensory/aromatic qualities takes place. By application of the EC Regulation, it is not possible to characterize virgin olive oil by its sensory properties and thereby discriminate between olive oil with a higher sensory quality (e.g. rather complex aromatic and harmonious impression) and olive oil with a lower quality, but still free from defects (e.g. rather flat and inharmonious).

http://dx.doi.org/10.5772/64727

This does not meet reality. When we have a look especially on the quality range of extra virgin olive oils (EVOOs), modality as well as intensity of the parameter values of different sensory descriptors show a very broad spectrum. This sensory diversity of olive oil is depending on many different factors like olive variety, soil properties, climate conditions, effective time of harvest, type of harvest and production, blending procedures, storage conditions. Additionally, the experience and expertise of experts and specialists, involved along the various steps of the value chain for olive oil production, does contribute to the final (sensory) quality of olive oil in the bottle. To make this sensory diversity among EVOOs transparent and visible, there is need for additional and appropriate sensory quality factors.

In order to point out differences between olive oils that show various and different sensory characteristics, the German Olive Oil Panel (DOP) and the Swiss Olive Oil Panel (SOP) together developed additional sensory parameters [4] and extended their profile sheet compared with the official panel test [3]. The focus of the enhancement lies especially in a detailed profiling of the oils fruitiness, respectively, the various aromatic impressions that are perceivable during tasting. Using this extended profile sheet, olive oils are not only grouped in a cluster of "green" and "ripe" oils (as it is done in the panel test), but are additionally described in more detail with respect to their relevant aromatic characteristics (descriptors) and their intensity. This detailed flavour description is an important foundation for the evaluation of an additional criterion, the so-called harmony value. The harmony value first of all describes the extent and degree of balance between the three positive sensory characteristics: fruitiness, bitterness and pungency. Moreover, the harmony value incorporates the evaluation of the clarity, intensity and complexity of the aromatic profile as well as the persistency of the overall positive impressions.

According to the existing legislation, extra virgin olive oils (EVOOs) in Europe more or less fulfil the required sensory quality criteria. But all these oils for sure show differences in a broad range with respect to their aromatic characteristics and their harmony value. Therefore, the current situation on the olive oil market is not sufficient. In order to make sensory differences between olive oils more transparent and comprehensible, the European authorities should facilitate the improvement of the current legislation by adding additional quality criteria like the harmony value to the official panel test.

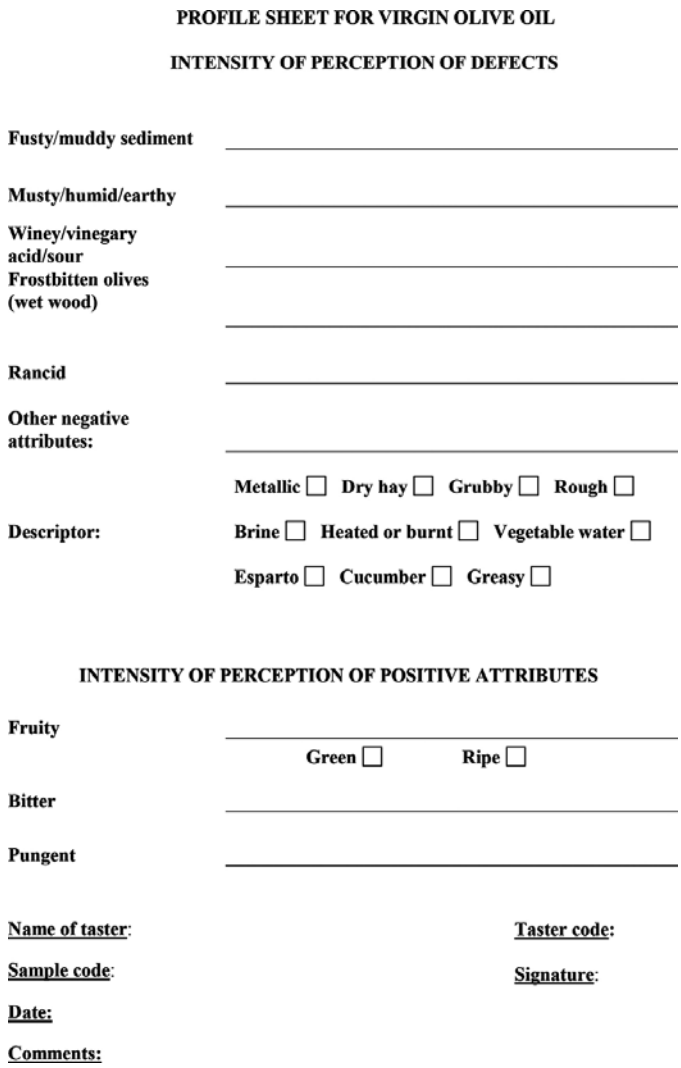
This scientific paper takes a closer look at this situation and the relevance of the harmony value as an additional quality factor for the discrimination of olive oils within the quality range of EVOOs.

2. Materials and methods

2.1. Sensory panellists (panel)

In order to undertake the sensory evaluation of extra virgin olive oils (EVOO), officially accredited panels are needed. These panels consist of at least 8–12 well-trained olive oil tasters. The accreditation according to the standard DIN EN ISO 17025 [5] and corresponding

guidelines [6] is required to receive the registration of the relevant national governmental institutions and thereby offers the possibility to take part in the annual proficiency tests of the International Olive Council (IOC) in Madrid. If a panel passes all the steps, it will be recognized by the IOC as an official test panel for the time period of one year. Round about 60 IOC-recognized panels do exist all over the world.



PROFILE SHEET FOR VIRGIN OLIVE OIL

INTENSITY OF PERCEPTION OF DEFECTS

Fusty/muddy sediment _____

Musty/humid/earthy _____

Winey/vinegary acid/sour _____

Frostbitten olives (wet wood) _____

Rancid _____

Other negative attributes: _____

Descriptor: Metallic ☐ Dry hay ☐ Grubby ☐ Rough ☐ Brine ☐ Heated or burnt ☐ Vegetable water ☐ Esparto ☐ Cucumber ☐ Greasy ☐

INTENSITY OF PERCEPTION OF POSITIVE ATTRIBUTES

Fruity _____ Green ☐ Ripe ☐

Bitter _____

Pungent _____

Name of taster: **Taster code:**

Sample code: **Signature**:

Date:

Comments:

Figure 1. Basic profile sheet [3].

Two of these panels are the German Olive Oil Panel (DOP, founded 1998) and the Swiss Olive Oil Panel (SOP, founded 2002). They carry out sensory evaluations of olive oil either as Central Location Tests (CLT) in a sensory laboratory, respectively, another adequate testing facility as described in common sensory science textbooks (e.g., see [7] or [8]) or the evaluation takes

http://dx.doi.org/10.5772/64727

place "virtually". In this case, the tasters work at home on their own test desks in strict accordance with the official regulations and submit single results online to the panel supervisor (PSV) for final calculation of the panel results.

Both panels cooperate closely concerning training units, exchange of samples and sensory experiences. Normally one of several training units per year takes place in an olive oil producing country for exchanging practical experiences with local test panels in combined training sessions as well as to share knowledge with local producers concerning sensory typicality of different olive oils produced from different varieties.

2.2. Basic sensory evaluation of virgin olive oils (panel test)

Inside the European Union (EU), two different quality levels can be distinguished for virgin olive oils, namely: "extra virgin olive oil" (EVOO) and "virgin olive oil" (VOO). According to the EC Regulation 2568/91 in its actual version [2], chemical limitations as well as sensory parameters (panel test, **Figure 1**) have to be respected in order to get classified in these categories. In **Table 1**, the sensory requirements for EVOO and VOO (and OO) are described. Here can be seen that passing the panel test with a median of fruitiness > 0 and a median of defects = 0 leads to the classification as EVOO, but from a sensory point of view this confirms only the fulfilment of minimal quality standard requirements.

Classification categories	Abbreviation	Sensory requirements and characteristics	Description
Extra virgin olive oil	EVOO	• Median of defects = 0 • Median of fruitiness > 0	• EVOO is the superior category olive oil, obtained directly from olives and solely by mechanical means. • EVOO shows a certain fruitiness, but no defects whatsoever
Virgin olive oil	VOO	• Median of defects < 3.5 • Median of fruitiness > 0	• VOO is olive oil obtained directly from olives and solely by mechanical means • VOO shows a certain fruitiness and may show defects up to an intensity of 3.5
Olive oil "lampante"	OO	• Median of defects > 3.5 • Median of fruitiness > 0 or • Median of defects > 6.0 • Median of fruitiness = 0	• "Lampante" olive oil is not marketable. It has to be further processed → OO • OO is oil comprising exclusively olive oils that have undergone refining and oils obtained directly from olives (EVOO or VOO)

Table 1. Classification categories [2].

Any aromatic differences or other objective descriptors are not taken into consideration, when classifying olive oil. Miscellaneous olive oils, no matter what kind of specific sensory characteristics the oils show, have only to meet the mentioned requirements in order to be branded within the very same quality-level "extra virgin".

2.3. Extended sensory evaluation of virgin olive oils

To discriminate between olive oils within the category of "extra virgin", the methodology, compared with the official panel test [3], was developed further by extending the profile sheet

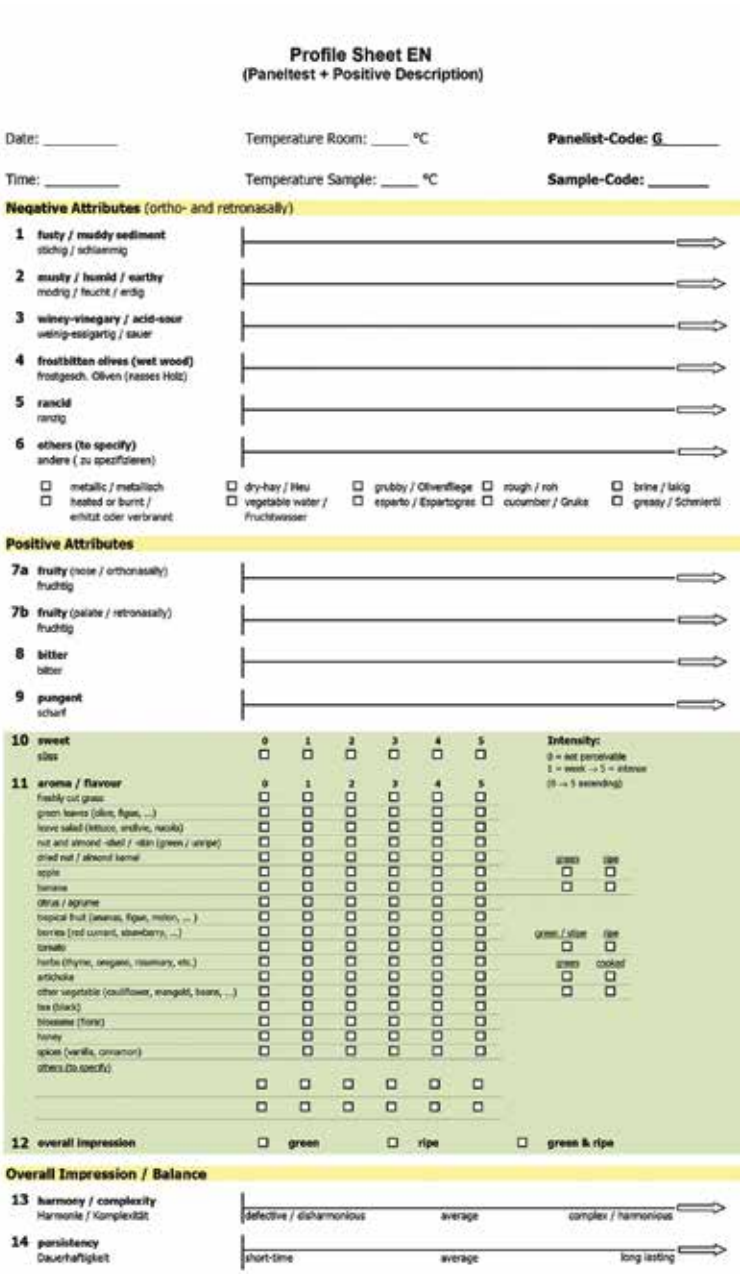


Figure 2. Extended profile sheet [4].

http://dx.doi.org/10.5772/64727

(**Figure 2**) with additional sensory parameters [4]. This methodology is well established within the accredited quality-management system of DOP and SOP.

First of all, a detailed profiling of the oils fruitiness with their various aromatic impressions that are perceivable during tasting is done. In order to characterize their aromatic specificity, tasters describe the oils in detail with respect to the relevant aromatic descriptors as well as their intensity (**Table 2**). Descriptors can be grouped as "green", "ripe" and "green and ripe". The aroma description is done on unipolar 6-point category scales (0–5), for one or more aroma components. Rating 0 (zero) means that there is no perceivable sensation. Ratings of 1 or 2 stand for a "slight" sensation. A rating of 3 represents a "noticeable" or "medium intensity". And finally the ratings of 4 or 5 describe an "intense" sensation of the respective aroma component.

Aroma descriptors	Specification	Examples
Freshly cut grass		
Green leaves		Olive leaves, fig leaves
Leaves salad		Lettuce, endive, arugula
Nut and almond shell/skin	Green/unripe	
Nut/almond kernel	Dried	
Apple	Green or ripe	
Banana	Green or ripe	
Citrus fruits		
Tropical fruit		Bananas, fig, melon
Berries		Red currant, strawberry
Tomato	Green or ripe	Green or ripe
Herbs		Thyme, oregano, rosemary
Artichoke	Green or cooked	
Other vegetables	Green or cooked	Cauliflower, mangold, beans
Tea		Black tea leaves
Blossoms		Floral impression
Honey		
Spices		Vanilla, cinnamon

Table 2. Relevant aroma-descriptors for olive oil.

To describe the aroma of olive oils, panellists can either use a flavour wheel for olive oil [9] (**Figure 3**) that shows relevant aroma descriptors, or they can memorize these descriptors in order to be able to do the harmony evaluation "directly" (this is the case for the DOP). The

other possibility is that panellists use a detailed profile sheet (**Figure 2**) including all descriptors and the related 6-point category scales for describing the aroma profile (this is the case for the SOP). Aroma description is an integral part of the evaluation of the harmony value.

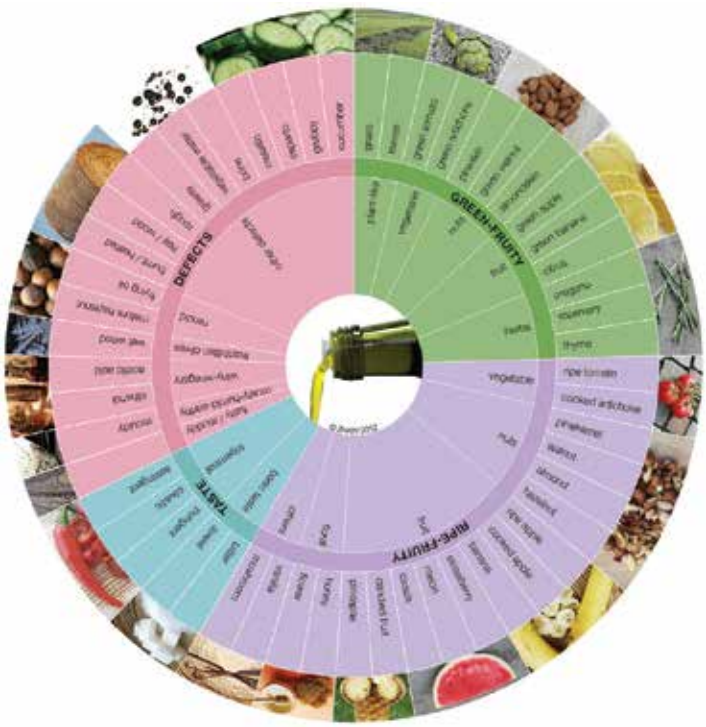


Figure 3. ZHAW flavour wheel for olive oil [9].

The harmony value first of all describes the relation of odour and taste. The extent and degree of balance between the three well-known positive characteristics for olive oil: fruitiness, bitterness and pungency, are very important. Knowing that olive oils fruitiness can be either one-dimensional or rather complex, the harmony value incorporates additionally a detailed description of the intensity and diversity of single aroma components (green and ripe). The recording of all these descriptors finally lead to an overall impression of clarity, intensity, complexity and persistency.

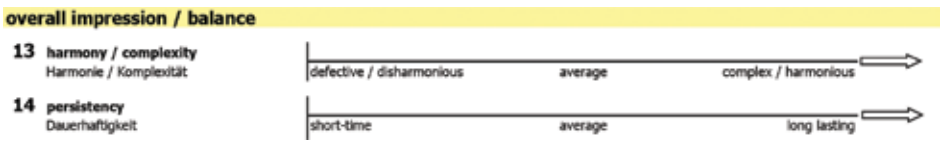


Figure 4. Harmony scale [4] (extract from **Figure 2**).

In order to evaluate the harmony value, a bipolar 10-cm scale is used (see **Figures 2** and **4**). The interpretation of harmony values is possible as shown in **Table 3**.

Median "harmony"	Rating	Definition/description
0	VOO	• Median of defects >0 (panel test)
0.1 – 3.0	EVOO/Not acceptable	• Median of defects = 0 (panel test), but notation of single defect-assumptions • Overall characteristics are absolutely unbalanced and inharmonious
3.1 – 4.4	EVOO/Not sufficient	• Overall characteristics are rather unbalanced and inharmonious • Flavour is rather one-sided (if any at all) • Rare pleasant aspects do not last very long respectively are not very persistent
4.5 – 5.0	EVOO/Lower standard	• Oil shows an average quality → "just in" • Overall characteristics are more or less balanced and quite harmonious • Flavour diversity is rather narrow → average • Some pleasant aspects do not last long respectively are not persistent
5.1 – 5.4	EVOO/Upper standard	• Oil shows an average quality → "well in" • Overall characteristics are balanced and harmonious • Flavour diversity is getting broader → still average • Some pleasant aspects last a bit longer respectively are a bit more persistent
5.5 – 6.4	EVOO/Good	• Overall characteristics are well balanced and harmonious • Flavour diversity is getting broader • Many pleasant aspects last a bit longer respectively are a bit more persistent
6.5 – 7.5	EVOO/Very good	• Overall characteristics are very well balanced and harmonious • Flavour diversity is broad • Many pleasant aspects last longer respectively are more persistent
7.6 – 10.0	EVOO/Excellent	• Characteristic of oil is perfectly balanced and harmonious • Flavour diversity is very complex • Many pleasant characteristics last very long respectively are very persistent

Table 3. Terminology and interpretation/rating of the harmony value.

3. Results and discussion

Olive oils within the category of EVOO that are traded as private labels or "low-price" brands do not raise high expectations concerning their sensory characteristics. The generally more expensive and the so-called premium class olive oils stimulate such imaginations. Despite that,

they are all declared as "extra virgin". This leads to a lack of objective transparency. There is no chance for the producer and retailer to promote differences concerning the sensory quality of his EVOOs. And consumers have no possibility to recognize these differences between EVOOs, except by buying and tasting the oils.

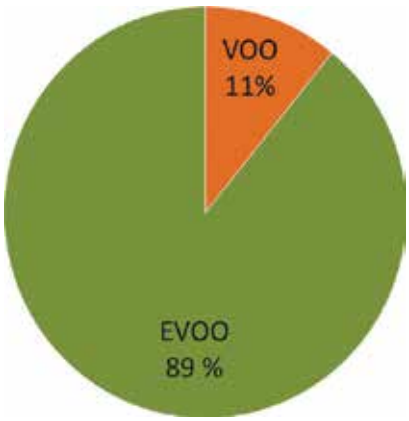


Figure 5. Percentage of samples (n = 2736), evaluated in the years 2011–2015, subdivided due to their classification into the official quality categories EVOO (n = 2434) and VOO (n = 302) [2].

In the years 2011 until 2015, the DOP and SOP evaluated overall 2736 olive oils, including 2161 oils directly from the market and 575 oils from the competition "Olive Oil Award (OOA)", concerning their sensory characteristics. **Figure 5** shows that 89% of all evaluated olive oils belonged to the category of EVOO and 11% could only be rated as VOO, despite their declaration as EVOO.

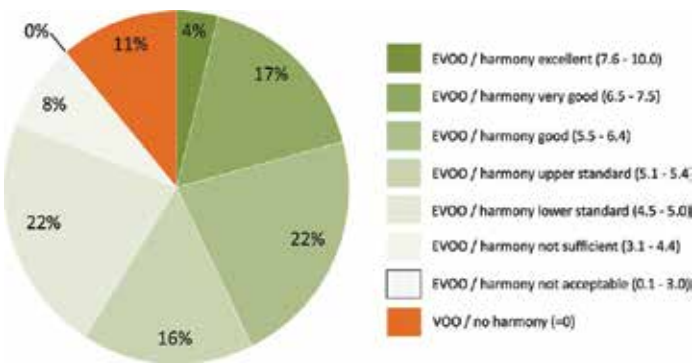


Figure 6. Percentage of oil samples (n = 2736), additionally discriminated within the category of EVOO, using the harmony value.

Looking at the different qualities within the range of EVOOs in the same time period, **Figure 6** explains the sensory diversity with respect to the harmony value.

http://dx.doi.org/10.5772/64727

3.1. Olive oil quality in European supermarkets/discounters (IGO study)

Low-price private labels and even some low-price brands of extra virgin olive oils generally sold in supermarkets and discounters on the European market have a rather bad image due to their low sensory quality. Within the European Union (EU), the share in private labels and low-price brands for olive oil (including the categories EVOO, VOO and OO) is assumed to be about 75%. This means that approx. 200 million consumers buy olive oil from these categories.

As described in Section 2, an olive oil belongs to the category EVOO if an IOC-recognized panel, undertaking an official panel test, states that it shows no defects (median = 0) and at least a certain fruitiness (median > 0). A confirmed classification as EVOO does not take into account the wide range of sensory qualities within the category of EVOO. The result is that discrimination between premium EVOOs and EVOOs with a lower quality standard is not possible. But, using the harmony value as an additional test criterion allows the differentiation of sensory qualities within the category of EVOO as excellent, very good, good, standard (upper/lower), sufficient and not acceptable (**Table 3**).

The study at hand took place during April and September 2015 [10]. Altogether, 70 olive oil samples, representing different origins (→ EU blends as well as oils from Spain, Italy and Portugal), were sampled in 15 different European olive oil markets/countries (→ Austria, Belgium, Denmark, Finland, France, Germany, Italy, Poland, Spain, Sweden, The Netherlands, United Kingdom and UK/Ireland and Switzerland). All olive oil samples did belong to the low-price offers in supermarkets/discounters and were labelled as EVOO. Best-before dates showed a range between October 2015 and February 2017.

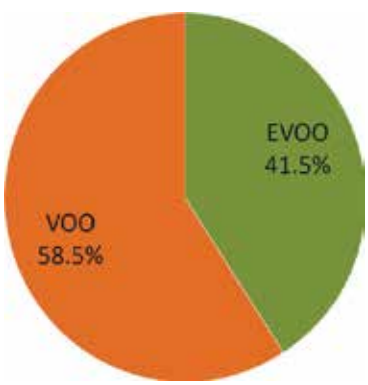


Figure 7. Percentage of samples (n = 70), subdivided into the official quality categories EVOO (n = 29) and VOO (n = 41) [2].

The samples were tested by five accredited and IOC-recognized sensory panels, which were Chemlab of the Ministry of Consumer Protection in Athens (GR); Chemiservice SrL in Monopoli (IT); German Olive Oil Panel, Wessling (DE); Swiss Olive Oil Panel, Waedenswil (CH); and Instituto Superiore de Agronomia, Lisboa (PT). Out of the panel tests, only 41.4%

(29/70) of the oil samples were confirmed as EVOOs. The majority of 58.6% (41/70) had to be downgraded to the category VOO (**Figure 7**), due to certain defects, of which 24.4% (10/41) could be traced back to oxidation processes. With respect to this, almost 50% (20/41) of all defective oils ensured rather unrealistic shelf life of 24 months and more.

Additionally, a chemical analysis was determined, carried out by near-infrared (NIR) spectroscopy. NIR spectroscopy is based on the absorption of electromagnetic radiation at wavelengths in the range 780–2500 nm and is a secondary method requiring calibration against a reference method for the constituent of interest. As a consequence of the physics of diffuse transmittance and reflectance and the complexity of the spectra, calibration is normally carried out using multivariate mathematics (chemometrics). In this case, the calibration included results from over 2500 olive oil samples and results, in comparison with chemical reference analyses, were excellent.

Altogether, only 15.7% (11/70) of all olive oil samples in the study did not meet the official chemical requirements, but at least 58.6% (41/70) of all samples did show sensory defects. This is only a portion of 26.8% (11/41). Thereby, the special importance and "power" of sensory evaluation (panel test) in clarifying the intrinsic product quality of olive oil is well confirmed.

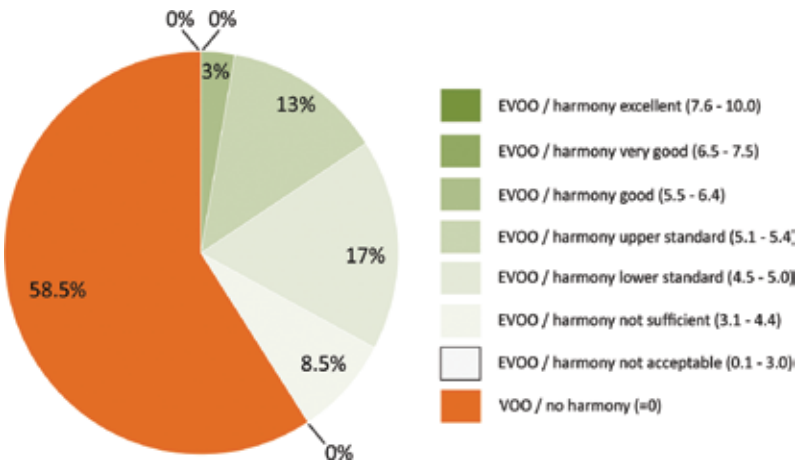


Figure 8. Percentage of oil samples (n = 70), additionally discriminated within the category of EVOO, using the harmony value.

Especially in the fought markets for low-price private labels and low-price brands of extra virgin olive oils, a lack of sensory controlling in various importing and exporting countries is responsible for this non-satisfying situation on the European market. Results of this study show that it is not possible to master the situation and to ensure a correct categorization with a focus on chemical analysis. On the contrary, there is urgent need for an additional sensory test criterion that is capable to differentiate relevant sensory quality in a comprehensible way right at the "borderline" between VOO and EVOO.

http://dx.doi.org/10.5772/64727

By using the quality factor "harmony" as an additional test criterion, 29 out of 70 confirmed EVOOs could be discriminated into different quality levels (41.4%). The results show even in this low-price EVOO segment offered in 15 distributing EU countries (including Switzerland) a wide range of different sensory quality ratings: 2.9% (2/29) good, 12.9% (9/29) upper standard, 17.1% (12/29) lower standard, 8.6% (6/29) not sufficient (**Figure 8**).

3.2. Impact of the harmony value on quality discrimination of EVOOs on the German market

EVOOs made from different olive varieties show in their different stages of maturity different intensities for fruitiness, bitterness and pungency. Moreover, many chemical parameters vary because of the same reason. The sensory evaluation (panel test) according to the EC Regulation [2] can confirm these differences in terms of intensity, but in the end will classify all olive oils that show no defects, as EVOO, no matter what kind of aromatic profile they show and how complex and well balanced the various positive characteristics of these oils are.

Approx. 70–80% of all imported olive oils of the Germany market are sold as "private label" olive oils (trade brands). Set up mainly as EU Blends, these oils are sold correctly as EVOOs. The remaining 20–30% of olive oils on the German market cover a higher up to premium quality level within the category EVOO, resulting in the assignment of these oils to a higher price segment.

The fact that large German importers and distributors became sensitized in order to set benchmarks for different quality levels had a big impact on the overall quality of olive oil on the German market. The study at hand shows the development during the years 2011–2014.

The ambitious aim to improve the olive oil quality on the German market first of all led to the necessity to expulse defective olive oils from the category EVOO and thereby from the German market. Back in the 1990s, too many of the olive oils declared as EVOO still had sensory defects and therefore were downgraded to the category of VOO. Defective oils in general are not rated on the harmony scale or in other words the harmony level of defective oils is set to "zero" (0). During the following years, still some EVOOs were just reaching values between 0 and 3.0 (not acceptable) as well as between 3.1 and 4.4 (not sufficient) on the harmony scale, and therefore lacked in the defined sensory quality from importers and distributers in Germany. The aimed harmony level was set to a value of >4.5, which is understood in minimum as lower standard quality. The more sensitized the involved partners (importers, distributors) got, the higher harmony values were set by them in order to achieve higher quality and to avoid too high risks of falling below the level of 4.5. In the meantime, most importers and distributers aim at harmony levels of >5, but still reasonably priced.

Results of the study at hand show that the quality factor "harmony" was capable to change olive oil quality in the lower price segments on the German market over time (**Figure 9**). For example in 2011, 40.2% (162/403) olive oil samples of the German market, analysed by the DOP, only reached a lower standard harmony of 4.5–5.0. Until 2014 and due to a continuous controlling, the amount of lower standard harmony oils could be reduced to 20.9% (77/368).

At the same time, the amount of harmony values >5 could be augmented up to 62.2% (229/368), which comprise upper standard harmony oils with 25.3% (93/368), good harmony olive oils with 31.3% (115/368), very good ones with 3.8% (14/368) and excellent ones with 1.9% (7/368). All stakeholders did contribute to this increase in olive oil quality in favour of the consumer.

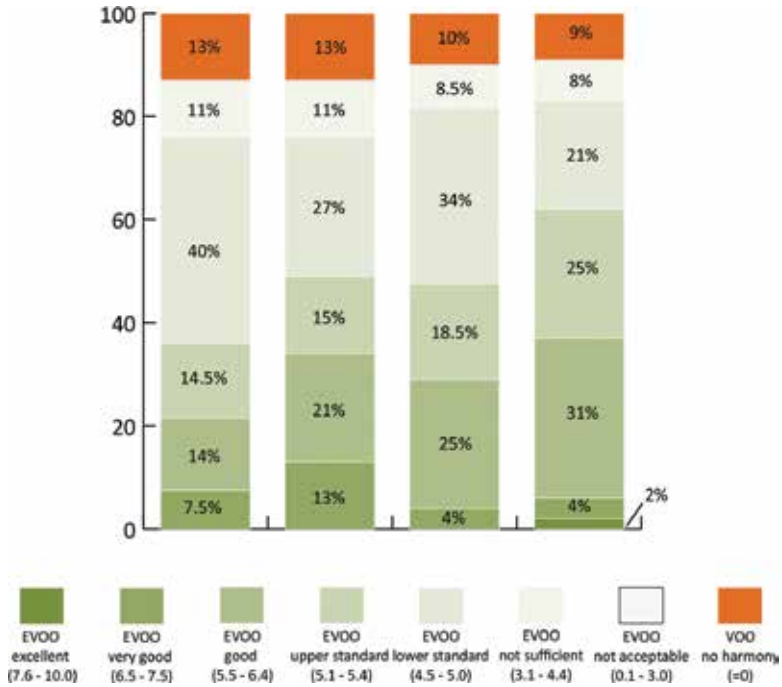


Figure 9. Improvement of sensory quality on the German olive oil market (years 2011–2014), using the harmony value.

In general, the DOP surveys 400–500 samples per year. The impact of this continuous controlling over the last years and thereby the improvement of the olive oil quality, especially in the private label segment, on the German market can be confirmed. A stable quality at lower harmony levels for a low-price market-segment and the horizontal enlargement of higher harmony values improved the olive oil market in general. This confirms the fact that olive oils in Germany are sold generally in a reasonable cost/performance ratio.

3.3. Potential of the harmony value to discriminate EVOOs by quality within the premium segment and/or olive oil competitions

As seen in Sections 3.1 and 3.2, olive oils that are, for example, on the market as private label olive oils and low-price brands can be discriminated within the quality range of EVOOs with the help of the quality factor "harmony".

Since 2002, the research project "Olive Oil Award—Zurich" (OOA) of the Zurich University of Applied Sciences (ZHAW) invites producers, importers and retailers to participate in an annual olive oil competition. Per year round about 100–150 olive oils from the Swiss (and other

http://dx.doi.org/10.5772/64727

northern European) markets as well as samples registered directly from producing countries are evaluated with a focus on their sensory quality.

The sensory assessment of the OOA is organized in a 3-step procedure. The first step has only orientating character to identify rough defects and the approximate intensity of fruitiness. This information is necessary to define correct presentation designs for the following test sessions but has no statistical influence on the final results. As second step, an extended panel test takes place. At least sensory panels of eight tasters each, recruited out of the SOP, do the sensory evaluation in blind tastings. The sensory assessment is based on the panel test according to the EC Regulation 2568/91 in its actual version [2] and is extended with an aroma description and the evaluation of the "harmony value". The third step of the procedure is the repetition of step two in order to confirm the sensory results and is at the same time the basis for granting awards in gold, silver and bronze for olive oils with very high harmony values.

Due to the evaluation of the quality factor "harmony," discrimination between different quality levels within the range of EVOO is possible. Compared with Sections 3.1 and 3.2, where a disproportionate number of oil samples were rated as "standard" (harmony levels of 4.5–5.4), here a disproportionate number of oils get higher ratings, for example good harmony levels (5.5–6.4), very good harmony levels (6.5–7.4) or even excellent harmony levels (>7.5).

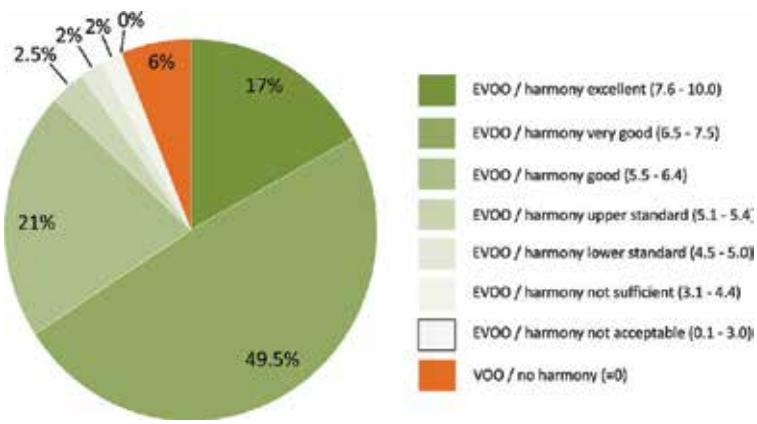


Figure 10. Percentage of oil samples (n = 575 of which n = 34 VOO and n = 551 EVOO) over the years 2011–2015, discriminated additionally within the category of EVOO, using the harmony value.

Results in **Figure 10** show that in the years 2011 until 2015 altogether 6% (34/575) of the oils evaluated within the OOA had to be downgraded as VOOs, but 94% of the oils (541/575) were classified as EVOOs and additionally could be discriminated to their different quality levels within the range of EVOO. Only 7% of the oils showed a quality level that could be characterized as not sufficient (11/575), lower standard (11/575) or upper standard (15/575). All other oils were rated with higher harmony values, beginning with 21% with a harmony level of 5.5–6.4 as good (121/575), 49% in the harmony range of 6.5–7.4 as very good (284/575) and finally 17% in the harmony range of >7.5 as excellent (99/575).

Within this highest category of olive oils, rated with excellent harmony values higher than 7.5, a discrimination of sensory quality is possible and leads to a justified decision in order to honour olive oils that really deserve it with an award in gold, silver or bronze.

3.4. Variability of harmony values within EVOOs produced as mono-variety oils from "Koroneiki" olives in 2015

The olive variety "Koroneiki" is the most spread variety in Greece with an approximate share of 50–60% [11] compared with other Greek varieties. Koroneiki olives grow throughout Greece (including Crete) and are now also cultivated in parts of Spain, France and Turkey.

Taking into account an average production of Koroneiki olive oil in Greece of around 220,000 t per year (excluding the high proportion of self-production by very small farmers), only 20,000–30,000 t are consumed by the Greek population themselves. It is well known that the Greek consumers are still ahead in consumption of olive oil, compared with all other European countries. As a consequence, plenty of the excess Greek extra virgin olive oil is sold as bulk to other countries for blending.

In 2015, the German and the Swiss Olive Oil Panels (DOP and SOP) did sensory evaluation of altogether 104 Koroneiki olive oil samples from various producers and distributors. In order to evaluate these mono-variety olive oils, both panels used their extended profile sheet including the quality factor "harmony".

Figure 11 shows that the biggest part of all evaluated olive oil samples, 40.4% (42/104), were rated as good (harmony level 5.5–6.4); 16.3% (17/104) of the oils were rated even better, that is very good, with harmony levels of 6.6–7.5. This means that well more than 50% of Koroneiki EVOOs are far above the average harmony values of low cost private label qualities.

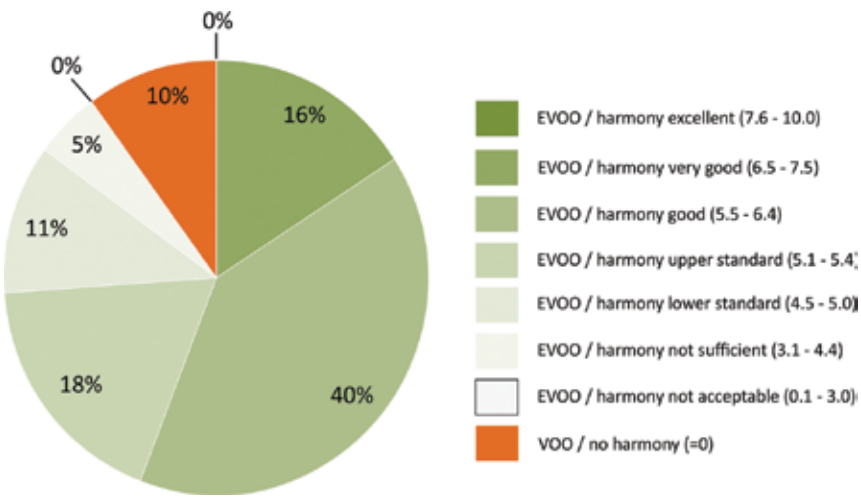


Figure 11. Percentage of mono-variety Koroneiki oil samples (n = 104 of which n = 10 VOO and n = 94 EVOO) in the year 2015, discriminated within the category of EVOO, using the harmony value.

http://dx.doi.org/10.5772/64727

It is important to know that 2015 was not the best year if one takes into account rather big problems during agricultural olive growing. Frost in some regions, less rain compared with other years and the fly problem did hurt some regions and orchards hardly. This might be part of the reason why some Koroneiki olive oils did not reach the classification "extra virgin" in 2015; 9.6% (10/104) samples did not reach extra virgin quality level and had to be downgraded as VOO. Five more samples (4.8%) were rated as not sufficient (harmony level 3.3–4.4). 10.6% of the samples (11/104) did reach a lower standard harmony level of 4.5–5.0 and 18.3% of the samples (19) did reach an upper standard harmony level of 5.1–5.4.

In general, Koroneiki olives, as it is for other olive varieties, reach their best sensory characteristics when harvested at an "ideal" time during the process of olive fruit maturation. In this case, the character of the resulting olive oil is rather green, with a grassy flavour of freshly cut grass, with aromas of green tomatoes, green apple, herbs and the aroma of bitter almonds, not very well known to many European consumers.

Results of the study at hand concerning on olive oils produced from Koroneiki olives show a remarkable difference in comparison with the results of olive oil evaluation of low-price private labels and private brands [see Section 3.1 (**Figure 8**)] in which Greek EVOOs probably were just a part of the EU blends). Only the harmony value is able to ensure an objective comparison between the sensory quality of a well-produced mono-variety EVOO and an average low-price private label blend.

4. Conclusion

Results from studies presented in Chapter 3 confirm the fact that the extension of the sensory evaluation of olive oil with additional sensory parameters, especially the quality factor "harmony", makes it possible to monitor and distinguish in a reproducible way between different quality levels within the range of EVOO on the market.

The development of the methodology for the evaluation of the quality factor "harmony" was primarily based on the necessity to discriminate EVOOs with a standard sensory quality from olive oils with a higher/premium sensory quality. This approach triggered the idea to use the same methodology for the entire olive oil market in order to create transparency for all stakeholders—producers, bottlers, retailers and consumers. The methodology is statistically validated and accredited by the national authorities in Germany and Switzerland.

The discrimination of different quality levels within the category of "extra virgin" olive oil facilitates a reliable, honest and reasonable pricing and commercialization of EVOOs. Moreover, a serious application and tailor-made dissemination of harmony values among all stakeholders is capable to create transparency in the field of olive oil quality. This helps to improve overall olive oil quality on the market. Additionally, the evaluation of the harmony value is a solid basis for crosswise comparisons of olive oils, done, for example, in olive oil competitions.

In Germany and Switzerland, the methodology is implemented and used already for the last 15 years by the German and the Swiss Olive Oil Panels (DOP, SOP). A positive development and effect on the olive oil quality could be achieved. In order to establish an even broader acceptance for the quality factor "harmony" among all stakeholders, the common use and application of this methodology in additional olive oil panels throughout Europe (as well as worldwide) would be necessary. To support this aim and to improve the quality levels of EVOO in the various markets and price segments, it would be meaningful to enter the discussion together with the International Olive Council (IOC) and the European Authorities in order to approve this procedure.

Author details

Annette Bongartz[1*], Martin Popp[1], Reinhard Schneller[1] and Dieter Oberg[2]

*Address all correspondence to: annette.bongartz@zhaw.ch

1 University of Applied Sciences Zurich (ZHAW), Food Sensory Science Group, Wädenswil, Switzerland

2 Association for Care and Support of the Value of Extra Virgin Olive Oil e. V./Information office Olive Oil (IGO), Wessling, Germany

References

[1] ISO 8402. Quality management and quality assurance – Vocabulary. 1994.

[2] EC Regulation. COMMISSION REGULATION (EEC) No. 2568/91 of 11 July 1991 on the characteristics of olive oil and olive residue oil and the relevant methods of analysis. 1991.

[3] EC Regulation. COMMISSION IMPLEMENTING REGULATION (EU) 2015/1833 of 12 October 2015 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. 2015.

[4] Bongartz A. Oberg D.G. Sensory Evaluation of Extra Virgin Olive Oil (EVOO) Extended to Include the Quality Factor "Harmony". Journal of Agricultural Science and Technology A 1, 422–435. 2011.

[5] DIN EN ISO 17025. General requirements for the competence of testing and calibration laboratories. 2005.

[6] EA 4/09. Accreditation for Sensory Testing Laboratories. 2003.

http://dx.doi.org/10.5772/64727

[7] Lawless H.T., Heymann H. Sensory Evaluation of Food – Principles and Practices. New York: Chapmann & Hall; 1998.

[8] Meilgaard M., Civille G.V., Carr B.T. Sensory Evaluation Techniques. Boca Raton: CRC Press; 2006.

[9] Bongartz A., Popp M. ZHAW Flavour-Wheel Olive Oil [Internet]. 2012. Available from: https://www.zhaw.ch/storage/lsfm/ueber-uns/oliveoil/bewertung-aromarad.pdf

[10] Oberg, D. IGO Study "Influence of the harmony value for reliable sensory data in private label quality of EVOO in the EU". EuroFedLipid Congress, Florence, 2015

[11] International Olive Council. World Catalogue of Olive Varieties. 2000; p. 95, ISBN 978-84-931663-1-1

Chapter 9

Consumer Perception, Attitudes, Liking and Preferences for Olive Oil

Sara Cicerale, Gie Liem and Russell S.J. Keast

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64554

Abstract

The consumption of healthful olive oil (OO) has grown considerably over the past 20 years, particularly in areas outside of Europe. To meet this demand, worldwide production of OO has doubled over this time period. Greece, Italy and Spain remain the major producers of this commodity; however, significant growth in production has also occurred in countries such as Australia and the US. OO consumption is closely associated with the traditional Mediterranean diet. It is likely that the potential health benefits of using OO as a primary dietary fat have been a driver of increased intake, but undoubtedly other factors will be involved. An understanding of the factors that influence consumers' perceptions, attitudes, liking and preferences for OO will be of benefit to the OO sector. Olive growers, OO manufacturers, packaging specialists and marketers, etc. can utilize these insights to aid in the development and delivery of OO products in line with consumer needs and wants, and help drive further growth in this sector particularly with regard to new and emerging markets. The following chapter details information on the intrinsic and extrinsic factors that have demonstrated an influence on consumer perception, attitudes, liking and preferences for OO.

Keywords: olive oil, consumer, perception, attitude, liking, preference, intrinsic factors, extrinsic factors

1. Introduction

The consumption of olive oil (OO) has grown considerably over the past 20 years. For instance, approximately 1.7 million tons of OO was consumed worldwide in 1990–1991 and this increased to approximately 3.1 million tons in 2013–2014. To meet the demand, worldwide production of OO has doubled over this time period. Greece, Italy and Spain are the major producers of OO

[1]; however, significant growth in production has also occurred in countries such as Australia and the USA [1, 2]. For instance in Australia, 500 tons of OO was produced in 1998–1999 and this increased to 13,500 tons in 2013–2014. A similar growth rate has also been noted for the USA, producing 1000 tons of OO in 1998–1999 and 12,000 tons by the years 2013–2014 [1, 2]. While dietary fats are often maligned in terms of health, OO, particularly extra virgin olive oil (EVOO) and virgin olive oil (VOO), holds a special place as consumption is closely associated with the traditional Mediterranean diet and the health benefits associated with this diet [3, 4]. It is likely that the potential health benefits of using OO as primary dietary fat have been a driver of increased intake, but undoubtedly other factors will be involved.

An understanding of the factors that influence consumers' perceptions, attitudes, liking and preferences for OO will be of benefit to the OO sector. Olive growers, OO manufacturers, packaging specialists and marketers, etc. can utilize these insights to develop and deliver OO products in line with consumer needs and wants, and help drive further growth in this sector particularly with regard to new and emerging markets.

Consumers make multiple judgments about the foods and beverages they choose to eat and drink on a daily basis and these evaluations are based on conscious reflection as well as automatic, habitual and subconscious decisions. Underlying these reflections and decisions are the quality evaluations consumers make using both intrinsic and extrinsic product cues. Intrinsic product cues relate to the physical attributes of a given product (i.e., color and flavor). Extrinsic product cues, on the other hand, are the attributes that are related but not contained within a given product such as brand and product origin [5]. The Total Food Quality Model by Grunert [5] provides a framework of how intrinsic and extrinsic product attributes influence consumer quality perception of products. This model distinguishes quality perception before and after a product's purchase and demonstrates that before purchase, consumers make a judgment of product quality using several intrinsic and extrinsic cues. These intrinsic and extrinsic quality cues are connected to consumer knowledge, expertise and beliefs about what is good quality. Furthermore, Grunert [5] proposes that for the majority of food purchases, major quality dimensions of a product (for example, taste) cannot be ascertained before the purchase, and for such purchase decisions to be made, consumers have to form quality expectations. Post-purchase, the product will lead to some type of quality experience.

It is also important to note that an OO sensory wheel has been developed, acting as a valuable tool in describing EVOO and VOO and establishing the importance of particular intrinsic product attributes for the perceived quality of the oil. The attribute profiles of EVOO and VOO can be linked to consumer preferences and can be used partly as a prediction model for certain consumer groups. Although the sensory aspects of OO have been deemed to be important, extrinsic product attributes have also been noted to be of importance [6].

The following chapter details information on the intrinsic and extrinsic factors that have demonstrated an influence on consumer perception, attitudes, liking and preferences for OO. Please note for the purpose of this chapter the term OO will be used to encompass EVOO, VOO and the more refined OO when discussing this oil more generally. When a specific type of OO has been examined in the literature reviewed, it will be specified.

http://dx.doi.org/10.5772/64554

2. Intrinsic product attributes

Intrinsic attributes provide a product's functionality and relate to the physical aspect of the product itself. In light of the previous research conducted, the intrinsic product cues that will be discussed in the section to follow are OO color and flavor.

2.1. Color

The coloring of OO can vary from a deep green to gold and is mostly dependent on the amount of chlorophyll and carotenoid pigments present in the oil. These pigments vary in OO as a result of a number of factors such as olive cultivar, maturation index, production zone, processing treatments and storage conditions. The color of OO can therefore stand as a quality index [7, 8].

The observation that refined OO is associated with a much lighter, paler color was highlighted in a study by McEwan [9]. The UK-based consumer cohort ($n = 9$) who participated in a focus group expressed the attitude that very pale oils signified a cheaper product with less flavor and highlighted the importance of OO color in their purchasing decision. In a face-to-face survey concerning a Tunisian cohort ($n = 296$), whereby consumers were asked questions about important criteria for choosing OO, the participants expressed a significantly higher preference for green over yellow colored OO, $p < 0.05$ [10]. In another face-to-face survey involving Italian consumers from central-north and south Italy ($n = 1000$), green EVOO was preferred over yellow EVOO to a greater degree in the southern consumer sample (71%) compared with the central-northern consumer sample (52%) [11].

Building on the aforementioned focus group and survey investigations, a consumer evaluation-based study by Vazquez-Araujo et al. [12] also demonstrated consumer preference for green EVOO. Spanish consumers ($n = 100$) were asked to evaluate five samples of EVOO and one sample of refined OO in terms of liking of the product color on a 9-point hedonic scale. Certain EVOOs with a dark green coloring were significantly favored over EVOO and refined OO possessing a light yellow coloring, $p < 0.05$. The US consumers ($n = 100$) also included in this study, however, expressed neutral opinions on the oil coloring. Lastly a study was conducted in a Finnish population ($n = 74$), whereby participants completed a survey and evaluated the color of four EVOOs, two considered excellent quality and two regular quality. Using a 9-point hedonic scale, a significantly lower liking for darker green EVOO compared to lighter colored EVOO was noted, $p < 0.001$ [13].

The color of food has been identified as an imperative intrinsic product cue with regard to consumer expectations of the likely taste and flavor and hence quality of food and beverages [14]. In general, consumers appear to associate more intensely colored foods with increased flavor [14] and the McEwan study [9] supports this. Differences in the acceptance for the darker colored OOs among the surveyed populations may in part be due to the experiences and exposure these populations have to OO in terms of volume and quality. Spain and Tunisia are major producers and consumers of EVOO; hence, EVOO is very much a staple of the Spanish

and Tunisian diet. For instance, in the 2013–2014 period, Spain produced 1,782,000 tons and consumed 525,000 tons of OO. Tunisia produced 340,000 tons and consumed 30,000 tons of OO [2]. Conversely for the British, US and Finnish populations, OO is fairly novel [1, 2]. The Spanish and Tunisian populations are more likely to consume fresher, higher quality OO compared with other populations such as the US, since they produce much of the OO they consume [1]. The USA currently imports 98% of the OO they consume [15] and majority of the vegetable oil used by US consumers has been noted to be light yellow in color (for instance, soybean, corn, canola, cottonseed) [13]. With regard to the Finnish population, consumption data demonstrates OO consumption to be low (4000 tons consumed in 2013–2014) [2]. The US and Finnish populations may therefore lack experience with fresh, higher quality OO and this may be an influencing factor in their preference for lightly colored OO. Although color was deemed important for the Brits when choosing OO, this observation was from a small ($n = 9$) study. Patterns of preference and liking appear in the literature examined and this information will be important for OO producers in terms of tailoring their OO for local and or export markets.

2.2. Flavor

The flavor profile of OO is clearly distinguishable from other vegetable oils. The components that contribute most to OO flavor have been noted as volatile and phenolic substances [16]. In particular, phenolic compounds largely contribute to the bitter, pungent and astringent qualities that are distinctive of high-quality EVOO and VOO [17]. OO's characteristic flavor has been identified as a contributing factor for its increasing popularity around the world [18]. Two survey-based studies support this notion. A study containing US participants ($n = 178$) demonstrated that flavor was a main driver for the purchase of the OO they were using at home [19]. Similarly, a study in UK consumers ($n = 151$) found that flavor was a main factor for OO purchase [20].

A handful of studies have also shown that consumers tend to appreciate fruity, floral and sweet attributes in EVOO more so than bitter and pungent attributes [11–13, 21–24]. However, the degree in liking of the EVOO attributes varies among differing populations. For instance, in a study whereby Spanish consumers ($n = 100$) tasted and rated five EVOOs and one refined OO (on a 9-point hedonic scale), EVOO that had higher flavor intensity and contained fruity, green, peppery notes was significantly preferred, $p < 0.05$. The US consumers ($n = 100$) of the same study expressed a liking for fruity, floral notes and bland-refined OO and found the EVOOs liked by the Spanish consumers to be too bitter, pungent and intense in flavor, $p < 0.05$. The frequency of OO consumption among the Spanish and US consumers was also investigated in this study and found to differ greatly. While 93% of the Spanish consumers surveyed noted a daily consumption of OO, only 8% of the US consumers also chose this option [12].

The differences noted among the Spanish and US consumers may be in part due to the differences in degree of exposure to not only OO itself but also the quality of OO. The Spanish consume much of the OO produced within Spain whereas Americans consume mostly imported OO which may have reduced quality (hence lowered bitterness and pungency) due to a variety of factors including the imported OO becoming rancid over time [15, 25]. According

http://dx.doi.org/10.5772/64554

to Tourila and Recchia [26], a learning process is required for an individual to develop a taste and appreciation for OO. Hence, preference for OO flavor may be dependent on the type of OO individuals have been accustomed to via exposure to them over time.

As seen with the Spanish consumers, a liking for stronger EVOO flavor and dislike for bland-flavored OOs has also been noted in a group of Tunisian consumers (n = 300) who participated in a face-to-face survey [10]. A similar finding has also been found for a group of Italian consumers (n = 1000) who participated in a face-to-face survey. In this study, the Italian consumers preferred their OO to possess a more intense flavor; however, differences in the percentage of preference were noted between the consumers from south Italy (84%) and those from central-north Italy (59%) [11]. The findings discussed may in part be explained by the fact that Tunisia and Italy are main producers of OO and Tunisian and Italian OO is well known for its high quality, therefore Tunisian and Italian consumers may be well accustomed to the flavor of such OO [10, 11].

A study by Delgado et al. [23] containing US consumers (n = 110) who evaluated EVOO samples (n = 22) via tasting found the following EVOO flavor attributes: nutty, ripe fruit, green tea, butter, green fruit and grassy to be positive drivers of liking. For a portion of the consumers surveyed, known defective flavor attributes such as rancidity, mustiness, fustiness and winey were also found to be drivers of liking. With regard to the latter finding, these consumers may have become used to such defects because they may, for example, choose to consume cheaper, imported and or mass-marketed refined OOs or retain a bottle of OO for an extended period of time which results in the OO developing defects over this period [23, 25].

Finally a study containing British (n = 50), Danish (n = 90) and French (n = 50) consumers evaluated a total of seven vegetable oils (including one VOO and one refined OO) by interview. Respondents were presented with the seven oils and were asked to rank them into three groups: a group consisting of the most preferred oils, a group of the least preferred oils and a group of oils the respondent would consider if they could not get any of the most preferred oils. Respondents were then asked to give reasons for the grouping made. Finally, the respondents were asked to rank the products within each group according to preference and to state reasons why. The seven oils were in this way ranked from 1, the most preferred oil, to 7, the least preferred oil. This study found that a portion of the British cohort did not express favorable views on the flavor of VOO. However, for the majority of Danish and French respondents, an attitude of VOO being a 'tasty product' and one that 'tastes good' was expressed [27]. Despite the UK's consumption of OO being 61,000 tons in 2013–2014, OO still remains a relatively new food product for this population with exposure being on the lower side. France's consumption of OO in 2013–2014 was 110,000 tons, almost double for that of the British. However, despite the Danish providing favorable opinions on VOO, they have quite a low consumption of OO at 7000 tons in 2013–2014 [2]. The Danish may have a lowered exposure to OO overall but what is available may be of higher quality.

A pattern appears to be emerging in terms of differing population perceptions, attitudes, liking and preferences for OO flavor. Consumers who are more likely to be exposed to high-quality OO appear more likely to appreciate flavor attributes typically associated with such oil. Consumers who have become accustomed to bland-flavored OO due to a variety of reasons

seem to prefer this OO and dislike more intensely flavored OO. As with any food, much variation in liking exists for OO and it is imperative that OO producers understand their target consumer and tailor their OO products to such demand.

3. Extrinsic product attributes

Unlike intrinsic product cues, extrinsic product attributes are not part of the physical product but are related to the aspects and information surrounding a product. Drawing on previous research, the extrinsic product attributes to be discussed in terms of OO include: label information, packaging, perceived health benefits and price.

3.1. Label information

Food labeling information with regard to product origin, nutritional qualities of a product, ingredients used and manufacturing process has been noted as an influential factor on hedonic expectations and food acceptability [28]. A small number of studies have examined the influence of labeling information on the perceptions, attitudes, liking and preferences for OO and the outcomes from such studies demonstrate that OO food labeling appears to influence quality and taste perception, and intention to purchase.

In a meta-analysis of 20 studies concerning consumer preferences for EVOO, origin labeling/certification was found to positively influence consumers' willingness to pay [29]. A study concerning French (n = 123) and Tunisian (n = 128) consumers, involved the tasting of four EVOOs (in a blinded fashion) and rating them on a 10-point hedonic scale as to how much they liked particular attributes such as taste, color and appearance. The test group was then supplied with origin information, including an image, and was asked to rate the perceived quality of the oils using a 10-point expected quality scale. After this, the test group was given the oils to taste again once knowing the origin information and rated liking as well as perceived quality. The test group was also asked what they were willing to pay for each of the oils under the differing conditions. Product origin information was found to influence perceived quality of the EVOOs included in the study, particularly in terms of expected prices more so than hedonic expectations. For example, there was a significant increase (p = 0.005–0.04) in the expected price for four out of five EVOO products under the test versus control condition. For hedonic score, only one out of the five EVOO products was rated significantly higher (p = 0.02) under the test versus control condition. Hence, consumers were willing to pay more for the oils upon being provided origin information [30].

A second experiment was conducted with the same cohort that further investigated which attributes related to regional image (olive variety—single or blend; natural conditions—soil or climate; human factors—modern or traditional mill) influenced perceived quality to the greatest degree. Price was also added to the investigation, where variations included: low, medium, high and very high. The consumers undertook 10 choice tasks whereby each task consisted of three alternatives for region of origin, olive variety, natural conditions, human factor and price level, plus a no option choice. The consumers were asked to imagine themselves in a supermarket faced with a number of OOs to choose from. Looking at the percentage

http://dx.doi.org/10.5772/64554

importance, region of origin and human factors were found to be more important to the French consumers (28% and 24% respectively) compared to the Tunisian consumers (4% and 3%). Olive variety was more important to the Tunisian consumers (17%) compared to their French counterparts (2%). Price labeling was deemed as the most important attribute for both the French (44%) and Tunisian (76%) consumers. Natural conditions were found to have little importance for both groups (below 2%). The results suggest that origin information is multi-dimensional and that perceptions may reflect differences in local experience and culture. The French are known to favor "terroir" and distrust industrially made foods. Tunisians purchase much of their OO at local mills, therefore may be a factor in their consideration of olive variety being important. Price was more important for the Tunisian consumers and this is not surprising given that although the researchers tried to choose consumers with equal income, there were less Tunisian consumers in the high income category and more in the low-medium income category compared to the French cohort [30].

In another study containing French (n = 123) and Tunisian (n = 122) consumers, the influence of product origin labeling (including country and region information) was examined using the "best-worst (B-W)" scale, whereby subjects choose the attributes they considered most and least important in their choice of OO and the B-W value is calculated by subtracting those that choose an attribute as least important from most important. The consumers (n = 245) who took part in the study attended multiple study sessions (13 in Tunisia and 10 in France) and in those sessions completed a series of questionnaires. The origin attributes investigated were found to be important determinants in consumer choice among the Tunisian and French consumers. However, there were slight differences in the origin information that was most important for these two population groups. Country of origin appeared to be more important to the French consumers (B-W 84 compared with B-W 38 for the Tunisian consumers) and this may be in part due to OOs from different countries being readily available in France. On the contrary, in Tunisia whereby OO is locally made and sold (for instance there are no imports), region of origin was deemed as more important (B-W 102 compared with B-W 14 for the French consumers) [31].

Additionally, a face-to-face survey investigating country of origin and region of origin labeling on Canadian consumer (n = 207) attitudes found that 81–86% of the Canadian consumers surveyed preferred Italian EVOO over Spanish EVOO. When comparing EVOOs with country of origin (Italy) information displayed versus region of origin information displayed, a greater percentage of Canadian consumers had a preference for the Italian origin EVOOs over the Italian region origin EVOOs (86% versus 70%) [32]. In a US-based study whereby consumers (n = 102) visually assessed EVOO labels as well as tasted the oils blinded (n = 18) and then indicated their liking and purchase intent for these oils, the results demonstrated that there was an increased liking for the oils when the labels were shown (without tasting) compared with when the oils were tasted blind ($p < 0.05$). Consumers were also prepared to pay more (up to $30) when they were presented with the EVOO labels compared with tasting the oils blind. Furthermore, there was an increased preference for EVOOs labeled as originating from California as opposed to those labeled as imports ($p < 0.05$) [33]. A study in Japanese consumers (n = 456) who completed a survey found that country or region of origin information was

the second most considered attribute (with 37% of respondents noting this) when buying OO. Furthermore, the evaluation of OOs differed according to the country of origin information supplied on the label. For instance, these consumers rated their preference for Italian originating oils higher than Spanish and Tunisian olive oils [34]. Finally, similar findings were noted for a study conducted in Italian consumers (n = 1000) who completed a face-to-face survey. Geographic origin was the second most important attribute, with an average importance of 25%. Following geographic origin, protected designation of origin (PDO) was found to be an important attribute among those surveyed (19–24%) [12].

With respect to other aspects of labeling, Delgado and colleagues [33] noted that in their study containing US consumers (n = 212), whereby the consumers either visually assessed 18 EVOO labels or tasted the EVOO blind, EVOO labeling was significantly correlated with the expected intensity of the EVOO flavor. The authors hypothesized that olive cultivar labeling could partly explain this finding [33]. For example, EVOOs made with the Californian olive cultivars: Arbequina, Frantoio and Picual were expected to possess a stronger flavor whereas EVOOs made with the Californian Horiblanca and Mission olive cultivars together with EVOOs made with olive cultivar blends from various countries were expected to possess a milder flavor. In the same study, nutritional expectations based on labeling were also investigated. Imported EVOO blends and a Spanish EVOO were expected to be less nutritious compared with those from California, Italy and Chile ($p < 0.05$). A potential reason for this finding is that the imported OO blends were a generic brand, providing the perception that they were of lower quality [35], hence less nutritious. Moreover, the EVOO rated to contain the highest nutritional profile by 77% of the consumers included in the study was a Californian EVOO that contained organic certification on its labeling. The reason for this finding may be due to certain consumers having expectations of organic products containing higher nutritional value compared with their conventional counterparts [33]. In a meta-analysis of 20 studies by Del Giudice et al. [29], organic certification of EVOOs was found to positively influence consumers' willingness to pay.

Conversely in another study containing Italian consumers (n = 60) who evaluated eight EVOOs by tasting and other parameters, organic farming information did not influence liking of the OOs tested [22]. A similar finding was noted in a face-to-face survey study where the Italian respondents (n = 1000) noted that the method of production (organic versus traditional) was lower in importance (17–19%) than for other product attributes [11]. Thøgersen [36] has noted that the gap between intention and behavior is larger in Southern European countries with regard to organic foods. This may in part be explained by a higher degree of uncertainty and the lower availability of organic food in these countries. Finally, Delgado and colleagues [23] found that the awards an oil had and certification of quality were important factors influencing OO purchasing decisions for a portion of the US-based consumers (n = 110) who took part in this survey and tasting-based study.

In summary, origin information appears to exert a positive influence on OO liking and preference; however, the degree of influence appears to depend very much on the cohort of consumers investigated. As Grunert [35] explains, consumers may use region of origin knowledge to form a quality evaluation. In the case of repeat purchases of a product, region

http://dx.doi.org/10.5772/64554

of origin information may help to re-identify a product, the quality of which they found satisfactory—a process that may be most relevant when the product does not carry a strong brand. Origin information will have no effect on quality evaluations when consumers have no knowledge about the region of origin, when the quality of the product is not in fact experienced as desirable by the consumer and/or when we are dealing with trial (as opposed to repeat) purchases. Other types of labeling information also appear to have an influence on the consumer perceptions of OO to a varying degree across populations due to a variety of potential factors such as local experience, culture, monetary considerations, etc. It is vital for OO producers and marketers to understand their target consumer and tailor their OO labeling to contain information the consumer considers most important and responds most to.

3.2. Packaging

The appearance of a product has been noted to play an integral role in shaping consumer expectations and packaging specifically, has been identified as a key element of the marketing mix, adding interest to a product [33]. Packaging has become an important extrinsic quality cue which provides information not only about the food but also about brand image or lifestyle. Packaging also aids in product differentiation, helping consumers to choose the product from a wide range of similar products. A small number of studies have examined the influence of packaging on the perceptions, attitudes, liking and preferences for OO and these are discussed below.

Delgado and colleagues [33] found among the participating US consumers ($n = 212$) who either rated 18 EVOOs based on packaging alone or tasted the same oils in a blind manner, that hedonic ratings of the EVOOs were higher when assessed solely on the package compared to a blind tasting of the oils ($p < 0.05$). Furthermore, Californian EVOOs in clear or black bottles or those inside a box increased purchase intentions and the consumers also expected to pay more for the EVOO based on packaging alone. Previous research has noted that clear packaging is preferred by consumers [37] and in the US, darker colored packaging is associated with high quality, elegance and richness [38].

In a focus group study investigating UK consumers' ($n = 130$) attitudes towards key product attributes of OO, packaging was noted as an important determinant of OO purchase. The study's findings revealed a linear relationship between packaging and price, in that as the price increased, a higher quality packaging was expected. These consumers also highlighted that EVOO was expected to possess a superior bottle presentation, however interestingly this did not apply to more refined OOs. The use of plastic packaging was generally associated with cheaper cooking oils. Regarding the size of the packaging, participants preferred to purchase OO in smaller sized bottles (500 ml being the most popular) in comparison to other cooking oils [39]. Additionally, Delgado et al. [23] noted a preference for glass versus plastic bottle packaging among a portion of the US consumers ($n = 110$) they investigated. In this study, the consumers evaluated 22 EVOOs based on a number of parameters including packaging. Interestingly, previous research has demonstrated that glass packaging is perceived to hold high-quality products [39]. In an Italian study that involved the interviewing of 1000 consumers, those from central-north Italy preferred to purchase their EVOO in packaging of 500 ml

and 750 ml. However, consumers based in South Italy preferred to purchase EVOO in 1 l bottles and larger. This finding may be partly explained by the additional finding that Northern Italians preferred to buy bottled EVOO (70.4%) over bulk EVOO (20.4%). Whereas Southern Italians preferred to buy bottled EVOO to a lesser extent (59%) and bulk EVOO (35.3%) to a greater degree [11].

Conversely, a face-to-face survey study concerning a Tunisian cohort (n = 300) found that packaging had no influence on consumer choice of OO. This finding may be related to the fact that the majority of Tunisian OO consumers buy the product in bulk with very few purchasing bottled OO from supermarkets [10].

Although packaging presents as a quality cue and appears to be an influential factor with regard to OO expectations and preferences, the degree of influence appears to differ among varying populations due to factors such as country and cultural expectations. Creating packaging that meets the expectations of consumers is therefore important and may increase the likelihood of OO purchase. OO packaging specialists and marketers should pay special attention to the findings thus far and for the populations where research has not been conducted, collection of such data may be necessary prior to the creation of packaging for OO in differing markets.

3.3. Perceived health benefits

Non-sensory product factors such as the perceived health benefits of a food have been noted as an important driver of food choice [40]. The handful of studies investigating the influence of this extrinsic cue with regard to OO further supports this.

Santosa and colleagues [19] noted that awareness of OO's health benefits prompted most of the US consumers who participated in this study (either via a focus group (n = 35) or survey (n = 178)) to begin consuming this oil. Delgado et al. [23] found that of the US consumers (n = 110) who were included in their study which involved a tasting of EVOOs and completion of a survey, 74% of them noted that a main motivator for their consumption of EVOO was health benefits. In a focus group study containing UK-based consumers (n = 9), OO was noted as possessing a healthful image with a number of these consumers also noting its low saturated fat profile (even though only a few knew what this meant) [9]. Furthermore, another study investigating UK consumers (n = 151) via a questionnaire found that OO health considerations were found to be strongly correlated with the intention to use OO (r = 0.5) [20]. Finally, a study by Nielsen et al. [27] noted a common attitude of VOO being a 'healthy oil' among the British, Danish and French consumers (n = 190) who participated in the evaluation of seven OOs by interview.

From the findings of these aforementioned studies, it appears that a great proportion of consumers are aware of OO's health associations and it clearly presents as a cue of quality and driver for its use. This is not surprising given OO consumption is closely associated with the traditional Mediterranean diet that has a reputation of being health benefiting [3]. OO marketers can utilize this consumer awareness to further market the health-promoting properties of OO.

http://dx.doi.org/10.5772/64554

3.4. Price

Individual dietary choices can be partly influenced by the price of food [41, 42]. The handful of studies investigating the influence of price on OO perceptions, attitudes, liking and preferences supports such a finding.

Santosa et al. [19] noted among the US-based consumers who took part in a focus group ($n = 35$) or completed a survey ($n = 178$), price or cost of OO was one of the main factors affecting their OO purchases. Participants expressed the fact that they were satisfied with a higher price for an OO providing the oil was deemed of good quality. Furthermore, consumers expressed that if the oil was intended for personal use, how the OO was going to be used and consumed affected the price these consumers were willing to pay for the oil. For example, consumers wanted to purchase less expensive OO to be used in cooking, but were willing to pay more for OO to be used to dress salads and for dipping purposes.

In a study by McEwan [9], whereby UK consumers ($n = 9$) took part in a focus group, price was also found to be a primary factor for OO purchase. The consumers in this study tended to purchase smaller volumes of OO as they were lower in price compared with larger volumes, but they also generally felt that cheaper oils were more likely to be inferior in quality compared with more expensive OOs. Similarly, a study investigating Japanese consumers ($n = 456$) who completed a survey found that a significant portion of those surveyed (77%) cited price as being the most important attribute when buying OO [34]. A study in an Albanian cohort ($n = 204$) who participated in a face-to-face survey also noted price to be among the most important attributes in terms of OO choice [43]. In a focus group-based study containing UK users of OO ($n = 130$), price was determined to be an important factor for choice and purchase of the more refined OO but the same was not found for the higher quality EVOO. The consumers in this study felt that although EVOO was the most expensive oil, it presented as better value for money [39]. An Italian face-to-face survey study ($n = 1000$) also found price to be a leading factor for EVOO choice, with 31–39% of the surveyed population choosing this factor as the most important attribute when choosing EVOO [11].

Moreover, in a study investigating French and Tunisian consumers ($n = 251$) who completed a survey, although price was identified as an important attribute for both populations, it was particularly important for the Tunisian consumers. As financial constraints are more prevalent among the Tunisian population compared with the French population, this may partly explain the difference observed [30]. These findings were also mirrored in another study investigating Tunisian consumers ($n = 296$) who undertook a face-to-face survey, whereby the authors noted a decrease in the likelihood of OO purchase as price increased [10]. Finally, a similar finding was highlighted in research conducted by Delgado and Guinard [23] where US consumers ($n = 110$) tasted and evaluated 22 EVOOs. Those with the lowest levels of income and education, made OO purchasing decisions based mainly on price.

Considering the evidence presented, price of OO appears to indeed be a quality cue. However, despite price appearing to be an influential factor with regard to OO purchase, financial constraints appear to impact the degree of influence. Price should therefore be a consideration for OO producers and marketers when tailoring OO products to differing markets.

4. Conclusion

Considering the worldwide growth of OO consumption over the past 20 years, an investigation into the intrinsic and extrinsic factors that influence OO perceptions, attitudes, liking and preferences is timely. The research conducted thus far demonstrates that there are clear population differences with regard to the degree of influence of the discussed intrinsic and extrinsic OO product attributes due to various factors including cultural and situational factors. An understanding of the factors that influence consumers' perceptions, attitudes, liking and preferences for OO will be of benefit to the OO sector. Olive growers, OO manufacturers, packaging specialists and marketers, etc. can utilize these insights to provide OO and information that meets and supports consumer needs and wants, thus helping to drive further growth in this sector particularly with regard to emerging markets.

Author details

Sara Cicerale*, Gie Liem and Russell S.J. Keast

*Address all correspondence to: sara.cicerale@deakin.edu.au

Centre for Advanced Sensory Science (CASS), Deakin University, Melbourne, Australia

References

[1] Zampounis V. Olive oil in the world market. In: Boskou D, editor. Olive Oil Chemistry and Technology. Illinois: AOCS Press; 2006. p. 21–40.

[2] International Olive Council 2015 [cited 2015 December]. 2015; Available from: http://www.internationaloliveoil.org/.

[3] Cicerale S, Conlan XA, Sinclair AJ, Keast RS. Chemistry and health of olive oil phenolics. Critical Reviews in Food Science and Nutrition. 2009;49(3):218–236.

[4] Schwingshackl L, Hoffmann G. Monounsaturated fatty acids, olive oil and health status: a systematic review and meta-analysis of cohort studies. Lipids in Health and Disease. 2014;13:154.

[5] Grunert KG. Current issues in the understanding of consumer food choice. Trends in Food Science and Technology. 2002;13:275–285.

[6] Mojet J, de Jong S. The sensory wheel of virgin olive oil. Grasas y Aceites. 1994;45:42–47.

[7] Moyano MJ, Heredia FJ, Meléndez-Martínez AJ. The color of olive oils: the pigments and their likely health benefits and visual and instrumental methods of analysis. Comprehensive Reviews in Food Science and Food Safety. 2010;9(3):278–291.

[8] Boskou D, Blekas G, Tsimidou M. Olive oil composition. In: Boskou D, editor. Olive Oil Chemistry and Technology. Illinois: AOCS Press; 2006. p. 41–72.

[9] McEwan JA. Consumer attitudes and olive oil acceptance: the potential consumer. Grasas y Aceites. 1994;45(1–1):9–15.

[10] Mtimet N, Zaibet L, Zairi C, Hzami H. Marketing olive oil products in the Tunisian local market: the importance of quality attributes and consumers' behavior. Journal of International Food & Agribusiness Marketing. 2013;25(2):134–145.

[11] Di Vita G, D'Amico M, La Via G, Caniglia E. Quality perception of PDO extra-virgin olive oil: Which attributes most influence Italian consumers? Agricultural Economics Review. 2013;14(2):46.

[12] Vazquez-Araujo L, Adhikari K, Chambers Et, Chambers DH, Carbonell-Barrachina AA. Cross-cultural perception of six commercial olive oils: a study with Spanish and US consumers. Food Science and Technology International. 2015;21(6):454–466.

[13] Recchia A, Monteleone E, Tuorila H. Responses to extra virgin olive oils in consumers with varying commitment to oils. Food Quality and Preference. 2012;24(1):153–161.

[14] Spence C. On the psychological impact of food colour. Flavour. 2015;4(1):1–16.

[15] American Olive Oil Producers Association [cited 17 April 2016]. Available from: http://www.aoopa.org/Home-i-1-1.html.

[16] Aparicio R, Luna G. Characterisation of monovarietal virgin olive oils. European Journal of Lipid Science and Technology. 2002;104(9–10):614–627.

[17] Boskou D, Tsimidou M, Blekas G. Polar phenolic compounds. In: Boskou D, editor. Olive Oil Chemistry and Technology. Illinois: AOCS Press; 2006. p. 73–92.

[18] Boskou D. Culinary applications. In: Boskou D, editor. Olive Oil Chemistry and Technology. Illinois: AOCS Press; 2006. p. 243–248.

[19] Santosa M, Clow EJ, Sturzenberger ND, Guinard J-X. Knowledge, beliefs, habits and attitudes of California consumers regarding extra virgin olive oil. Food Research International. 2013;54(2):2104–2111.

[20] Thompson KE, Haziris N, Alekos PJ. Attitudes and food choice behaviour. British Food Journal. 1994;96(11):9–13.

[21] Valli E, Bendini A, Popp M, Bongartz A. Sensory analysis and consumer acceptance of 140 high-quality extra virgin olive oils. Journal of the Science of Food and Agriculture. 2014;94(10):2124–2132.

[22] Barbieri S, Bendini A, Valli E, Gallina Toschi T. Do consumers recognize the positive sensorial attributes of extra virgin olive oils related with their composition? A case

study on conventional and organic products. Journal of Food Composition and Analysis. 2015;44:186–195.

[23] Delgado C, Guinard J-X. How do consumer hedonic ratings for extra virgin olive oil relate to quality ratings by experts and descriptive analysis ratings? Food Quality and Preference. 2011;22(2):213–225.

[24] Predieri S, Medoro C, Magli M, Gatti E, Rotondi A. Virgin olive oil sensory properties: comparing trained panel evaluation and consumer preferences. Food Research International. 2013;54(2):2091–2094.

[25] Delgado C, Guinard J-X. Sensory properties of Californian and imported extra virgin olive oils. Journal of Food Science. 2011;76(3):S170-S176.

[26] Tourila H, Recchia A. Sensory perception and other factors affecting consumer choice of olive oil. In: Monteleone E, Langstaff S, editors. Olive Oil Sensory Science. John Wiley & Sons Ltd; 2014.

[27] Nielsen NA, Bech-Larsen T, Grunert KG. Consumer purchase motives and product perceptions: a laddering study on vegetable oil in three countries. Food Quality and Preference. 1998;9(6):455–466.

[28] Caporale G, Policastro S, Carlucci A, Monteleone E. Consumer expectations for sensory properties in virgin olive oils. Food Quality and Preference. 2006;17(1–2):116–125.

[29] Del Giudice T, Caracciolo F, Cicia G. What attributes of extra virgin olive oil are really important for consumers: a meta-analysis of consumers' stated preferences. Agricultural and Food Economics. 2015;3(20):1–15.

[30] Dekhili S, d'Hauteville F. Effect of the region of origin on the perceived quality of olive oil: an experimental approach using a control group. Food Quality and Preference. 2009;20(7):525–532.

[31] Dekhili S, Sirieix L, Cohen E. How consumers choose olive oil: the importance of origin cues. Food Quality and Preference. 2011;22(8):757–762.

[32] Menapace L, Colson G, Grebitus C, Facendola M. Consumer preferences for extra virgin olive oil with country-of-origin and geographical indication labels in Canada. American Agricultural Economics Association Annual Meeting. 2008:1–19.

[33] Delgado C, Gómez-Rico A, Guinard J-X. Evaluating bottles and labels versus tasting the oils blind: effects of packaging and labeling on consumer preferences, purchase intentions and expectations for extra virgin olive oil. Food Research International. 2013;54(2):2112–2121.

[34] Mtimet N, Ujiie K, Kashiwagi K, Zaibet L, Nagaki M, editors. The effects of information and country of origin on Japanese olive oil consumer selection. EAAE 2011 Congress; 2011; Zurish, Switzerland.

[35] Grunert KG. Food quality and safety: consumer perception and demand. European Review of Agricultural Economics. 2005;32(3):369–391.

[36] Thøgersen J. Direct experience and the strength of the personal norm–behavior relationship. Psychology and Marketing. 2002;19(10):881–893.

[37] Eldesouky A, Pulido AF, Mesias FJ. The role of packaging and presentation format in consumers' preferences for food: an application of projective techniques. Journal of Sensory Studies. 2015;30(5):360–369.

[38] Chandra Lal R, Yambrach F, McProud L. Consumer perception toward package designs: a cross cultural study. Journal of Applied Packaging Research. 2015;7(2):61–94.

[39] Martínez MG, Aragonés Z, Poole N. A repositioning strategy for olive oil in the UK market. Agribusiness. 2002;18(2):163–180.

[40] MacFie HJH, Thomson DMH. Measurement of food preferences. London: Blackie Academic, 1994.

[41] French SA. Pricing effects on food choices. The Journal of Nutrition. 2003;133(3):841s–843s.

[42] Zeithaml VA. Consumer perceptions of price, quality, and value: a means-end model and synthesis of evidence. Journal of Marketing Research. 1988;52(3):2–22.

[43] Chan-Halbrendt C, Zhllima E, Sisior G, Imami D, Leonetti L. Consumer preferences for olive oil in Tirana, Albania. International Food Agribus Man. 2010;13(3):55–74.

Oils with Protected Designation of Origin

Chapter 10

Olive Oils with Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI)

Zisimos Likudis

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64909

Abstract

The consumers' demand for excellence in agricultural products has led to the introduction of certification labels. Among others, the European Commission enforces two types of certification labels: protected designation of origin (PDO) and protected geographical indication (PGI) (EEC, No. 2082/92). Olive oil, as a typical high-value agricultural product, is included in PDO/PGI labeling. The latter constitutes a great motivation for a considerable range of consumers, as it is considered to be associated with high-quality olive oil. However, a misunderstanding and/or unawareness of PDO/PGI and "organic" certification labels is often observed. Limited investigations in PDO/PGI olive oils demonstrated lower occurrence and lower levels of agrochemical residues compared to conventional olive oils. Future investigations are required in this field, in order to confirm that the better cultivation and industrial processes associated with PDO/PGI certification result in lower levels of agrochemicals in the final products. Analytical and Bioanalytical Chemistry will play a vital role in the traceability of PDO/PGI olive oils and the confirmation of their geographical origin and authenticity.

Keywords: olive oil, protected geographical indication (PGI), protected designation of origin (PDO), pesticide residues, agrochemicals, traceability of olive oil, authenticity

1. Introduction

The EU quality scheme is known as PDO, PGI, and TSG, and it identifies agricultural products and foodstuffs farmed and produced to exacting specifications. This scheme was established in 1992 in order to allow producers to use the added value of their products, to protect the names of their products, to provide consumers with clear information on the product origin or specialty character linked to the region, and enables them to make more

informed purchases [1]. The implementation of the above legislative initiative, particularly enables farmers in disadvantaged areas switch to forms of integrated countryside development and improve their income due to better prices. In addition, consumers can buy quality-guaranteed products, based on origin.

2. The certification labels PDO and PGI in olive oils

In 1992, with Regulation 2081/92 [2], the European Union adopted, for the first time, the regime for the protection of geographical indications and designations of origin for agricultural products and foodstuffs. In 2006, this regulation was replaced by 510/06 [3], without any changes in scope and feasibility. In 2012, the European Commission introduced a new regulation on quality systems for agricultural products, Regulation (EU) No. 1151/2012 [4]. This regulation replaced previous ones, and concerned schemes including PDO (Protected Designation of Origin), PGI (Protected Geographical Indication), and TSG (Traditional Specialty Guaranteed), while introducing the definition of "mountain products." This regulation entered into force in December 2012 and is supplemented by the Delegated Act (EU) No. 665/2014, approved in July 2014. Regulation 1308/2013 attempts to justify the peculiarities of PDO and PGI products. According to researcher Martín, it is necessary to consider their legal nature as genuine intellectual property rights [5].

These labels were introduced in order to encourage diverse agricultural production, to protect names from misuse and imitation, and to help consumers better understand the specific character of the products.

The Protected Designation of Origin (PDO) is the European recognition (Reg. 510/06) [3] for an agricultural product or food, whose entire production cycle—from raw material to final product (processing, preparation, and packaging)—is carried out in a specific territory. Thus, PDO olive oils are produced, processed and prepared in a specific region, using the traditional regional production method. The combination of natural factors—including environmental characteristics, location, and human influence—makes the product unique. All production rules should be strictly adhered to, in order to ensure high quality and reproducibility of the characteristics of the product. In the case of Spanish Olive Oils, the term "D.O.," standing for "Denominación de Origen" (Denomination of Origin) is also used, while for French Olive Oils the term "AOC," standing for "Appellation d'origine contrôlée" (Controlled Destination of Origin) also exists.

The geographical indication is associated with at least one stage of production, preparation, or processing. For example, olives may come from another region and produced in the specified geographical location. This implies that PGI labeling provides a more flexible connection between the olive oil and the specific region, focusing on quality, reputation, and on specific characteristics attributed to the geographical origin.

It should be noted that PDO/PGI products adhere to strict production specifications during the whole processing cycle from harvesting to bottling, including among others, the methods

http://dx.doi.org/10.5772/64909

of cultivation, varieties of olives, and processing approaches. PDO/PGI labeling encourages agricultural production, protects regional products from misuse and imitation, and prevents consumers from being misled by providing them with information about the specific character of the products.

The specifications for PDO/PGI olive oils are dictated by the corresponding Ministerial Decisions or the Executive Regulations of the European Commission. According to these regulations, the olive-crop of a specific variety that is used for the production of PDO/PGI olive oil, exclusively emanates from PDO/PGI regions. Moreover, the processing of the olive fruit takes place within the geographical area, in classic or centrifugal olive mills, which ensure olive-oil paste temperatures of less than 30°C or even lower, during all phases of processing. Finally, particular mention is made to farming techniques in the methods of fighting entomological offences and the short transport of the olive-crop to the olive press. It should be noted that another EU certification is the "Traditional Speciality Guaranteed" (TSG) scheme, which highlights the traditional character of the oil, either in the olive oil's composition or in its production (EC) No. 509/2006 [6].

Countries of the Mediterranean basin are responsible for 98% of globally produced olive oil; it is therefore plausible that these countries have registered the largest number of PDO/PGI olive oils. Indeed, until the 27th of March 2016, according to the EC's DOOR database (Database Of Origin & Registration), Italy had registered 43 PDO/PGI olive oils, while Spain, Greece, France, and Portugal had respectively registered 31, 30, 10, and 6, respectively [7]. From the aforementioned countries, geographical regions producing PDO/PGI olive oils are localized in Greece and France in specific locations, while they are almost uniformly spread in Portugal and Italy. In Greece, the majority (about 70%) of PDO/PGI olive oils are produced in the Peloponnese and Crete. In France, all PDO olive oils are produced in the South East region (Provence, Corsica, Nimes, Nyons, and Nice). Finally, in Spain, approximately half of PDO olive oils are produced in the southern region (EC, Agriculture and Rural Development) [7].

As mentioned above, PDO and PGI labeling of olive oil is connected only to its geographical origin, and not to holistic or ecologically balanced approaches of organic farming, which avoid synthetic chemicals and genetically modified organisms. Organic olive oil complies with EC Regulation 834/07 [8]. In a recent study concerning the Andalusian olive oil industry, the adoption of PDO certifications revealed no link with the implementation of better industrial practices but, rather, with better marketing practices [9].

3. PDO/PGI labeling as a promotion factor for olive oil

Factors that influence consumer behavior may be divided into three groups [10]: (a) properties of foods; (b) individual, related factors (e.g., biological, psychological, and demographic); and (c) environmental factors (i.e., economic, cultural factors, and marketing aspects. Trust and good knowledge of the product [16, 11, 12] are important factors for consumers [13], since they reduce complexity and uncertainty when it comes to making a purchasing decision [14]. The

impact of trust and its correlation to the willingness to pay, is higher among consumers of PDO/PGI products [14, 15]. Factors which seem to play an important role with respect to the consumer's perceived health risk of the olive oil product are the possible low credibility of the media through which it is promoted, the asymmetric information on the quality of the product and the consumer's concerns about the negative impact of agricultural products on health [16]. In the aspect of trust, a study revealed that Greek consumers tend to trust personal information provided by friends and relatives much more than written information on the product's label. This leads to the considerable quantities of unpacked olive oil which are being sold in Greece [17].

Other researchers [18] found that these "official cues" are more important for consumers who live in nonproducing olive oil countries. In countries which do produce olive oil, consumers tend to select olive oil based on origin and "sensory cues." Furthermore, elements related to the origin of olive oil, are gradually becoming more important in the consumer's decision process [19]. In this respect, there is a growing segment of consumers who prefer quality food with certification of origin (PDO/PGI). Sanz and Macias [20] confirmed the strategic role of Spanish PDO olive oils. These PDOs add greater value to local production systems and so enhance the final quality and market differentiation of a specific origin olive oil. Similar results were obtained by Scarpa et al. [21], who studied the PDO label in olive oil, along with two other products (table grapes and oranges). According to these authors, the role of PDOs was stronger for olive oil compared to the other two categories. Chaniotakis et al. [22] focused on the case of own-label olive oil (PDO, PGI, BIO, etc.). This study explores the factors affecting consumers' intentions to buy an own-label olive oil. These factors include the consumers' perceived benefits, economic situation, brand loyalty, and trust. The level of income has a negative impact on both consumer attitudes and purchase intention [22].

Panico et al. [23] investigated consumer preferences in extra virgin olive oil in Italy. Results showed that information on origin, both in terms of the adoption of PDO or PGI certification and labeling of the origin, production method and organoleptic characteristics, crucially affect consumer preferences. Market segmentation shows that there are consumers who are particularly sensitive to origin and organic certification, as well as labeling clarity [23]. Yangui et al. studied the effect of personality traits on consumer preferences in extra virgin olive oil [24]. The results demonstrate that Catalan consumers have unclear knowledge regarding organic attributes as correlating to other production system alternatives (conventional and PDO). The organic attribute is not perceived as a significant quality cue, and the price is not a relevant factor to interpret this result, as organic olive oils are cheaper than PDO olive oils on average. The Catalan consumers who are looking for quality, select PDO extra virgin olive oil. In another investigation performed in Andalusia (Spain) in a sample of 439 olive oil consumers, results demonstrated that origin labeling (PDO labeling), along with the price, affected the preferences of most consumers [25].

Vlontzos and Duquenne [26] investigated the impact of subjective norms of consumer behavior in the Greek olive oil market. The study indicates that consumers are aware of, and accept paying premium prices for organic 66.4% olive oil, as well as for other 30.9% certification protocols (PDO, PGI, etc.).

http://dx.doi.org/10.5772/64909

Similar results were demonstrated in a study by Di Vita et al. [27], revealing that the three main factors affecting consumer preferences toward olive oil involve its area of origin, geographical designation (PDO and PGI), organic certification, and price. With regard to the price factor, consumers from traditionally nonolive oil producing countries, consider price to be an indicator of quality. Other studies indicate that Italian olive oil consumers are positively affected by PDO and BIO (Biological) labels [27, 28]. Other studies found that the PDO label was a more important factor than price [29–31]. Van der Lans et al. found [32]—also concerning olive oil—that price and color were more important than the PDO label.

Espejel et al. [33, 34], studied consumer buying intention for a PDO olive oil from Bajo, Aragon. The results show that the PDO label helps bring out feelings of satisfaction and loyalty.

Despite the fact that customers are strongly motivated to buy PDO/PGI products, many surveys [35–38] indicate that most consumers only have a vague knowledge of the definition and characteristics of PDO/PGI [29, 39, 40]. Percentages of correct understanding vary between 3% for both PDO and PGI in the Italian study by Aprile et al. [36], and 70% for PDO and 40% for PGI in the study by Likoudis et al. [40]. In this study, consumers indicated awareness of the fact that PDO/PGI certified products, including olive oil, were of better quality and safer compared with conventional ones. There is also further confusion regarding different grades of olive oil. For example, Greek consumers often falsely consider that olive oils sold in the market are all virgin [17].

4. Verification of the authenticity of PDO/PGI olive oils

Comprehensive control of PDO/PGI labeling is of vital importance for the protection of a high quality (or even high reputation) olive oil, from unfair competition with similar, but lower value, products. Thus, a verification of origin is the key parameter in establishing the authenticity of a PDO/PGI olive oil. The verification of the authenticity of a product is, however, a difficult challenge in analytical science and the valid strategy lies in the consideration of the product as a complex entity, rather than in measuring a simple property [41, 42]. It should be noted that olive oil composition may vary in soil characteristics, vegetal variety, growing conditions, climate, and/or fertilization. Systems of comparative indicators should be developed, and perhaps the most suitable word for such investigations is "discrimination" between original and fraudulent products rather than real "identification" of the geographical origin. This approach explains to a large extent the multivariate methods used very often in order to classify chemicals and physicochemical properties related to the geographical origin of olive oils [41].

Numerous approaches have been developed and proposed for the assessment of the traceability of olive oils. Classical chemical analysis, particularly determination of moisture content and peroxide index as well as quantification of volatile compounds, fatty acids, sterols, and triterpenic alcohols combined with an appropriate chemometric pattern recognition strategy [43] or neural networks [44] have been proven to be, among others, effective tools for the

recognition of the geographical origin of olive oils. However, this approach has major drawbacks such as the requirement of extensive high-skilled labor resources and the destruction of the sample, while it provides results in a lengthy turnaround time [45]. As alternatives, a wide range of instrumental methods of analysis, permitting rapid screening of olive oils under investigation have been proposed. The geographical identification of olive oils can be performed by studying their phenolic profile by means of liquid chromatography coupled to mass spectrometry and multivariate analysis tools [46]. A lower-cost photo-diode array detector can also be used for the simultaneous detection of the eluted phenolic compounds at different wavelengths and results should be submitted to chemometric techniques in order to create a chromatographic fingerprint [47]. Powerful chemometric tools include, among others, partial least squares discriminant analysis (PLS-DA) [47], linear and stepwise-linear discriminant analysis [48], and principal component analysis [49]. It should be noted that in some cases it is difficult to distinguish a high-quality, family-farmed olive oil, from a PDO extra-virgin olive oil. For instance, Antonini et al. [50] carried out a comparative investigation of several parameters including acidity, peroxide index, UV spectrum and levels of hydroxytyrosol, tyrosol, and dialdehydic forms of decarboxymethylelenolic acid linked to hydroxytyrosol and tyrosol. No statistically significant values between family-farmed and PDO olive oils were observed [50]. Another instrumental method of analysis with applications in the chemical and genetic characterization of PDO olive oils is nuclear magnetic resonance (NMR). Del Coco et al. revealed the possibility of tracing extra olive oils from the same PDO to different cultivars and, partially, to different subareas, by using ^{1}H NMR [51]. Camin et al. combined isotopic composition with ^{1}H NMR data, using multivariate statistical techniques to discriminate Italian olive oil from olive oil imported from Tunisia, with about 98.5% predictive success rate [49]. There are also several studies reporting the use of UV–visible [52, 53], near-infrared spectroscopy (NIS) [52–55] and artificial nose [56]. Due to its wide use in such investigations, the employment of NIR spectroscopy to predict the geographical origin of olive oil has been reviewed [57]. The combination of NIR and UV-visible spectroscopy, along with artificial nose, has also been reported [48].

Another possibility of the geographical characterization of olive oils is related to their metal content, such as Cu, Cr, Fe, and Ni. Distribution of trace elements in virgin olive oils varies according to their geographical origin and, therefore, a "metal content fingerprint" can be provided by appropriate statistical treatment of the levels of metals, allowing for a geographical characterization of different virgin olive oils [58–60]. The determination of metals can be carried out, using appropriate pre-treatment of an olive oil sample—mainly digestion—and detection by spectrometric techniques, such as electrothermal atomic spectrometry (ET-AAS) [60], inductively coupled plasma-optical emission spectrometry (ICP-OES) [59], and inductively coupled plasma-mass spectrometry (ICP-MS) [61]. It should be taken into account that variability of each trace element in olive oils from the same geographical area can be considerable. In such cases, the appropriate chemometric approach can lead to their accurate characterization [60].

Isotopic ratio analysis of certain elements can also be used as a geographical traceability marker. Indeed, the isotopic fractionation of C, H, and O is linked to factors such as soil,

http://dx.doi.org/10.5772/64909

climate, latitude, and rain and, therefore, it is associated with the geographical area of olive trees [62]. Faberi et al. developed a PDO/PGI olive oil authentication, based on the isotope ratio of C in bulk oils and in their fatty acid methyl esters [63]. Another isotopic ratio for possible olive oil authentication purposes, refers to $^{87}Sr/^{86}Sr$, which has been successfully employed as an identification parameter for foodstuffs, such as rice [64], wines [65], and coffee [66]. This ratio would be a promising tool in the case of olive oils [67]. However, such isotopic analysis is only possible via ICP-MS, whose cost is a serious drawback for this approach. Finally, biological and immunochemical techniques can also be employed for the investigation of the geographical origination of olive oil [68]. More information can be found in other chapters in this book.

5. Agrochemicals in PDO/PGI olive oils

Despite the large arsenal of investigations on the levels of agrochemicals in olive oil samples, a systematic comparative study between olive oils with and without PDO/PGI labeling is missing. Among other agrochemicals, pesticide residues are of particular interest, because they are applied to large amounts to olive groves for the control of diseases and pests, such as *Dacus oleae, Saissetia oleae*, and *Prays oleae* in olive trees. They are also utilized in order to increase the number and/or size of olives and subsequent yields. Due to their lipophilic nature, pesticides possess considerable affinity with the lipid matrix of olive oil [69–71]. Thus, a wide range of pesticides can be accumulated in olive oil [72]. In order to serve the purpose of consumer health protection, the European Union has established maximum residue limits, MRLs, of pesticides in olives [73]. Along with Codex Alimentarius [74] of the Food and Agriculture Organization, the European Commission has extended legislation, establishing MRLs for several pesticides in olive oil [75]. With the purpose of comparing the levels of pesticide residues in PDO/PGI olive oils with conventional ones, Likudis et al. analyzed 70 commercially available PDO/PGI Greek olive oil samples for 51 target pesticides [76]. In 30 samples (46% of the analyzed samples), no detectable pesticide residues were found. In the positive samples, penconazole ($n = 20$), α-endosulfan ($n = 18$), β-endosulfan ($n = 16$), and flufenoxuron ($n = 12$) possessed the highest detection rates. Seven other pesticides, namely azinphos-methyl, chlorpyrifos, endosulfan sulfate, fenthion, parathion, parathion-methyl, and quinalphos were detected in fewer samples. The number of different pesticide residues detected in the positive samples ranged from 1 to 4 [76]. The presence of these pesticide residues is in agreement with previous studies concerning olive oil samples in Greece without PDO/PGI labeling [77, 78]. However, detection rates and concentration levels of pesticide residues in PDO/PGI olive oil samples reported by Likudis et al. [76] were significantly lower compared to previous investigations in conventional and organic cultivations [77, 78]. The case of fenthion, detected in 74% of the conventional olive oil samples investigated by Amvrazi and Albanis in a concentration range between 4.6 and 767 $\mu g\ kg^{-1}$ [78] is indicative. The same pesticide was detected only in 4% of the investigated PDO/PGI olive oils with a concentration range in the positive samples between 16.9 and 23.9 $\mu g\ kg^{-1}$ [76]. It should be noted that some pesticide residues gave statistically significant correlation, such as α- and β-endosulfan [76, 78]. Possible explanations are their

simultaneous use or the presence of intercropping activities (olive trees with apple or orange trees, vineyards, etc.

Except for the pesticide residues, heavy metals are also of specific importance. In general, crude oils manufactured without refining, such as extra virgin olive oils, may contain concentrations of trace elements [60]. Among heavy metals, As and Pb are of primary importance due to their high toxicity. Less toxic metals, such as Cu and Fe are also undesirable due to their adverse effects on the oxidative stability of olive oils. The presence of metals in olive oils is attributed to the environmental contamination of soils and air, the use of pesticides and fertilizers, as well as contamination from metallic surfaces during extraction from olive fruits [60, 61]. For this purpose, the International Olive Council (IOC) and the European Union have established criteria for the presence of metal residues. According to IOC, the maximum levels of As, Cu, Fe, and Pb in virgin olive oil are 0.1, 0.1, 3, and 0.1 μg g^{-1}, respectively [79]. The maximum level of 0.1 μg g^{-1} for Pb has also been established by the European Commission in Regulation (EC) No. 1881/2006 [80]. Studies concerning the presence of heavy metals in virgin olive oils have given rather controversial results. However, relevant studies showed that metals extracted from virgin olive oil, only amount to a very small portion compared to their initial concentration in the olive fruits [61, 81]. Over 90% of the metal content present in the olive fruits remained in the olive pomace and, subsequently, they are transferred in great extent to the olive pomace oil [61]. However, such a trend was evident only for Cu in a relevant study reported by Bakkali et al. [82]. This study also revealed high Pb concentrations in extra virgin oils, very close to the maximum level of 0.1 μg g^{-1}. Unfortunately, today, there is a shortage of research on trace metal content in PDO/PGI olive oils. Future systematic studies and PDO/PGI certifications will need to explore the effect of good manufacturing practice on quality, also including levels of heavy metals.

6. Conclusions

PDO/PGI labeling of olive oil constitutes a great motivation for a wide range of consumers, even though a misunderstanding and/or lack of knowledge concerning their meaning is observed. To a significant extent, PDO/PGI labels seem to act as a link to better marketing policies rather than better industrial practices. In some studies, PDO/PGI labeling seems to have no impact concerning their personal characteristics and opinions. Consumer attitude toward certifications needs to be further investigated. The consumer is generally willing to pay a premium in order to buy high-quality olive oil, but there is a rather substantial lack of knowledge about how this quality is expressed and what PDO/PGI and "organic" certification labels stand for. As a result of consumer misunderstanding about certification labels and the confusion surrounding the different qualities of olive oil, significant amounts of unpacked olive oil—of questionable quality—are being sold. In this respect, information should be provided by local authorities and made available to the olive oil consumer. Despite the strict production rules of PGO/PGI foodstuffs, these labels do not necessarily imply that PDO/PGI olive oils are free of pesticide residues. What is required is a "green" and ecologically balanced approach, as expressed by organic farming, which is not the case for PDO/PGI labeling. More

http://dx.doi.org/10.5772/64909

research may indeed confirm that the better cultivation and industrial processes associated with PDO/PGI certifications, result in lower levels of agrochemicals in the final products. The relevant investigations should not only focus on pesticide residues, but be extended to include metals (As, Pb, Cu, Fe), which also affect olive oil quality. Analytical and Bioanalytical Chemistry will continue to be vital in terms of determining the traceability of PDO/PGI olive oils and in confirming their geographical origin and authenticity. Although instrument advances have led to great success in the identification and quantification of chemical compounds with validated methods, an internationally accepted methodology for the identification of the geographical origin of olive oil samples is still missing. New analytical approaches for in situ, rapid screening of olive oil samples are also required.

Author details

Zisimos Likudis

Address all correspondence to: zisimos10@gmail.com

Unit of Public Health, Department of Public Health and Community, Technological Institute of Athens, Egaleo, Athens, Greece

References

[1] Verbeke W, Pieniak Z, Guerrero L, Hersleth H. Consumers' awareness and attitudinal determinants of European Union quality label use on traditional foods. Bio-based and Applied Economics. 2012; 1: 213–229. ISSN: 2280-6180 (print), 2280-6172 (on-line).

[2] European Commission, Regulation (EC) No. 2081/1992 of 14 July 1992. Official Journal European Union 1992; L208: 1–8.

[3] European Commission, Regulation No. 510/2006 of 20 March 2006. Official Journal European Union 2006; L93: 12–25.

[4] European Commission, Regulation No. 1151/2012 of 21 November 2012. Official Journal European Union 2012; L343: 1–29.

[5] Martín JMC. Designations of origin and competition law. Revista de Estudios Regionales Issue N° 102, I.S.S.N.: 0213-7585 (2015), pp. 149–175; 1 January 2015.

[6] European Commission, Regulation (EC) No. 509/2006 of 20 March 2006. Official Journal European Union 2006; L93: 1–11.

[7] European Commission, Agriculture and Rural Development, DOOR database. http://ec.europa.eu/agriculture/quality/door/list.html (accessed in 27-3-2016).

[8] European Commission, Regulation (EC) No. 834/2007 of 28 June 2007. Official Journal European Union 2007; L189: 1–23.

[9] Parra- Lopez C, Hinojosa- Rodriquez A, Sayadi S, Carmona- Torres C. Protected designation of origin as a certified quality system in the Andalusian olive oil industry: Adoption factors and management practices. Food Control. 2015; 51: 321–332. DOI: 10.1016/j.foodcont.2014.11.044.

[10] Steenkamp JB. Dynamics in consumer behaviour with respect to agricultural and food products. In: Agricultural Marketing and Consumer Behaviour in a Changing World. Kluwer Academic Publishers; 1997.

[11] Fearne A, Hornibrook S, Dedman S. The management of perceived risk in the food supply chain: a comparative study of retailer-led beef quality assurance schemes in Germany and Italy. International Food and Agribusiness Management Review, 2001; 4: 19–36.

[12] Velcovska S, Sadilek T. Certification of cheeses and cheese products origin by EU countries. British Food Journal. 2015; 117: 1843–1858. DOI 10.1108/BFJ-10-2014-0350.

[13] Bredahl L. Determinants of consumer attitudes and purchase intentions with regard to genetically modified foods: results of a cross national survey. Journal of Consumer Policy, 2001; 24, 23–61.

[14] Herrera CF, Blanco CF. Consequences of consumer trust in PDO food products: the role of familiarity. Journal of Product and Brand Management, 2011; 20, 282–296.

[15] Yi Y, La, S. What influences the relationship between customer satisfaction and repurchase intention? Investigating the effects of adjusted expectations and customer loyalty. Psychology and Marketing, 2004; 21, 351–373.

[16] Calvo D. Analysis of Quality and Perceived Risk in the Buying Decision-Making Process of Food Products. Doctoral Thesis, Universidad de Coruna, Coruna, Spain; 2001.

[17] Vassiliou A, Kabourakis E, Papadopoulos D. Traceability and ethical traceability in the Greek olive oil chain. In: Coff C, Barling D, Korthals M, Nielsen T (eds.). Ethical Traceability and Communicating Food. The International Library of Environmental, Agricultural and Food Ethics, Vol. 15. Springer; 2008.

[18] Dekhili S, Siriex, L, Cohen, E. How consumers choose olive oil: the importance of origin cues. Food Quality and Preference, doi:10.1016/j.foodqual.2011.06.005; 2011.

[19] Guerrero-Jiménez José Felipe, Juan Carlos Gázquez-Abad, Juan Antonio Mondéjar-Jiménez, Rubén Huertas-García. In: Dimitrios Boskou (ed.). Consumer Preferences for Olive-Oil Attributes: A Review of the Empirical Literature Using a Conjoint Approach, Olive Oil-Constituents, Quality, Health Properties

and Bioconversions, ISBN: 978-953-307-921-9. InTech. Available from: http://www.intechopen.com/books/olive-oil-constituents-quality-health-properties-andbio-conversions/consumer-preferences-for-olive-oil-attributes-a-review-of-the-empirical-literature-using-aconjoint-.

[20] Sanz J, Macías A. Quality certification, institutions and innovation in local agrofood systems: protected designations of origin of olive oil in Spain. Journal of Rural Studies, 2005; 21: 475–486.

[21] Scarpa R, Philippidis G, Spalatro F. Product-country images and preference heterogeneity for Mediterranean food products: a discrete choice framework. Agribusiness, 2005; 21(3): 329–349.

[22] Chaniotakis IE, Lymperopoulos C, Soureli M. Consumers' intentions of buying own-label premium food products. Journal of Product & Brand Management, 2010; 19(5): 327–334.

[23] Panico T, Del Giudice T, Caracciolo, F. Quality dimensions and consumer preferences: a choice experiment in the Italian extra-virgin olive oil market. Agricultural Economics Review 2014; 15(2): 100–112.

[24] Yangui A, Montserrat C-F, José MG. The effect of personality traits on consumers' preferences for extra virgin olive oil. Food Quality and Preference 2016; 51: 27–38. doi: 10.1016/j.foodqual.2016.02.012.

[25] Erraach Y, Sayadi S, Gomez AC, Parra-Lopez C. Consumer-stated preferences towards Protected Designation of Origin (PDO) labels in a traditional olive-oil-producing country: the case of Spain. New Medit 2014; 13: 11–19.

[26] Vlontzos G, Duquenne MN. Assess the impact of subjective norms of consumers' behaviour in the Greek olive oil market. Journal of Retailing and Consumer Services, 2014; 21: 148–157.

[27] Di Vita G, D'Amico M, La Via G, Caniglia E. Quality perception of PDO extra-virgin olive oil: which attributes most influence Italian consumers? Agricultural Economics Review 2014; 14(2).

[28] Vecchio R, Annunziata A. The role of PDO/PGI labelling in Italian consumers' food choices. Agricultural Economics Review 2011; 12: 80–98.

[29] Fotopoulos C, Krystallis A. Are quality labels a real marketing advantage? A conjoint application on Greek PDO protected olive oil. Journal of International Food and Agribusiness Marketing, 2001; 12: 1–22.

[30] Monteiro D, Lucas M. Conjoint measurement of preferences for traditional cheeses in Lisbon. British Food Journal, 2001; 103: 414–424.

[31] Resano H, Sanjuán A, Albisu L. Consumers' response to the EU quality policy allowing for heterogeneous preferences. Food Policy 2012; 37(4): 355–365.

[32] Van der Lans I.A, Ittersum K, Cicco A., Loseby M. The role of the region of origin and EU certificates of origin in consumer evaluation of food products. European Review of Agricultural Economics, 2001; 28: 451–477.

[33] Espejel J, Fandos C, Flavián C. The influence of consumer degree of knowledge on consumer behavior: the case of Spanish olive oil. Journal of Food Products Marketing 2008; 15(1): 15–37. DOI:10.1080/10454440802470565.

[34] Espejel J, Fandos C, Flavián C. Consumer satisfaction: a key factor of consumer loyalty and buying intention of a PDO food product. British Food Journal, 2008; 110(9): 865–881.

[35] Alcalde MJ, Ripoll G, Panea B. Consumer attitudes towards meat consumption in Spain with special reference to quality marks and kid meat. In: Klopčič M, Kuipers A, Hocquette J-F (eds.). Consumer Attitudes to Food Quality Products: Emphasis on Southern Europe. EAAP Publication No. 133. Wageningen Academic Publishers, The Netherlands; 2013. pp. 97–107.

[36] Aprile, MC, Caputo V, Gallina G. Attitude and awareness of EU quality labels: an analysis of Italian consumers. Rivista di Economia Agraria, 2009; LXIV: 269–289.

[37] Aprile MC, Caputo V, Nayga RM Jr. Consumers' valuation of food quality labels: the case of the European geographic indication and organic farming labels. International Journal of Consumer Studies 2012; 36: 158–165.

[38] Menapace L, Colson G, Grebitus C, Facendola M. Consumers' preferences for geographical origin labels: evidence from the Canadian olive oil market. European Review of Agricultural Economics 2011; 38: 193–212.

[39] Philippidis G, Sanjuan A. Territorial product associations in Greece: the case of olive oil. Journal of International Food and Agribusiness Marketing 2003; 14: 25–46.

[40] Likoudis Z, Sdrali D, Costarelli V, Apostolopoulos C. Consumers' intention to buy protected designation of origin and protected geographical indication foodstuffs: the case of Greece. International Journal of Consumer Studies ISSN 1470-6423, doi: 10.1111/ijcs.12253 2015.

[41] Bevilacqua M, Bucci R, Magri AD, Magri AL, Marini F. Tracing the origin of extra virgin oils by infrared spectroscopy and chemometrics: a case study. Analytica Chmica Acta 2012; 717: 39–51.

[42] Aparicio R, Harwood J. Handbook of Olive Oil. Analysis and Properties. Springer Science-Business Media, 2nd edition, New York; 2013.

[43] Armanino C, Leardi R, Lanteri S, Modi G. Chemometric analysis of Tuscan olive oils. Chemometrics and Intelligent Laboratory Systems 1989; 5: 343–354.

[44] Torrecilla JS, Cancilla JC, Matute G, Diaz- Rodriguez P. Neural network models to classify olive oils within the protected denomination of origin framework. International Journal of Food Science and Technology 2013; 48: 2528–2534.

[45] Valcarcel M, Cardenas S. Vanguard-rearguard analytical strategies. Trends in Analytical Chemistry 2005; 24: 278–280.

[46] Bajoub A, Carrasco- Pancorbo A, Ajal EE, Ouazzani N, Fernandez- Gutierrez A. Potential of LC-MS phenolic profiling combined with multivariate analysis as an approach for the determination of the geographical origin of north Moroccan virgin olive oils. Food Chemistry 2015; 166: 292–300.

[47] Nescatelli R, Bonanni RC, Bucci R, Magri AL, Magri AD, Marini F. Geographical traceability of extra virgin olive oils from Sabina PDO by chromatographic fingerprinting of the phenolic fraction coupled to chemometrics. Chemometrics and Intelligent Laboratory Systems 2014;139: 175–180.

[48] Forina M, Oliveri P, Bagnasco L, Simonetti R, Casolino MC, Nizzi Grifi F, Casale M. Artificial nose, NIR and UV-visible spectroscopy for the characterization of the PDO Chianti Classico olive oil. Talanta 2015; 144: 1070–1078.

[49] Camin F, Pavone A, Bontempo L, Wehrens R, Paolini M, Faberi A, Marianella RM, Capitani D, Vista S, Mannina L. The use of IRMS, ^{1}H NMR and chemical analysis to characterize Italian and imported Tunisian olive oils. Food Chemistry 2016; 196: 98–105.

[50] Antonini E, Farina A, Leone A, Mazzara E, Urbani S, Selvaggini R, Servili M, Ninfali P. Phenolic compounds and quality parameters of family farming versus protected designation of origin (PDO) extra-virgin olive oils. Journal of Food Composition and Analysis 2015; 43: 75–81.

[51] Del Coco L, Mondelli D, Mezzapesa GN, Miano T, de Pascali SA, Girelli CR, Fanizzi FP. Protected designation of origin extra virgin olive oils assessment by nuclear magnetic resonance and multivariate statistical analysis: "Terra di Bari", an Apulian (Southeast Italy) case study. JAOCS Journal of the American Oil Chemists' Society 2016; in press. DOI: 10.1007/s11746-015-2778-1.

[52] Casale M, Casolino C, Oliveri P, Forina M. The potential of coupling information using three analytical techniques for identifying the geographical origin of Liguria extra virgin olive oil. Food Chemistry 2010; 118: 163–170.

[53] Casale M, Oliveri P, Casolino C, Sinelli N, Zunin P, Armanino C, Forina M, Lanteri S. Characterisation of PDO olive oil chianti classico by non-selective (UV-visible, NIR and MIR spectroscopy) and selective (fatty acid composition) analytical techniques. Analytica Chimica Acta 2012; 712: 56–63.

[54] Casale M, Armanino C, Casolino C, Forina M. Combining information from headspace mass spectrometry and visible spectroscopy in the classification of the Ligurian olive oils. Analytica Chimica Acta 2007; 589: 89–95.

[55] Galtier O, Dupuya N, Le Dr'eau Y, Ollivier D, Pinatel C, Kister J, Artaud J. Geographic origins and compositions of virgin olive oils determinated by chemometric analysis of NIR spectra. Analytica Chimica Acta 2007; 595: 136–144.

[56] Cosio MS, Ballabio D, Benedetti S, Gigliotti C. Geographical origin and authentication of extra olive oils by an electronic nose in combination with artificial neural networks. Analytica Chimica Acta 2006; 567: 202–210.

[57] Casale M, Simonetti R. Review: near infrared spectroscopy for analyzing olive oils. Journal of Near Infrared Spectroscopy 2014; 22: 59–80.

[58] Mendil D, Uluozlu OD, Tuzen M, Soylak M. Investigation of the levels of some element in edible oil samples produced in Turkey by atomic absorption spectrometry. Journal of Hazardous Materials 2009; 15: 724–728.

[59] Zeiner M, Juranovic-Cindric I, Skevin D. Characterization of extra virgin olive oils derived from the Croatian cultivar Oblica. European Journal of Lipid Science and Technology 2010; 112: 1248–1252.

[60] Cabrera- Vique C, Bouzas PR, Oliveras-Lopez MJ. Determination of trace elements in extra virgin olive oils: a pilot study on the geographical characterization. Food Chemistry 2012; 134: 434–439.

[61] Llorent-Martinez EJ, Fernandez-de Cordova ML, Ortega-Barrales P, Ruiz-Medina A. Quantitation of metals during the extraction of virgin olive oil from olives using ICP-MS after microwave-assisted acid digestion. Journal of American Oil Chemical Society 2014; 91: 1823–1830.

[62] Angerosa F, Breas O, Contento S, Guillou C, Reniero F, Sada E. Application of stable isotope ratio analysis to the characterization of the geographical origin of olive oils. Journal of Agricultural and Food Chemistry 1999; 47: 1013–1017.

[63] Faberi A, Marianella RM, Fuselli F, La Mantia A, Ciardiello F, Montesano C, Mascini M, Sergi M, Compagnone D. Fatty acid composition and $\delta^{13}C$ of bulk and individual fatty acids as marker for authenticating Italian PDO/PGI extra virgin olive oils by means of isotopic ratio mass spectrometry. Journal of Mass Spectrometry 2014; 49: 840–849.

[64] Kawasaki A, Oda H, Hirata T. Determination of strontium isotope ratio of brown rice for estimating its provenance. Soil Science and Plant Nutrition 2002; 48: 635–640.

[65] Durante C, Baschieri C, Bertacchini L, Cocchi M, Sighinolfi S, Silvestri M, Marhetti A. Geographical traceability based on $^{87}Sr/^{86}Sr$ indicator: a first approach for PDO Lambrusco wines from Modena. Food Chemistry 2013; 141: 2779–2787.

[66] Techer I, Lancelot J, Descroix F, Guot B. About Sr isotopes in coffee "Bourbon Pointu" of the Reunion Island. Food Chemistry 2011; 126: 718–724.

[67] Janin M, Medini S, Techer I. Methods for PDO olive oils traceability: state of art and discussion about the possible contribution of strontium isotopic tool. European Food Research and Technology 2014; 239: 745–754.

[68] De La Guardia M, Gonzalvez A. Food protected designation of origin. Methodologies and applications. In: Barcelo D (ed). Comprehensive Analytical Chemistry, Vol. 60. Elsevier, UK; 2013.

[69] Van de Waterbeemd H, Testa B. The parametrization of lipophilicity and other structural properties in drug design. In: Testa B (ed.). Advances in Drug Research, Vol. 16. Academic Press, New York; 1987.

[70] Tsopelas F, Ochsenkühn-Petropoulou M, Tsantili- Kakoulidou A, Ochsenkühn KM. Study of the lipophilicity of selenium species. Analytical and Bioanalytical Chemistry 2005; 381: 420–426. DOI: 10.1007/s00216-004-2822-8.

[71] Tsopelas F, Tsantili-Kakoulidou A, Ochsenkühn-Petropoulou M. Lipophilicity, biomimetic retention profile and antioxidant activity of selenium species. Microchemical Journal 2013; 110: 711–718. DOI:10.1016/j.microc.2013.08.009.

[72] Tsoutsi C, Konstantinou I, Hela D, Albanis T. Screening method for organophosporus insecticides and their metabolites in olive oil samples based on headspace solid-phase microextraction coupled with gas chromatography. Analytica Chimica Acta 2006; 573–574: 216–222.

[73] European Communities, Council Directive No. 76/ 895 of 23-11-1976. Official Journal European Union 1976; L340/26.

[74] Codex Alimentarius Commission International Food Standards, http://www.codexalimentarius.org (assessed 12-3-2016).

[75] European Commission, Regulation (EC) No. 396/2005 of 23 February 2005. Official Journal European Union 2005; L70/1.

[76] Likudis Z, Costarelli V, Vitoratos A, Apostolopoulos C. Determination of pesticide residues in olive oils with protected geographical indication or designation of origin. International Journal of Food Science and Technology 2014; 49: 484–492.

[77] Hiskia AE, Atmajidou ME, Tsipi DF. Determination of organopshosphorus pesticide residues in Greek virgin olive oil by capillary gas chromatography. Journal of Agricultural and Food Chemistry 1998; 46: 570–574.

[78] Amvrazi EG, Albanis TA. Pesticide residue assessment in different types of olive oil and preliminary exposure assessment of Greek consumers to the pesticide residues detected. Food Chemistry 2009; 113: 253–261.

[79] International Olive Council. Trade standard applying to olive oils and olive-pomace oils. COI/T. 15/ NC No. 3/Rev. 7, 2012.

[80] European Commission. Regulation No. 1881/ 2006 of 19 December 2006 setting maximum values for certain contaminants in foodstuffs. Official Journal European Union 2006; L364: 5–24.

[81] Llorent-Martinez EJ, Ortega-Barrales P, Fernandez-de Cordova ML, Ruiz-Medina A. Analysis of the legislated metals in different categories of oil and olive-pomace oils. Food Control 2011; 22: 221–225.

[82] Bakkali K, Ramos Martos N, Souhail B, Ballesteros E. Determination of heavy metal content in vegetables and oils from Spain and Morocco by Inductively Coupled Plasma Mass Spectrometry. Analytical Letters 2012; 45: 907–919.

Chapter 11

Geographical Indication Labels in Moroccan Olive Oil Sector: Territorial Dimension and Characterization of Typicality: A Case Study of Meknès Region

Aadil Bajoub, Lucía Olmo-García,
Noureddine Ouazzani, Romina Paula Monasterio,
Gabriel Beltrán and Alegría Carrasco-Pancorbo

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64538

Abstract

Geographical indications (GIs) implementation is, nowadays, one of the most prominent differentiation strategies used in olive oil market. The proliferation of these labels, however, causes debate and controversy, in particular regarding their usefulness, effectiveness, and suitability of some protected areas to acquire them. This chapter discusses the use of GI labels in olive oil market, and proposes a four-stage methodological approach to examine the potential of Meknès region—a Moroccan olive growing area—to acquire a GI label. Based on this approach, Meknès region territorial dimensions were defined, the typicality of its olive oil was characterized, a general scheme for the GI recognition was proposed, and the adopted strategy to enhance the meaning of this label on domestic, national, and international markets was highlighted. The main findings of this study justify the suitability of Meknès region to protect its olive oil with a GI label.

Keywords: olive oil, geographical indication, chemical characterization, differentiation marketing strategy, Meknès region

1. Introduction

In the Mediterranean Basin, olive oil production can be considered as a science, but also a combination of creativity and innovation; it is also a lifestyle and a unique and ancestral tradition

passed down through history from one generation to another. This historical interaction between Mediterranean populations, environment, and olive tree cultivation has created a specific cultural identity, which is crucial to understand the emergence and spread of olive tree cultivation and oil production all over the world [1]. It is also very important to mention the fundamental socioeconomic role that olive oil production and trade have played in different parts of the Mediterranean area throughout history.

In many Mediterranean regions, nowadays, an integral form of revitalization, enhancement, and appreciation of the historical legacy of olive oil production is the establishment of geographical indications (GIs). They are often perceived as valuable tools that allow not only the safeguard of their cultural identity, but also to gain market benefits and profitability and competitiveness in a growing olive oil globalized market [2].

GIs are names of places or regions used to brand goods (olive oil in this case) with a distinct geographical connotation, which means that their specific quality attributes are considered to be inherently linked to (or determined by) their geographical origin's characteristics. In this way, olive oil companies, besides using private trademarks, have an excellent opportunity to promote the uniqueness of their products through the use of these labels. Olive oil producers generally demonstrate the quality of this product by regulatory standardized parameters (mainly content of free fatty acids, peroxide value, ultraviolet specific extinction coefficients, and sensory characteristics); in contrast, GIs broaden the olive oil quality concept to added-value attributes such as provenance, local know-how, cultural traditions, and distinctive quality, which can help to differentiate the origin-labeled olive oil among similar products. By adopting these labels, added-value, premium price and a competitive positioning on either traditional or emerging olive oil markets are, obviously, expected. It is also assumed that they can provide rural areas (where they are established) with additional social and economic benefits [3, 4]. Given their importance, an unprecedented recognition of these labels was recorded over the last years in many parts of the Mediterranean area. According to the information published by different organizations [5–9], it is possible to state that till 2015, about 123 olive growing regions have registered their olive oil production under GI schemes; all of them are located in Mediterranean countries, with Italy (43 GIs), Greece (29 GIs), and Spain (28 GIs) at the highest positions of the list.

Because of the rapid proliferation of GIs in the olive oil sector, their usefulness and effectiveness are currently lively debated topics. Within this context, some very successful stories of olive oil GIs can be told (e.g., the Spanish Baena olive oil [10], French GIs olive oils [11], and Italian Toscana olive oil [12], that have achieved a considerably higher price (premium price) than other no GI labeled olive oils). Nevertheless, these cases represent a limited number of examples if compared with the high number of existing GI olive oils. Some authors have expressed reservations in this respect, pointing out the lack of reputation and notoriety of several of these olive oils produced under GI schemes. This would obviously have negative consequences on the overall value of the olive oil GI system [13, 14]. Therefore, it is very important to identify the moment in which implementing an olive oil GI is appropriate, as well as how to benefit from it. As properly stated by Aubard [15], GIs should be adopted as response to an identified (product chain) need, and must be designed pragmatically and

http://dx.doi.org/10.5772/64538

realistically, so as to be useful to the business. Furthermore, the author criticizes the wrong idea that many producers have about GIs, expecting that the label will automatically make the markets more accessible to them. This is not true and any procedure aiming to increase the value of products (such as GI) necessary requires a properly planned management and considerable marketing efforts [15].

Morocco has traditionally been a land of olive tree cultivation and olive oil production, and it currently stands out as one of the most important olive oil producing countries, ranking sixth worldwide [16]. In this country, the olive oil sector is increasingly considered as an economic and social development engine of various regions. Under that perspective, olive oil sector modernization, yield increasing, and olive oil quality enhancement, have been—and remain—a priority in Moroccan agricultural policy [17]. In this respect, the establishment of GIs is the cornerstone of the current Moroccan olive oil quality policy. Protected designations of origin (PDO), protected geographical indications (PGIs), and traditional specialities guaranteed (TSG) are the instruments created for this purpose [18]. Indeed, till 2016, two PDOs and three PGIs have been registered, while some others are in the scrutiny process.

Meknès region is one of these Moroccan olive growing areas that aim to provide their olive oil production with a GI label (particularly, a PDO). With the purpose to contribute to the implementation of this GI label for Meknès olive oil, our research group has carried out a multidisciplinary and pluri-annual study. Other Mediterranean experiences in this field, and the assets and constraints abovementioned that have led to the current scenario (in which some olive oil GIs have experienced exceptional success while others have failed) have been considered. In our study, we basically tackled the following broad questions:

-is setting up a PDO in Meknès region appropriate?

-what should it be done to assure that this label will be long-lastingly effective and not only one more without any interest and benefit?

2. Setting up a Meknès olive oil PDO: methodological approach and main findings

The approach in this study was based on a comprehensive literature review of what drives to successful processes of implementation and valorization of different GIs. Every success factor identified in literature was listed and adapted during our research, with additional insights gained over the study period.

It was found that no universal model exists for a successful GI labels implementation and valorization; there is, indeed, a wide diversity of practices for implementing registered GIs all over the world [19–23]. In addition, literature clearly points out that the process of determining the suitability of a product in a given region to pursue a GI label should be scientifically grounded and built on a robust methodology taking into account the current knowledge in the field. It should be also able to stimulate the participation of all the product supply chain actors in order to integrate different points of view and interests. The concept of GI necessarily

requires the efforts and skills of the different producers and/or processors to build a common vision concerning the quality of the product and the specific characteristics of its production process.

From the beginning, our team was convinced about the fact that the implication of the future Meknès olive oil PDO stakeholders was strongly encouraged and could represent the key to success. We refer to the members of the association "Union pour le Développement de l'Olivier de Meknès (UDOM)", including farmers, olive oil producers and processors. The scientific approach was designed around the basic definition of a PDO (also called "geographical designation of origin" or "appellations of origin"): "*the geographical denomination of a country, region, or locality, which serves to designate a product originating therein, the quality or characteristics of which are due exclusively or essentially to the geographical environment, including natural and human factors*" [24]. Two features are noteworthy in this definition:

- The first is the fact that a PDO identifies a geographical entity, including natural and human factors. This is commonly designed by the term "terroir".
- The second is the existence of a link between the quality, characteristics and reputation of the PDO product and the "terroir" where is produced. This refers to the term "typicality".

Consequently, the implementation of a PDO label should be certainly built on the delimitation of the terroir, the definition of its factors, and the characterization of the typicality of the product.

2.1. A first step toward Meknès virgin olive oil PDO: characterization of "terroir" dimensions

Terroir is a derivative of the French word for soil or land "terre", that can be conceptualized in several different disciplines [25, 26]. This concept is practically the base of any GI system and many researchers have worked on its definition and the determination of its components [19, 27]. Terroir could be defined as: *a delimited geographical space, where a human community has constructed a collective intellectual or tacit production know-how, based on physical and biological milieu and a set of human factors, which confer a typicality and induce a reputation for a product that originates in that terroir* [28, 29]. What comes out from this definition is that an efficient way of approaching terroir should basically imply the determination of its three dimensions:

- *Geographical dimension:* the natural environment (mainly climate, topography, geology, and soil);
- *Social dimension:* the local know-how, traditions and cultural aspects in relation to the production, trade and use of the product; and
- *Technical dimension:* the agronomical and technological techniques used in the elaboration of the product.

Overall, it can be said that it is the combination and the strong interaction among these three dimensions, which is reflected on the special quality and particular characteristics of a GI product.

http://dx.doi.org/10.5772/64538

2.1.1. Meknès terroir geographical dimension

Meknès region is located in the North-Center of Morocco (33°53′36N, 5°32′50W), covering an area of approximately 400,700 ha, including a total olive growing area of 43,000 ha. It is a region with dramatic topographic contrasts and its landscape has a complex geomorphology (**Figure 1**). Climatically, Meknès terroir is situated in a Mediterranean subhumid to semi-arid climatic zone, with cool winter and warm dry summer. Rainfall is mostly uniformly distributed over the year. On average, this terroir annually receives 400–600 mm of rainfall, which is favorable for various crops, including olive tree. Meknès soils are constituted by fluvisols, regosols, lithosols, rendzinas, yermosols, xerosols, vertisols, kastanozems, chernozems, phaezems, luvisols, and acrisols, varying in thickness, depending on the depth of the substrate and the old and recent manifestations of anthropogenic erosion and runoff.

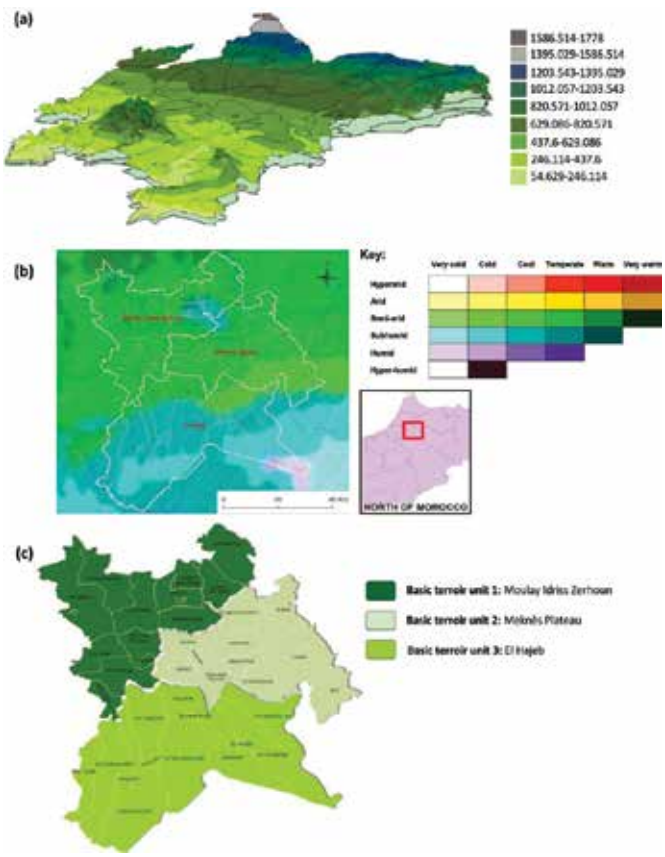


Figure 1. Meknès terroir geographical dimension construction: (a) altitude map; (b) climate map; (c) Meknès terroir delimitation showing the localization of the three basic terroir units identified.

The first step in the characterization of the geographical dimension of Meknès terroir has revealed a relatively complex variability of natural environmental factors in this area,

which can affect the homogeneity of qualitative and compositional profile of the olive oils obtained in this terroir. Therefore, the identification of basic terroir units (homogenous geographical areas, from the environmental point of view) inside this region was the following step. The model proposed by Morlat [30] was used for that. Practically, each unit is defined by three associated components: a geological component, a pedoclimatic element and a landscape component. Therefore, the workflow of this methodology involved three main tasks:

- Delimitation, characterization and cartography of Meknès terroir.
- Characterization of the landscape and pedoclimatic conditions in this terroir.
- A multifactorial analysis which integrates all the results obtained from the first two activities and allows the determination of the basic terroir units.

Thus, by applying this methodology, it was possible to identify three basic terroir units in Meknès region: Moulay Idriss Zerhoun, Meknès Plateau, and El Hajeb (**Figure 1**).

2.1.2. Social dimension of Meknès terroir

Once the geographical dimension of Meknès terroir was defined, a careful investigation of ancient documents, archive maps and books that report the history of olive tree and oil production in this territory was made. Gastronomy habits and ancient practices and uses of this product throughout the history of Meknès were also documented. Finally, historical structures and archeological evidences which testify the long-standing olive growing and oil production practices in this zone were explored and inventoried.

It was clearly demonstrated that Meknès region constitutes the cradle of olive tree cultivation and oil production in Morocco, since the oldest evidence of these practices in this country (dating for the roman era, about 2000 years ago) are found in this area (Moulay Idriss Zerhoun area and the archaeological site of Volubilis).

2.1.3. Technical dimension of Meknès terroir

At this point, we analyzed the olive-growing and olive oil processing sector characteristics of Meknès terroir. The work was firstly based on the study of data coming from the national and local institutions in charge of agriculture development, and, then, several surveys were performed among farmers and olive oil processors. The surveys focused on olive-growing farms were conducted to characterize the practiced agronomical techniques for the management of olive orchards (mainly planted olive varieties, plantation density, soil management, irrigation, fertilization, pruning, disease management, and harvesting), and also to evaluate the productivity of the olive orchard. The collected data were analyzed in depth and allowed to distinguish four main different olive growing cultivation systems: traditional rainfed, intensive rainfed, intensive irrigated, and superintensive system (**Figure 2**), with 93.4% of Meknès olive orchard planted using "Picholine Marocaine" cultivar.

http://dx.doi.org/10.5772/64538

Figure 2. Olive growing cultivation systems in Meknès: (a) traditional rainfed; (b) intensive rainfed; (c) intensive irrigated; (d) superintensive system.

A second type of surveys focused on olive oil mills. The collected data were compiled in a geodatabase and integrated with digital maps of Meknès region (**Figure 3**). A total of 245 olive oil mills were listed; 102 of them were traditional processing mills, so-called massars, 91 semimoderns oil mills, and 52 moderns oil mills (28 two-phase and 24 three-phase). Regardless of the number of mills of each type, most of the produced olive oil is made by modern mills, showing a mean processing capacity of 3533.6 t/day. Furthermore, all the olive oils commercialized in package are coming from these mills.

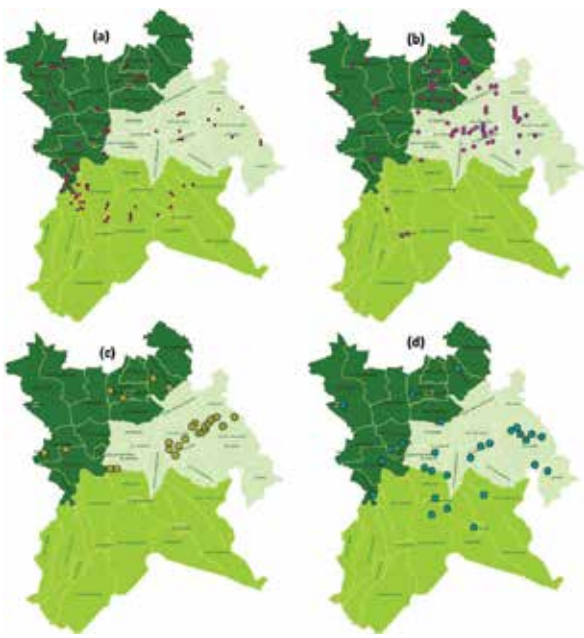


Figure 3. Geographical localization maps of Meknès olive oil mills: (a) traditional; (b) semimodern; (c) three-phase; (d) two-phase.

The first phase of the study was, therefore, accomplished, and, the territorial dimensions of Meknès region had been characterized. The obtained results logically conditioned the selected methodological approach to carry out the second stage of the project, in particular, regarding the selection and collection of olive oil samples for the determination of the typicality of Meknès olive oil.

2.2. Characterization of typicality of Meknès virgin olive oil

2.2.1. The typicality concept: definition, dimensions and attributes

According to the definition of the concept of typicality of an agricultural product, this term means: *"the property of belonging to a particular type defined and recognized as such by a specific human group, the different members of which have acquired areas of knowledge or know-how relative to their role in the production process. Know-how, then, as regards the setting up of the process, and the making, testing and tasting of the product. […] It is a particular construction that aims to materialize the effect of the land on a given product"* [28]. Inspired by this definition, various authors describe as mandatory the determination of a set of properties of belonging (level of representativeness of an item in a category) and distinction (properties that make possible to differentiate, identify, and recognize the product among others similar) for the ascertainment of the typically of a product [31, 32].

2.2.2. Typicality of Meknès virgin olive oil: findings of the main studies

Based on the previous definition, the evaluation of the typicality of Meknès olive oil was performed by determining:

-*Its properties of belonging:* by assessing the qualitative and compositional profile of olive oils produced within Meknès region. It was expected that the olive oils produced in this area would show some differences among them, but sharing certain common characteristics. Indeed, the determination of these common features could allow defining the "typical" olive oil produced in this region. If this "typical profile" would differ among the three basic terroir units identified, there will be no way to consider the whole Meknès region as an eligible area to be certified by a PDO scheme.

-*Its properties of distinction:* which involves the characterization and identification of qualitative and compositional properties of Meknès olive oils that distinguish them among others oils produced outside this terroir. It is a question of identifying the uniqueness and singularity of the oils produced in Meknès region.

In the coming paragraphs, we will try to briefly summarize the most relevant results from some of the research projects which have been carried out (or are ongoing) within our lab involving the use of different analytical techniques and the determination of the physico-chemical and sensory quality, as well as other compositional parameters (such as phenolic compounds, triacylglycerols, and volatile compounds).

http://dx.doi.org/10.5772/64538

For the determination of Meknès olive oil properties of belonging, two pluri-annual studies were carried out. The first work aimed the characterization of the physicochemical and organoleptic quality and compositional profile of 298 olive oil samples from "Picholine Marocaine" cultivar, obtained from 12 industrial olive oil mills, located in the three identified basic terroir units. Both variations induced by crop season and those expected between the three basic terroir units were assessed over four consecutive crop seasons (from 2010 to 2013). The results obtained reveal that, besides an interannual variation, olive oils produced in Meknès region are characterized by high physicochemical and sensory quality, as well as a homogeneous composition regardless of the production subarea. Considering their sensory quality, all the analyzed samples were classified as extra virgin olive oils, according to IOC regulations. **Figure 4** depicts the standard/average sensory profile of the studied samples, having two categories: intense fruitiness and medium fruitiness. More details can be found in [33].

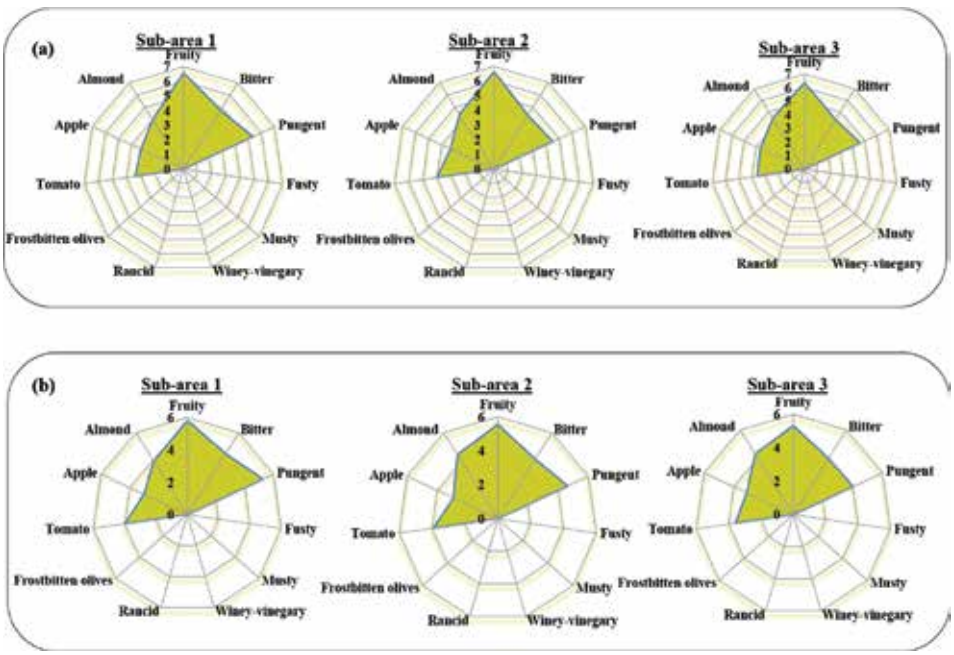


Figure 4. Sensory averaged profiles of Meknès monovarietal olive oils: (a) intense fruitiness profile; (b) medium fruitiness profile (reproduced with permission from [33]).

In the second study [34], particular attention was paid to the characterization of phenolic compounds from oils produced in Meknès region. These compounds are of unquestionable importance since they have a noticeable influence on some olive oil sensory characteristics and biological properties [35, 36]. These reasons made us going for their characterization in Meknès olive oils. The study was conducted over three consecutive crop seasons (2011, 2012, and 2013) on 142 "Picholine Marocaine" olive oil samples obtained by extracting olive fruits collected from orchards located on the three Meknès basic terroir units. A liquid chromatography-mass

spectrometry platform was used to this purpose. A total of 28 phenolic compounds (and quinic acid) were determined, revealing the complex profile of Meknès virgin olive oil, composed, in order of abundance, by secoiridoids, phenolic alcohols, lignans, flavonoids, and phenolic acids. Results showed that the variation of the content of phenolic compounds was mainly related to the crop season, which proves, once again, the homogenous character of the profile of olive oils produced in the entire Meknès terroir. **Table 1** shows some of the quantitative results obtained in this study, showing the mean ± standard deviation (mg/kg) of phenolic compounds, grouped by chemical categories.

Chemical category	Crop season 2011			Crop season 2012			Crop season 2013		
	Subarea 1 (n = 13)	Subarea 2 (n = 15)	Subarea 3 (n = 15)	Subarea 1 (n = 11)	Subarea 2 (n = 16)	Subarea 3 (n = 7)	Subarea 1 (n = 24)	Subarea 2 (n = 9)	Subarea 3 (n = 32)
Simple phenols	10.22^{a}a ± 7.97	13.07^{a}a ± 6.98	11.19^{a}a ± 7.02	10.19^{a}a ± 2.63	8.77^{a}ab ± 2.98	7.64^{a}ab ± 1.78	8.11^{a}a ± 4.83	7.51^{a}b ± 3.95	6.90^{a}b ± 3.15
Lignans	5.34^{a}a ± 2.16	4.08^{a}a ± 2.23	4.41^{a}a ± 2.96	2.99^{a}b ± 3.25	4.09^{a}ab ± 5.83	2.19^{a}a ± 2.12	1.43^{a}b ± 0.87	1.58^{a}b ± 0.42	3.08^{a}a ± 4.77
Flavonoids	1.55^{a}a ± 1.17	3.23^{a}a ± 3.18	3.15^{a}a ± 2.47	1.40^{a}a ± 0.24	1.57^{a}ab ± 0.76	2.10^{a}ab ± 1.1	1.38^{ab}a ± 0.5	1.10^{a}b ± 0.29	1.63^{b}b ± 0.67
Phenolic acids	0.13^{a}a ± 0.09	0.22^{a}a ± 0.15	0.22^{a}a ± 0.27	0.18^{a}a ± 0.07	0.19^{a}a ± 0.14	0.22^{a}a ± 0.15	0.18^{a}a ± 0.09	0.17^{a}a ± 0.07	0.21^{a}a ± 0.15
Secoiridoids	704.59^{a}a ± 361.56	1018.01^{a}a ± 524.23	1106.96^{a}a ± 553.85	594.50^{a}a ± 175.16	706.15^{a}ab ± 218.98	831.46^{a}ab ± 363.83	684.64^{a}a ± 177.52	540.12^{a}b ± 158.91	688.14^{a}b ± 253.54
Other compounds	1.04^{a}a ± 1.05	12.87^{b}a ± 16.60	9.34^{b}a ± 15.2	2.80^{a}a ± 3.89	0.51^{b}b ± 0.53	1.32^{ab}a ± 1.18	1.84^{a}a ± 2.35	5.48^{a}a ± 12.38	2.34^{a}b ± 3.83

-Significant differences in the same row are indicated with different lowercase letters (comparison among crop seasons, $p < 0.05$) and with different superscript letters (comparison among subareas at the same crop season, $p < 0.05$).
-The different categories included the sum of the individual amount of the following compounds: simple phenols (hydroxytyrosol, tyrosol, and oxidized hydroxytyrosol), lignans (pinoresinol, acetoxypinoresinol, and syringaresinol), flavonoids (luteolin and apigenin), phenolic acids (*p*-coumaric acid), secoiridoids (decarboxymethylated form of elenolic acid, desoxy elenolic acid, elenolic acid, decarboxymethyl oleuropein aglycone, methyl decarboxymethyl oleuropein aglycone, decarboxymethyl ligstroside aglycone, dehydro oleuropein aglycone, oleuropein aglycone (and its isomers), methyl oleuropein aglycone, and ligstroside aglycone (and its isomers)), and other compounds (quinic acid).
-Quinic acid, hydroxytyrosol, tyrosol, pinoresinol, luteolin, apigenin, and *p*-coumaric acid were quantified in terms of their commercial pure standards. Oxidized hydroxytyrosol was quantified in terms of hydroxytyrosol; lignans, in terms of pinoresinol; and secoiridoids with oleuropein.
-Mean values are those calculated for all the samples coming from the same subarea and crop season, therefore, standard deviation gives to the reader only an idea about the variability of the olive oils in terms of composition, and obviously not about the repeatability of the analytical methods used. (The same is applicable to **Table 2** regarding the different provenance regions.)

Table 1. Mean ± standard deviation (mg/kg) of phenolic compounds determined in Meknès monovarietal virgin olive oils.

With regard to the properties of distinction of Meknès olive oils, various phenolic compounds profiling studies (combining compositional data and chemometric treatments) were performed in an attempt to discriminate the olive oils produced in Meknès terroir from others

http://dx.doi.org/10.5772/64538

produced outside this region. Within this context, the potential of merging quality and chemical profiles data and multivariate statistical analysis was tested on 279 olive oil samples (among which 69 were from Meknès region and the others were collected from six North Moroccan regions). The obtained chemometric models were able to correctly discriminate Meknès olive oils from the rest, with rate of 100% and 91.30% in recognition and prediction abilities, respectively [37].

In another work carried out on the same samples set, the triacylglycerols (TAG) fraction was determined and chemometric data analysis (including principal components analysis (PCA), linear discriminant analysis (LDA), partial least squares-discriminant analysis (PLS-DA), and soft independent modeling of class analogies (SIMCA)) was used to differentiate the studied samples according to their geographical origin. Twenty one TAGs were characterized and the variability observed among the studied samples could be related to the production area. The mean concentration and standard deviation (mean ± SD (%)) for the TAGs identified in the studied North Moroccan olive oil samples are listed in **Table 2**.

	Chefchaouane	**Fès**	**Meknès**	**Ouazzane**	**Sefrou**	**Taounate**	**Taza**
	Mean ± SD						
ECN42							
LLL	0.05a ± 0.02	0.41b ± 0.14	0.14a ± 0.06	0.28c ± 0.13	0.11a ± 0.03	0.25c ± 0.09	0.14a ± 0.06
OLLn + PoLL	0.35a ± 0.13	0.52b ± 0.17	0.37a ± 0.09	0.50b ± 0.03	0.37a ± 0.06	0.38a ± 0.07	0.48b ± 0.09
PLLn	0.07ac d ± 0.03	0.11b ± 0.03	0.08c ± 0.03	0.10b ± 0.01	0.06d ± 0.01	0.08c ± 0.02	0.07acd ± 0.02
Total ECN 42	0.58ade ± 0.29	1.04b ± 0.30	0.59ad ± 0.14	0.88c ± 0.16	0.54d ± 0.09	0.71e ± 0.15	0.70ae ± 0.16
ECN44							
OLL	2.96ade ± 0.88	4.80b ± 0.88	2.41cd ± 0.71	3.45a ± 0.91	2.08c ± 0.51	3.52a ± 0.75	2.90e ± 0.63
OOLn	1.58ab ± 0.21	1.79a ± 0.26	1.68a ± 0.32	1.77a ± 0.04	1.76a ± 0.06	1.47b ± 0.17	2.13c ± 0.19
PLL	0.46a ± 0.27	0.90b ± 0.54	0.48a ± 0.14	0.85b ± 0.14	0.45a ± 0.1	0.46a ± 0.07	0.76b ± 0.17
POLn	0.63ab ± 0.13	0.66a ± 0.16	0.66a ± 0.14	0.66ab ± 0.10	0.58b ± 0.03	0.43c ± 0.08	0.76d ± 0.11
Total ECN 44	5.03ade ± 1.64	8.16b ± 1.61	5.24ad ± 0.99	6.73c ± 0.72	4.87d ± 0.64	5.88e ± 0.83	6.54ec ± 0.80
ECN46							
OOL + PPLn	14.23ace ± 3.79	19.03b ± 1.19	14.91ce ± 1.68	16.87dfg ± 1.49	15.01eg ± 1.58	17.72f ± 1.42	16.07g ± 1.34
PoOO	1.19abde ± 0.38	1.28abe ± 0.22	1.17ad ± 0.14	1.12acd ± 0.18	0.91f ± 0.12	1.31ae ± 0.14	1.01acf ± 0.16
SLL+PLO	4.62acd ± 1.43	5.85e ± 1.00	5.41ae ± 1.26	6.58b ± 0.46	4.71cd ± 0.46	4.57d ± 0.49	5.41ae ± 0.67
PoOP	0.72abcde ± 0.35	0.84abc ± 0.50	0.96b ± 0.42	0.67c ± 0.30	0.42de ± 0.06	1.01b ± 0.20	0.37e ± 0.24

	Chefchaouane	Fès	Meknès	Ouazzane	Sefrou	Taounate	Taza
	Mean ± SD						
Total ECN 46	20.76ace ± 4.84	27.00b ± 1.64	22.45c ± 2.85	25.24d ± 1.65	21.05e ± 1.93	24.61d ± 1.94	22.86ac ± 1.89
ECN48							
PLP	0.44ad ± 0.05	0.38b ± 0.04	0.42a ± 0.04	0.38c ± 0.01	0.45d ± 0.02	0.44d ± 0.02	0.40a ± 0.03
OOO	45.62ac ± 4.71	39.33b ± 4.11	43.20ac ± 4.00	39.62b ± 0.83	46.67ad ± 2.03	45.33ad ± 2.54	41.91c ± 2.77
SOL	0.55ace ± 0.08	0.47b ± 0.06	0.57ce ± 0.06	0.55ac ± 0.05	0.52ae ± 0.03	0.43d ± 0.03	0.55e ± 0.04
POP	2.63abde ± 0.84	2.25b ± 0.64	2.94ade ± 0.76	2.89de ± 0.57	2.42ab ± 0.20	1.67c ± 0.35	2.94d ± 0.50
POO	18.37ace ± 2.81	15.79b ± 1.86	19.33ce ± 1.90	18.54ace ± 1.73	17.61ae ± 0.84	14.64d ± 0.97	18.58e ± 1.28
Total ECN 48	67.61ac ± 6.42	58.22b ± 2.69	66.46a ± 3.58	61.99d ± 2.48	67.68a ± 2.77	62.51cd ± 2.79	64.39c ± 2.72
ECN50							
SOO	5.12ac ± 1.30	4.81ad ± 0.82	4.47a ± 0.63	4.28b ± 0.24	5.04ad ± 0.55	5.53c ± 0.81	4.66abd ± 0.62
POS	0.79abcd ± 0.09	0.75ad ± 0.08	0.79abcd ± 0.11	0.85bc ± 0.1	0.82c ± 0.07	0.76d ± 0.14	0.78acd ± 0.09
Total ECN 50	5.91abc ± 1.35	5.56ac ± 0.85	5.26c ± 0.70	5.13c ± 0.27	5.86ab ± 0.61	6.29b ± 0.90	5.44ac ± 0.68

Reproduced with permission from [38].
-ECN: equivalent carbon number.
-TAG names are abbreviated by means of three letters corresponding to the FA bound to the glycerol backbone. In alphabetic order: L, linoleic acid (C18:2); Ln, linolenic acid (C18:3); O, oleic acid (C18:1); P, palmitic acid (C16:0); Po, palmitoleic acid (C16:1); and S, stearic acid (C18:0).
-Significant differences within the same line are indicated with different lowercase letters (comparison between studied regions, $p < 0.05$).

Table 2. Quantitative results (mean ± standard deviation, %) for TAGs in the studied samples considering the provenance region.

LDA and PLS-DA were the statistical treatments which gave the best results (in terms of accuracy for both training and test sets), but also the other classification procedure used (SIMCA) achieved a satisfactory and correct geographical classification. It was demonstrated that, for most of the considered regions in the study, useful information could be extracted from TAG data for the geographical discrimination of their virgin olive oils. When considering Meknès terroir samples, rates of 93.48% in both classification and cross-validation and 82.61%, in external validation, were obtained [38].

In addition, a phenolic compounds profiling approach was applied to discriminate Meknès olive oils (36 samples) from those produced in six North Moroccan regions (120 samples) [39]. The developed methodology (using liquid chromatography coupled to mass spectrometry and a discriminant data analysis treatment) allowed differentiating 100% of Meknès samples in

http://dx.doi.org/10.5772/64538

recognition and 91.67% of them in prediction. Besides, a similar approach was used to discriminate Meknès olive oil from those coming from two existing olive oil GIs in Morocco (PGI Ouazzane and PDO Tyout-Chiadma) [40]. In the just cited contribution, 136 commercial extra virgin olive oil samples were collected and their phenolic profile was characterized. Typical Base Peak Chromatograms of the methanolic extracts of representative samples from the three studied regions are shown in **Figure 5**.

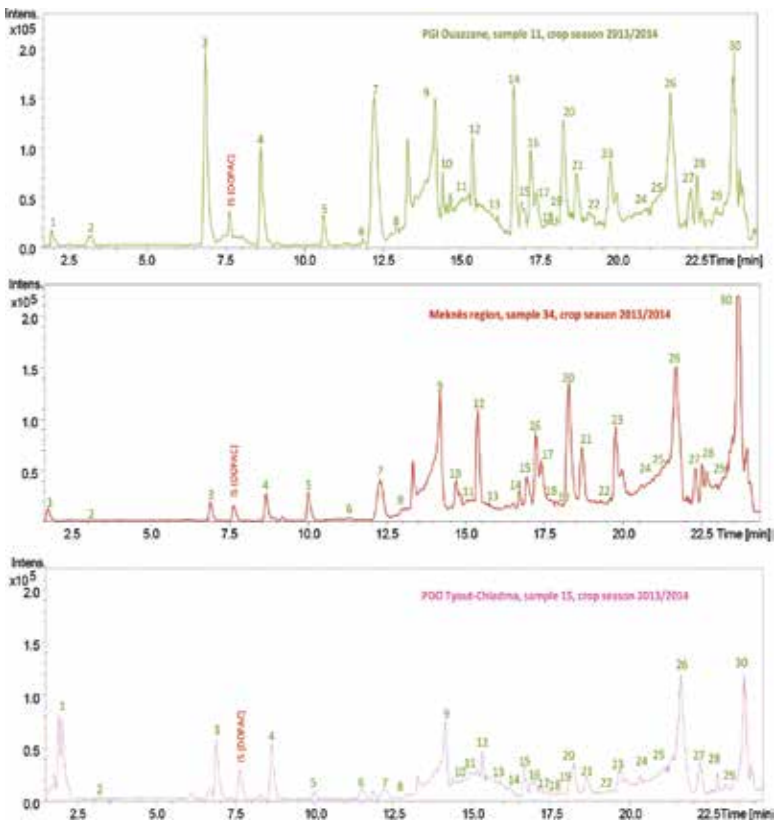


Figure 5. Base peak chromatograms typical for Meknès, PGI Ouazzane, and PDO Tyout-Chiadma extra virgin olive oils samples. Peak identification: (1) quinic acid; (2) oxidized hydroxytyrosol; (3) hydroxytyrosol; (4) tyrosol; (5) dialdehydic form of decarboxymethyl elenolic acid; (6) *p*-coumaric acid; (7) desoxy elenolic acid; (8) hydroxy elenolic acid; (9) elenolic acid; (10) oleuropein aglycone isomer 1; (11) dialdehydic form of decarboxymethyl oleuropein aglycone; (12) oleuropein aglycone isomer 2; (13) syringaresinol; (14) luteolin; (15) oleuropein aglycone isomer 3; (16) ligstroside aglycone isomer 1; (17) pinoresinol; (18) acetoxypinoresinol; (19) methyl decarboxymethyl oleuropein aglycone; (20) oleuropein aglycone isomer 4; (21) dialdehydic form of decarboxymethyl ligstroside aglycone; (22) apigenin; (23) oleuropein aglycone isomer 5; (24) ligstroside aglycone isomer 2; (25) dehydro oleuropein aglycone; (26) oleuropein aglycone; (27) oleuropein aglycone isomer 6; (28) ligstroside aglycone isomer 3; (29) methyl oleuropein aglycone; (30) ligstroside aglycone (reproduced with permission from [40]).

When statistical tools were applied for data treatment, the results were very satisfactory, since the 57 samples belonging to Meknès terroir were 100% correctly classified and 94.70% accurately predicted.

The potential of volatile compounds (determined by gas chromatography coupled to mass spectrometry) combined to chemometric data analysis was also tested to distinguish Meknès olive oils from other olive oil samples produced in diverse Moroccan zones. Among the 92 samples analyzed, very good rates of classification (100%), and prediction (90.48%) were obtained for the 21 studied samples from Meknès region [41].

In general, the good discriminant rates achieved within all the above-mentioned studies, as well as the identified geographical markers demonstrate, from our point of view, the uniqueness and specificity of the olive oil produced in Meknès region.

We can conclude this section stating that, in light of the results obtained by the summarized studies in which about 967 samples were analyzed, the typicality of Meknès olive oil was properly characterized, defining, at the same time, an average qualitative and compositional profile of these oils. Their distinctive characteristics where compared to other Moroccan olive oils. The information obtained within these studies was (and is being) of great practical use in redacting the specifications report.

3. General scheme for the recognition of Meknès virgin olive oil PDO

Once the potential of Meknès olive oil to be certified under a PDO scheme was verified, the next step was the elaboration of a general scheme to be applied for registration in front of the relevant authorities. **Figure 6** briefly illustrates the main activities undertaken within this stage of the study. Working sessions were arranged with the producers of the future PDO and articulated around four actions:

- *Preparation of the production specifications:* a manual containing all the relevant information about Meknès olive oil, as well as a clear description of the practices to be complied with (and those not permitted) along the production chain of this product was prepared. This manual includes the name of the product "Meknès olive oil"; definition of the geographical area of Meknès region; description of the raw materials and the main organoleptic and physicochemical characteristics of Meknès olive oil; information justifying the link with the geographical area, etc.
- *Elaboration of the internal monitoring system:* corresponding to a plan which details *in-situ* controls and documents that should be adopted (and filled in) by Meknès olive oil producers to check the activities, techniques and processes employed during the elaboration of this product.
- *Elaboration of the external monitoring system:* details the main points to be checked and the relevant evaluation methods to be used for ensuring compliance with specifications (logically made with the assistance of an accredited certification body).
- *Definition of specific rules* concerning packaging and labeling of the future Meknès olive oil PDO.

http://dx.doi.org/10.5772/64538

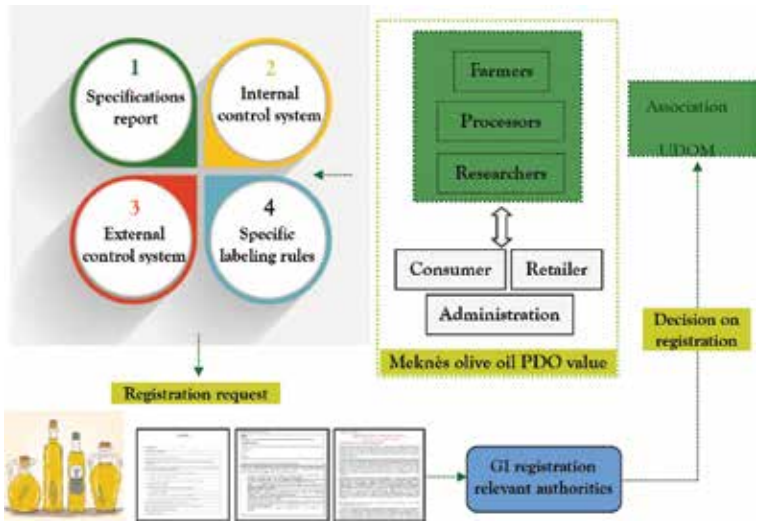


Figure 6. Proposed scheme for Meknès virgin olive oil PDO recognition.

All the elaborated documents were given to the Meknès olive oil PDO stakeholders to start the procedure to apply for the official registration (in front of the relevant authorities). The further procedure for the registration is set out in Food and Agriculture Organization of the United Nations [42].

The official recognition of Meknès olive oil PDO cannot be, however, considered the end of the process, it is rather the beginning of a huge amount of work to maintain, monitor, and promote this PDO. The acquisition of this label is not the goal itself; the final objective is the creation of added-value products and benefits for Meknès farmers and olive oil producers, enhancing, therefore, the access to national and international markets.

As clearly emphasized within this chapter, the success of the PDO label—in Meknès or anywhere else—widely depends on proper implementation, management and further marketing and promotional strategies to intensify its effectiveness. To that end, specific emphasis has to be made on the promotion of Meknès olive oil PDO within the communication charter and action plans jointly elaborated by the association UDOM and other public and private organisms.

4. Enhancement of the effectiveness of PDO Meknès: promoting a terroir through olive tree and olive oil

The regional charter for promoting Meknès terroir through the olive tree and its products reflects a common ambition (shared by all the participants) to make olive growing and oil production activities the basis of the economic regional development. It summarizes a collective vision derived from the alliance between different institutions that take part in the

olive oil sector advancement in this region. Their intention is to stimulate cultural, touristic and commercial activities in a way which will be advantageous for all regional partners. Mechanisms to accomplish these objectives could be principally structured around the following actions:

-Development of common activities to improve cost-effectiveness of management, marketing and promotion of Meknès olive oil;

-Implementation of a cooperative communication (regional, national, and international) strategy;

-Organization of promotional events around the culinary uses of olive oil with special emphasis on Meknès olive oil characteristics;

-Promotion of the health benefits of olive oil, standing out the Meknès olive oil composition and its richness on bioactive compounds;

-Enhancement of the reputation of Meknès PDO olive oil on domestic, regional and international markets (by means of promotional video materials and publicity spots, flyers, Meknès olive oil route map, websites, international fairs, exhibitions, sensory competitions, or other events to promote a more appealing image of Meknès olive oil);

-Organization of Meknès olive oil festival (trade show);

-Organization of a national sensory quality competition with the participation of well-known international experts;

-Membership in other related domestic and foreign olive oil organizations;

-Supporting the development of olive oil tourism in Meknès region.

The strategy is already getting positive effects on Meknès olive oil regarding its recognition on the international market (oils from this region are listed among the best ones worldwide in specialized manuals and guides) and the premium prices achieved by regional producers in national and international markets.

5. Conclusions

The current situation of the use of GI labels all over the world has been discussed, paying particular attention on their usefulness for achieving consumers' recognition, quality signaling, control, and differentiation, and competitive benefits to producers. Moreover, the main factors that affect the success or failure of these geographical labeling schemes and the support needed to make them effective have been underlined. After a general analysis, we asserted that what makes GIs both feasible and operationally effective is the methodological approach followed for their setting up and the capacity of stakeholders to collectively manage, promote, and transform territorial resources into quality attributes recognized by consumers.

We have used Meknès olive oil as example to contextualize the matter and suggest a methodology to define the properties of belonging and distinction of the oils from this region,

http://dx.doi.org/10.5772/64538

supporting the implementation of a PDO label. Our approach involved the use of different analytical techniques and the determination of the physicochemical and sensory quality, as well as other compositional parameters (such as phenolic compounds, triacylglycerols, and volatile compounds) of the oils. Within this plan of action, the suitability of this region to acquire a PDO label was verified and a cooperative management, marketing, and promotion strategy adopted. We really believe that the proposed methodology could be of great importance and assistance to better guide olive oil GI labels implementation, both inside and outside Morocco.

Acknowledgements

This chapter is based on researches funded by grants from the Spanish Agency for International Development Cooperation (AECID) (predoctoral grant), the Vice-Rector's Office for International Relations and Development Cooperation of the University of Granada, the IAMZ-CIHEAM Mediterranean Agronomic Institute of Zaragoza (Master fellowship) and the Millennium Challenge Account Morocco, Fruit Tree Productivity Project (PAF)/Olive cultivation register Project/North Moroccan Regions. The authors want to express their sincere gratitude to the UDOM association members and Agro-pôle Olivier ENA-Meknès team and partners for their support and contribution to the attainment of this work.

This work was also partially supported by the Spanish Government (Ministry of Education, Culture and Sport) by means of a FPU fellowship (FPU13/06438).

Author details

Aadil Bajoub[1], Lucía Olmo-García[1], Noureddine Ouazzani[2], Romina Paula Monasterio[3], Gabriel Beltrán[4] and Alegría Carrasco-Pancorbo[1*]

*Address all correspondence to: alegriac@ugr.es

1 Department of Analytical Chemistry, Faculty of Science, University of Granada, Granada, Spain

2 Agro-pôle Olivier, National School of Agriculture in Meknes, Meknès, Morocco

3 Agricultural Biology Institute of Mendoza (IBAM), National Scientific and Technical Research Council (CONICET), UNCuyo, Mendoza, Argentina

4 The Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA), Mengíbar, Jaén, Spain

References

[1] Efe R, Soykan A, Cürebal I, Sönmez S. Olive and olive oil culture in the Mediterranean Basin. In: Efe R, Öztürk M, Ghazanfar S, editors. Environment and Ecology in the Mediterranean Region. 1st ed. Newcastle, UK: Cambridge Scholars Publishing; 2012. p. 54–64.

[2] Parra-López C, Hinojosa-Rodríguez A, Sayadi S, Carmona-Torres C. Protected designation of origin as a certified quality system in the Andalusian olive oil industry: adoption factors and management practices. Food Control. 2015;51:321–32.

[3] Lamani O, Khadari B. Stratégies de différenciation par l'origine des huiles d'olive en Méditerranée (Differentiation strategies by origin of olive oil in the Mediterranean region). Cah Agric. 2015;24:145–50.

[4] Menozzi D. Extra-virgin olive oil production sustainability in northern Italy: a preliminary study. Br Food J. 2014;116:1942–59.

[5] International Olive Council (IOC). Technical-legal study on geographical designations for olive oil and table olives. 2010. 171 p.

[6] Olive oils recognized by the European Union [Internet]. 2015. Available from: http://www.oliveoilmarket.eu/origins/europian-pdos [Accessed: 2015-12-05].

[7] PDO and PGI oils in Italy [Internet]. 2015. Available from: http://www.portfoil.com/en/index.php?option=com_content&task=view&id=17&Itemid=348 [Accessed: 2015-12-05].

[8] Association Française Interprofessionnelle de l'Olive. Étude sur la différenciation et la valorisation des produits oléicoles français sur les marchés France et export (Study on the differentiation and valorization of French olive products on French and export markets). 2011. 56 p.

[9] Spanish Ministry of Agriculture Food and Environment. Mapa de los Aceites con Denominación de Origen Protegida [Internet]. 2014. Available from: http://www.magrama.gob.es/es/cartografia-ysig/publicaciones/alimentacion/mapa_dop_aceites.aspx [Accessed: 2015-12-05].

[10] Clavero FC, Riccioli C, Navarro EM, Collado RG. Case Study: "Baena" PDO Extra Virgin Olive Oil. European Commission DG IRC-IPTS, Junta de Andalucía. 2006. 55 p.

[11] Association Française Interprofessionnelle de l'Olive. Etude de faisabilité d'une huile d'olive up-premium (Feasibility study of an ultra-premium olive oil). 2010. 49 p.

[12] Consorzio per la tutela dell'Olio Extravergine di Oliva Toscano IGP (Consortium for the protection of Tuscan extra virgin olive oil PGI) [Internet]. 2015. Available from: http://www.oliotoscanoigp.it/ [Accessed: 2015-12-11].

[13] Avilés PR, Navarro GL, Barea BF, Vázquez C. La calidad y las denominaciones de origen en los aceites de oliva andaluces (The quality and origin denominations of Andalusian

http://dx.doi.org/10.5772/64538

olive oils). Distrib y Consum. 2007;96:42–50. Available from: http://www.mercasa.es/files/multimedios/1288206151_DYC_2007_96_42_50.pdf [Accessed: 2016-04-25]

[14] Sanz CJ. Las Denominaciones de Origen Protegidas de Aceite de Oliva en España: sistemas agroalimentarios locales, gobernanza y externalidades territoriales (The olive oil protected designations of origin in Spain: local agro-food systems, management and territorial externalities). In: Vilar Hernández, J editor. Algunas Contribuciones sobre Olivicultura y Elaiotecnia desde la Perspectiva de la Experiencia (Some contributions on oliviculture and elaiotechnic from the perspective of experience). 1st ed. Jaén, Spain: GEA Westfalia Separator Andalucía; 2009. p. 225–41. Available from: http://digital.csic.es/bitstream/10261/16471/1/Cap%C3%ADtulo%2011%20-%20Javier%20Sanz.pdf [Accessed: 2016-04-25]

[15] Aubard A. Setting up a GI: requirements and difficulties at the producer level. In: Blakeney M, Coulet T, Mengistie G, Mahop MT, editors. Extending the Protection of Geographical Indications: Case Studies of Agricultural Products in Africa. 1st ed. London, UK: Earthscan from Routledge; 2012. p. 35–50.

[16] International Olive Council (IOC). General description of olive growing in Morocco. 2013. 10 p.

[17] Moroccan Ministry of Agriculture and Marine Fisheries (MAPM). Note stratégique n °95, Veille économique-Secteur oléicole. 2013. 14 p.

[18] Moroccan Ministry of Agriculture and Marine Fisheries (MAPM). Law No. 25-06 concerning distinctive signs of origin and quality for foodstuffs, agricultural and halieutics products. 2008. 12 p.

[19] Bramley C, Bienabe E, Kirsten J. Developing Geographical Indications in the South: The Southern African Experience. 1st ed. London, UK: Springer; 2013. 142 p.

[20] Blakeney M, Coulet T, Mengistie GA, Mahop MT. Extending the Protection of Geographical Indications: Case Studies of Agricultural Products in Africa. 1st ed. London, UK: Earthscan from Routledge; 2012. 386 p.

[21] Ngo Bagal M, Vittori M. Practical Manual on Geographical Indications for ACP countries. Agridea, Switzerland: CTA/oriGIn; 2011. 265 p.

[22] Giovannucci D, Josling T, Kerr W, O'Connor B, Yeung MT. Guide to geographical indications: linking products and their origins. Genova: International Trade Centre; 2009. 207 p.

[23] Worldwide Symposium on Geographical Indications; 22–24 June 2011; Lima, Peru. 174 p.

[24] World Intellectual Property Organization (WIPO). Lisbon Agreement for the Protection of Appellations of Origin and their International Registration [Internet]. 2015. Available from: http://www.wipo.int/lisbon/en/legal_texts/lisbon_agreement.html [Accessed: 2015-12-15].

[25] Lenglet F. Influence of terroir products meaning on consumer's expectations and likings. Food Qual Prefer. 2014;32:264–70.

[26] Elaydi R, McLaughlin J. Cultivating terroir in subsistence markets: development of terroir strategy through harmony-with-community framework. J Bus Res. 2012;65:1743–8.

[27] Cadot Y, Caillé S, Thiollet-Scholtus M, Samson A, Barbeau G, Cheynier V. Characterisation of typicality for wines related to terroir by conceptual and by perceptual representations. An application to red wines from the Loire Valley. Food Qual Prefer. 2012; 24:48–58.

[28] Casabianca F, Sylvander B, Coulon JB, Béranger C, Roncin F. Terroir et typicité: deux concepts-clés des appellations d'origine contrôlées. Essai de définitions scientifiques et opérationnelles. Produits agricoles et alimentaires d'origine: enjeux et acquis scientifiques. In: Proceedings of Colloque international de restitution des travaux de recherche sur les indications et appellations d'origine géographiques; 17–18 November 2005; Paris, France. p. 199–213.

[29] Barham E. Translating terroir: the global challenge of French AOC labeling. J Rural Stud. 2003;19:127–38.

[30] Morlat R. Éléments importants d'une méthodologie de caractérisation des facteurs naturels du terroir, en relation avec la réponse de la vigne à travers le vin (Important elements of a methodology for the characterization of the natural factors of terroir, in relation with the reaction of the vineyard through the wine). In: Proceeedings of 1er Colloque international sur les terroirs viticoles; 17–18 Jully 1996; Angers, France. p. 17–31.

[31] Cadot Y, Caillé S, Samson A, Barbeau G, Cheynier V. Sensory dimension of wine typicality related to a terroir by quantitative descriptive analysis, just about right analysis and typicality assessment. Anal Chim Acta. 2010;660:53–62.

[32] Barham E, Sylvander B. Labels of Origin for Food. 1st ed. Oxfordshire, UK: CABI; 2011. 240 p.

[33] Bajoub A, Carrasco-Pancorbo A, Ajal EA, Beltrán G, Fernández-gutiérrez A, Ouazzani N. Contribution to the establishment of a protected designation of origin for Meknès virgin olive oil: a 4-years study of its typicality. Food Res Int. 2014;66:332–43.

[34] Bajoub A, Hurtado-Fernández E, Ajal EA, Ouazzani N, Fernández-Gutiérrez A, Carrasco-Pancorbo A. Comprehensive 3-year study of the phenolic profile of Moroccan monovarietal virgin olive oils from the Meknès region. J Agric Food Chem. 2015;63:4376–85.

[35] Bendini A, Cerretani L, Carrasco-Pancorbo A, Gómez-caravaca AM, Segura-carretero A, Fernández-gutiérrez A, Giovanni L. Phenolic molecules in virgin olive oils: a survey

http://dx.doi.org/10.5772/64538

of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. Molecules. 2007;12:1679–719.

[36] Servili M, Esposto S, Fabiani R, Urbani S, Taticchi A, Mariucci F, Selvaggini R, Montedoro F. Phenolic compounds in olive oil: antioxidant, health and organoleptic activities according to their chemical structure. Inflammopharmacology. 2009;17:76–84.

[37] Bajoub A, Hurtado-Fernández E, Ajal EA, Fernández-gutiérrez A, Carrasco-Pancorbo A, Ouazzani N. Quality and chemical profiles of monovarietal north Moroccan olive oils from "Picholine Marocaine" cultivar: registration database development and geographical discrimination. Food Chem. 2015;179:127–36.

[38] Bajoub A, Medina-Rodriguez S, Hurtado-Fernández E, Ajal EA, Ouazzani N, Fernández-Gutiérrez A, Carrasco-Pancorbo A. A first approach towards the development of geographical origin tracing models for North Moroccan olive oils based on triacylglycerols profiles. Eur J Lipid Sci Technol. DOI: 10.1002/ejlt.201500251.

[39] Bajoub A, Carrasco-Pancorbo A, Ajal EA, Ouazzani N, Fernández-Gutiérrez A. Potential of LC-MS phenolic profiling combined with multivariate analysis as an approach for the determination of the geographical origin of North Moroccan virgin olive oils. Food Chem. 2014; 166:292–300.

[40] Bajoub, A, Ajal, EA, Fernández-Gutiérrez, A, Carrasco-Pancorbo, A. Evaluating the potential of phenolic profiles as discriminant features among extra virgin olive oils from Moroccan controlled designations of origin. Food Res Int. 2016;84:41–51.

[41] Bajoub A, Sánchez-Ortiz A, Ajal EA, Ouazzani N, Fernández-Gutiérrez A, Beltrán G, Carrasco-Pancorbo A. First comprehensive characterization of volatile profile of north Moroccan olive oils: a geographic discriminant approach. Food Res Int. 2015;76:410–7.

[42] Food and Agriculture Organization of the United Nations (FAO). Manuel de Procédures pour la Commission Nationale des Signes Distinctifs d' Origine et de Qualité (Procedures Manual for the National Commission on Specific Quality and Origin-linked Signs). 2010. 78 p.

Section 5

Innovations in Table Olives Production

Chapter 12

Modern Techniques in the Production of Table Olives

Nurcan Değirmencioğlu

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64988

Abstract

The olive tree (*Olea europaea* L.) is one of the most important trees in the world, and olive oil and table olives are consumed extensively as a basic ingredient of the Mediterranean diet. Table olives are prepared from the fruit of a variety of cultivated olive trees, and, after removing their bitterness by several methods, they are preserved by natural fermentation or other methods before packing. Currently, scientists and consumers alike are interested in and prefer fresh and healthy table olives that have been minimally and safely processed. The aim of this chapter is to provide information about the modern food-processing techniques that are used to improve the quality characteristics of table olives.

Keywords: table olives, washing, pesticide, debittering, non-thermal processing, salt reduction, packaging

1. Introduction

Table olives are the most important and popular fermented vegetable in the food industry, especially in Spain, Turkey, Italy, Egypt, Morocco, and Greece. They are produced in two ways, that is, (1) by treating green olives with an alkaline solution, which is known as the Spanish style of treatment and (2) by treating olives in an alkaline oxidation process, which is known as the California style of treatment. The olives may be directly brined, or they can be used untreated [1–4]. Also, they can be prepared by other traditional methods (e.g., dry-salting and cracking), industrial processing, or homemade production using various fermentation conditions (e.g., temperature, aeration, and salt content) based on their degree of maturation (i.e., green, turning color, or black) [5–8]. Modern food-processing technologies often rely on non-thermal processes, and fresh fruit, such as olives, are passed through various processing steps to remove soil and pesticide residues and to reduce the microbial load. These processing

steps include cleaning, trimming, peeling, coring, slicing, shredding, washing, sanitizing, packaging, and storage. The final product can be stored at a low temperature or packaged using vacuum packaging (VP) and/or modified atmosphere packaging (MAP). These techniques control the growth of fungi, thereby minimizing the potential for producing mycotoxins. The use of low-temperature storage (<4°C) can increase the product's shelf life [4, 5, 9]. In recent years, scientists and consumers have been interested in fresh, healthy foods that are safe and have been minimally processed by novel preservation technologies. In this chapter, we describe the current methods that are used to process table olives.

2. Preprocessing of olives

2.1. Surface disinfection processes

Surface disinfection processes have the potential for eliminating undesirable microbial species by (i) modification of the composition of the surfaces of the olives by dipping/spraying with antimicrobial solutions or with substances that prevent enzymatic or physical deterioration, (ii) modification of the microecology of the food's surface by curing or bioprotection, (iii) isolation of the food's surface from the environment by packaging or coating with an edible substance, (iv) removal of contaminants from the surfaces of the olives by washing or blowing them with air, (v) modification of the redox potential of the food's surface by washing with aqueous solutions that contain chlorine or hydrogen peroxide, or by ultraviolet radiation (UV) or pulsed light [10], and (vi) using modified atmosphere packaging (MAP) or active packaging to modify the composition and redox potential of the atmosphere in contact with the surfaces of the olives [5, 6].

2.2. Chlorine-based agents

Chlorine-based agents are often used to disinfect olives due to their bactericidal properties and cost efficiency [4, 11]. Such agents include sodium hypochlorite (NaClO), calcium hypochlorite ($Ca(ClO)_2$), and chlorine gas (Cl_2). Industrial applications of chlorinated water, at the conditions of 50–200 ppm free chlorine, 1–2 min, and pH = 6.0–7.5, are used extensively to wash fruits and vegetables, but its effectiveness in reducing the population of microorganisms is limited (<2 log colony-forming unit—cfu), and it has the potential to react with organic materials to form harmful by-products.

2.3. Gaseous chlorine dioxide (ClO_2)

Gaseous chlorine dioxide (ClO_2) at the concentration of 0.1 ppm in high-pH solutions can reach and penetrate microorganisms better than aqueous sanitizers, and it has about 2.5 times the oxidation capacity of chlorine [11]. However, the residual concentration of ClO_2 in foods should not exceed 3 mg/L [12], because it can cause sensory changes [11].

2.4. Ozone (O_3)

Ozone (O_3) is a natural and strong oxidant in the atmosphere, and it is used for sterilization, virus inactivation, deodorization, decolorization, decomposition of organic matter, degradation of mycotoxins, and oxidizing pesticides to reduce their adverse effects on people [13]. Ozone is 1.5 times stronger than chlorine as an oxidizing agent [14], and, to disinfect food products, it is recommended that water containing 2–10 ppm of ozone be used for up to 5 min at a slightly acidic pH [11]. The Code of Federal Regulations indicates that "ozone may be safely used in the treatment, storage, and processing of foods" [15]. Weak organic acids may inhibit microorganisms to a greater extent than strong acids, depending on the type of acid used, the pH of the medium, and the concentration and temperature of the acid solution [4]. However, high concentrations of organic acids, such as acetic acid and lactic acid, must be used to affect the undesirable microorganisms, but even the minimum effective doses of these acids are likely to have adverse effects on the sensory quality of the produce [11].

In considering other non-thermal disinfectant approaches, Gök and Pazır [16] evaluated the effects of tap water, NaClO (15, 30, 50, and 80 ppm for 1 min), electrolyzed oxidizing water (EOW), and ultraviolet (UV) irradiation processes (at distances of 10, 15, and 20 cm and UV irradiation times of 5, 10, 15, 20, and 30 min) for disinfecting the surfaces of black Gemlik olives. According to the results of their research, the highest efficiency was obtained at a chlorine concentration of 80 ppm for 20 min with the product at a distance of 10 cm from the UV lamp. However, the use of UV light may not be practical because the water used to wash the fresh produce would have considerable UV absorbance due to the presence of organic matter or suspended particles that could absorb or shield the UV rays [11].

2.5. Pesticides

Most pesticide residues are retained on the surface of the peels of fruits and vegetables, and whether the pesticides are removed, reduced, or retained depends on their solubility in water. The pesticides also can be removed by various other processes, such as peeling, brushing, blanching, juicing, cooking, milling, hydrostatic pressure, boiling, baking, drying, pasteurization, malting, brewing, fermentation, canning, oil extraction, and refining [13].

Agrochemicals, which include insecticides, herbicides, and fungicides, are used extensively in the olive plantations of Mediterranean countries, and they are intended to decrease losses during production and at harvest time by protecting the olive trees from insects, such as *Daucus oleae* [17–19]. However, the residues of these pesticides can persist to the harvest stage, so they may contaminate the olives that are used to produce table olives and olive oil [17, 18, 20]. The possible contamination of olives by pesticides generally is due to the inappropriate use of the pesticides, for example, using dosages that are too high. This often occurs because the producers do not respect the guidelines for the use of pesticides, resulting in a contamination if flight olives mix with soil olives during harvesting [18, 21]. Therefore, to protect human health and to improve the quality of olive products, different regulations that establish maximum residue limits (MRLs) in olives have been established by both the European Union (EU) and the Food and Agriculture Organization (FAO) of the United Nations [17, 18].

Olives that have fallen from the tree to the ground are used as table olives and to produce olive oil, but they have higher levels of pesticide residue than olives that are collected directly from the tree. Thus, washing the olives by dipping them into water to remove or reduce pesticide residues is a preliminary step that is used when producing table olives and olive oil. The most commonly recommended chemical agents for removing hydrophobic pesticide residues and for improving the effectiveness of washing procedures are chlorine (10–100 ppm), chlorine dioxide (10–500 ppm), ozone (1–3 ppm), hydrogen peroxide (10–100 ppm), weak and strong acids, calcium hypochlorite ($Ca(OCl)_2$, 500 ppm), potassium permanganate ($KMnO_4$, 0.001%), NaCl solution (5–10%), baking soda ($NaHCO_3$, 5–10%), vinegar (0.1%) for several minutes, and ultrasonic cleaners [13, 22–24]. Among these chemical agents, chlorine dioxide (ClO_2) is the most powerful oxidizing agent, and several researchers have shown that it can remove significant amounts of pesticide residues from several foods. It has been observed that several parameters, including ozone dosage, treatment time, temperature, bubble size, oil content, thickness of the surface, the concentration of the pesticide on the olives, and the structural properties of pesticide, can affect the efficiency of pesticide removal. Obviously, better removal efficiency can be obtained if these parameters are optimized [14, 22]. The application of intense UV light also can promote the degradation of some pesticides by direct photolysis due to their potential to absorb light [18]. Nieto et al. [18] attempted to develop a simple UV immersion system (200–280 nm, 150 W) to reduce the amount of pesticides in virgin olive oil depending on the treatment time and temperature (15, 20, 25, and 30°C). While these results indicated the possibility of using UV light as an effective, low-cost process for the destruction of pesticides in olive oil and in table olives, no further progress has been reported in this regard.

3. Debittering process for table olives

Generally, oleuropein is the major phenolic compound in olive cultivars, and it is responsible for the well-known bitterness of olives [25, 26]. However, there are significant decreases in the amount of oleuropein as the olives ripen and are processed [25, 27, 28]. Olives can be consumed only after debittering, which consists of the removal or degradation of oleuropein by the action of lye, some microorganisms, or enzymes. In the natural processing, olives are placed directly in brine without prior debittering with lye solutions, and their bitterness diminishes during storage. Then, the olives are fermented to have their characteristic texture and aroma [27–34]. The action of strains of lactic acid bacteria (LAB) has been proposed as a way to biologically debitter olives, and the direct oxidation of oleuropein also has been proposed [28, 30–33]. Table olives can be debittered with an NaOH solution (1–3%) that hydrolyzes the ester bond of hydroxytyrosol before brining [25, 28, 29]. The debittering treatment is followed by washing with tap water. Then, the olives are placed in a brine solution (6–11%), in which they undergo lactic acid fermentation, which depends on the cultivar, salt content, and temperature [2, 28, 29, 31, 35].

In the rapidly expanding food industry, new and alternative technologies are needed to reduce the debittering process time and to completely replace the use of NaOH and the subsequent

http://dx.doi.org/10.5772/64988

neutralizing washes or brine debittering processes. Ultra sound (US) is one of the newest, fastest-growing, non-thermal food analysis and processing methods, and it has no known negative side effects; it uses the energy generated by sound waves (at frequencies too high to be detected by the human ear) [36]. In order to scale up the debittering of olives using US, large tanks are equipped with power US generators at different conditions of power and amplitude [25, 33]. Habibi et al. [36] studied the effects of US-accelerated debittering (UAD, 35 kHz frequency, 40 W power, 10–50 min) of olives at different concentrations of NaOH that is, 1.50, 1.75, and 2.00% (w/v), and at different temperatures, that is, 25, 30, and 35°C. They stated that UAD was a suitable and applicable technique to minimize the time required to debitter olives and to reduce the NaOH concentration [26].

The use of starter cultures, usually based on autochthonous microbiota, still is not a common practice in the fermentation of vegetables or table olives in Europe [37]. The starter cultures for the fermentation of table olives have the following attributes, that is, rapid and predominant growth at low temperatures with increased acid production, homofermentative metabolism, tolerance to salt and phenolic glucosides, and an inhibitory effect on foodborne pathogens [38–40]. At the beginning of the fermentation process, olives that have not been treated with alkali and oleuropeinolytic LAB strains are recommended as the starter for the fermentation of olives and the production of olive oil [35, 41–43]. This enzymatic hydrolysis could be taken into consideration as an alternative processing method to replace lye and/or brine treatment [27, 44–49]. Lactic acid starters should be identified and selected according to their potential for biologically debittering fermented olives and improving their sensorial characteristics [27, 45, 50, 51].

Lactobacillus plantarum showed the highest percentage of strains producing β-glucosidase and esterases, and it was followed by *L. pentosus, Pediococcus pentosaceus*, and *L. brevis* [35, 41–43, 47, 52–54]. The growth of LAB can be increased based on the simultaneous inoculation of yeasts [55] and their technological properties, which can remove the natural bitterness of fermented olives [1, 8, 55]. In fact, yeasts can produce some substances that promote the growth of *Lactobacillus* spp. [54], such as vitamins B_1 and B_6, amino acids, and purines. They also can break down the complex carbohydrates that are essential for promoting self-growth, thereby contributing the organoleptic properties of table olives by the production of desirable metabolites and volatile compounds [3, 8, 37, 56, 57]. Also, due to the presence of phenolics [55], yeasts can be used as biocontrol agents for non-desirable yeast species and for the inhibition of pathogens [39, 55]. As a result, the concentrations of salt and preservatives can be reduced, the stability of the packaging conditions can be improved, and the nutritional quality (antioxidant capacity), shelf life of the processed olives, and their beneficial effects on consumers' health can be enhanced [1, 27, 40, 45–49, 51–55]. Several authors have emphasized the importance of the appropriate selection of yeasts and their use in factory conditions, with and without LAB [1, 8, 55, 58–62]. They have reported that the following criteria should be considered for the selection of yeasts and LAB: (i) presence of microbial β-glucosidase and esterases hydrolyse oleuropein [49]; (ii) no production of biogenic amines [63]; (iii) presence of proteolytic and lipolytic activity [64, 65]; and (iv) absence of pectolytic activity [57, 59]. It was reported that, among yeasts, the following exhibited potential for use

as starters, that is, *Wickerhamomyces anomalus, Saccharomyces cerevisiae, Kluyveromyces lactis, Debaryomyces hansenii, Candida norvegica, C. diddensiae, C. oleophila, C. boidinii* and *Pichia membranifaciens, P. galeiformis, and P. anomala* [1, 8, 39, 59, 60, 66–72]. Some authors have proposed the use of *Enteroccus* spp., such as *Enterococcus faecium, E. casseliflavus*, and *E. hirae*, as starter cultures for the Spanish-style fermentation of green olives [73–75] with *L. plantarum, L. pentosus*, or *S. cerevisiae*, respectively [39, 73–75]. It should be noted that the use of enterococci, which can cause infections in people, is not recommended by the European Food Safety Authority (EFSA) [75].

4. Reducing salt in the processing of table olives

Sodium is the only mineral element added during the processing of table olives, and the habitual consumption of table olives may be responsible for a significant proportion of daily intake. It has been recommended that the intake of sodium be limited to a maximum of 2400 mg/day [76]. However, the average total daily sodium intake per individual in developed countries is 4000–5000 mg of Na (10,000–12,000 mg of NaCl), which is about 25 times greater than the minimum adult requirement (500 mg of NaCl) [77]. Therefore, a diet that is low in sodium and high in potassium and calcium is recommended to lower blood pressure and to protect against osteoporosis, colon cancer, and cardiovascular diseases [32, 77–80].

Storage and fermentation of vegetable products in brine or dry salt are traditional methods for the preservation of food, and NaCl is used mainly as a preservative, since it causes a reduction of water activity (a_w) to inhibit the growth of undesirable microorganisms, increases the ionic strength of the brine to reduce the solubility of oxygen in water, initiates competitive and selective microbiological growth process, ensures the microbial safety of the final product during storage, and improves the organoleptic properties of food [75, 79, 81]. To date, several studies have investigated the partial or complete substitution for NaCl in fermented green and black olives, and olive juice [58, 76, 80–84]. Products were obtained that had a balanced mineral composition and enhanced nutritional value based on a controlled process that prevented deterioration and spoilage and improved the sensorial characteristics of the products [58, 82–88].

The reduction in Na can be made possible by using substitutes for NaCl, such as potassium chloride (KCl), magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), zinc chloride ($ZnCl_2$), zinc sulfate, and/or zinc perchlorate. Each of these compounds has an antimicrobial effect on pathogens, yeasts, and toxigenic fungi, and each of them also is permitted for the preparation of fortified foods within the current EU legislation (Commission Regulation EU 432/2012) [76, 80–82, 87–90].

Several researchers have partially or completely replaced some of the NaCl with KCl, $CaCl_2$, $ZnCl_2$, and various combinations of these three compounds as preservatives, and they observed better physicochemical and sensory attributes when the non-NaCl proportions were low, and they also reported good fermentation kinetics without any spoilage in green and black table olives [2, 29, 54, 76, 80–83, 86, 92–95].

http://dx.doi.org/10.5772/64988

5. Increasing the shelf life of table olives by a non-thermal process

Shelf life and food quality are related closely to microbial quality and biochemical and enzymatic reactions; managers and researchers in the food industry are very interested in non-thermal preservation methods [96] that can improve the quality and safety of foods. Some of the non-thermal processes used in the food industry are high-intensity, pulsed electric field (HIPEF); high hydrostatic pressure (HHP); high pressure homogenization (HPH); ultraviolet (UV); ultrasound; osmotic dehydration; supercritical fluid extraction; high field strength electrical pulses; and irradiation. These methods affect the viability of microorganisms and the structure of proteins/enzymes during food processing and storage, but they generally do not have any significant effects on the sensory, nutritional, and health-related qualities of the food [96, 97].

Among the non-thermal technologies, high hydrostatic pressure (HHP) applies pressure to foods (liquid and solid) in the range of 50–1000 MPa, depending on the particular food being processed [96, 98]. Generally, a moderate pressure (up to 200–300 MPa) decreases the rate of reproduction and growth of microorganisms, whereas higher pressures (300–700 MPa) inactivate microbial activity [97]. The ingredients that foods contain and the physical conditions of the food can provide a baroprotective effect on microorganisms [99], and they also can lower water activity (a_w), pH (≤4.5), the nature of the solute (i.e., sugar or salt), and temperature (above or below the ambient temperature), thereby influencing the extent to which food must be treated to eliminate/inactivate vegetative cells and to control pathogenic microorganisms [95, 99].

The pressure resistance of microorganisms is at its maximum value in the temperature range of 15–30°C, particularly with regard to bacterial spores [99], and the pressure resistance of bacteria is highly variable [100, 101]. The first research on the effect of high pressure on food was conducted in the nineteenth century describing an increase in the shelf life of food products that were stored at pressures far in excess of atmospheric pressure; however, there are very few studies on the application of HHP on fermented vegetable foods [96, 97, 100, 102, 103].

Contamination of olives may be due to olive harvesting directly from the soil, poor hygiene and unsanitary procedures by field and processing personnel, inadequate cleaning and sanitizing of processing equipment, and failure to wash the olives prior to brining [98]. At the end of these processing steps and irrespective of the packaging material, the industry usually uses a thermal pasteurization step to extent shelf life and to stabilize table olives microbiologically [104]. The protective effect of different levels of HHP (250–600 MPa for 5–30 min) reduced the yeast and mold populations, the mycotoxin level (citrinin), and extended the shelf lives of table olive products [96, 98, 102, 105]. Also, Tokuşoğlu et al. [97] reported that no hazardous microorganisms were found on the olives, with the exceptions of yeasts and molds that were found to be less than 10^6 CFU/g, which was in compliance with the International Olive Oil Council's (IOOC's) trade standard for table olives.

Olives have a high functional potential due to the presence of essential micronutrients, essential fatty acids (oleic acid), and biologically active phytochemicals, such as phenols,

tocopherols, and phytosterols [96, 97], but thermal processing of table olives induces some deterioration in their quality, resulting in softening of their tissue, changing their green color to brown, developing a cooking taste, and degrading active biocompounds [102]. Optimization of the HHP conditions is important for bioactive compounds' stability, quality, and quantity. Very limited information is available in the literature about the use of the non-thermal method, HHP, as an alternative to thermal processing, on the quality of table olives [98]. According to the research results, the total phenolic and hydroxytyrosol levels were increased by factors of 2.1–2.5 and 0.8–2.0, respectively, while oleuropein decreased after HHP [97]. Similar results also were reported for Cornezuelo olives treated by HHP. However, olives treated by HHP had higher stability in terms of pH and free acidity values, and the HHP treatment can be used to prevent the formation of gas in the packed olives and to improve the sensory characteristics of Cornezuelo dressed olives [103].

The color makes a key contribution to the marketability of table olives, and since a vivid green color is an essential characteristic of the product, especially in Spanish-style processing [98, 106]; it must be noted that HHP treatments caused a moderate degradation of the color of the processed olives [96, 98, 102, 103]. Possible techniques for preserving and improving the color of HHP-treated olives include the addition of ascorbic acid (15 g/L) and purging with gaseous nitrogen [8].

6. Effect of packaging methods on the shelf life of table olives

Packaging of table olives is a way to improve their economic value and expand markets. Table olives, as a final product, may be marketed in bulk to local markets or exported abroad. There is a tendency to pack olives in glass or plastic containers, tins, and polyethylene, aluminum, or multi-laminated pouches. These materials are filled with brine that contains pH regulators, preservatives, antioxidants, anti-softening additives, and, in some cases, gases (CO_2, N_2). Subsequently, pasteurization or sterilization is used to stabilize the product microbiologically [4, 5, 107, 108]. Alternatively, physical treatments can be used [103]. In vacuum packaging, the product is placed in a pack with low oxygen permeability, the air is evacuated, and the package is sealed. Since it is not possible to evacuate all of the air (0.3–3% of it may remain after sealing), the gaseous atmosphere of the vacuum package is likely to change during storage (due to microbial and product metabolism and gas permeation); therefore, the atmosphere in the package over time may be different from the original atmosphere [4]. MAP or vacuum packaging (VP) can be applied to increase the quality and shelf life of products that are designed to be "natural" or reduced in preservatives by "hurdle effect" technologies; improved presentation and visibility of the product; and reduced production, storage, and transport costs due to better utilization of labor and equipment. Nevertheless, the costs of gas packaging machinery, gases, packaging materials, and analytical equipment to measure gas mixtures are many disadvantages of MAP [109]. The use of MAP and VP for the storage of table olives at ambient or low temperature has been well noted and proven in several studies [4–6, 107, 108, 110–113]. As reported by these researchers, dipping the olives in anti-microbial solutions (potassium sorbate—1.0%, chlorine dioxide—10 ppm, or organic acid—1.0–2.0 acetic or lactic)

http://dx.doi.org/10.5772/64988

before packaging, storing them at 4°C, and storing them under pressure in a CO_2 atmosphere-controlled microbial activity effectively, especially the population of yeasts, and it minimized the production of mycotoxins as well as obtaining the best quality characteristics of the final products.

7. Improving functionality of table olives by probiotic cultures

Fermented vegetables are being considered as a splendid source and vehicle of probiotic microorganisms [41, 114]. The fermentation of table olives usually is the result of the competitive and synergic metabolic activities of the autochthonous microbiota, together with a variety of contaminating microorganisms from the fermentation environment [8, 115]. The LAB microbiota of table olives also are characterized by the presence of *Lactobacillus plantarum, L. rhamnosus, L. pentosus, L. casei, L. paracasei*, and heterofermentative cocci, such as *Leuconostoc mesenteroides* [41, 62, 102, 115–119]. The use of table olives as a probiotic source has been explored in several studies [98, 102, 116, 120–123], which, through *in vitro* methods, has evaluated the probiotic and technological characteristics of autochthonous LAB isolated from the fermentation of table olives. In addition to the probiotic characteristics of LAB, and yeasts as adjunct culture, these starters must possess appropriate technological characteristics, that is, adequate growth rate, rapid and high lactic acid production, ability to adhere to the outer peeling of the olives, sugar consumption and tolerance, or synergy with other components of the starter, to produce functional olives [1, 55, 59, 66, 98, 114, 120, 122–125]. To deliver health benefits, probiotic foods must contain an adequate amount of live bacteria (at least 10^6–10^7 CFU/g) and must prolong their viability at the end of the fermentation process [98, 122] and after long-term storage of the fermented product, provided that the latter has acceptable organoleptic characteristics. Viewed from this perspective, an edible portion of about 80–100 g of olives must contain $>10^9$ live cells of selected LAB strains in order to be considered as a probiotic [114]. In this volume, also see chapter how biotechnology can improve a traditional product as table olives.

Author details

Nurcan Değirmencioğlu*

Address all correspondence to: ndegirmencioglu@bandirma.edu.tr

Department of Food Processing, Bandırma Vocational High School, Bandırma Onyedi Eylül University, Bandırma-Balıkesir, Turkey

References

[1] Arroyo-López FN, Romero-Gil V, Bautista-Gallego J, Rodríguez-Go´mez F, Jiménez-Díaz R, García-García P, Querol A, Garrido-Fernández A. Yeasts in table olive processing: desirable or spoilage microorganisms? Int J Food Microbiol. 2012;160:42-49. DOI: 10.1016/j.ijfoodmicro.2012.08.003

[2] Tassou CC, Panagou EZ, Katsaboxakis KZ. Microbiological and physicochemical changes of naturally black olives fermented at different temperatures and NaCl levels in the brines. Food Microbiol. 2002;19:605-615. DOI:10.1006/fmic.2002.0480

[3] Garrido-Fernández A, Fernandez-Diez MJ, Adam MR. 1997. Olives and table olives. In A. Garrido Fernández, M. J. Fernandez-Diaz, and M. R. Adams (ed.), Table olives: production and processing, 1st ed. Chapman & Hall, London. 1997. p. 10-21. DOI: 10.1007/978-1-4899-4683-6

[4] Değirmencioğlu N, Gürbüz O, Değirmencioğlu A, Yıldız S. Effect of pre-treatments on microbial growth and sensory properties of dry-salted olives. J Food Prot. 2014;77:1527-1537. DOI:10.4315/0362-028X.JFP-14-036.

[5] Değirmencioğlu N. Influence of temperature and modified atmosphere on the microbial profile of packed Gemlik dry-Salted olives. J Food Saf. 2011;31:115-124. DOI: 10.1111/j.1745-4565.2010.00274.x

[6] Panagou EZ. Greek dry-salted olives: monitoring the dry-salting process and subsequent physicochemical and microbiological profile during storage under different packing conditions at 4 and 20 degrees C. LWT-Food Sci Technol. 2006;39:323-330. DOI: 10.1016/j.lwt.2005.02.017

[7] Arroyo-Lopez FN, Duran-Quintana MC, Romero C, Rodriguez-Gomez F, Garrido-Fernandez A. Effect of storage process on the sugars, polyphenols, color and microbiological changes in cracked Manzanilla-Alorena table olives. J Agric Food Chem. 2007;55:7434-7444. DOI:10.1021/jf070571b

[8] Arroyo-Lopez FN, Bautista-Gallego J, Duran-Quintana MC, Rodriguez-Gomez F, Romero-Barranco C, Garrido-Fernandez A. Improvement of the storage process for cracked table olives. J Food Eng. 2008a;89:479-487. DOI:10.1016/j.jfoodeng.2008.06.001

[9] Değirmencioğlu N, Gürbüz O, Değirmencioğlu A, Şahan Y, Özbey H. Effect of MAP and vacuum sealing on sensory qualities of dry salted olive. Food Sci Biotechnol. 2011;20:1307-1313. DOI:10.1007/s10068-011-0180-9

[10] Manzocco L, Nicoli MC. Surface processing: existing and potential applications of ultraviolet light. Crit Rev Food Sci Nutr. 2015;55(4):469-484. DOI: 10.1080/10408398.2012.658460.

[11] Banach JL, Sampers I, Van Haute S, van der Fels-Klex HJ. Effect of disinfectants on preventing the cross-contamination of pathogens in fresh produce washing water. Int J Environ Res Public Health. 2015;12:8658-8677. DOI:10.3390/ijerph120808658

[12] Food and Drug Administration (FDA). Code of Federal Regulations. Sec. 173.300 Chlorine dioxide. Available online: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/ CFRSearch.cfm?fr=173.300 (accessed on 21 June 2016).

[13] Bajwa U, Sandhu KS. Effect of handling and processing on pesticide residues in food—a review. J Food Sci Technol. 2014;51:201-220. DOI:10.1007/s13197-011-0499-5

[14] Kırış S, Velioğlu YS. Reduction in pesticide residue levels in olives by ozonated and tap water treatments and their transfer into olive oil. Food Addit Contam. Part A. 2016;33:128-136. DOI: 10.1080/19440049.2015.1114683

[15] Food and Drug Administration (FDA). Code of Federal Regulations. Sec. 173.368 Ozone. Available online: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=173.368 (accessed on 21 June 2016).

[16] Gök S, Pazır F. Efficacy of some non-thermal operations on shelf life of packaged black olive in the absence of brine. 11th International Congress on Engineering and Food, Athens, Greece, May 22-26, 2011.

[17] Amvrazi EG, Albanis TA. Pesticide residue assessment in different types of olive oil and preliminary exposure assessment of Greek consumers to the pesticide residues detected. Food Chem. 2009;113:253-261. DOI:10.1016/j.foodchem.2008.06.073

[18] Nieto LM, Hodaifa G, Casanova MS. Elimination of pesticide residues from virgin olive oil by ultraviolet light: Preliminary results. J Hazard Mater. 2009;168:555-559. DOI: 10.1016/j.jhazmat.2009.02.030.

[19] Misra NN. The contribution of non-thermal and advanced oxidation technologies towards dissipation of pesticide residues. Trends Food Sci Technol. 2015;45:229-244. DOI:10.1016/j.tifs.2015.06.005

[20] Ikeura H, Kobayashi F, Tamaki M. Removal of residual pesticide, fenitrothion, in vegetables by using ozone microbubbles generated by different methods. J Food Eng. 2011;103:345-349. DOI:10.1016/j.jfoodeng.2010.11.002

[21] Kaushik G, Satya S, Naik SN. Food processing a tool to pesticide residue dissipation—A review. Food Res Int. 2009;42:26-40. DOI:10.1016/j.foodres.2008.09.009

[22] García-Reyes JF, Ferrer C, Gómez-Ramos MJ, Fernández-Alba AR, Molina-Díaz A. Determination of pesticide residues in olive oil and olives. Trends Analyt Chem. 2007;26:239-251. DOI:10.1016/j.trac.2007.01.004

[23] Gómez-Almenar MC, García-Mesa JA. Determination of pesticide residues in olives by liquid extraction surface analysis followed by liquid chromatography/tandem mass spectrometry. Grasas y Aceitas. 2015;66:e078. DOI:10.3989/gya.0828142

[24] Guardia-Rubio M, Ayora-Cañada MJ, Ruiz-Medina A. Effect of washing on pesticide residues in olives. J Food Sci. 2007;72:139-143. DOI:10.1111/j.1750-3841.2006.00252.x

[25] Cocolin L, Alessandria V, Botta C, Gorra R, De Filippis F, Ercolini D, Rantsiou K. NaOH-debittering induces changes in bacterial ecology during table olives fermentation. PloS One. 2013;8:e69074. DOI:10.1371/journal.pone.0069074.

[26] Habibi M, Golmakani MT, Farahnaky A, Mesbahi G, Majzoobi M. NaOH-free debittering of table olives using power ultrasound. Food Chem. 2016;192:775-781. DOI: 10.1016/j.foodchem.2015.07.086.

[27] Ozdemir Y, Guven E, Ozturk A. Understanding the characteristics of oleuropein for table olive processing. J Food Process Technol. 2014;5;328. DOI: 10.4172/2157-7110.1000328.

[28] Ramírez E, García-García P, de Castro A, Romero C, Brenes M. Debittering of black dry-salted olives. Eur J Lipid Sci Technol. 2013;115:1319-1324. DOI:10.1002/ejlt.201300167

[29] Moreno-Baquero JM, Bautista-Gallego J, Garrido-Fernández A, López-López A. Mineral and sensory profile of seasoned cracked olives packed in diverse salt mixtures. Food Chem. 2013;138:1-8. DOI:10.1016/j.foodchem.2012.10.027.

[30] Brenes M, de Castro A. Transformation of oleuropein and its hydrolysis products during Spanish-style green olive processing. J Sci Food Agric. 1998;77:353-358. DOI: 10.1002/(SICI)1097-0010(199807)

[31] Medina E, Romero C, Brenes M, García P, de Castro A, García A. Profile of anti-lactic acid bacteria compounds during storage of olives which are not treated with alkali. Eur Food Res Technol. 2008;228:133-138. DOI:10.1007/s00217-008-0916-9

[32] Servili M, Settani L, Veneziani G, Espoto S, Massitti O., Taticchi A, Urbani S, Montedoro GF, Corsetti A. The use of Lactobacillus plantarum 1MO to shorten the debittering process time of black table olives (Cv. Itrana and Leccino): a pilot-scale application. J Agric Food Chem. 2006;54:3869-3875. DOI:10.1021/jf053206y

[33] García A, Romero C, Medina E, García P, de Castro A, Brenes M. Debittering of olives by polyphenol oxidation. J Agric Food Chem. 2008;56:11862-11867. DOI:10.1021/jf802967y.

[34] Lanza B, Di Serio MG, Iannucci E, Russi F, Marfisi P. Nutritional, textural and sensorial characterisation of Italian table olives (Olea europaea L. cv.'Intosso d'Abruzzo'). Int J Food Sci Technol. 2010; 45:67-74. DOI: 10.1111/j.1365-2621.2009.02104.x

[35] Hurtado A, Reguant C, Bordons A, Rozès N. Lactic acid bacteria from fermented table olives. Food Microbiol. 2012;31:1-8. DOI:10.1016/j.fm.2012.01.006

[36] Habibi M, Golmakani MT, Mesbahi G, Majzoobi M, Farahnaky A. Ultrasound-accelerated debittering of olive fruits. Innov Food Sci Emerg Technol. 2015;31:105-115. DOI:10.1016/j.ifset.2015.06.014

[37] Aponte M, Blaiotta G, La Croce F, Mazzaglia A, Farina V, Settanni L, Moschetti G. Use of selected autochthonous lactic acid bacteria for Spanish style table olive fermentation. Food Microbiol. 2012;30:8-16. DOI:10.1016/j.fm.2011.10.005

[38] Randazzo CL, Todaro A, Pino A, Pitino I, Corona O, Mazzaglia A, Caggia C. Giarraffa and Grossa di Spagna naturally fermented table olives: Effect of starter and probiotic cultures on chemical, microbiological and sensory traits. Food Res Int. 2014;62:1154-1164. DOI:10.1016/j.foodres.2014.05.056

[39] Corsetti A, Perpetuini G, Schirone M, Tofalo R, Suzzi G. Application of starter cultures to table olive fermentation: an overview on the experimental studies. Front Microbiol. 2012;3:248. DOI:10.3389/fmicb.2012.00248

[40] Tataridou M, Kotzekidou P. Fermentation of table olives by oleuropeinolytic starter culture in reduced salt brines and inactivation of Escherichia coli O157: H7 and Listeria monocytogenes. Int J Food Microbiol. 2015; 208:122-130. DOI:10.1016/j.ijfoodmicro.2015.06.001.

[41] Kaltsa A, Papaliaga D, Papaioannou E, Kotzekidou P. Characteristics of oleuropeinolytic strains of Lactobacillus plantarum group and influence on phenolic compounds in table olives elaborated under reduced salt conditions. Food Microbiol. 2015;48:58-62. DOI:10.1016/j.fm.2014.10.016

[42] Sabatini N, Mucciarella MR, Marsilio V. Volatile compounds in uninoculated and inoculated table olives with Lactobacillus plantarum (Olea europea L., cv. Moresca and Kalamata). LWT— Food Sci Technol. 2008;41:2017-2022. DOI:10.1016/j.lwt.2007.12.002

[43] Leal-Sánchez MV, Ruíz-Barba JL, Sánchez AH, Rejano L, Jiménez-Díaz R, Garrido A. Fermentation profile and optimization of green olive fermentation using Lactobacillus plantarum LPCO10 as a starter culture. Food Microbiol. 2003;20:421-430. DOI:10.1016/S0740-0020(02)00147-8

[44] Ciafardini G, Zullo B. β-Glucosidase activity in olive brine during the microbiological debittering process. Adv Food Sci. 2000;22:100-106.

[45] Tuna S, Akpinar-Bayizit A. The use of ß-glucosidase enzyme in black table olives fermentation. Not Bot Hort Agrobot Cluj. 2009;37:182-189.

[46] Aponte M, Ventorino V, Blaiotta G, Volpe G, Farina V, Avellone G, Lanza CM, Moschetti G. Study of green Sicilian table olive fermentations through microbiological, chemical and sensory analyses. Food Microbiol. 2010;27:162-170. DOI:10.1016/j.fm.2009.09.010.

[47] Ciafardini G, Zullo BA. Survival of micro-organisms in extra virgin olive oil during storage. Food Microbiol. 2002a;19:105-109. DOI:10.1006/fmic.2001.0458

[48] Bevilacqua A, Beneduce L, Sinigaglia M, Corbo MR. Selection of yeasts as starter cultures for table olives. J Food Sci. 2013;78:742-751. DOI: 10.1111/1750-3841.12117.

[49] Restuccia C, Muccilli S, Palmeri R, Randazzo CL, Caggia C, Spagna G. An alkaline β-glucosidase isolated from an olive brine strain of Wickerhamomyces anomalus. FEMS Yeast Res. 2011;11:487-493. DOI:10.1111/j.1567-1364.2011.00738.x.

[50] Othman NB, Roblain D, Chammen N, Thonart P, Hamdi M. Antioxidant phenolic compounds loss during the fermentation of Chétoui olives. Food Chem. 2009;116:662-669. DOI:10.1016/j.foodchem.2009.02.084

[51] Ghabbour N, Lamzira Z, Thonart P, Cidalia P, Markaoui M, Asehraou A. Selection of oleuropein-degrading lactic acid bacteria strains isolated from fermenting Moroccan green olives. Grasas y Aceites. 2011;62:84-89. DOI:10.3989/gya.055510

[52] Ciafardini G, Marsillio V, Lanza B, Pozzi N. Hydrolysis of oleuropein by Lactobacillus plantarum strains associated with olive fermentation. Appl Environ Microbiol. 1994;60:4142-4147.

[53] Benincasa C, Muccilli S, Amenta M, Perri E, Romeo FV. Phenolic trend and hygienic quality of green table olives fermented with Lactobacillus plantarum starter culture. Food Chem. 2015;186:271-276. DOI:10.1016/j.foodchem.2015.02.010.

[54] Tsapatsaris S, Kotzekidou P. Application of central composite design and response surface methodology to the fermentation of olive juice by Lactobacillus plantarum and Debaryomyces hansenii. Int J Food Microbiol. 2004;95:157-168. DOI:10.1016/j.ijfoodmicro.2004.02.011

[55] Arroyo-López FN, Romero-Gil V, Bautista-Gallego J, Rodríguez-Gómez F, Jiménez-Díaz R,García-García P, Querol A, Garrido-Fernández A. Potential benefits of the application of yeast starters in table olive processing. Front Microbiol. 2012a;27:34. DOI: 10.3389/fmicb.2012.00161.

[56] Lamzira Z, Asehraou A, Brito D, Oliveira M, Faid M, Peres C. Reducing the bloater spoilage during lactic fermentation of Moroccan green olives. Food Technol Biotechnol. 2005;43:373-377. DOI:10.3989/gya.2002.v53.i3.325

[57] Hernández A, Martín A, Aranda E, Pérez-Nevado F, Córdoba MG. Identification and characterization of yeast isolated from the elaboration of seasoned green table olives. Food Microbiol. 2007;24:346-351. DOI:10.1016/j.fm.2006.07.022

[58] Silva T, Reto M, Sol M, Peito A, Peres CM, Peres C, Malcata FX. Characterization of yeasts from Portuguese brined olives, with a focus on their potentially probiotic behavior. LWT-Food Sci Technol. 2011;44:1349-1354. DOI:10.1016/j.lwt.2011.01.029

[59] Bevilacqua A, Corbo MR, Sinigaglia M. Selection of yeasts as starter cultures for table olives: a step-by-step procedure. Front Microbiol. 2012; 31:DOI:10.3389/fmicb. 2012.00194.

[60] Tofalo R, Perpetuini G, Schirone M, Suzzi G, Corsetti A. Yeast biota associated to naturally fermented table olives from different Italian cultivars. Int J Food Microbiol. 2013;161(3):203-208. DOI:10.1016/j.ijfoodmicro.2012.12.011.

[61] Rodríguez-Gómez F, Romero-Gil V, Bautista-Gallego J, Garrido-Fernández A, Arroyo-López FN. Multivariate analysis to discriminate yeast strains with technological applications in table olive processing. World J Microbiol Biotechnol. 2012a; 28:1761-1770. DOI:10.1007/s11274-011-0990-1.

[62] Bonatsou S, Benítez A, Rodríguez-Gómez F, Panagou EZ, Arroyo-López FN. Selection of yeasts with multifunctional features for application as starters in natural black table olive processing. Food Microbiol. 2015;46:66-73. DOI:10.1016/j.fm.2014.07.011.

[63] García-García PG, Barranco CR, Quintana MC, Fernández AG. Biogenic amine formation and "Zapatera" spoilage of fermented green olive: effect of storage temperature and debittering process. J Food Prot. 2004;67:117-123.

[64] Marsilio V, Lanza B. Characterisation of an oleuropein degrading strain of Lactobacillus plantarum. Combined effects of compounds present in olive fermenting brine (phenols, glucose and NaCl) on bacterial activity. J Sci Food Agr.1998;76:520-524. DOI:10.1002/(SICI)1097-0010(199804)

[65] Rodríguez-Gómez F, Arroyo-López FN, López-López A, Bautista-Gallego J, Garrido-Fernández A. Lipolytic activity of the yeast species associated with the fermentation/storage phase of ripe olive processing. Food Microbiol. 2010;27:604-612. DOI:10.1016/j.fm.2010.02.003.

[66] Silva T, Reto M, Sol M, Peito A, Peres CM, Peres C, Malcata XF. Characterization of yeasts from Portuguese brined olives, with a focus on their potentially probiotic behavior. LWT Food Science and Technology. 2011;44:1349-1354. DOI: 10.1016/j.lwt.2011.01.029

[67] Hurtado A, Ben Othman N, Chammem N, Hamdi M, Ferrer S, Reguant C, et al. Characterization of Lactobacillus isolates from fermented olives and their bacteriocin gene profiles. Food Microbiol. 2011;28:1514-1518. DOI:10.1016/j.fm.2011.07.010

[68] Tufariello M, Durante M, Ramires FA, Grieco F, Tommasi L, Perbellini E, Falco V, Tasioula-Margari M, Logrieco AF, Mita G, Bleve, G. New process for production of fermented black table olives using selected autochthonous microbial resources. Front Microbiol. 2015;24:6. DOI:10.1016/j.ijfoodmicro.2011.03.013.

[69] Bleve G, Tufariello M, Durante M, Grieco F, Ramires FA, Mita G, Margari MT, Logrieco AF. Physico-chemical characterization of natural fermentation process of Conservolea and Kalamata table olives and developement of a protocol for the pre-selection of fermentation starters. Food Microbiol. 2015a;46:368-382. DOI:10.1016/j.fm.2014.08.021

[70] Bleve G, Tufariello M, Durante M, Perbellini E, Ramires FA, Grieco F, Cappello MS, De Domenico S, Mita G, Tasioula-Margari M, Logrieco AF. Physico-chemical and microbiological characterization of spontaneous fermentation of Cellina di Nardò and Leccino table olives. Frontiers in Microbiology. 2014;5:570, 1-18. DOI: 10.3389/fmicb.2014.00570

[71] Bleve G, Tufariello M, Durante M, Grieco F, Ramires FA, Mita G, Tasioula-Margari M, Logrieco AF. Physico-chemical and microbiological characterization of natural fermentation process of Conservolea and Kalamata table olives and developement of a protocol for the pre-selection of fermentation starters. Food Microbiology. 2015;46:368-382. DOI: 10.1016/j.fm.2014.08.021

[72] Heperkan D, Erol-Meriç B, Sismanoglu G, Dalkiliç G, Güler FK. Mint: Mycobiota, Mycotoxigenic fungi and citrinin production in black olives. Advances in Experimental Medicine and Biology. 2006;571:203-210. DOI: 10.1007/0-387-28391-9_13

[73] De Castro A, Montano A, Casado FJ, Sánchez A H, Rejano L. Utilization of Enterococcus casseliflavus and Lactobacillus pentosus as starter cultures for Spanish-style green olive fermentation. Food Microbiol. 2002;19:637-644. DOI:10.1006/fmic.2002.0466

[74] Lavermicocca P, Gobbetti M, Corsetti A, Caputo L. Characterization of lactic acid bacteria isolated from olive phylloplane and table olive brines. Ital J Food Sci. 1998;10:27-39.

[75] Heperkan D. Microbiota of table olive fermentations and criteria of selection for their use as starters. Front Microbiol. 2013;4:10-3389. DOI:10.3389/fmicb. 2013.00143

[76] Panagou EZ, Hondrodimou O, Mallouchos A, Nychas GJ. A study on the implications of NaCl reduction in the fermentation profile of Conservolea natural black olives. Food Microbiol. 2011;28:1301-1307. DOI:10.1016/j.fm.2011.05.008.

[77] Katsiari MC, Voutsinas LP, Alichanidis E, Roussis IG. Lipolysis in reduced sodium Kefalograviera cheese made by partial replacement of NaCl with KCl. Food Chem. 2011;72:193-197. DOI:10.1016/S0308-8146(00)00218-1

[78] López A, García P, Garrido Fernández A, Multivariate characterization of table olives according to their mineral composition. Food Chem. 2008;106:369-378. DOI:10.1016/j.foodchem.2007.05.055

[79] Albarracín W, Sánchez IC, Grau R, Barat JM. Salt in food processing; usage and reduction: a review. Int J Food Sci Technol. 2011;46:1329-1336. DOI:10.1111/j. 1365-2621.2010.02492.x

[80] Bautista-Gallego J, Arroyo-López FN, Romero-Gil V, Rodríguez-Gómez F, García-García P, Garrido-Fernández A. Chloride salt mixtures affect Gordal cv. green Spanish-style table olive fermentation. Food Microbiol. 2011b;28:1316-1325. DOI:10.1016/j.fm. 2011.06.002.

[81] Bautista-Gallego J, Arroyo-López FN, Romero-Gil V, Rodríguez-Gómez F, García-García P, Garrido-Fernández A. Microbial stability and quality of seasoned cracked green Aloreña table olives packed in diverse chloride salt mixtures. J Food Prot. 2013a; 76:1923-1932. DOI: 10.4315/0362-028X.JFP-12-504.

[82] Bautista-Gallego J, Arroyo-López FN, Durán-Quintana MC, Garrido-Fernández A. Fermentation profiles of Manzanilla-Aloreña cracked table olives in different chloride salt mixtures. Food Microbiol. 2010;27:403-412. DOI:10.1016/j.fm.2009.11.015.

[83] Kanavouras A, Gazouli M, Tzouvelekis Leonidas L, Petrakis C. Evaluation of Greek-style black table olives in salt varying brines. Grasas y Aceites. 2005;56:106-115. DOI: 10.3989/gya.2005.v56.i2.117

[84] Di Silva A. Preliminary results of a new processing in order to obtain green table olives with a low sodium content. Ind Aliment. 2000;39:844-847.

[85] Bautista-Gallego J, Arroyo-López FN, López-López A, Garrido-Fernández A. Effect of chloride salt mixtures on selected attributes and mineral content of fermented cracked Aloreña olives. LWT-Food Sci Technol. 2011a;44:120-129. DOI:10.1016/j.lwt.2010.06.027

[86] Mulé R, Fodale AS, Bati CB, Tucci A, di Pisa A. Preliminary results of a new processing in order to obtain green table olives with a low sodium content. Ind Aliment. 2000;39:844-847.

[87] Bautista-Gallego J, Moreno-Baquero JM, Garrido-Fernández A, López-López A. 2013c. Development of a novel Zn fortified table olive product. LWT-Food Sci Technol 50:264-271. DOI:10.1016/j.lwt.2012.05.017

[88] Bautista-Gallego J, Arroyo-López FN, Romero-Gil V, Rodríguez-Gómez F, Garrido-Fernández A. The effect of $ZnCl_2$ on green Spanish-style table olive packaging, a presentation style dependent behaviour. J Sci Food Agric. 2014;95:1670-1677. DOI: 10.1002/jsfa.6870.

[89] Bautista-Gallego J, Romero-Gil V, Garrido-Fernández A, Arroyo-López FN. Modeling the inhibitory effects of zinc chloride on table olive related yeasts. Food Control. 2012;23:499-505. DOI:10.1016/j.foodcont.2011.08.020

[90] Savi GD, Bortoluzzi AJ and Scussel VM. Antifungal properties of zinc compounds against toxigenic fungi and mycotoxin. Int J Food Sci Technol. 2013;48:1834-1840. DOI: 10.1002/jsfa.6870.

[91] Papoff CM, Agabbio M, Vocredt A, Farris GA. Influence of some biotechnological combinations on the sensory quality of "Manna" green table olives. Ind Aliment. 1996;35:375-381.

[92] Marsilio V, Campestre C, Lanza B, de Angelis M, Russi F. Sensory analysis of green table olives fermented in different saline solutions. Acta Horticulture. 2002;586:617-620. DOI:10.17660/ActaHortic.2002.586.130

[93] Rodríguez-Gómez F, Bautista-Gallego J, Romero-Gil V, Arroyo-López FN, Garrido-Fernández A, García-García P. Effects of salt mixtures on Spanish green table olive fermentation performance. LWT-Food Sci Technol. 2012b;46:56-63. DOI:10.1016/j.lwt. 2011.11.002

[94] Bautista-Gallego J, Arroyo-López FN, Romero-Gil V, Rodríguez-Gómez F, García-García P, Garrido-Fernández A. Fermentation profile of green Spanish-style Manzanilla olives according to NaCl content in brine. Food Microbiol. 2015a;49:56-64. DOI:10.1016/j.fm.2015.01.012.

[95] Bautista Gallego J, Arroyo López FN, Romero Gil V, Rodríguez Gómez F and Garrido Fernández A, Evaluating the affect on Zn chloride as a preservative in cracked table olive packing. J Food Protect. 2011c;74:2169-2176. DOI:10.4315/0362-028X.JFP-11-201.

[96] Delgado-Adamez J, Franco MN, Sánchez J, De Miguel C, Ramírez MR, Martín-Vertedor D. Comparative effect of high pressure processing and traditional thermal treatment on the physicochemical, microbiology, and sensory analysis of olive jam. Grasas y Aceites. 2013;64:432-441. DOI:10.3989/gya.023613

[97] Tokuşoğlu Ö, Alpas H, Bozoğlu F. High hydrostatic pressure effects on mold flora, citrinin mycotoxin, hydroxytyrosol, oleuropein phenolics and antioxidant activity of black table olives. Innov Food Sci Emerg Technol. 2010;11:250-258. DOI:10.1016/j.ifset.2009.11.005

[98] Argyri AA, Panagou EZ, Nychas GJE, Tassou CC. Nonthermal pasteurization of fermented green table olives by means of high hydrostatic pressure processing. BioMed Res Int. 2014;515623. DOI:10.1155/2014/515623.

[99] Considine KM, Kelly AL, Fitzgerald GF, Hill C, Sleator RD. High-pressure processing—effects on microbial food safety and food quality. FEMS Microbiol Lett. 2008;281:1-9. DOI:10.1111/j.1574-6968.2008.01084.x.

[100] Rendueles E, Omer MK, Alvseike O, Alonso-Calleja C, Capita R, Prieto M. Microbiological food safety assessment of high hydrostatic pressure processing: a review. LWT-Food Sci Technol. 2011;44:1251-1260. DOI:10.1016/j.lwt.2010.11.001

[101] Pilavtepe-Çelik M, Balaban MO, Alpas H, Yousef AE. Image analysis based quantification of bacterial volume change with high hydrostatic pressure. J Food Sci. 2008;73:423-429. DOI:10.1111/j.1750-3841.2008.00947.x.

[102] Abriouel H, Benomar N, Gálvez A, Pulido RP. Preservation of Manzanilla Alorena cracked green table olives by high hydrostatic pressure treatments singly or in combination with natural antimicrobials. LWT-Food Sci Technol. 2014;56:427-431. DOI:10.1016/j.lwt.2013.09.012

[103] Pradas I, Del Pino B, Peña F, Ortiz V, Moreno-Rojas JM, Fernández-Hernández A, García-Mesa JA. The use of high hydrostatic pressure (HHP) treatments for table olives preservation. Innov Food Sci Emerg Technol. 2012;13:64-68. DOI:10.1016/j.ifset.2011.10.011

[104] Kadam PS, Jadhav BA, Salve RV, Machewad GM. Review on the high pressure technology (HPT) for food preservation. J Food Process Technol 3:1–5. DOI:10.4172/2157-7110.1000135

[105] Sánchez J, De Miguel C, Ramirez MR, Delgado J, Franco MN, Martín D. Effect of high hydrostatic pressure versus thermal pasteurization on the microbiological, sensory aspects and oxidative stability of olive pate. Grasas y Aceites. 2012;63:100-108. DOI: 10.3989/gya.071211

[106] Sánchez-Gómez AH, García-García P, Garrido Fernández A. Spanish-style green table olive shelf-life. Int J Food Sci Technol. 2013;48:1559-1568. DOI:10.1111/ijfs.12124

[107] Lavermicocca P, Valerio F, Lonigro SL, de Angelis M, Morelli L, Callegari ML. Study of adhesion and survival of lactobacilli and bifidobacteria on table olives with the aim of formulating a new probiotic food. Appl Environ Microbiol. 2005;71:4233-4240. DOI: 10.1128/AEM.71.8.4233-4240.2005

[108] Panagou EZ. Effect of different packing treatments on the microbiological and physicochemical characteristics of untreated green olives of the Conservolea cultivar. J Sci Food Agric. 2004;84:757–764. DOI:10.1002/jsfa.1732

[109] Mattos LM, Moretti CL, Ferreira MD. Modified Atmosphere Packaging for Perishable Plant Products, Polypropylene, Dr. Fatih Dogan (Ed.), InTech Europe, Rijeka, Croatia ISBN: 978-953-51-0636-4, 2012, InTech, Available from: http://www.intechopen.com/books/polypropylene/modified-atmosphere-for-perishable-plant-products

[110] Argyri AA, Nisiotou AA, Pramateftaki P, Doulgeraki AI, Panagou EZ, Tassou CC. Preservation of green table olives fermented with lactic acid bacteria with probiotic potential under modified atmosphere packaging. LWT-Food Sci Technol. 2015;62:783-790. DOI:10.1016/j.lwt.2014.11.046

[111] Panagou EZ, Tassou CC. Katsaboxakis KZ. Microbiological, physicochemical and organoleptic changes in dry-salted olives of Thasos variety stored under different modified atmospheres at 4°C and 20°C. Int J Food Sci Technol. 2002;37:635-641. DOI: 10.1046/j.1365-2621.2002.00590.x

[112] Mantzouridou F, Tsimidou MZ. Microbiological quality and biophenol content of hot air-dried Thassos cv. table olives upon storage. Eur J Lipid Sci Technol. 2011;113:786-795. DOI:10.1002/ejlt.201000453

[113] Doulgeraki AI, Hondrodimou O, Iliopoulos V, Panagou EZ. Lactic acid bacteria and yeast heterogeneity during aerobic and modified atmosphere packaging storage of natural black Conservolea olives in polyethylene pouches. Food Control. 2012;26:49-57. DOI:10.1016/j.foodcont.2012.01.006

[114] Lavermicocca P, Rossi M, Russo F, Srirajaskanthan R. Table olives: a carrier for delivering probiotic bacteria to humans. In Preedy VR, Watson RR, editors. Olives and Olive Oil in Health and Disease Prevention. Oxford: Academic Press; 2010. p.735–743

[115] Botta C, Langerholc T, Cencič A, Cocolin L. In vitro selection and characterization of new probiotic candidates from table olive microbiota. PloS One. 2014;9:e94457. DOI: 10.1371/journal.pone.0094457.

[116] De Bellis P, Valerio F, Sisto A, Lonigro SL, Lavermicocca P. Probiotic table olives: microbial populations adhering on the olive surface in fermentation sets inoculated with the probiotic strains Lactobacillus paracasei IMPC2.1 in an industrial plant. Int J Food Microbiol. 2010;140:6–13. DOI:10.1016/j.ijfoodmicro.2010. 02.024

[117] Botta C, Cocolin L. Microbial dynamics and biodiversity in table olive fermentation: culture-dependent and independent approaches. Front Microbiol. 2002;3:245–245. DOI:10.3389/fmicb.2012.00245

[118] Rodríguez-Gómez F, Romero-Gil V, Bautista-Gallego J, García-García P, Garrido-Fernández A, Arroyo-López FN. Production of potential probiotic Spanish-style green table olives at pilot plant scale using multifunctional starters. Food Microbiol. 2014a; 44:278-287. DOI:10.1016/j.fm.2014.03.023.

[119] Domínguez-Manzano J, Olmo-Ruiz, C, Bautista-Gallego J, Arroyo-López FN, Garrido-Fernández A, Jiménez-Díaz R. Biofilm formation on abiotic and biotic surfaces during Spanish style green table olive fermentation. Int J Food Microbiol. 2012;157:230-238. DOI:10.1016/j.ijfoodmicro.2012.05.011.

[120] Bautista-Gallego J, Arroyo-López FN, Rantsiou K, Jiménez-Díaz R, Garrido-Fernández A, Cocolin L. Screening of lactic acid bacteria isolated from fermented table olives with probiotic potential. Food Res Int. 2013;50:135-142. DOI:10.1016/j.foodres.2012.10.004

[121] Bevilacqua A, Altieri C, Corbo MR, Sinigaglia M, Ouoba LI. Characterization of lactic acid bacteria isolated from italian Bella di Cerignola table olives: selection of potential multifunctional starter cultures. J Food Sci. 2010;75:536–544. DOI:10.1111/j. 1750-3841.2010.01793.x.

[122] Blana VA, Grounta A, Tassou CC, Nychas G.J, Panagou EZ. Inoculated fermentation of green olives with potential probiotic Lactobacillus pentosus and Lactobacillus plantarum starter cultures isolated from industrially fermented olives. Food Microbiol. 2014;38:208-218. DOI:10.1016/j.fm.2013.09.007.

[123] Peres CM, Alves M, Hernandez-Mendoza A, Moreira L, Silva S, Bronze MR, … Malcata FX. Novel isolates of lactobacilli from fermented Portuguese olive as potential probiotics. LWT-Food Sci Technol. 2014;59:234-246. DOI:10.1016/j.lwt.2014.03.003

[124] Psani M, Kotzekidou P. Technological characteristics of yeast strains and their potential as starter adjuncts in Greek-style black olive fermentation. World J Microbiol Biotechnol. 2006;22:1329-1336. DOI: 10.1007/s11274-006-9180-y

[125] Rodríguez-Gómez F, Bautista-Gallego J, Arroyo-López FN, Romero-Gil V, Jiménez-Díaz R, Garrido-Fernández A, García-García P. Table olive fermentation with multifunctional Lactobacillus pentosus strains. Food Control. 2013;34:96-105. DOI:10.1016/ j.foodcont.2013.04.010

Chapter 13

Biotechnology can Improve a Traditional Product as Table Olives

Maria Tufariello, Giovanni Mita and Gianluca Bleve

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64687

Abstract

Table olives are fermented vegetables very popular in the world and especially in the Mediterranean countries. Five main styles (Spanish or Sevillian, Castelvetrano, Siciliano, Californian, and Greek) are diffused to produce commercial products, beside several traditional styles. Although the main preparation methods of table olives are known for a long time, they are not yet optimized systems, and each of them is characterized by advantages and disadvantages. The use of NaOH for green olive debittering is responsible for the elimination of many aroma compounds and nutritionally important molecules. High volumes of heavily contaminated wastewaters are produced during olive processing. Spontaneous fermentation processes used to ferment black or green olives are difficult either to monitor or control. Microbial starters, selected for specific bio/technological and safety traits, can be useful to (i) improve the table olives organoleptic characteristics, (ii) control the fermentation process and significantly reduce the time to obtain a final product, (iii) monitor the correct evolution of the process, (iv) ensure the maintenance and/or improvement of nutritional and healthy features of the product, (v) protect table olives from undesired spoilage and pathogenic microorganisms, (vi) produce table olives as a carrier of microorganisms with probiotics characters, and (vii) enhance product stability and shelf life.

Keywords: table olives, starters, organoleptic traits, nutritional characteristics, probiotics

1. Introduction

Table olives are one of the most important and popular fermented vegetables in Western world and in particular in Southern European countries. Table olives world production was estimated to be 2,742,500 tons in 2015–2016 season (**Figure 1**).

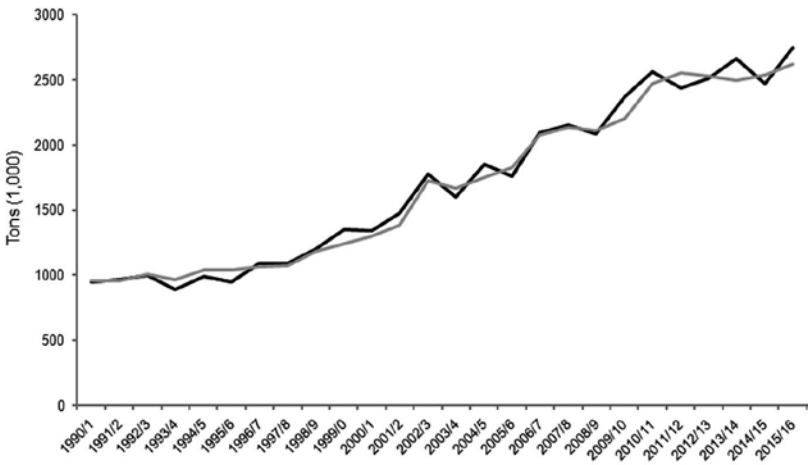


Figure 1. Table olives world production (▬) and consumption (▬). Adapted from data reported in [1].

The 29% of this production (796,000 tons) is located in the European Union (EU). Spain has a leading position in table olive production with 514,000 tons, followed by Greece (210,000 tons), Italy (50,000 tons), and Portugal (17,500 tons) [1]. Among the countries of the Mediterranean Basin, Egypt, Turkey, Algeria, and Morocco are the main producers and consumers (**Figure 2**).

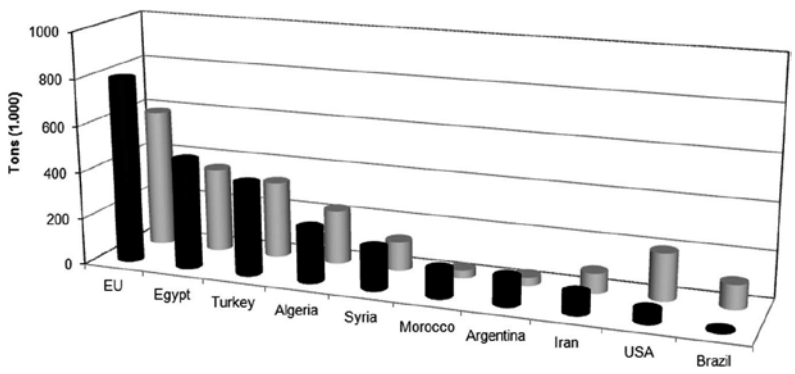


Figure 2. Table olives production ( ) and imports ( ) in main producing and importer countries. Adapted from data reported in [1].

http://dx.doi.org/10.5772/64687

According to International Olive Council Standard, the term "table olive" means the product prepared from the sound fruits of varieties of the cultivated olive trees that are chosen for their production of olives whose volume, shape, flesh-to-stone ratio, fine flesh, taste, firmness, and ease of detachment from the stone make them particularly suitable for processing; treated to remove its bitterness and preserved by natural fermentation, or by heat treatment with or without the addition of preservatives; packed with or without covering liquid.

Table olives are classified according to the degree of ripeness of the drupes (green olives, olives turning color, and black olives), trade preparations (treated olives, natural olives, dehydrated and/or shriveled olives, olives darkened by oxidation, and specialties), and styles (whole, pitted, stuffed, salad, and other). They are produced by processing raw olives with the objective of eliminating their natural bitterness, which is mainly due to oleuropein and other phenolics [2]. The main commercial types of table olives are processed according to five styles: Spanish (or Sevillian), Castelvetrano, Siciliano, Californian, and Greek [3], although several other traditional styles also exist for the preparation of treated and natural table olives [4].

The two main commercial table olives preparations, lye-treated olives (Spanish and Castelvetrano styles) and brine-soaked olives (Greek style) are industrially produced by spontaneous fermentation, but, currently, these processes are difficult to be monitored and controlled [5]. The spontaneous process cannot ensure either the correct evolution of the process or the good quality and safety standards of the final product. Controls of the presence of biogenic amines and toxins in table olives commercial preparations need to be increased [6].

In green olives productions, the NaOH is used as chemical debittering system. This treatment is economic, characterized by a simple implementation, and an easy standardization. However, simultaneously to the debittering effect, it causes the elimination of many aroma compounds together with nutritional and health important molecules. The process produces also high volumes of heavily contaminated wastewaters. Besides, the use of NaOH for debittering organic table olives is prohibited in many countries [7].

The employment of starter cultures of *Lactobacillus plantarum* and *L. pentosus* can be used as an alternative to NaOH for debittering. This strategy has the advantage to control the fermentation process and to improve the quality of the final product [8]. Lactic acid fermentation is considered the key step in spontaneous fermentation processes. It promotes (i) debittering of the olives through oleuropein hydrolysis, (ii) lowering of brine pH, which prevents the growth of spoilage and pathogenic microorganisms, and (iii) the enhancement of a correct flavor and texture profile in the final product [9, 10].

It has also been demonstrated that yeasts, producing desirable metabolites and volatile compounds, are able to improve the organoleptic properties. Yeasts can also enhance the growth of lactic acid bacteria (LAB) and degrade phenolic compounds. A role of yeasts as starters has been recently proposed for production of table olive [11–15].

There is an increasing interest in lowering NaCl concentration (now 8–10%) and in shortening the fermentation time (8–12 months) in order to obtain a healthier product suitable to reach the market very soon.

Recently, Bleve et al. described a novel method based on the sequential use of autochthonous yeast and bacterial strains to shorten the time of fermentation, to standardize the process, and to improve organoleptic and nutritional properties of olives [16].

The future challenges will be to investigate some strains for their probiotic characteristics, in order to produce functional olives. Indeed, several studies demonstrated that the use of LAB as starter for table olive production can produce beneficial effects on human health [17, 18]. Also yeast strains have been evaluated for their probiotic properties [13, 15]. The nutritional and health-related compounds associated to fermented table olives (or derivatives) could be assessed by *in vitro* and *in vivo* analyses. The results of these assays can produce precise information on the importance of these compounds for the prevention and/or treatment of several human and animal diseases (i.e., gastrointestinal, cardiovascular, neurodegenerative diseases, and tumors).

Table olives are considered by many food scientists as the "food of the future" owing to the healthy bioactive compounds they contain. In fact, table olives, together with olive oil, represent an important food of the Mediterranean diet and are perceived to have positive nutritional and therapeutic effects. Monounsaturated fatty acids, as found in olives are known to be healthier than polyunsaturated and saturated fats. In addition, epidemiological studies indicate that olive biophenols have a role in lowering incidence of several chronic and heart diseases [19, 20].

2. Production methods

Table olives, directly harvested from trees, need to be processed in order to reduce or eliminate their bitter taste and to obtain a product ready to be consumed. Different commercial preparations of table olives are produced using procedures inherited and opportunely modified from traditional methods. As previously extensively described by Boskou et al. [21], the main methods including fermentation steps to obtain the final product are water-cured olives produced by soaking olives in water over a week or more and then placing them in brine where a fermentation process can occur; Greek-style or "natural" olives and Sicilian-style green olives spontaneously fermented in brines; lye-treated olives (Spanish or Sevillian style, Castelvetrano method) produced by a first treatment with alkali (NaOH) and, after washing olives with water to remove NaOH, by a second step in brine to obtain a partial or complete fermentation of the drupes.

Other methods for black olives not involving fermentation are known as Californian and Spanish styles. The drupes are debittered by lye and soaking in brine. They are also aerated insufflating air to oxidize the pigments and immersed in ferrous gluconate or ferrous lactate solution in order to stabilize a uniform black color. Table olives can also be produced by traditional methods diffused in Mediterranean Basin using lime (CaO) and olive wood ash, or they can be dried and debittered without chemicals by using salt or heat treatment. Some cultivars of olives resulted naturally debittered also by parasite fungi directly on the tree without necessity of further treatment. They can also undergo a natural sweetening during

ripening on the tree, although genetic and biochemical mechanisms involved in this last phenomenon are until now unknown.

3. Biotechnological approaches to produce table olives

The fermentation process, generally performed by indigenous microorganisms, is one of the best and oldest procedures of treating food products to transform and preserve them. However, as already demonstrated in several food products (wine, beer, bread, yogurt, cheese, sake, chocolate, etc.), spontaneous fermentations are uncontrolled and not predictable. These spontaneous processes are inefficient since they do not ensure the expected quality and safety characteristics of the final product, the sensorial and structure features, the limitation, or absence of growth of harmful or undesired spoilage organisms [22].

In order to obtain a more controlled process and to improve the quality and safety levels of table olives, the selection and use of starter cultures is diffusing. In fact, several studies demonstrated the usefulness and the benefits of starters in table olives production [5, 9, 15, 23].

3.1. Starter selection

The microbiota associated to olives can be different among the different cultivars. Microorganisms detected in table olives and brines belong to members of bacteria (lactic acid bacteria, Enterobacteriaceae, *Pseudomonas, Staphylococcus, Clostridium*, etc.), yeasts, and moulds. Enterobacteriaceae, *Clostridium*, and *Pseudomonas* are generally associated to raw olives and to the beginning of fermentation. They are completely eliminated at the end of the process, especially due to the low pH [24–26] (**Figure 3**).

The presence of hazardous pathogens such as *C. botulinum* has to be adduced to incorrect processing, heat treatment, packaging, and transportation [27].

The most studied group of bacteria is lactic acid bacteria (homo and hetero fermentative), since they are responsible for the sugar conversion to organic acids and in particular to lactic acid. The different table olives production methods can influence microbial population present in raw olives and their evolution during fermentation (**Figure 3**). *Lactobacillus coryniformis, L. plantarum, L. pentosus*, and *Leuconostoc mesenteroides* have been detected and isolated in Spanish style green olives across the process. Also *Enterococcus* spp., *Pseudomonas* spp., and *Staphylococcus* spp. are associated to olives produced by this method. Bacterial biodiversity associated to natural green and natural cracked green olives is richer than that present in Spanish style olives, treated with NaOH [28] (**Figure 3**). In black olives, bacteria belonging to Enterobacteriaceae, *Kocuria, Swaminathania, Acetobacter*, and *Pseudomonas* were detected only at the initial stage of fermentation, except for *Swaminathania* that has been found, in some cases, also at the end of fermentation. Bleve et al. [29, 30] reported the presence of LAB (*Lactobacillus* sp., *L. plantarum, L. pentosus, Leuconostoc mesenteroides*) associated to the final stage of fermentation (120–180 days) in Leccino and Kalamàta cultivars (**Figure 3**).

Yeasts found in olives belong to the genera *Candida, Debaryomyces, Hanseniaspora, Issatchenkia, Kluyveromyces, Pichia, Rhodotorula, Saccharomyces, Torulaspora, Wickerhamomyces, Zygosaccharomyces, Zygotorulaspora*, with some differences between green and black olives (**Figure 4**). Yeasts are detectable throughout the fermentation process in all table olive cultivars.

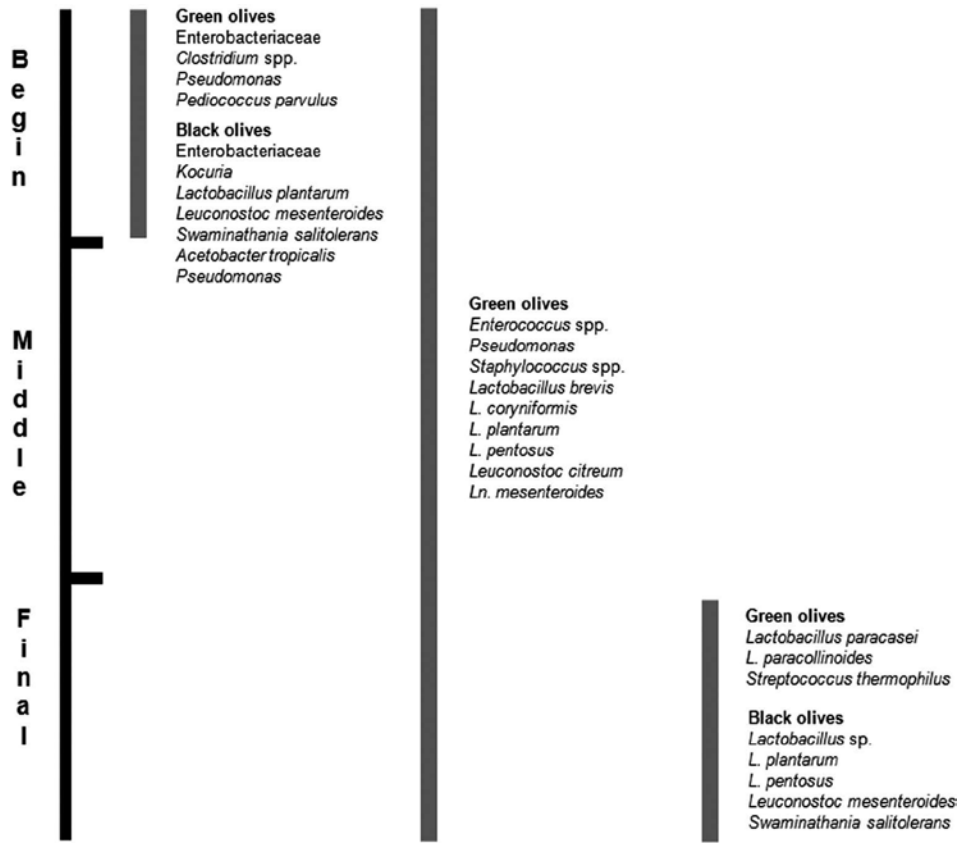


Figure 3. Main genera and species of bacteria associated to different production stages of green and black table olives. Adapted from Heperkan et al. [32]; Bleve et al. [29], [30].

Also the mould genera *Aureobasidium* and *Geotrichum* have been isolated from green and black naturally fermented olives, whereas isolates belonging to the genus *Penicillium* and *Aspergillus* were isolated by naturally fermented black olives [21, 31, 32].

The introduction of LAB and yeasts starter cultures in table olives production can also be motivated by the difficulty to monitor and control spontaneous fermentation in the industrially production of black as well as several cultivars of green olives [33–35]. Starter cultures are preparations of microorganisms, live, or resting, generally present in high cell number, which can be added to enhance, accelerate, and improve a fermentation process by their metabolic activities.

LAB have been considered very important since they are able to debitter olives, low brine pH, limit the spoilage and the presence of pathogens, and develop a correct flavor and texture in the final product. Several studies proposed the use of *Lactobacillus plantarum* and/or of *L. pentosus* as starter cultures among the possible available technological approaches [9, 10, 15, 33, 36–38].

The use of yeasts as starters cultures has been recently proposed for production of table olive [11, 13, 14, 35, 39], since they can improve the organoleptic properties [11, 40], enhance the growth of LAB [12, 41], and biodegrade phenolic compounds [42].

Moreover, the possibility to use simultaneous or sequential inocula of yeasts and LAB in green and black olives has been proposed [12, 15, 37, 41, 43]. The presence of yeasts together with LAB can produce a significant improvement of the sensorial quality of olives. They can also favor LAB growth rate, help in Enterobacteriaceae reduction, sensitively shorten the time needed to obtain the final product.

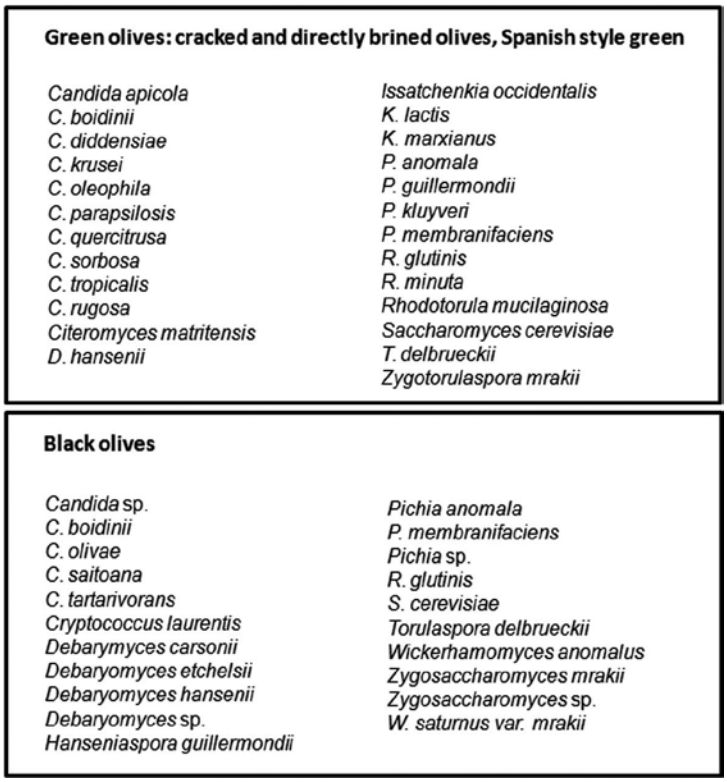


Figure 4. Main species of yeasts associated to green and black table olives. Adapted from Heperkan et al. [32]; Bleve et al. [29], [30].

Moulds can be responsible of undesirable effects on table olives quality. They can alter olive taste and appearance and can be responsible of mycotoxins production [32]. These microor-

ganisms need to be deeply studied in order to evaluate their possible positive role in table olive processing.

LAB and yeast strains to be used as starter cultures can be selected among microorganisms associated to a spontaneous fermentation. In a first step, they can be selected on the basis of their characteristics and abilities: (i) to lower pH (by homo or hetero-fermentative metabolism for LAB, by fermentative metabolism for yeasts); (ii) to survive and to grow in the presence of different constraints (poor nutrient substrate like olives in brine, low pH, high salt level, presence of phenols, wide range of temperatures), (iii) to produce lactic acid and other organic acids; (iv) to metabolize phenols and, in particular, to degrade oleuropein, which is the main compound responsible for the bitter taste in olives; (v) to develop desired flavors (volatile compounds); (vi) to produce no biogenic amines, which represent an emerging concern in table olives, wine and other fermented products [44–46]; (vii) to possess esterase and lipase activities that have a role in improving the aromatic profile of fermented olives (by increasing their free fatty acid content); (viii) to have no proteolytic and pectolytic activities, which could have a negative impact on olive quality since they are related to olive softening; (ix) to have functional (probiotics and health-promoting) properties. Several laboratory tests have been developed to select yeasts and LAB for all of these features. In a laboratory-scale, the most promising isolates can be tested for their ability to dominate the indigenous microbiota by predominant growth or by production of antagonistic substances during table olives fermentation.

The selected LAB and yeast isolates can be then tested in a pilot-scale fermentation (200 kg) in order to mimic the industrial conditions of fermentation. The best performing isolates can be proposed for industrial-scale fermentation in tanks of 3–8 tons.

3.2. Influence of starter cultures on table olives chemical and aromatic profile

The distribution and structure of the chemical constituents of olive fruit is complex and depend on variety, cultivation practices, geographical origin, and the level of maturation. Olive fruit's average composition is water (50%), protein (1.6%), fat (22%), carbohydrate (19.1%), cellulose (5.8%), inorganic substances (1.5%) and phenolic compounds (1–3%).

Both in green and black olive fermentations, lactic, citric, tartaric, and acetic acids were found to be the major metabolic products in drupes and in the brines [9, 47, 48], responsible for a decrease in pH value (about 4.0), satisfactory for naturally black olive fermentation [29, 30].

Although in the literature there are many data about aroma compounds in olive oil, very little is known about the quali-quantitative composition of volatile compounds in table olives. Among table olives, more attention has been placed on the characterization of the volatile fraction of the fermented black olives. Little is known about volatile fraction of green olives, probably because their volatile profiles are less rich, due to the NaOH treatment; the latter affects many precursors of the volatile compounds.

The formation of flavor compounds in table olives is a dynamic process mainly occurring during fermentation carried out by LAB and yeasts, along with a variety of contaminating microorganisms, which produce a variety of volatile compounds [49]. Volatile and semivolatile organic compounds are responsible for the olive complex flavor that in turn can influence the

http://dx.doi.org/10.5772/64687

consumer's preference. The "green odor" of unripe olives was associated to the presence of C5 and C6 volatile compounds (alcohols and aldehydes) originating from the activity of lypoxygenase metabolic pathway [50]. Hexanol and 2-hexenal are the major contributors to the characteristic green odor of olives and of many fruit and vegetable fermented foods. In spontaneous fermentation of black olives, the main product is ethanol that derives from the metabolic activity of different yeasts and hetero-fermentative LAB and is very important for the organoleptic properties of the final product [51].

C6 alcohols such as 1-hexanol and *cis*-3-hexen-1-ol, characterized by a "vegetal" and "herbaceous" aromas, seem to be linked to the different yeast strain used [52]. As already observed in wines, the relevant presence of the ethyl-acetate ester at the end of fermentation adds complexity to the aroma of the final product [53]. The high level of isoamyl alcohols indicates the role of yeasts in driving the process. In particular, 2 + 3 methyl-1-butanol (isoamyl alcohol, fruity-winey notes), hexanol (fruity-green notes) and *cis*-3-hexen-1-ol (green notes) are very important both in olives and brines. Other higher alcohols (1-propanol and 2-methyl-1 propanol) derive from the reduction process of aldehydes, but can also be linked to the microbial deamination process of amino acids [54]. Hexanal, (*Z*)-hex-3-enol, hexanol, (*Z*)-hex-3-enol acetate and hexyl acetate, detectable at various concentrations were reported to be related to the lipoxygenase activity [29, 30, 49].

Fatty acids, formed enzymatically during fermentations constitute an important group of aroma compounds that can contribute to the aroma complexity of table olives [29, 30]. Terpenes production is closely linked to cultivars, geographical area, climatic conditions and proliferation of specific pests and microorganisms characteristic of a given production area [55]. The presence of styrene can increase during fermentation [15]. This compound could be linked to an environmental contaminants and/or produced by L-phenylalanine deamination and decarboxylation of *trans*-cinammic acid [60] or by the dehydration of 2-phenylethanol.

The use of selected starter cultures has been proposed for Spanish- and Greek-type, green and black, olives to improve fermentation performance. Starters can accelerate and control the process, reduce undesired off-flavors and enhance quality of the final product by the development of typical and peculiar sensorial and taste characteristics.

In the evolution of volatile compounds during spontaneous fermentation of different black olive cultivars (Leccino, Cellina di Nardò, Conservolea and Kalamàta), Tufariello et al. [15] identified three main temporary steps characterized by the presence of chemical descriptors: aldehydes at the first stage (30 days), higher alcohols and styrene in the middle (90 days), and ethyl esters and fatty acids at the end of fermentation (third fermentation stage, 180 days). These descriptors could help in monitoring the fermentation process of other black olive cultivars as well as of naturally fermented green olives.

In starter-driven fermentations carried out by sequential inoculum of yeast LAB strain, three main stages have been described. The first stage (30 days) is characterized by high aldehydes content, compounds responsible of herbaceous flavors in fruits and vegetables. The second stage (60 days) is characterized by the presence of higher alcohols, styrene [56] and terpenes, compounds correlated with the metabolic activities of inoculated yeast starter strains. The third

final fermentation stage (90 days), mainly characterized by the presence of acetate esters (isoamyl acetate, ethyl acetate), esters (ethyl hexanoate and ethyl octanoate), and acids, probably due to the different pathways undertaken by LAB enzymes.

The use of starter microorganisms significantly reduced the time of fermentation process from 180 to 90 days. The first stage of fermentation shifted from 90 (spontaneous fermentation) to 60 days (starter-driven fermentation) and the second step shifted from 180 (spontaneous fermentation) to 90 days (starter-driven fermentation). The use of sequential inoculation strategy of selected yeast and LAB starters produced a volatile profile richer in compounds that can be associated to attributes such as fruity, winey-sweet and herbaceous. A significant reduction of volatile phenols and hydrocarbons was observed.

For Moresca and Kalamàta table olives inoculated with selected starter cultures of *L. plantarum*, a shift from herbal notes to fruity, sweet, and floral profile has been reported. In inoculated samples, a significant increase of higher alcohols (isoamylalcohols, 1-propanol, 2-methyl-1-propanol, phenylethylalcohol), esters (ethyl butanoate), and acetate esters (isoamyl acetate, ethyl acetate) was also observed [57].

Grounta et al. [58] demonstrated that the use of *L. pentosus* B281 produced table olives with good physical and chemical features and sensory properties highly appreciated by expert panelists. The coinoculation of *L. pentosus* B281 and *P. membranifaciens* M3A in brines of Conservolea olives developed a proper fermentation process, producing a final product with good sensory attributes and a milder acid gustatory sensation. This product could be suitable for consumers who do not appreciate the acid taste of natural black fermented olives and prefer milder tastes. No off-odors associated to abnormal fermentation (i.e., butyric, putrid fermentation, or zapateria spoilage) were detected by the panelists [58].

In green olives, most studies have been carried out for the selection of starter cultures able to control fermentation in brines after lye treatment. This process generally needs 3–7 months to be completed. It is mainly driven by LAB belonging to lactobacilli, *Leuconostoc* and *Pediococcus* spp. There are few studies on the volatile fraction and its evolution during the fermentation process. Panagou and Tassou [59] studied the evolution of the volatiles in green table olives (Conservolea cv.) treated with NaOH and then inoculated with *L. plantarum* or *L. pentosus*. The use of starters produced an acceleration of fermentation process. The final products contained increased concentrations of lactic and acetic acid as well as volatile molecules such as ethanol, methanol, acetate esters and isobutyric, isovaleric, and propionic acids. The sensorial characteristics ascribable to typical lactic fermentation were obtained in the final product also inoculating the strains *L. pentosus* B281 and *L. plantarum* B282, as single or combined cultures to ferment Spanish-style produced Halkidiki green olives [8].

In order to improve the fermentation of directly brined green olives, the application of the "pied de cuve" technology has been proposed [38]. Partially fermented brines deriving from a previous spontaneous fermentation were used to produce green olives with improved aroma and taste complexity. In comparison with brines deriving from previous fermentations performed with the starter *L. pentosus* OM13, undesired off-odors and off-flavors were not detected and a good control of microorganism spoilage was obtained. The use of selected

http://dx.doi.org/10.5772/64687

strains of lactobacilli and yeasts accelerated the fermentation process of directly brined Bella di Cerignola green olives [37], a cultivar traditionally debittered in the Spanish style. In inoculated samples, major compounds that significantly increased were ethanol, acetic acid and ethyl acetate. There was also an increase in the level of esters (fruity nuances), alcohols (fruity, floral and sweet notes), and acids except for propanoic acid. A decrease of aldehydes content was also observed [37].

Volatile compounds confer peculiar sensorial characteristics and contribute to the aroma "fingerprint" of single table olives variety. Then, it could be important to promote the use of new descriptors other than those linked to taste (crispness, sourness, bitterness, and astringency), appearance (brightness, intense green color, etc.) [2]. New descriptors should be able to describe floral, fruity, green, winey as well other similar notes (**Figure 5**).

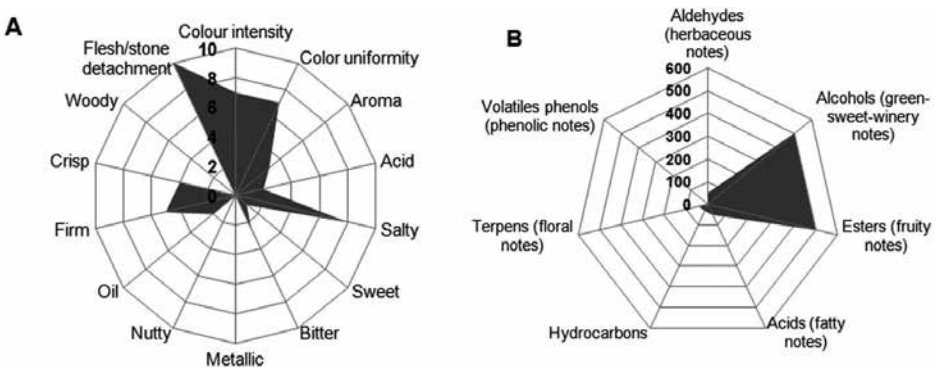


Figure 5. Spider plot showing the main organoleptic attribute intensities identified by trained panel (A) and (B) radar plot of all volatiles classes associated to black olives fermented by yeast and LAB starters.

To link chemical data to sensory data, it is necessary to evaluate the perception thresholds of the volatiles, defined as the lowest concentration capable of producing a sensation. The contribution of each volatile compound to odor profile can be quantified by its odor activity value (OAV). OAV is the ratio of the compound concentration to its odor threshold. In the table olives sector, these thresholds are not available, so it is not still possible to establish the role of each volatile compound as odorant in the multiplicity of olives aroma components.

3.3. Influence of microbial fermentation on table olives nutritional profile

In the past decades, olive oil and table olives have been attracting interest, mostly due to their beneficial effects on health. Table olives contain several nutritional components that largely depend on the olive variety, the cultivation conditions, the maturation stage of the olive fruit, and the processing method. The consumption of table olives thus allows the dietary introduction of bioactive components, such as triterpenic acids, α-tocopherol, biophenols, and fatty acids. These compounds are known to be responsible for a variety of health benefits. More specifically, olive fruits are remarkably rich in maslinic and oleanolic acids [60]. These triterpenic acids are located in the epicarp of the olive fruit and they constitute the main substances

of the surface waxes [61, 62]. Some studies indicate that maslinic and oleanolic acids possess health beneficial activities such as anti-inflammatory, antioxidant [63–65], antimicrobial [66], antiviral [67], cardioprotective [68], antihypertensive [69], antihyperlipidemic [70, 71], antidiabetic [72, 73], and even antitumor [73–77]. It is worthwhile noting that the content of these bioactive compounds in table olives is significantly higher than in olive oil [78].

NaOH treated green and black olives contain low levels of these compounds in comparison with naturally fermented olives [78]. Indeed, the NaOH treatment leads to the solubilization of maslinic and oleanolic acids into the alkaline and washing solutions. The resulting final product contained significantly reduced levels of these compounds.

In Greek-style preparations, the fermentation of black table olives driven by selected starter cultures can preserve the triterpenic acid content. The amount of these molecules was around 1000–2000 mg/kg olive flesh, much higher than the values observed in extra virgin olive oils [Bleve G., unpublished]. These observations confirm that table olives can be considered a dietary natural source of triterpenic acids.

The health benefits of olive oil and table olives are also attributed to their high content in monounsaturated fatty acid (MUFA). Commonly recognized as a high-fat food (about 80–85% of the calories in olives come from fat), olives provide a high content of oleic acid. Linoleic acid and α-linolenic acid are present in small amounts. Owing to the content of MUFA, the consumption of table olives can prevent and reduce the risk of cardiovascular diseases, regulate cholesterol levels, stimulate transcription of LDL-cholesterol receptor mRNA, and reduce breast cancer risks [79–81]. In Spanish-, Californian-, and Greek-style processes, triglylglycerols composition remains unaffected, although fatty acid composition of both green and black olives, shows differences depending on the ripeness degree. The concentration of oleic acid, the most abundant fatty acid in green and black olives, showed differences depending on the stage of maturity the producing methods [82]. When considering the PUFA/SFA ratio, green and directly brined table olives showed a value >0.4. This is a value recommended by the nutritional guidelines [83]. In particular, a significantly high PUFA/SFA ratio was found in directly brined olives [84, 85]. The use of selected starter cultures for black olives fermentation ensured a PUFA/SFA ratio >0.4 in the final product.

The olive fruit is also highly valuable for the presence of α-tocopherol (TC), β-carotene (BC) [80], and biophenols. TC acts as the major radical scavenging antioxidant and efficiently interrupts the propagation of lipid oxidation chain [86]. Several works described a protective action of TC on human health against different pathologies. It contributes to reduce the effects of inflammations and it defends the body against the negative effects of free radicals [87]. When cultivars of black table olives were fermented using selected starter cultures, Vitamin E and carotenes levels were found more constant.

Among biophenols, tyrosol, hydroxytyrosol, luteolin, and oleuropein are the main species found in olives [88–93]. The latter compound is mainly responsible for the bitter taste of unprocessed olives. Other phenolic compounds are verbascoside, 3,4-dihydroxyphenylglycol [94], anthocyanins, flavonoids, and phenolic acids [88]. Mechanisms postulated for chemical

http://dx.doi.org/10.5772/64687

and microbial oleuropein degradation and the effects on valuable phenols caused by different table olives processing methods are described in details by Boskou et al. [21].

In Greek-style fermented olives (driven by natural microbiota or by starter cultures), a higher content of total phenols was detected than that observed in lye treated olives [15, 29, 30, 37, 95, 96]. During the process, a complete hydrolysis of oleuropein and its aglycone takes place in olive flesh by yeasts and LAB β-glycosidase and esterase activities. In fact, high levels of hydroxytyrosol and tyrosol, together with verbascoside, caffeic acid, vanillic acid, and hydrocaffeic acid were detected in the final products. Table olives are a very good source of hydroxytyrosol which is known to possess a high antioxidant and free radical scavenging activity [95, 97]. Table olives together with virgin olive oil are the only edible source of hydroxytyrosol; in olive oil, however, the bound forms of this compound prevail.

Changes in the profiles of bioactive compounds caused by metabolic activities of microbial starters can produce variations in the bioaccessibility and/or bioavailability of these metabolites. The role of microorganisms and the effects of their activities on these bioactive compounds need to be further elucidated. Table olives can be used to ensure a "positive" or "optimal" dietary intake of these compounds. They represent a source of phytochemicals useful for the prevention of several diseases and the promotion of human health (**Figure 6**).

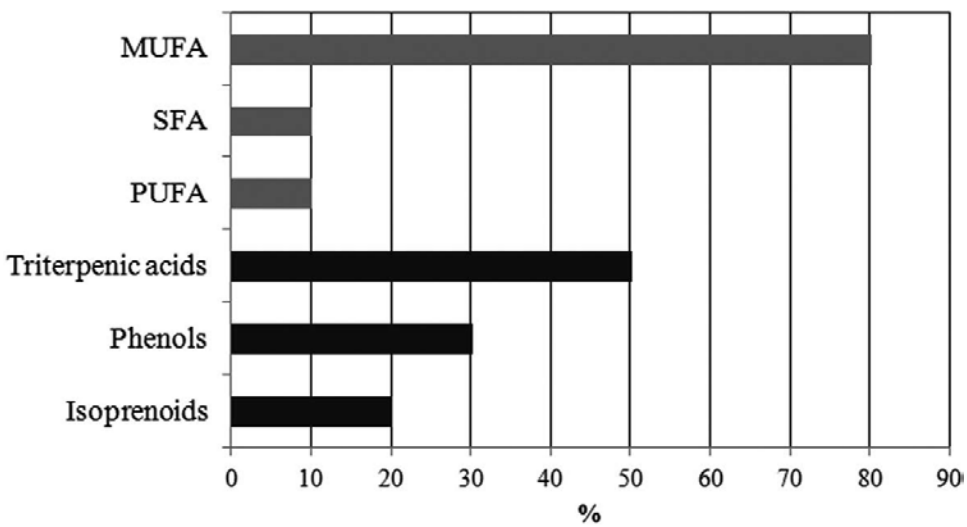


Figure 6. Profile of the main nutritional traits (% on drupe fresh weight) associated to black table olives fermented by yeast and LAB starters.

3.4. Use of starter cultures as probiotics

Another important character for the selection of potential starter cultures is referred to probiotic traits that beneficially influence intestinal microflora and health [98]. LAB, mainly the strains belonging to genera *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*, are microor-

ganisms generally considered for probiotics preparations. The term "probiotic" is defined by a United Nations and World Health Organization Expert Panel as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [99]. Probiotics are live microorganisms that have a beneficial effect on the host by influencing the composition and or metabolic activity of the flora of the gastrointestinal (GI) tract. Selected LAB probiotics strains can have beneficial properties. They can enhance the immune system responses, improve resistance to infection, protect against certain types of cancer, lower serum cholesterol levels, and reduce the incidence of coronary heart disease. They are also involved in the prevention or treatment of peptic ulcer disease, treatment of intractable diarrhea during antibiotic therapy, reduction of allergic inflammation, production of antimicrobial substances, reduction of symptoms of lactose intolerance, and the enhancement of the nutrients bioavailability [100–102].

Probiotic characteristics are strongly required for microorganisms to be used for food production. These attributes can be conferred to a food by microbial preparations different or by the same microorganisms used as starter cultures. In the first case, the interaction between probiotics strains and traditional starter cultures must be considered, since some probiotics strains may have effects on organoleptic properties of the food product or can influence negatively the starter culture bacteria [103]. In the latter case, strains to be proposed as starters need to be selected also for probiotics traits.

Recent studies have focused on the use of table olives as a carrier of LAB probiotic strains as well as on the evaluation of the fermentation performances of these probiotics [39, 101, 104, 105]. *L. paracasei* strain (IMPC2.1) was able to successfully colonize both the olive surface [102] and human gut [104], also driving the fermentation [106]. Moreover, LAB strains directly isolated from fermented olives have been proposed as probiotics starters instead of bacteria from human and animal sources. By this approach, some strain of *L. pentosus, L. plantarum*, and *L. paracasei* ssp. *paracasei* isolated from fermented olives showed desirable *in vitro* probiotic properties as well as good aptitude to be employed as starter cultures [8, 18].

During the past few years, some researchers have identified yeast species with potential probiotic properties, such as *C. boidinii, C. oleophila, D. hansenii*, and *P. membranifaciens* [35, 107, 108].

As proposed by different authors [14, 23, 109], for these microorganisms, probiotics traits are: (i) the resistance and/or survival to gastric pH conditions and to bile salts, (ii) the capacity to adhere to intestinal mucosa, and (iii) the antimicrobial activities against intestinal and foodborne pathogens. In addition, several other health promoting factors can be considered in order to promote yeast starters as probiotics. They are the production of B vitamins and the reduction of the intestinal proinflammatory response as an antagonistic effect of yeasts or probiotic bacteria toward pathogen; the ability to reduce cholesterol serum levels [110]; the ability to biodegrade phytate complexes, responsible for sequestering nutritional divalent minerals; the capability to synthesize natural folates, essential cofactors in the biosynthesis of nucleotides and crucial for cellular replication and growth [111]; the ability to produce a number of bioactive compounds. Anyway, in order to obtain probiotics and healthy olives, it is necessary that yeasts adhere to olive skin and survive during storage/packaging. The copresence of yeasts

and LAB in the biofilm associated to epidermis of natural black olives (Greek-style fermented Conservolea) and Spanish-style olives (Gordal and Manzanilla) indicate that the coinoculation of yeasts and LAB as multifunctional starter is a good strategy for carrying probiotics by table olives [35, 58].

3.5. Influence of starters in bioremediation of table olive processing wastewaters (TOPW)

During table olives processing, clean water is used and a large quantity of wastewater is produced depending on cultivar, maturity, and type of treatment from 0.5 l/kg to 6 l/kg. In 2013/2014, 2.7 million tons of table olives were produced in the world. The production of 1.2–14 million tons of wastewaters can be estimated. The volumes of wastewaters depends on the different table olives processing methods: for Spanish style 2–3.5 l/kg of olives; for California green ripe olives 1.5–3.5 l/kg of olives, for California black ripe olives 2–6.5 l/kg of olives, for naturally black olives (Greek style) 1 l/kg of olives [112]. The availability of the water as a resource and the environmental impact deriving from its use are very important matters for many table olives producer countries. The main problems associated to TOPW are their high chemical oxygen demand (COD) up to 35 g/l, biological oxygen demand (BOD) ranging from 0.6 to 38.3 g/l, different pH values (alkaline, up to 9–13, for waters deriving by lye-treatment and acidic, 3.6–4.4, for fermentation brines), the presence of several water-soluble phenols and polyphenols, the high salt levels (56–77 g/l) in Greek-style olives.

The different composition of TOPW (produced by different table olives processing methods) requires the development of diverse approaches for their management and treatment. The contemporary presence of high organic matter content (reducing sugars, organic acids), suitable to be used by microorganisms, and of compounds that affect microbial growth and metabolism (phenols), renders biological approaches for their remediation very difficult.

There are different methods based on the use of aerobic and anaerobic processes of TOPW [113, 114]. There are also strategies that combine chemical and biological processes, using a pretreatment with *Aspergillus* sp. in order to degrade phenols, very toxic, and able to limit the activities of anaerobic digestion [115, 116]. Several studies demonstrated that the use of aerobic and anaerobic biodegradation of wastewaters can significantly reduce organic load expressed by COD (aerobic treatment between 50 and 70% and anaerobic between 81 and 94%) [116–119].

The use of starter microorganisms in table olive processing can represent a useful system to mitigate the presence of chemical pollutants in TOPW. They are phenols (such as oleuropein and derivates, anthocyanins), NaOH in the lye and sodium content in brine. Selected microorganisms, by their metabolic activities can reduce the presence of phenols. They can also efficiently dominate the spontaneous microflora and facilitate reduction of NaCl concentration in brines. Concerning NaOH in lye, a future challenge can be to develop new systems able to reduce or eliminate the use of NaOH in lye. These strategies can allow to perform a more natural transformation process by the combination of technological and biotechnological approaches.

Author details

Maria Tufariello, Giovanni Mita and Gianluca Bleve*

*Address all correspondence to: gianluca.bleve@ispa.cnr.it

National Research Council, Institute of Sciences of Food Production, Section of Lecce, Lecce, Italy

References

[1] http://www.internationaloliveoil.org/estaticos/view/132-world-table-olive-figures [Accessed: 04/03/2016].

[2] International Olive oil Council (2004). Trade Standard Applying to Table Olives. Resolution No. RES-2/91-IV/04.

[3] Garrido-Fernandez A, Fernandez Diez MJ, Adams MR. Olives and table olives. In: Garrido A, Fernandez MJ, Fernandez D, Adams MR, editors. Table Olives: Production and Processing. London: Chapman and Hall; 1997. p. 10–22, 289–367.

[4] Lanza B. Nutritional and sensory quality of table olives. In: Muzzalupo I, editor. Olive Germplasm – The Olive Cultivation, Table Olive and Olive Oil Industry in Italy, 2012. InTech. ISBN 978-953-51-0883-2. DOI: 10.5772/51723.

[5] Corsetti A, Perpetuini G, Schirone M, Tofalo R, Suzzi G. Application of starter cultures to table olive fermentation: an overview on the experimental studies. Frontiers in Microbiology. 2012; 3: 248–253. DOI: 10.3389/fmicb.2012.00248.

[6] Medina-Pradas E, Arroyo-López FN. Presence of toxic microbial metabolites in table olives. Frontiers in Microbiology. 2015; 6: 1–6. DOI: 10.3389/fmicb.2015.00873.

[7] García A, Romero C, Medina E, García P, De Castro A, Brenes M. Debittering of olives by polyphenol oxidation. Journal of Agricultural and Food Chemistry. 2008; 56: 11862–11867. DOI: 10.1021/jf802967y.

[8] Blana VA, Grounta A, Tassou CC, Nychas GJ, Panagou EZ. Inoculated fermentation of green olives with potential probiotic Lactobacillus pentosus and Lactobacillus plantarum starter cultures isolated from industrially fermented olives. Food Microbiology. 2014; 38: 208–218. DOI: 10.1016/j.fm.2013. 09.007.

[9] Panagou EZ, Schillinger U, Franz CMAP, Nychasa GJE. Microbiological and biochemical profile of cv. Conservolea naturally black olives during controlled fermentation with selected strains of lactic acid bacteria. Food Microbiology. 2008; 25: 348–358. DOI: 10.1016/j.fm.2007.10.005.

[10] Servili M, Settanni L, Veneziani G, Esposto S, Massitti O, Taticchi A, Urbani S, Montedoro GF, Corsetti A. The Use of Lactobacillus pentosus 1MO to shorten the debittering process time of black table olives (Cv. Itrana and Leccino): a pilot-scale application. Journal of Agricultural and Food Chemistry. 2006; 54: 3869–3875. DOI: 10.1021/jf053206y.

[11] Arroyo-López FN, Querol A, Bautista-Gallego J, Garrido-Fernández A. Role of yeasts in table olive production. International Journal of Food Microbiology. 2008; 128:189–196. DOI: 10.1016/j.ijfoodmicro.2008.08.018.

[12] Segovia Bravo KA, Arroyo Lopez FN, García García P, Duran Quintana MC, Garrido Fernandez A. Treatment of green table olive solutions with ozone. Effect on their polyphenol content and on Lactobacillus pentosus and Saccharomyces cerevisiae growth. International Journal of Food Microbiology. 2007; 114: 60–68. DOI: 10.1016/j.ijfoodmicro.2006.09.032.

[13] Bevilacqua A, Beneduce L, Sinigaglia M, Corbo MR. Selection of yeasts as starter cultures for table olives. Journal of Food Science. 2013; 78: M742–M751. DOI: 10.1111/1750-3841.12117.

[14] Bonatsou S, Benítez A, Rodríguez-Gómez F, Panagou EZ, Arroyo-López FN. Selection of yeasts with multi functional features for application as starters in natural black table olive processing. Food Microbiology. 2015; 46: 66–73. DOI: 10.1016/j.fm.2014.07.011.

[15] Tufariello M, Durante M, Ramires FA, Grieco F, Tommasi L, Perbellini E, Falco V, Tasioula-Margari M, Logrieco AF, Mita G, Bleve G. New process for production of fermented black table olives using selected autochthonous microbial resources. Frontiers in Microbiology. 2015; 6: 1007–1022. DOI: 10.3389/fmicb.2015.01007.

[16] Bleve G, Tufariello M, Durante M, Perbellini E, Mita G, Ramires AF, Grieco F, Logrieco AF. Metodo per la Produzione di Olive da Tavola Fermentate. 2015. European Patent Application No. 14197402.2. Munich: European Patent Office (EPO).

[17] Lavermicocca P, Valerio F, Lonigro SL, De Angelis M, Morelli L, Callegari ML, Rizzello GC, Visconti A. Study of the adhesion and survival of lactobacilli and bifidobacteria on table olives with the aim of formulating a new probiotics food. Applied Environmental Microbiology. 2005; 71: 4233–4240. DOI: 10.1128/AEM.71.8.4233-4240.2005.

[18] Argyri A, Zoumpopoulou G, Karatzas KA, Tsakalidou E, Nychas GJE, Panagou EZ, Tassou CC. Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. Food Microbiology. 2013; 33: 282–291. DOI: 10.1016/j.fm.2012.10.005.

[19] Barbaro B, Toietta G, Maggio R, Arciello M, Tarocchi M, Galli A, Balsano C. Effects of the Olive-Derived Polyphenol Oleuropein on Human Health. International Journal of Molecular Sciences. 2014; 15: 18508–18524. DOI: 10.3390/ijms151018508.

[20] Charoenprasert S, Mitchell A. Factors influencing phenolic compounds in table olives (Olea europaea). Journal of Agricultural and Food Chemistry. 2012; 60: 7081–7095. DOI: 10.1021/jf3017699.

[21] Boskou D, Camposeo S, Clodoveo ML. Table olives as sources of bioactive compounds. In: Boskou D, editor. Olives and Olive Oil Bioactive Constituents,; 2015.Urbana, IL, USA: AOCS Press. p 179–216, 217—260. ISBN: 978-1-630670-41-2.

[22] Steensels J, Verstrepen KJ. Taming wild yeast: potential of conventional and nonconventional yeasts in industrial fermentations. Annual Review of Microbiology. 2014; 68: 61–80. DOI: 10.1146/annurev-micro-091213-113025.

[23] Heperkan D. Microbiota of table olive fermentations and criteria of selection for their use as starters. Frontiers in Microbiology. 2013; 4: 1–11. DOI: 10.3389/fmicb.2013.00143.

[24] Tassou CC, Panagou EZ, Nychas GJE. Microbial colonization of naturally fermented olives. In: Preedy VR, Watson RR, editors. Olives and Olive Oil in Health and Disease. Singapore: Academic Press is an imprint of Elsevier; 2010. p. 397–406.

[25] Randazzo CL, Ribbera A, Pitino I, Romeo FV, Caggia C. Diversity of bacterial population of table olives assessed by PCR-DGGE analysis. Food Microbiology. 2012; 32: 87–96. DOI: 10.1016/j.fm.2012.04.013.

[26] Alves M, Gonçalves T, Quintas T. Microbial quality and yeast population dynamics in cracked green table olives fermentations. Food Control. 2012; 23: 363–368. DOI: 10.1016/j.foodcont.2011.07.033.

[27] Jalava K, Selby K, Pihlajasaari A, Kolho E, Dahlsten E, Forss N, Bäcklund T, Korkeala H, Honkanen-Buzalski T, Hulkko T, Derman Y, Järvinen A, Kotilainen H, Kultanen L, Ruutu P, Lyytikaïnen O, Lindström M. Two cases of food-borne botulism in Finland caused by conserved olives. Eurosurveillance. 2011; 16: 1–3.

[28] Cocolin L, Alessandria V, Botta C, Gorra R, De Filippis F, Ercolini D, Rantsiou K. NaOH-debittering induces changes in bacterial ecology during table olives fermentation. PLoS One. 2013; 8: 1–11. DOI: 10.1371/journal.pone.0069074.

[29] Bleve G, Tufariello M, Durante M, Perbellini E, Ramires FA, Grieco F, Cappello MS, De Domenico S, Mita G, Tasioula-Margari M, Logrieco AF. Physico-chemical and microbiological characterization of spontaneous fermentation of Cellina di Nardò and Leccino table olives. Frontiers in Microbiology. 2014; 5: 570, 1–18. DOI: 10.3389/fmicb.2014.00570.

[30] Bleve G, Tufariello M, Durante M, Grieco F, Ramires FA, Mita G, Tasioula-Margari M, Logrieco AF. Physico-chemical and microbiological characterization of natural fermentation process of Conservolea and Kalamata table olives and developement of a protocol for the pre-selection of fermentation starters. Food Microbiology. 2015; 46: 368–382. DOI: 10.1016/j.fm.2014.08.021.

[31] Nisiotou AA, Chorianopoulos N, Nychas GJ, Panagou EZ. 2010. Yeast heterogeneity during spontaneous fermentation of black conservolea olives in different brine

solutions. Journal of Applied Microbiology. 2010; 108: 396–405. DOI: 10.1111/j. 1365-2672.2009.04424.

[32] Heperkan D, Erol-Meriç B, Sismanoglu G, Dalkiliç G, Güler FK. Mint: mycobiota, mycotoxigenic fungi and citrinin production in black olives. Advances in Experimental Medicine and Biology. 2006; 571: 203–210. DOI: 10.1007/0-387-28391-9_13.

[33] De Castro A, Montano A, Casado FJ, Sanchez AH, Rejano L. Utilization of Enterococcus casselifavus and Lactobacillus pentosus as starter cultures for Spanish-style green olive fermentation. Food Microbiology. 2002. 19: 637–644. DOI: 10.1006/fmic.2002.0466.

[34] Abriouel H, Benomar N, Lucas R, Gálvez A. Culture-independent study of the diversity of microbial populations in brines during fermentation of naturally fermented Aloreña green table olives. International Journal of Food Microbiology. 2011; 144: 487–496. DOI: 10.1016/j. ijfoodmicro.2010.11.006.

[35] Arroyo-López FN, Romero-Gil V, Bautista-Gallego J, Rodriguez-Gómez F, Jiménez-Díaz R, García-García P, Querol A, Garrido-Fernandez A. Yeasts in table olive processing: desirable or spoilage microorganisms? International Journal of Food Microbiology. 2012; 160: 42–49. DOI: 10.1016/j.ijfoodmicro.2012.08.003.

[36] Leal-Sánchez MV, Ruiz-Barba JL, Sánchez AH, Rejano L, Jiménez-Díaz R, Garrido A. Fermentation profile and optimization of green olive fermentation using Lactobacillus plantarum LPCO10 as a starter culture. Food Microbiology. 2003; 20: 421–430. DOI: 10.1016/S0740-0020(02)00147-8.

[37] De Angelis M, Campanella D, Cosmai L, Summo C, Rizzello CG, Caponio F. Microbiota and metabolome of un-started and started Greek-type fermentation of Bella di Cerignola table olives. Food Microbiology. 2015; 52: 18-30. DOI: 0.1016/j.fm.2015.06.002.

[38] Martorana A, Alfonzo A, Settanni L, Corona O, La Croce F, Caruso T, Moschetti G, Francesca N. An innovative method to produce green table olives based on "pied de cuve" technology. Food Microbiology. 2015; 50: 126–140. DOI: 10.1016/j.fm.2015.03.008.

[39] Arroyo-López FN, Romero-Gil V, Bautista-Gallego J, Rodriguez-Gómez F, Jiménez-Díaz R, García-García P, Querol A, Garrido-Fernández A. Potential benefits of the application of yeast starters in table olive processing. Frontiers in Microbiology. 2012; 3: 161–164. DOI: 10.3389/fmicb.2012.00161.

[40] Garrido Fernández A, Garcia P, Brenes M. Olive fermentations. In: Remand HJ, Reed G, editors. Biotechnology. Weinheim: VCH Press; 1995. p. 539–627.

[41] Tsapatsaris S, Kotzekidou P. Application of central composite design and response surface methodology to the fermentation of olive juice by Lactobacillus plantarum and Debaryomyces hansenii. International Journal of Food Microbiology. 2004; 95: 157–163. DOI: 10.1016/ j.ijfoodmicro.2004.02.011.

[42] Ettayebi K, Errachidi F, Jamai L, Tahri-Jouti MA, Sendile K, Ettayebi M. Biodegradation of polyphenols with immobilized Candida tropicalis under metabolic induction. FEMS Microbiology Letters. 2003; 223: 215–219. DOI: 10.1016/S0378-1097(03)00380-X.

[43] Hurtado A, Ben Othman N, Chammem N, Hamdi M, Ferrer S, Reguant C, Bordons A, Rozés N. Characterization of Lactobacillus isolates from fermented olives and their bacteriocin gene profiles. Food Microbiology. 2011; 28: 1514–1518. DOI: 10.1016/j.fm.2011.07.010.

[44] Garcia PG, Barranco CR, Quintana MC, Fernandez AG. Biogenic amine formation and "zapatera" spoilage of fermented green olives: effect of storage temperature and debittering process. Journal of Food Protection. 2004; 67: 117–123. DOI: 10.1016/j.fm.2007.10.005.

[45] Spano G, Russo P, Lonvaud-Funel A, Lucas P, Alexandre H, Grandvalet C, Coton E, Coton M, Barnavon L, Bach B, Rattray F, Bunte A, Magni C, Ladero V, Alvarez M, Fernández M, Lopez P, de Palencia PF, Corbi A, Trip H, Lolkema JS. Biogenic amines in fermented foods. European Journal of Clinical Nutritional. 2010; 64: 95–100. DOI: 10.1038/ejcn.2010.218.

[46] Tristezza M, Vetrano C, Bleve G, Spano G, Capozzi V, Logrieco A, Mita G, Grieco F. Biodiversity and safety aspects of yeast strains characterized from vineyards and spontaneous fermentations in the Apulia Region, Italy. Food microbiology. 2013; 36: 335–342. DOI: 10.1016/J.FM.2013.07.001.

[47] Nychas GJN, Panagou EZ, Parker ML, Waldron KW, Tassou CC. Microbial colonization of naturally black olives during fermentation and associated biochemical activities in the cover brine. Letters in Applied Microbiology. 2002; 34: 173–177. DOI: 10.1046/j.1472-765x.2002.01077.x.

[48] Chorianopoulos NG, Boziaris IS, Stamatiou A, Nuchas GJE. Microbial association and acidity development of unheated and pasteurised green table olives fermented using glucose or sucrose supplements at various levels. Food Microbiology. 2005; 22: 117–124. DOI: 10.1016/j.fm.2004. 04.010.

[49] Sabatini N, Marsilio V. Volatile compounds in table olives (Olea europaea L., Nocellara del Belice cultivar). Food Chemistry. 2008; 107: 1522–1528. DOI: 10.1016/j.foodchem.2007.10.008.

[50] Angerosa F, Servili M, Selvaggini R, Taticchi A, Esposto S, Montedoro G. Volatile compounds in virgin olive oil: occurrence and their relationship with the quality. Journal of Chromatography A. 2004; 1054: 17–31.

[51] Fleming HP, Etchells JL, Bell TA. Vapor analysis of fermented Spanish-type green olives by gas chromatography. Journal of Food Science. 1969; 34: 419–422. DOI: 10.1111/j.1365-2621.1969.tb12794.x.

[52] Torrens J, Urpi P, Montserrat RA, Vichi S, López-Tamames E, Buxaderas S. Different commercial yeast strains affecting the volatile and sensory profile of cava base wine.

International Journal of Food Microbiology. 2008; 124: 48-57. DOI: 10.1016/j.ijfoodmicro.2008.02.023.

[53] Mallouchos A, Skandamis P, Loukatos P, Komaitis M, Koutinas A, Kanellaki M. Volatile compounds of wines produced by cells immobilized on grape skins. Journal of Agricultural and Food Chemistry. 2003; 51: 3060–3066. DOI: 10.1021/jf026177p.

[54] McFeeters RF. Fermentation microorganisms and flavor changes in fermented foods. Journal of Food Science. 2004; 69: 35–37. DOI: 10.1111/j.1365-2621.2004.tb17876.x.

[55] Damascelli A, Palmisano F. 2013 Sesquiterpene fingerprinting by headspace SPME–GC–MS: preliminary study for a simple and powerful analytical tool for traceability of olive oils. Food Analytical Methods. 2012; 6: 900–905. DOI: 10.1007/s12161-012-9500-9.

[56] Gilbert-López B, Robles-Molina J, García-Reyes JF, Molina-Díaz A. Rapid determination of BTEXS in olives and olive oil by headspace-gas chromatography/mass spectrometry (HS-GC-MS). Talanta. 2010; 83: 391–399. DOI: 10.1016/j.talanta.2010.09.052.

[57] Sabatini N, Mucciarella M, Marsilio V. Volatile compounds in uninoculated and inoculated table olives with Lactobacillus plantarum (Olea europaea L., cv. Morescaand Kalamata). Food Science and Technology. 2008; 41: 2017–2022. DOI: 10.1016/j.lwt.2007.12.002.

[58] Grounta A, Doulgeraki AI, Nychas GJE, Panagou EZ. Biofilm formation on Conservolea natural black olives during single and combined inoculation with a functional Lactobacillus pentosus starter culture. Food Microbiology. 2016; 56: 35–44. DOI: 10.1016/j.fm.2015.12.002.

[59] Panagou EZ, Tassou CC. Changes in volatile compounds and related biochemical profile during controlled fermentation of cv. Conservolea green olives. Food Microbiology. 2006; 23: 738–746. DOI: 0.1016/j.fm.2006.02.005.

[60] Stiti N, Triki S, Hartmann MA. Formation of triterpenoids throughout Olea europaea fruit ontogeny. Lipids. 2007; 42: 55–67.

[61] Bianchi G. Lipids and phenols in table olives. European Journal of Lipid Science and Technology. 2003; 105: 229–242. DOI: 10.1002/ejlt.200390046.

[62] Guinda Á, Rada M, Delgado T, Gutiérrez-Adánez P, Castellano JM. Pentacyclic triterpenoids from olive fruit and leaf. Journal of Agriculture of Food Chemistry. 2010; 58: 9685–9691. DOI: 10.1021/jf102039t.

[63] Ismaili H, Milella L, Fkih-Tetouani S, Ilidrissi A, Camporese A, Sosa S, Altinier G, Della Loggia R, Aquino R. In vivo topical anti-inflammatory and in vitro antioxidant activities of two extracts of Thymus satureioides leaves. Journal of Ethnopharmacology. 2004; 91: 31–36. DOI: 10.1016/j.jep.2003.11.013.

[64] Liu J. Pharmacology of oleanolic acid and ursolic acid. Journal of Ethnopharmacology. 1995; 49: 57–68. DOI: 10.1016/0378-8741(95)90032-2.

[65] Tsai SJ, Yin MC. Antioxidative and anti-inflammatory protection of oleanolic acid and ursolic acid in PC12 cells. Journal of Food Science. 2008; 73: 174–178. DOI: 10.1111/j.1750-3841.2008.00864.x.

[66] Horiuchi K, Shiota S, Hatano T, Yoshida T, Kuroda T, Tsuchiya T. Antimicrobial activity of oleanolic acid from Salvia officinalis and related compounds on vancomycin-resistant Enterococci (VRE). Biological and Pharmaceutical Bulletin. 2007; 30: 1147–1149.

[67] Parra A, Rivas F, Lopez PE, Garcia-Granados A, Martinez A, Albericio F, Marquez N, Muñoz E. Solution- and solid-phase synthesis and anti-HIV activity of maslinic acid derivatives containing amino acids and peptides. Bioorganic & Medicinal Chemistry. 2009; 17: 1139–1145.

[68] Allouche Y, Beltran G, Gaforio JJ, Uceda M, Mesa MD. Antioxidant and antiatherogenic activities of pentacyclic triterpenic diols and acids. Food and Chemical Toxicology. 2010; 48: 2885–2890. DOI: 10.1016/j.fct.2010.07.022.

[69] Rodriguez-Rodriguez R, Perona JS, Herrera MD, Ruiz-Gutierrez V. Triterpenic compounds from "orujo" olive oil elicit vasorelaxation in aorta from spontaneously hypertensive rats. Journal of Agricultural and Food Chemistry. 2006; 54: 2096–2102. DOI: 10.1021/jf0528512.

[70] Liu J, Sun H, Duan W, Mu D, Zhang L. Maslinic acid reduces blood glucose in KK-Ay mice. Biological and Pharmaceutical Bulletin. 2007; 30: 2075–2078. DOI: 10.1248/bpb.30.2075.

[71] Liu J, Sun H, Wang X, Mu D, Liao H, Zhang L. Effects of oleanolic acid and maslinic acid on hyperlipidemia. Drug Development Research. 2007; 68: 261–266. DOI: 10.1002/ddr.20187.

[72] Sato H, Genet C, Strehle A, Thomas C, Lobstein A, Wagner A, Mioskowski C, Auwerx J, Saladin R. Anti-hyperglycemic activity of a TGR5 agonist isolated from Olea europaea. Biochemical and Biophysical Research Communications. 2007; 362: 793–798.

[73] Hsum YW, Yew WT, Hong PLV, Soo KK, Hoon LS, Chieng YC, Mooi LY. Cancer chemopreventive activity of maslinic acid: suppression of COX-2 expression and inhibition of NF-κB and AP-1 activation in Raji cells. Planta Medica. 2011; 77: 152–157. DOI: 10.1055/s-0030-1250203.

[74] Juan ME, Planas JM. Effects of pentacyclic triterpenes from olives on colon cancer. In: Watson RR, Preedy VR, editors. Bioactive Foods and Extracts: Cancer Treatment and Prevention. New York: CRC Press; 2010. p. 403–413.

[75] Li C, Yang Z, Zhai C, Qiu W, Li D, Yi Z, Wang L, Tang J, Qian M, Luo J, Liu M. Maslinic acid potentiates the anti-tumor activity of tumor necrosis factor a by inhibiting NF-κB signaling pathway. Molecular Cancer. 2010; 9: 1–13. DOI: 10.1186/1476-4598-9-73.

[76] Reyes FJ, Centelles JJ, Lupianez JA, Cascante M. (2Alpha, 3beta)-2,3-dihydroxyolean-12-en-28-oic acid, a new natural triterpene from Olea europea, induces caspase

dependent apoptosis selectively in colon adenocarcinoma cells. FEBS Letters. 2006; 580: 6302–6310. DOI: 10.1017/S0007114508882979.

[77] Reyes-Zurita FJ, Rufino-Palomares EE, Lupianez JA, Cascante M. Maslinic acid, a natural triterpene from Olea europaea L., induces apoptosis in HT29 human colon cancer cells via the mitochondrial apoptotic pathway. Cancer Letters. 2009; 273: 44–54. DOI: 10.1016/j.canlet.2008.07.033.

[78] Romero C, García A, Medina E, Ruíz-Méndez Mª V, de Castro A, Brenes M. Triterpenic acids in table olives. Food Chemistry. 2010; 118: 670–674. DOI: 10.1016/j.foodchem.2009.05.037.

[79] Owen RW, Haubner R, Würtele G, Hull WE, Spiegelhalder B, Bartsch H. Olives and olive oil in cancer prevention. European Journal of Cancer Prevention. 2004; 13: 319–326. DOI: 10.1097/01.cej.0000130221.19480.7e.

[80] Sakouhi F, Harrabi S, Absalon C, Sbei K, Boukhchina Kallel H. α-Tocopherol and fatty acids contents of some Tunisian table olives (Olea europea L.): changes in their composition during ripening and processing. Food Chemistry. 2008; 108: 833–839. DOI: 10.1016/j.foodchem.2007.11.043.

[81] Kastorini CM, Milionis HJ, Goudevenos JA, Panagiotakos DB. Mediterranean diet and coronary heart disease: is obesity a link—a systematic review. Nutrition, Metabolism and Cardiovascular Diseases. 2010; 20: 536–551. DOI: 10.1016/j.numecd.2010.04.006.

[82] Ünal K, Nergiz C. The effect of table olive preparing methods and storage on the composition and nutritive value of olives. Grasas Aceites. 2003; 54: 71–76. DOI: 10.3989/gya.2003.v54.i1.280.

[83] Wood JD, Richardson RI, Nute GR, Fisher AV, Campo MM, Kasapidou E, Sheard PR, Enser M. Effects of fatty acids on meat quality: a review. Meat Science. 2003; 66: 21–32. DOI: 10.1016/S0309-1740(03)00022-6.

[84] Lopez A, Montano A, Garcia P, Garrido A. Fatty acid profile of table olives and its multivariate characterization using unsupervised (PCA) and supervised (DA) chemometrics. Journal of Agricultural and Food Chemistry. 2006; 54: 6747–6753. DOI: 10.1021/jf0612474.

[85] Uylaser V, Yildiz G. Fatty acid profile and mineral content of commercial table olives from Turkey. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2013; 41: 518–523.

[86] Kalman A, Mujahid C, Mottier P, Heudi O. Determination of α-tocopherol in infant foods by liquid chromatography combined with atmospheric pressure chemical ionisation mass spectrometry. Rapid Communications in Mass Spectrometry. 2003; 17: 723–727. DOI: 10.1002/rcm.970.

[87] Bogani P, Galli C, Villa M, Visioli F. Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil. Atherosclerosis. 2007; 190: 181–186. DOI: 10.1016/j.atherosclerosis.2006.01.011.

[88] Macheix, JJ, Fleuriet A, Billot J. Fruit phenolics, vol. 92.Boca Raton, FL: CRC Press; 1990. p.111–112.

[89] Ryan D, Robards K. Phenolic compounds in olives. Analyst. 1998; 123: 31R–44R.

[90] Borzillo A, Iannotta N, Uccella N. Oinotria table olives: quality evaluation during ripening and processing by biomolecular components. European Food Research and Technology. 2000; 212: 113–121. DOI: 10.1007/s002170000178.

[91] Uccella N. Olive biophenols: novel ethnic and technological approach. Trends Food Science and Technology. 2001; 11: 328–339. DOI: 10.3989/gya.095811.

[92] Saija A, Ucella N. Olive biophenols; functional effects on human wellbeing. Trends Food Science and Technology. 2001; 11: 357–363.

[93] Panizzi L, Scarpati ML, Oriente G. The constitution of oleuropein, a bitter glucoside of the olive with hypotensive action. Gazzetta Chimica Italiana. 1960; 90: 1449–1485.

[94] Bianchi G, Pozzi N. 3,4-Dihydroxyphenylglycol, a major C6–C2 phenolic in Olea europaea fruits. Phytochemistry. 1994; 35: 1335–1337.

[95] Blekas G, Psomiadou E, Tsimidou M, Boskou D. On the importance of total polar phenols to monitor the stability of Greek virgin olive oil. European Journal of Lipid Science and Technology. 2002; 140: 340–346. DOI: 10.1002/1438-9312(200206)104:6<340.

[96] Pistarino E, Aliakbarian B, Casazza AA, Paini M, Cosulich ME, Perego P. Combined effect of starter culture and temperature on phenolic compounds during fermentation of Taggiasca black olives. Food Chemistry. 2013; 138: 2043–2049. DOI: 10.1016/j.foodchem.2012.11.021.

[97] Leenen R, Roodenburg AJC, Vissers MN, Schuurbiers JAE, van Putte KPAM, Wiseman SA, van de Put FHMM. Supplementation of plasma with olive oil phenols and extracts: influence on LDL oxidation. Journal of Agricultural and Food Chemistry. 2002; 50: 1290–1297. DOI: 10.1021/jf010968u.

[98] Doulgeraki AI, Hondrodimou O, Iliopoulos V, Panagou EZ. Lactic acid bacteria and yeast heterogeneity during aerobic and modified atmosphere packaging storage of natural black Conservolea olives in polyethylene pouches. Food Control. 2012; 26: 49–57. DOI: 10.1016/j.foodcont.2012.01.006.

[99] FAO/WHO. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria.Cordoba (Argentina): American Córdoba Park Hotel; 2001.

[100] Parvez S, Kang M, Chung HS, Cho C, Hong M-C, Shin M-K, Bae H. Survey and mechanism of skin depigmenting and lightening agents. Review. Phytotherapy Research. 2006; 20: 921–934. DOI: 10.1002/ptr.1954.

[101] De Bellis P, Valerio F, Sisto A, Lonigro SL, Lavermicocca P. Probiotic table olives: microbial populations adhering on olive surface in fermentation sets inoculated with

the probiotic strain Lactobacillus paracasei IMPC2.1 in an industrial plant. International Journal of Food Microbiology. 2010; 140: 6–13. DOI: 10.1016/j.ijfoodmicro.2010.02.024.

[102] Mena B, Aryana KJ. Influence of ethanol on probiotic and culture bacteria Lactobacillus bulgaricus and Streptococcus thermophilus within a therapeutic product. Journal of Medical Microbiology. 2012; 2: 70–76. DOI: 10.4236/ojmm.2012.23010.

[103] Tamime AY, Saarela M, Korslund Sondergaard A, Mistry VV, Shah NP. Production and maintenance of viability of probiotic micro-organisms in dairy products. In: Tamime AY, editor. Probiotic Dairy Products. London: Blackwell Publishing Ltd; 2005. p. 39–72.

[104] Lavermicocca P, Valerio F, Lisa Lonigro S, De Angelis M, Morelli L, Callegari ML, Rizzello CG, Visconti A. Study of adhesion and survival of Lactobacilli and Bifidobacteria on table olives with the aim of formulating a new probiotic food. Applied Environmental Microbiology. 2005; 71: 4233–4240. DOI: 10.1128/AEM.71.8.4233-4240.2005.

[105] Saravanos E, Kagli D, Zoumpopoulou G, Panagou EZ, Tassou CC. Use of probiotic lactic acid bacteria as starter cultures in Spanish-style green olive fermentation and determination of their survival using PFGE. Food Microbiology. 2008; 1–4 September, Aberdeen, UK.

[106] Valerio A, D'Antona G, Nisoli E. Branched-chain amino acids, mitochondrial biogenesis, and healthspan: an evolutionary perspective. Aging. 2011; 3: 464–478.

[107] Psani M, Kotzekidou P. Technological characteristics of yeast strains and their potential as starter adjuncts in Greek-style black olive fermentation. World Journal of Microbiology and Biotechnology. 2006; 22: 1329–1336. DOI: 10.1007/s11274-006-9180-y.

[108] Silva T, Reto M, Sol M, Peito A, Peres CM, Peres C, Malcata XF. Characterization of yeasts from Portuguese brined olives, with a focus on their potentially probiotic behavior. LWT Food Science and Technology. 2011; 44: 1349–1354. DOI: 10.1016/j.lwt.2011.01.029.

[109] Bevilacqua A, Corbo MR, Sinigaglia M. Selection of yeasts as starter cultures for table olives: a step-by-step procedure. Frontiers in Microbiology. 2012; 3: 194–202. DOI: 10.3389/ fmicb.2012.00194.

[110] Kourelis A, Kotzamanidis C, Litopoulou-Tzanetaki E, Scouras ZG, Tzanetakis N, Yiangou M. Preliminary probiotic selection of dairy and human 227 yeast strains. Journal of Biological Research Thessaloniki. 2010; 13: 93–104.

[111] Moslehi-Jenabian S, Lindegaard Pedersen L, Jespersen L. Beneficial effects of probiotic and food borne yeasts on human health. Nutrients. 2010; 2: 449–473. DOI: 10.3390/nu2040449.

[112] Cappelletti GM, Nicoletti GM, Russo C. Wastewater from table olive industries. In: Fernando Sebastian Garcia Einschlag, editor. Waste Water – Evaluation and Manage-

ment. InTech; 2011. ISBN: 978-953-307-233-3. Available from: http://www.intechopen.com/books/waste-water-evaluation-and-management/wastewater-from-table-olive-industries.

[113] Aggelis GG, Gavala HN, Lyberatos G. Combined and separate aerobic and anaerobic biotreatment of green olive debittering wastewater. Journal of Agricultural Engineering Research. 2001; 80: 283–92.

[114] Rivas FJ, Beltran FJ, Alvarez P, Frades J, Gimeno O. Joint aerobic biodegradation of wastewater from table olive manufacturing industries and urban wastewater. Bioprocess Engineering. 2000; 23: 283–286.

[115] Borja R, Martin A, Alonso V, Garcia I, Banks CJ. Influence of different aerobic pretreatments on the kinetics of anaerobic digestion of olive-mill wastewater. Water Research. 1995; 29: 489–495.

[116] Kyriacou A, Lasaridi KE, Kotsou M, Balis C, Pilidis G. Combined bioremediation and advanced oxidation of green table olive processing wastewater. Process Biochemistry. 2005; 40: 1401–1408.

[117] Beltran J, Gonzalez T, Garcia J. Kinetics of the biodegradation of green table olive wastewaters by aerobic and anaerobic treatments. Journal of Hazardous Materials. 2008; 154: 839–845.

[118] Brenes M, Garcìa P, Romero C, Garrido A. Treatment of green table olive wastewaters by an activated-sludge process. Journal of Chemical Technology and Biotechnology. 2000; 75: 459–463.

[119] Kotsou M, Kyriacou A, Lasaridi K, Pilidis G. Integrated aerobic biological treatment and chemical oxidation with Fenton's reagent for the processing of green table olive wastewater. Process Biochemistry. 2004; 39: 1653–1660.

Section 6

Olive Processing Wastes

Chapter 14

The Possibility of Recovering of Hydroxytyrosol from Olive Milling Wastewater by Enzymatic Bioconversion

Manel Hamza and Sami Sayadi

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64774

Abstract

This chapter discusses an innovative approach to obtain liquid fractions from olive mill wastewater (OMW) rich in hydroxytyrosol. The method is based on bioconversion combined with membrane separation techniques. An enzymatic bioconversion of three types of OMW was tested. TheS total volumes of OMW are 15 and 40 L. The reaction was monitored in mechanically stirred systems for 2 h at 50°C. Maximum hydroxytyrosol concentrations of about 1.53, 0.83 and 0.46 g/L in the presence of 5 IU *Aspergillus niger* β-glucosidase per milliliter from North OMW and South OMW were procured by two different olive millings, which are milling super press (MSP) and milling continuous chain (MCC), respectively. Enzymatic pretreatment was followed by two tangential flow membrane separation stages, microfiltration (MF) and ultrafiltration (UF). The ultrafiltration permeate was concentrated by evaporation at 45°C for 2 h. The latter exhibited a chemical oxygen demand (COD) level of 48.44 g/L. The UF permeate dehydration increased the hydroxytyrosol concentration to 7.2 g/L. A new natural product that contains some minerals beneficial to health and devoid of heavy metals or chemicals was obtained by this innovative work which describes an environmentally friendly process at pilot-scale.

Keywords: olive milling waste water, bioconversion, membrane separation, hydroxytyrosol, antioxydants

1. Introduction

Ancestral food of the Mediterranean, olive oil saw its production increases steadily, due to the recognition of its high dietetic nutritional value. After milling of the olive by pressing, the oil phase and the aqueous phase are separated and the liquid organic residue called vegetable

water is discarded. This by-product poses significant pollution problems. The olive mill wastewater (OMW) treatment is difficult due to the high concentration of pollution load toxicity microflora and low biodegradability. The pollution load of olive industry output is exceptional: it usually exceeds 120 g COD/L and can reach up to 200 g COD/L. The toxicity of this effluent is very high due to its high content of phenolic compounds. Each year, 40 million m^3 of vegetable water is produced around the Mediterranean basin. Most of these liquid wastes are treated by gathering in evaporation ponds built in the open. This produces the accumulation and concentration of the bulk of the organic matter present. Various methods have been tested for the treatment of vegetable waters.

The olive mill wastewater (OMW), with a high concentration of aromatic compounds (2.5–20 g/L), is a potential source of molecules or precursors to valuable molecules, particularly ortho-diphenols. These compounds, of 10–100 times higher concentration than in the vegetation water of olive oil [1], are known for their antioxidant properties and their beneficial role in preventing certain diseases such as cardiovascular disease. Ortho-diphenols are of major industrial interest for the food company, antioxidants as potential natural alternates to synthetic antioxidants. The ortho-diphenol hydroxytyrosol is a very powerful antioxidant. It also has a wide range of biological activities, in particular antibacterial, antiinflammatory and antihypertensive.

The process of producing hydroxytyrosol from the OMW has been developed in recent years [2]. A period of storage of OMW is necessary for the hydroxytyrosol recovery after its spontaneous fermentation. This storage time sometimes exceeds 100 days [3]. However, the stability of hydroxytyrosol and resistance to its oxidation and its fungal and microbial degradation require reflection and technical practices such as the search for the concentration of ethanol to be added to the effluent throughout the enrichment period. Indeed, the search for new, more attractive techniques overcoming these problems will be mandatory.

The theme of our chapter is related to a part of the search for a gentle and inexpensive method for increasing hydroxytyrosol concentration in OMW [4]. We were interested in finding an optimal method of enrichment hydroxytyrosol. A good approach is that the polymerized phenols in the OMW can be modified by bioconversion, thus producing an extract rich in simple phenolic compounds. The latter are of practical interest for the pharmaceutical, food and cosmetics. Besides, the process significantly reduces the chemical oxygen demand (COD) required for the degradation of these compounds.

2. Olive mill wastewater: a source of natural phenolic compounds

Ninety-seven percent of the total olive production of the world was delivered in Mediterranean basin. Nine percent of the world's olive oil was produced in the olive oil industry of Mediterranean basin [5]. The industrial method of olive oil has undertaken many changes. The traditional discontinuous pressing process was initially switched by continuous centrifugation, using a three-phase system and, subsequently, a two-phase system. The different olive oil production methods would certainly yield different waste materials.

http://dx.doi.org/10.5772/64774

2.1. Composition of olive mill wastewater

Three phases and two wastes materials: olive oil (20%), solid waste (30%) and aqueous liquor (50%) were produced by classic production of olive oil. The olive pulp and stones were mixed in solid waste. The aqueous liquor originates from the vegetation water and from the soft tissues of the olive fruits, with water added during the modification process, the so-called olive mill wastewater (OMW). High chemical oxygen demand (COD) values (up to 220 g/L) and mineral salts are a major problem for the wastewater treatment which is due to the presence of large amounts of organic substances such as oil, olive oil phenol, protein and polysaccharides [6]. A very important category of antioxidant is present in OMW which is phenolic compounds that are useful for the pharmaceutical and cosmetic industries [7]; 50–1000 μg/g of phenol compounds are present in olive oil ranges. This variation depends on the olive variety and the extraction system. The antioxidants quantity in olive oil is only 1–2% of the available pool of antioxidants in the olive fruit [8]. In OMW, more than 40 biophenols have been identified. Potential antioxidant, cardioprotective and cancer preventive actions in humans were manifested in these compounds [9]. Hydroxytyrosol, tyrosol and caffeic acid are among prominent components [9] (**Figure 1**). The recovery process of these compounds from OMW is more economical and more practical than other OMW valorization method [7]. Besides, recycling OMW is an alternative to diminish its impact on the environment and the ecological system in general. It would also allow the repositioning of the olive oil industry in highly competitive levels by considering OMW as by-products [6, 7]. Therefore, OMW can be converted into value-added products. Many possible applications were tested in this study: (i) bioconversion into useful products; (ii) recovery of natural components; and (iii) enrichment of OMW in hydroxytyrosol.

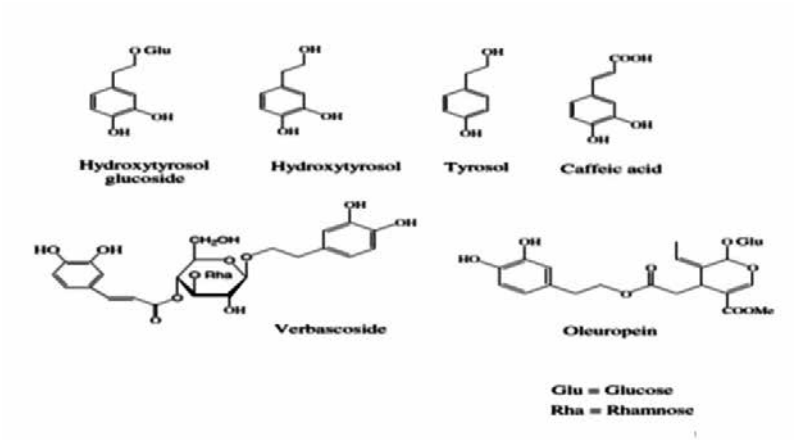
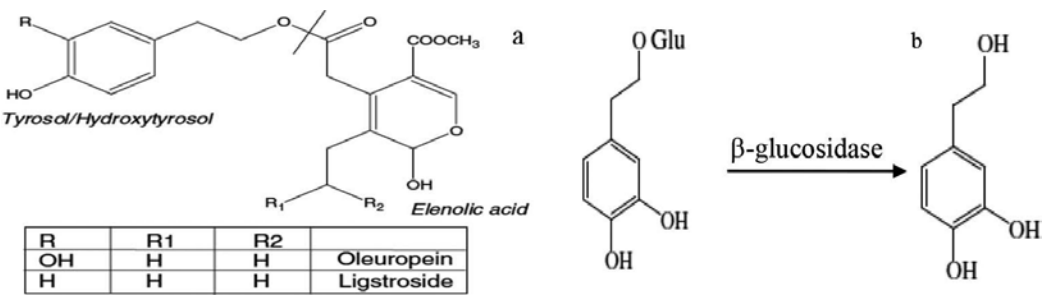


Figure 1. Chemical structures of phenolic compounds of OMW [9].

Hydroxytyrosol is the most valuable because of its amazing pharmacological and antioxidant actions, and it belongs to the major phenolic compounds present in olive fruit [10, 11]. Through

concern to the positive possessions of hydroxytyrosol, numerous approaches have been developed to produce this compound by means of chemical synthesis and enzymatic conversion [12, 13]. Further biological procedures have also been developed to produce hydroxytyrosol [14, 15]. Capasso et al. [12], Briante et al. [13] and Liebgott et al. [14] have tried the bioformation of pure substrate (oleuropein and tyrosol) to hydroxytyrosol. In the other hand, an ethyl acetate fraction and the corresponding aqueous exhausted fraction of dry olive mill residue were used as substrate for the culture of some saprobe fungi that led to the production of hydroxytyrosol [15].

The use of raw OMW as a natural source of hydroxytyrosol is very important because it is widespread in nature to leaf extract or synthetic oleuropein [16–18]. Indeed, hydroxytyrosol is present in OMW in two forms, free and combined. Combined molecules are oleuropein, demethyloleuropein, verbascoside and hydroxytyrosol glucosides [19–21] (**Figures 1** and **2**). Conjugated hydroxytyrosol cannot be recovered by membrane technology or solvent extraction. Furthermore, high amount of chemicals (acids, bases) for acidification to pH2 followed by neutralization was needed in chemical hydrolysis of OMW. Then, the label "BIO" would disappear. Consequently, this study purposes at discovery procedure to hydrolyze olive mill wastewater by a β-glucosidase preparation to acquire maximum hydroxytyrosol regaining. The key functioning variables governing the enzymatic hydrolysis process (temperature, time, pH, agitation) are studied. The amount among the enzyme and the substrate has also been estimated. The proposed enzymatic reaction has been practical to two diverse categories of OMW: the first is produced from the traditional discontinuous pressing process (milling super press, MSP), and the second is made from a continuous centrifugation using a three-phase system (milling continuous chain, MCC).



R	R1	R2	
OH	H	H	Oleuropein
H	H	H	Ligstroside

Figure 2. Release of hydroxytyrosol and tyrosol after degradation of oleuropein and ligstroside, respectively, [22] (a); hydrolyzate of hydroxytyrosol 4-β-D-glucoside (4-beta-D-glucosyl-hydroxyphenylethanol 3) (b).

2.2. Properties of hydroxytyrosol

Hydroxytyrosol is characterized by a strong antioxidant activity, which is similar to that of several synthetic and antioxidants, namely 2,6-di-tert-butyl-p-hydroxytoluene (BHT) and 3-tert-butyl-6-hydroxyanisole (BHA) [23]. Aeschbach et al. [24] showed that the antioxidant activity of this molecule was comparable to that of thymol, carvacrol, 6-gingerol and zingerone. It contributes to the stability of virgin olive oil [25]. It also inhibits low-density lipoprotein

http://dx.doi.org/10.5772/64774

(LDL) oxidation and also confers good cell protection and the dietary properties of virgin olive oil [25]. In addition to its antioxidant activity, hydroxytyrosol has an important interest in relation to human health. It has been shown that this ortho-diphenol opposes the cytotoxic effect of reactive oxygen metabolites in the cell, which prevents cell damage [26]. Hydroxytyrosol also exerts a marked antiinflammatory action. Petroni and his team showed that hydroxytyrosol inhibits the formation of a pro-inflammatory eicosanoide referred to as "leukotriene B4" [27]. De la Puerta and his team [27] found that hydroxytyrosol, tyrosol, oleuropein and caffeic acid inhibit the formation of leukotriene B4 by reducing the activity of 5-lipoxygenase, the enzyme that catalyzes this synthesis. Furthermore, it was reported that this enzyme is inhibited by olive extract, and the substances responsible for this effect are hydroxytyrosol, oleuropein and caffeic acid [28].

2.3. Synthesis of hydroxytyrosol

The only natural source of hydroxytyrosol remains for the moment the olive and olive oil by-products [29, 30]. Olive oil contains only a small amount of hydroxytyrosol concentrations of 0.01–1 mg/100 g of olive oil. This is due to the high solubility of hydroxytyrosol in water (about 5 g/100 mL); it is present in the OMW, which is in many cases discharged into the environment. A first pathway of hydroxytyrosol production relies on its purification from the olive mill wastewater. This process offers an impure product given that the great diversity and the large amount of polyphenols listed in the OMW (2.5–3% by weight, about a hundred different phenols [31]). Moreover, obtaining a purified fraction containing the hydroxytyrosol from the waste involves several chromatographic steps using large amounts of solvents [29]. Therefore, researchers have attempted to find a less expensive process for the purification of hydroxytyrosol. Other production pathways based on chemical methods include the synthesis of hydroxytyrosol by chemical reduction of the 3,4-dihydroxyphenylacetic acid [32, 33] and by catalytic conversion of tyrosol to hydroxytyrosol with a mixture of methylrhenium trioxide (MTO) and hydrogen peroxide. Two enzymatic production routes for hydroxytyrosol have also been described:

1. A hydroxytyrosol production by hydrolysis of oleuropein in the presence of β-glucosidase [34]. The β-glucosidase used in the process of Briante et al. is produced by a recombinant strain of *Escherichia coli*; the β-glucosidase from *Sulfolobus solfataricus* is a hyperthermophilic bacterium. This method has several disadvantages: the enzyme and oleuropein must first be purified from the bacterial culture and from olive leaves, respectively. The final extract is constituted by a mixture of hydroxytyrosol and two forms of the elenolic acid.

2. An enzymatic synthesis by conversion of tyrosol in the presence of a tyrosinase extracted from a fungus [35]. This second enzymatic method also has its limitations: firstly, the method requires the preliminary purification of tyrosinase with several steps which further increase the cost of production; secondly, the method requires the presence of ascorbic acid for inhibiting cresolase activity of tyrosinase and to avoid the formation of quinones; an additional final purification step must be implemented to eliminate the ascorbic acid from the reaction mixture.

Another type of production was approved by whole cells grown on tyrosol. Producing bacteria *Serratia marscecens* [36], *Pseudomonas aeruginosa* [2, 37], *Pseudomonas putida* F6 [38] and *Halomonas* sp. HTB24 [14], the enzyme system responsible for the bioconversion of tyrosol hydroxytyrosol has never been identified in these different bacterial species.

3. *A. niger* choice: generally recognized as a safe "GRAS" microorganism

One of the most important Ascomycota multi-uses in biotechnological applications is *A. niger*. Its use fascinated production of extracellular enzymes, such as glucoamylase [39], pectinase [40], the acidic lipase [41], esterase feruloyl [42] and xylanase [43]. This species is also known for the production of some organic acids such as gluconic acid [44] and citric acid [45]. Citric acid and several enzymes produced by *A. niger* are considered as generally recognized as a safe (GRAS) by the "Food and Drug Administration" of the United States [46]. In addition, various biotransformation of ferulic acid in vanilla [47], progesterone in polyethylene [48], isosteviol in diperpenoide and isosteviole [49], terpenes in "2-alpha, 3-beta, 13-trihydroxystemodane" [50], linalool oxide mixture of cis-and trans-linalool furanoid and cis-and trans-pyranoid oxide linalool are formed through *A. niger* [51]. During the past two decades, *A. niger* was the most broadly used food enzyme [46].

Besides its many applications in industry, *A. niger* also has an important role as environmental microorganism. It is involved in the biodegradation of the toxic chemicals, e.g., dioxins [52], in the treatment of waste molasses from beet and OMW [53, 54] and the bioconversion of sewage sludge [55]. *A. niger* biomass is also used in the biosorption toxic heavy metals such as cadmium, chromium and copper [56, 57].

The natural by-product was acknowledged as a solid support and a source of energy and carbon. Agricultural wastes were used for mushroom cultivation; this offers the advantage of combining the use of an inexpensive substrate and an interesting road to recover these by-products. Many by-products generated by the food industry were used as substrate for fungi such as sugar cane residue, wheat bran, rice and barley straw, beet pulp and pulp coffee [44, 58–61].

4. Bioconversion

4.1. Microbiological synthesis

This production method, also called "fermentation," uses bacteria or fungi cultured in the presence of the reagent selected as a precursor to synthesize the molecule. This type of biological reaction is relatively easy to implement. The proposed culture can be improved by optimizing the composition of the culture media of strains and experimental conditions [62]. Bioproduction processes involve various reactions such as hydroxylation, oxidation, reduction, hydrolysis, esterification, decarboxylation, methylation, condensation and isomerization.

http://dx.doi.org/10.5772/64774

Bioproduction reactions have been applied to the production of several types of molecules, in particular flavorings and aromatic antioxidants [63]. A typical example for the production of aroma is the synthesis of vanilla. Several studies have shown that some bacteria (*Pseudomonas putida*, *Streptomyces setonii*, *Amycolatopsis*, etc.) and some fungi (*Pycnoporus cinnabarinus*, *A. niger*, etc.) are able to convert ferulic acid to vanillin [64]. Vanillin was also produced by the bioconversion of isoeugenol by means of a *Bacillus* sp. type of bacteria [65].

4.2. Enzymatic synthesis

This method involves enzymes purified and immobilized on a suitable support. The choice of the nature of the enzyme depends on the chemical structures of the precursor and the product to be synthesized. Thus, to produce an ortho-diphenol, it is necessary to use a monooxygenase. Starting from a precursor free of hydroxyl group, it is necessary to employ a dioxygenase. This type of bioconversion reaction requires the use of cofactors such as NADH, NADPH and ATP [37]. In practical terms, the bioconversion reactions using cells (stationary growth) seem to be more interesting than those involving purified enzymes. Furthermore, regeneration of cofactors generally constitutes a handicap in the use of many enzymes. Besides, enzymatic hydrolysis of oleuropein by β-glycosidase has been studied [66]. It generates oleuropein aglycone. Immobilization by inclusion of a recombinant β-glucosidase and thermophilic *Sulfolobus solfataricus* of chitosan on a support so as to hydrolyze oleuropein enzymatically at 60 and 70°C was contemplated by Briante et al. [67]. The enzymatic hydrolysis attacks only the glycosidic linkage; the biotransformation generates unstable aglycone species by cleavage at a temperature above 60°C and releases hydroxytyrosol. An immobilized thermophilic enzyme in a bioreactor can solve the technical problems of hydrolysis of different substrates. Indeed, the elevated temperatures used limit microbial growth and assist in the solubilization of the substrate [68].

5. Analytical methods

5.1. High-performance liquid chromatography (HPLC) analysis

The identification and quantification of phenolic monomers were carried out by HPLC. The assays were achieved on a Shimadzu apparatus composed of an LC-10ATvp pump and an SPD-10Avp UV/visible detector. The column was a C-18 (4.6 × 250 mm; Shim-pack VP-ODS), and its temperature was kept at 40°C. The flow rate was 0.5 mL/min. The mobile phase used was 0.1% phosphoric acid in water (A) versus 70% acetonitrile in water (B) for a total running time of 50 min, and the next amounts of solvent B were used for the elution: 0–30 min, 20–50%; 30–35 min, 50%; and 35–50 min, 50–20%. The flow rate was 0.6 mL/min, and the injection volume was 20 μL. Identification and quantification of HT were based on its spectrum and on its retention time in comparison with standard analyzed under the same conditions.

5.2. Enzyme activities assays

The β-glucosidase activity was determined using 1 mM p-nitrophenyl-β-glucoside (pNPG) as substrate (in 100 mM citrate buffer pH 4.8). An aliquot of 0.2 mL of 1 mM pNPG in 50 mM sodium citrate buffer (pH 4.8) was incubated with 0.280 mL of citrate buffer and 20 μL of an appropriate diluted enzymatic preparation at 50°C for 15 min. The reaction was stopped by adding 0.5 mL Na_2Co_3 1 M; the liberated p-nitrophenol (pNP) was measured at 400 nm [68]. In the blank, 20 μL of water was used in place of the enzyme sample. The activity was defined as μM of p-nitrophenol produced per minute below the analyzed conditions (IU). Activities were measured in triplicate and were expressed in International Unit per milliliter (IU/mL) with 1 IU being defined as the amount of enzyme that catalyzes the release of 1.0 μM of p-nitrophenol per minute.

6. Scale-up of bioconversion of OMW phenolic compounds

The basic assumption of the method proposed by Hamza et al. [4, 16, 17] is that the polyphenols contained in OMW could be selectively hydrolyzed by enzymes to generate extracts or free compounds useful for the pharmaceutical and cosmetic industry and produce a wastewater that is free from polyphenols and with significantly reduced COD.

6.1. Enzymatic bioconversion of OMW

Figure 3a, **c**, and **e** shows the HPLC chromatogram of the ethyl acetate extract of raw MCC, MSP and North OMW. This profile shows that fresh OMW is rich in oleuropein (peak 4) and luteolin (peak 3). Simple phenolic bioactive compounds, such as HT (peak 1) and tyrosol (peak 2), were too present at moderate concentrations. The HT concentrations recorded in raw MCC, MSP and North OMW were 0.17, 0.23 and 0.86 g/L, respectively. This content corresponded to the free fraction of HT in raw OMW [4].

Further enzymatic analysis revealed that the β-glucosidase activities in the raw OMW of MCC, MSP and North OMW were 0.7, 0.7 and 1.03 IU/mL, respectively (**Figure 3**). This enzyme could be derived from olive fruit [20] and from the activity of endogenous microorganisms [69]. The enzyme concentration described above (as well as of other hydrolytic enzymes) is not plenty for the hydrolysis of molecules present in fresh OMW. With the time of storage of OMW, the HT concentration was previously shown to increase as a result of natural fermentation and cleavage of HT conjugates [3]. The increase in bioactive compound concentrations was tested through the enzymatic hydrolysis of OMW during 2.0 h at 50°C using 5 IU of *A. niger* β-glucosidase per milliliter of OMW [16]. As shown in **Figure 3**, important quantities of HT were free after the enzymatic pretreatments of OMW. A progressive rise was detected for the HT concentration in OMW over the response time of the β-glucosidase action in subsequent OMW enhancement by this enzyme. Blank controls deprived of enzyme were also run, and no significant HT production rates were detected (**Figure 2**). The HT quantities in the blank controls of MCC, MSP and North OMW were 0.2, 0.23 and 0.8 g/L, respectively. Several factors are known to affect the quantitative phenolic profiles of olive fruits. Among those factors, the

http://dx.doi.org/10.5772/64774

geographical origin, cultivar type, irrigation treatment and ripeness degree have the most pronounced impacts on phenolic composition [20]. The initial hydroxytyrosol concentrations recorded in this study corresponded to an average of about 41.14% of their final value in the hydrolyzed OMW. In the absence of broth culture, the substrate was not converted into HT even at high temperature (50°C). HPLC chromatograms indicated an increase in the HT peak (1) and tyrosol peak (2) with a simultaneous reduction in the oleuropein peak (4) and luteolin peak (3), respectively, subsequently the enzymatic pretreatment of OMW (**Figure 3**). These results established that β-glucosidase played a significant role in cleavage of the glycosidic bonds in the molecules present in OMW. Hence, HT could have created from oleuropein, via its aglycone, by the opening of the elenolic acid loop with a final reorganization into the secoiridoid compound and numerous procedures of elenolic acid [70]. This result is in agreement with the findings previously reported in the studies of Capasso et al. [29], Briante et al. [71], Hamza et al. [4, 16, 17] and Khoufi et al. [18].

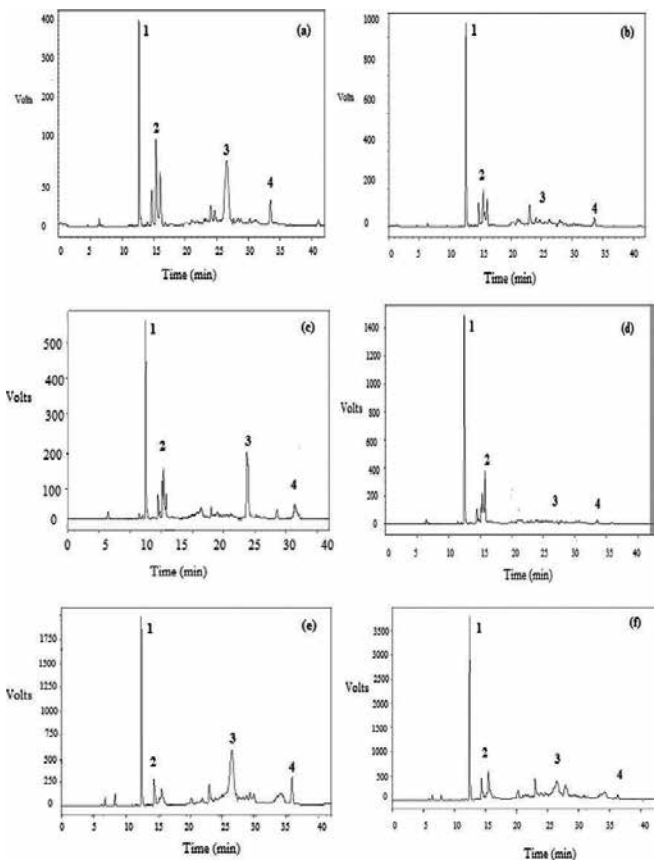


Figure 3. HPLC chromatogram of phenolic compounds [4] (detection at $\lambda = 280$ nm) extracted from raw MCC (a) and hydrolyzed MCC by *A. niger* β-glucosidase (b), raw MSP (c), hydrolyzed MSP by *A. niger* β-glucosidase (d), raw OMW North Tunisia (e) and hydrolyzed OMW North Tunisia by *A. niger* b-glucosidase (f). 1, hydroxytyrosol; 2, tyrosol; 3, luteolin; 4, oleuropein.

During enzymatic experiments, maximum HT concentrations of about 1.53, 0.83 and 0.46 g/L were obtained in the presence of 5 IU *A. niger* β-glucosidase per milliliter of OMW in North OMW, MSP and MCC, respectively. The results of this relatively large-scale study were inferior than those described in a preceding small-scale study by the authors [17]. So the initial HT concentration in large volumes of fresh OMW is much higher than in small volumes of fresh OMW acquired from separators in oil mills. The findings from this phase of the study suggested that OMW was a source of hydrolysable phenols, including oleuropein, ligstroside and verbascoside, which possess glycosidic and ester links between the main phenol components, polysaccharides and/or lignin (**Figure 1**) [70, 72]. Consequently, a higher quantity of HT was released when enzymatic hydrolysis was carried out on OMW using specific enzyme preparations. The proposed enzymatic pretreatment proved useful not only on a laboratory scale but also on large-scale applications involving OMW recycling.

6.2. Membrane separation technologies

Several studies have recently shown that membrane filtration is preferably applied in a cross flow mode. In cross flow or tangential flow filtration, the feed is pumped into the membrane module where it is separated into two streams, namely the filtrate (or permeate) and the retentate, in which the retained species has been concentrated. The tangential movement of the fluid targets removes most of the rejected materials from the membrane surface and, consequently, minimizes their accumulation at the membrane surface. Membrane technologies are known to employ special filters (membrane) that operate in a special fluido-dynamic condition (tangential flow), which reduces the filter fouling and, consequently, assures a high permeate flux as a function of time [73]. These technologies are commonly applied worldwide not only for the treatment of wastewaters but also for the recovery of dispersed solutes, often pollutants and generation of purified water.

The present study opted for the application of membrane technology for the treatment of OMW to enhance the extraction and recovery of its valuable phenol content and to facilitate its proper disposal in the environment. According to the proposed process, the OMW was submitted to enzymatic hydrolysis by a β-glucosidase enzyme to remove oleuropein and, thus, enhance the free HT in OMW (**Figure 3**). The liquid fraction separated from the degradation products was then used in a membrane system including tangential microfiltration (MF), tangential ultrafiltration (UF) and evaporation units in sequence. Both membrane retentate fractions constituted the new refined products with the addition of purified water obtained after the evaporation steps and the last concentrate recuperated which is phenol enriched, respectively. The ultimate concentrate of the system after evaporation was rich in hydroxytyrosol.

The initial fluxes of OMW processing by MF and UF were 42.85 and 103.89 L/m^2h in transmembrane pressures of 1.8 and 2.2 bar, separately. The determination of chemical oxygen demand (COD), pH, conductivity, hydroxytyrosol, total solids (TS) and total phenolic content were evaluated at different operating conditions. The COD load was due to high organic contents, which are nitrogen compounds, sugars, organic acids, oils, cellulose and phenolic compounds, with a concentration of 165 g/L in the raw OMW. A substantial decrease in COD and total solids (TS) was detected. The membrane technology under study generated a slightly

colored permeate, with less COD for its oxidation (3.42% of the initial COD) (**Table 1**). Microfiltration was prominent to eliminate 72.12% of COD. In fact, all the organic matter of the OMW in the form of an insoluble material suspension was retendered by microfiltration. However, the MF permeate contains the hydrophilic compounds, which are reducing sugars, hydroxytyrosol and minerals. Only 24% of hydroxytyrosol was recovered in the ultimate concentrate. This proportion was considerably enhanced by diluting the MF and UF retentates with pure water. Accordingly, diafiltration was required to recuperate more hydroxytyrosol. The utilization of moderate temperatures in evaporation step led to a hydroxytyrosol-rich concentrate. The results provided evidence that the process of this study can be used as an efficient treatment of OMW at mild conditions. Besides, 41% of the enhancement in total phenols was due to the discharge of hydroxytyrosol (**Table 1**). A ecological production of great quantities of hydroxytyrosol can, consequently, be realized. A promising alternative method was funded by the use of a pilot-scale method for the recovery of natural hydroxytyrosol. An additive in the food industry or an anaerobic substrate for methane production can be recycled by the retentate of MF and UF units which are rich in polyphenolic compounds. The total recovery of the chemical components in OMW using membrane technologies was previously reported on a similar process by Pizzichini and Russo [74].

Raw OMW		Hydrolyzed OMW		MF	UF	Cc
P	R	P	R			
pH						
5.03 ± 0.2	4.93 ± 0.2	4.78 ± 0.2	4.73 ± 0.2	4.81 ± 0.2	4.79 ± 0.2	4.84 ± 0.2
Total solids (g 100/g)						
9.33 ± 0.5	9.23 ± 0.5	6.28 ± 0.5	11.12 ± 0.5	4.71 ± 0.5	5.72 ± 0.5	41.95 ± 0.5
Conductivity (ms/cm)						
11.26 ± 0.5	11.25 ± 0.5	11.17 ± 0.5	10.62 ± 0.5	10.29 ± 0.5	10.7 ± 0.5	15.11 ± 0.5
COD (g/L)						
165.76 ± 1.5	165.83 ± 1.5	57.76 ± 1.5	119.25 ± 1.5	48.44 ± 1.5	59.62 ± 1.5	nd
Total phenols (g/L)						
4.5 ± 1.2	5.5 ± 1.2	3.31 ± 0.5	7.27 ± 1.2	1.96 ± 0.2	2.7 ± 0.5	17.6 ± 2.5
Reducing sugar (g/L)						
26 ± 3.2	60 ± 4.2	nd	nd	nd	nd	158.21 ± 8.5
Hydroxytyrosol (g/L)						
0.23 ± 0.2	0.93 ± 0.2	0.85 ± 0.1	0.72 ± 0.1	0.79 ± 0.1	0.807 ± 0.1	7.2 ± 1
Mineral composition of ultimate concentrate (mg/kg)						
Zn (240 ± 0.05); Cd (0.0264 ± 0.01); Ca (1156 ± 0.2); Fe (46 ± 0.01); K (61.36 ± 5.1); Mg (617.2 ± 6.3); Na (46.4 ± 0.5); Cr; (0); Pb (0); Ni (0); Cu (0)						

P, permeate; COD, chemical oxygen demand; R, retentate; nd, not determined; MF, microfiltration; UF, ultrafiltration; Cc, concentration with evaporation.

Table 1. pH, total solids, conductivity, total simple phenol, chemical oxygen demand (COD) content and hydroxytyrosol concentration [4] before and after the enzymatic hydrolysis of olive mill wastewater and after microfiltration, ultrafiltration and concentration; the mineral composition of ultimate concentrate from purified OMW.

6.3. Characterization of final product

Table 1 shows the characteristics of the OMW fractions at the different steps. The final product had a pH value of 4.84 which remains constant during the process. Conductivity was reserved constant, except after concentration step was increased. The ultimate concentrate contains a high concentration of reducing sugar (about 158.21 g/L) (**Table 1**). The inorganic content of this new product was found to consist mainly of metals. The metal content of the ultimate concentrate is shown in **Table 1**. This product was exempt of heavy metals but rich in calcium, iron, potassium, manganese and sodium (**Table 1**).

Metals remain the key from nutritional and toxicological perspectives. Certain metals, principally iron, copper and zinc, are indispensable constituents for the human body, and their deficiency can have chronic and severe effects [75]. OMW comprises certain added significant metal ions, such as magnesium and calcium, which have often been reported to diminish the danger of heart sicknesses [76]. Trace elements, such as copper and iron, should not be eliminated when they are in short dietary supply. Elements, such as cadmium and lead, which can accumulate in the body, should be minimized. Consequently, the characteristics of the raw material and extraction techniques have a significant effect on the composition of the final extracts, and, in industrial applications, the composition of olive phenol extracts must be standardized. In fact, an 'aqueous extract' of OMW suitable for foods and beverages can be easily obtained by simple ultrafiltration [77]. The commercialization scenarios will, therefore, depend on the intended use, whether an individual compound, a multicomponent mixture or crude aqueous extract of OMW are to be recovered.

7. Conclusion

This chapter discusses the potential of a scale-up enzymatic treatment for OMW with the aim of increasing HT concentration. Hydrolytic treatments were investigated using a culture broth of *A. niger* on wheat bran in a pilot-scale 100-L fermentor for 7 days. The use of the filtrate from the *A. niger* culture broth as a biocatalyst released 1.53, 0.83 and 0.46 g of HT per liter of North OMW, MSP and MCC, respectively. This process produced a natural and a bioactive product from a vegetal source as opposed to the molecule obtainable through chemical synthesis. Taken together, the findings suggested that the proposed enzymatic pretreatment may be considered useful not only at laboratory-scale applications but also at pilot-scale applications involving OMW recycling. The application of membrane filtration processes allowed for the recovery of four main liquid fractions in different volumetric percentages, all of which may have a potential for commercial use in the food, nutraceutical and cosmetic industries. In the ultimate concentrate obtained, only 24% of HT was recovered. The new product obtained met principally the dietary and other requirements: rich in HT, slightly acidic, with a reduced sugar content, devoid of heavy metals and chemicals and rich in minerals.

http://dx.doi.org/10.5772/64774

Author details

Manel Hamza* and Sami Sayadi

*Address all correspondence to: manel.hamza@yahoo.fr

Laboratory for Environmental Bioprocess, Laboratory Mixed International (LMI) (Cosys-Med), Sfax Biotechnology Center, Sfax, Tunisia

References

[1] Lesage-Meessen, L., Navarro, D., Maunier, S., Sigoillot, J. C., Lorquin, J., Delattre, M. (2001). Simple phenolic content in olive oil residues as a function of extraction systems. Food Chem. 75, 501–507.

[2] Allouche, N., Damak, A., Ellouz, R., Sayadi, S. (2004). Use of whole cells of Pseudomonas aeruginosa for synthesis of the antioxidant hydroxytyrosol via conversion of tyrosol. Appl. Environ. Microb. 70, 2105–2109.

[3] Feki, M., Allouche, N., Bouaziz, M., Gargoubi, A., Sayadi, S. (2006). Effect of storage of olive mill wastewaters on hydroxytyrosol concentration. Eur. J. Lipid Sci. Technol. 108, 1021–1027.

[4] Hamza, M., Sayadi, S. (2015). Valorisation of olive mill wastewater by enhancement of natural hydroxytyrosol recovery. Inter. J. Food Sci. Technol., 50, 826–833.

[5] Fiorentino, A., Gentili, A., Isidori, M., Monaco, P., Nardelli, A., Parrella, A., Temussi, F. (2003). Environmental effects caused by olive mill wastewaters: toxicity comparison of low-molecular-weight phenol components. J. Agric. Food Chem. 51, 1005–1009.

[6] Khoufi, S., Aloui, F., Sayadi, S. (2008). Extraction of antioxidants from olive mill wastewater and electro-coagulation of exhausted fraction to reduce its toxicity on anaerobic digestion. J. Hazard. Mater. 151, 531–539.

[7] Vlyssides, A. G., Loizides, M., Karlis, P. K. (2004). Integrated strategic approach for reusing olive oil extraction. J. Clean Prod. 12, 603–611.

[8] Rodis, P. S., Karathanos, V. T., Mantzavinou, A. (2002). Partitioning of olive oil antioxidants between oil and water phases. J. Agric. Food Chem. 50, 596–601.

[9] Obied, H. K., Allen, M. S., Bedgood, D. R., Prenzler, P. D., Robards, K., Stockmann, R. (2005). Bioactivity and analysis of biophenols recovered from olive mill waste. J. Agric. Food Chem. 53, 823–837.

[10] Obied, H. K., Bedgood, Jr., D. R., Prenzler, P. D., Robards, K. (2007). Bioscreening of Australian olive mill waste extracts: Biophenol content, antioxidant, antimicrobial and molluscicidal activities. Food Chem. Toxicol. 45, 1238–1248.

[11] Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gomez-Caravaca, A. M., Segura-Carretero, A., Fernandez-Gutierrez, A., Lercker, G. (2007). Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. Molecules. 12, 1679–1719.

[12] Capasso, R., Evidente, A., Visca, C., Gianfreda, L., Marmonti, M., Greco, G. J. R. (1996). Production of glucose and bioactive aglycone by chemical and enzymatic hydrolysis of purified oleuropein from Olea europaea. Appl. Biochem. Biotechnol. 61, 365–377.

[13] Briante, R., Patumi, M., Febbraio, F., Nucci, R. (2004). Production of highly purified hydroxytyrosol from Olea europaea leaf extract biotransformed by hyperthermophilic β-glycosidase. J. Biotechnol. 111, 67–77.

[14] Liebgott, P.-P., Amouric, A., Comte, A., Tholozan, J.-L., Lorquin, J. (2009). Hydroxytyrosol from tyrosol using hydroxyphenylacetic acid-induced bacterial cultures and evidence of the role of 4-HPA 3-hydroxylase. Res. Microbiol. 160, 757–766.

[15] Aranda, E., Garcia-Romera, I., Ocampo, J. A., Carbone, V., Malorni, A., Sannino, F., De Martino, A., Capasso, R. (2007). Reusing ethyl acetate and aqueous exhausted fractions of dry olive mill residue by saprobe fungi. Chemosphere. 66, 67–74.

[16] Hamza, M., Khoufi, S., Sayadi, S. (2012). Changes in the content of bioactive polyphenolic compounds of olive mill wastewater by the action of exogenous enzymes. J. Agric. Food Chem. 60(1), 66–73.

[17] Hamza, M., Khoufi, S., Sayadi, S. (2012). Fungal enzymes as a powerful tool to release antioxidants from olive mill wastewater. Food Chem. 131, 1430–1436.

[18] Khoufi, S., Hamza, M., Sayadi, S. (2011). Enzymatic hydrolysis of olive wastewater for hydroxytyrosol enrichment. Bioresource Technol. 102(19), 9050–9058.

[19] Romero, C., Brenes, M., Garcïa, P., Garrido, A. (2002). Hydroxytyrosol-4-β-d-glucoside, an important phenolic compound in olive fruits and derived products. J. Agric. Food Chem. 50, 3835–3839.

[20] Jemai, H., Bouaziz, M., Sayadi, S. (2009). Phenolic composition, sugar contents and antioxidant activity of Tunisian sweet olive cultivar with regard to fruit ripening. J. Agric. Food Chem. 57, 2961–2968.

[21] Ziogas, V., Tanou, G., Molassiotis, A., Diamantidis, G., Vasilakakis, M. (2010). Antioxidant and free radical-scavenging activities of phenolic extracts of olive fruits. Food Chem 120, 1097–1103.

[22] Visioli, F., Galli, C. (2003). Olives and their production waste products as sources of bioactive compounds. Curr. Top. Nutr. Res. 1, 85–88.

[23] Chimi, H., Sadik, A., Le Tutour, B., Rahmani, M. (1988). Contribution to the comparative study of antioxidant power in the olive oil tyrosol, hydroxytyrosol, caffeic acid, oleuropein and B.H.T. Rev. FR. Transfus Immu. 35, 339–344.

[24] Aeschbach, R., Loeliger, J., Scott, B., Murcia, A., Butler, J., Halliwell, B., Aruoma, O. L. (1994). Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. Food Chem. Toxicol. 32, 31–36.

[25] Attya, M., Benabdelkamel, H., Perri, E., Russo, A., Sindona, G. (2010). Effects of conventional heating on the stability of major olive oil phenolic compounds by tandem mass spectrometry and isotope dilution assay. Molecules. 15, 8734–8746. doi:10.3390/molecules15128734

[26] Manna, C, Galletti, P, Cucciolla, V, Moltedo, O, Leone, A, Zappia, V. (1997). The protective effect of the olive oil polyphenols (3-4-dyhydroxyphenyl)-ethanol counteracts reactive oxygen metabolite induced cytotoxicity in caco-2-cells. J. Nutr. 12, 286–292.

[27] Petroni, A., Blasevich, M., Salami, M., Papini, N., Montedero, G. F., Galli, C. (1995). Inhibition of platelet aggregation and eicosanoid production by phenolic components of olive oil. Thromb. Res. 78, 151–160.

[28] Kohyama, N., Nagata, T., Fujimoto, S., Sekiya, K. (1997). Inhibition of arachidonate lipoxygenase activities by 2-(3,4-dihydroxyphenyl) ethanol, a phenolic compound from olives. Biosci. Biotechnol. Biochem. 61, 347–350.

[29] Capasso, R., Evidente, A., Avolio, S., Solla, F. (1999). A highly convenient synthesis of hydroxytyrosol and its recovery from agricultural waste waters. J. Agric. Food Chem. 47, 1745–1748.

[30] Bezançon, P., Debosque, S., Delpeuch, F., Descomps, B., Gerber, M., Léger, C. L., Padilla, M., Puygrenier, M. (2000). Alimentation Mediterranean diet and health: present and future. Edited by John Libbey Eurotext, Montrouge, France. Martine Padilla (Ciheam-IAM Montpellier) 170 pp.

[31] Labat, M., Augur, C., Rio, B., Perraud-Gainé, I., Sayadi, S. (2000). Biotechnological potentialities of polyphenolic compounds of coffee and comparison with olive. In Coffee Biotechnology and Quality. Sera T, Soccol CR, Pandey A and Roussos S (Eds), Kluwer Acad. Publ., Dordrecht, Chap. 46, 517–531.

[32] Tuck, K. L., Tan, H. W., Hayball, P. J., (2000). Synthesis of tritium-labeled hydroxytyrosol, a phenolic compound found in olive oil. J. Agric. Food Chem. 48(9), 4087–4090.

[33] Verhe, R., Papadopoulos, G., Boskou, D. (1992). Preparation of hydroxytyrosol. Bull. Liason Groupe Polyphenols. 15, 237–244.

[34] Briante, R., La Cara, F., Tonziello, M. P., Febbraio, F., Nucci, R. (2001). Antioxidant activity of the main bioactive derivatives from oleuropein hydrolysis by hyperthermophilic beta-glycosidase. J. Agric. Food. Chem. 49, 3198–3203.

[35] Espin, J. C., Soler-Rivas, C., Cantos, E., Tomàs-Barberàn, F. A., Wichers, H. J. (2001). Synthesis of the antioxidant hydroxytyrosol using tyrosinase as biocatalyst. J. Agric. Food Chem. 49, 1187–1193.

[36] Allouche, N., Sayadi, S. (2005). Synthesis of hydroxytyrosol, 2-hydroxyphenylacetic acid and 3-hydroxyphenylacetic acid by differential conversion of tyrosol isomers using Serratia marcescens Strain. J. Agric. Food Chem. 53, 625–6530.

[37] Bouallagui, Z., Sayadi, S. (2006). Production of high hydroxytyrosol yields via tyrosol conversion by Pseudomonas aeruginosa immobilized resting cells. J. Agric. Food Chem. 54, 9906–9911.

[38] Brooks, S. J., Doyle, E. M., O'Connor, K. E. (2006). Tyrosol to hydroxytyrosol biotransformation by immobilized cell extracts of Pseudomonas putida F6. Enzyme Microb. Techol. 39, 191–196.

[39] Selvakumar, P., Ashakumary, L., Pandey, A. (1998). Biosynthesis of glucoamylase from Aspergillus niger by solid-state fermentation using tea waste as the basis of a solid substrate. Bioresource Technol. 65, 83–85.

[40] Castilhoa, L. R., Medronhob, R. A., Alvesa, T. L. M. (2000). Production and extraction of pectinases obtained by solid-state fermentation of agro-industrial residues with Aspergillus niger. Bioresource Technol. 71, 45–50.

[41] Mahadik, N. D., Puntambekar, U. S., Bastawde, K. B., Khire, J. M., Gokhale, D. V. (2002). Production of acidic lipase by Aspergillus niger in solid-state fermentation. rocess Biochem. 38, 715–721.

[42] Asther, M., Haon, M., Roussos, S., Record, E., Michel Delattre, M., Lesage-Meessen, L., Labat, M., Asther, M. (2002). Feruloyl esterase from Aspergillus niger a comparison of the production in solid state and submerged fermentation. Process Biochem. 38, 685–691.

[43] Duarte, J. C., Costa-Ferreira, M. (1994). Aspergilli and lignocellulosics: Enzymology and biotechnological applications. FEMS Microbiol. Rev. 13, 377–386.

[44] Pandey, A., Soccol, C. R., Nigam, P., Soccol, V. T. (2000). Biotechnologica potential of agro-industrial residues. I: sugarcane bagasse. Bioresource Technol. 4, 69–80.

[45] Kumar, D., Jain, V. K., Shanker, G., Srivastava, A. (2003). Citric acid production by solid-state fermentation using sugarcane bagasse. Process Biochem. 38(12), 1731–1738.

[46] Schuster, E., Dunn-Coleman, N., Frisvad, J. C., Van Dijck, P. W. M. (2002). On the safety of spergillus niger. A review. Appl. Microbiol. Biotechnol. 59, 426–435.

[47] Bonnin, E., Brunel, M., Gouy, Y., Lesage-Meessen, L., Asther, M., Thibault, J.-F. (2001). Aspergillus niger I–1472 and Pycnoporus cinnabarinus MUCL39533, selected for the biotransformation of ferulic acid to vanillin, are also able to produce cell wall polysac-

http://dx.doi.org/10.5772/64774

charide-degrading enzymes and feruloyl esterases. Enzyme Microb. Technol. 28, 70–80.

[48] Kulkarni, A. G., Lele, S. S., Kulkarni, P. R. (1998). Improved adsorption of Aspergillus niger 589 spores on high-density polyethylene for progesterone biotransformation. J Ferment. Bioeng 86, 510–512.

[49] De Oliveira, B. H., Dos Santos, M. C., Leal, P. C. (1999). Biotransformation of the diperpenoid, isosteviol, by Aspergillus niger, Penicillium chrysogenum and Rhizopus arrhizus. Phytochemistry. 51, 737–741.

[50] Chen, A. R. M., Reese, P. B. (2002). Biotransformation of terpenes from Stemodia maritima by Aspergillus niger ATCC 9142. Phytochemistry. 59, 57–62.

[51] Demyttenaere, J. C. R., Willemen, H. M. (1998). Biotransformation of linalool to furanoid and pyranoid linalool oxides by Aspergillus niger. Phytochemistry. 47, 1029–1036.

[52] Volke-Sepulveda, T. L., Gutierrez-Rojas, M., Favela-Torres, E. (2003). Biodegradation of hexadecane in liquid and solid-state fermentations by Aspergillus niger. Bioresource Technol. 87, 81–86.

[53] Jiménez, A. M., Borja, R., Martin, A. (2003). Aerobic-anaerobic biodegradation of beet molasses alcoholic fermentation wastewater. Process Biochem 38, 1275–1284.

[54] Vassilev, N., Fenice, M., Federeci, F., Azcon, R. (1997). Olive mill wastewater treatment by immobilized cells of Aspergillus niger and its enrichment with soluble phosphate. Process Biochem. 32, 617–620.

[55] Molla, A. H., Fakhru'l-Razi, A., Abd-Aziz, S., Hanafi, M. M., Roychoudhury, P. K., Alam, M. Z. (2002). A potential resource for bioconversion of domestic wastewater sludge. Bioresource Technol. 85, 263–272.

[56] Dursun, A. Y., Uslu, G., Cuci, Y., Aksu, Z. (2003). Bioaccumulation of copper(II), lead(II) and chromium(VI) by growing Aspergillus niger. Process Biochem. 38, 1647–1651.

[57] Kapoor, A., Viraraghavan, T. (1997). Heavy metal biosorption sites in Aspergillus niger. Bioresource Technol. 61, 221–227.

[58] Cordova, J., Nemmaoui, M., Ismaïli-Alaoui, M., Morin, A., Roussos, S., Raimbault, M., Benjilali, B. (1998). Lipase production by solid state fermentation of olive cake and sugar cane bagasse. J. Mol. Catal. 5, 75–78.

[59] Mandviwala, T. N., Khire, J. M. (2000). Production of high activity thermostable phytase from thermotolerant Aspergillus niger in solid state fermentation. J Ind. Microbiol. Biotechnol. 24, 237–243.

[60] Brand, D., Pandey, A., Roussos, S., Soccol, C. R. (2000). Biological detoxification of coffee husk by filamentous fungi using a solid state fermentation system. Enzyme Microb. Technol. 27, 127–33.

[61] Roussos, S., Lonsane, B. K., Raimbault, M., Viniegra-Gonzalez, G. (1995). Advances in solid state fermentation. In Proceedings of the 2nd International Symposium on Solid State Fermentation, FMS-95, Kluwer Academic Publishers. Montpellier, France

[62] Barhini, P., Montebove, F., Ruzzi, M., Scheisser, A. (1998). Optimal conditions for bioconversion of ferulic acid into vanillic acid by Pseudomonas fluorescens BF13 cells. Appl. Microbiol. Biotechnol. 49, 309–314.

[63] Edlin, D. A. N., Narbad; A., Dickinson, J. R., Lloyds, D. (1995). The biotransformation of simple phenolic compounds by Brettanomyces anomalus. FEMS Microbiol. Lett. 125, 311–316.

[64] Muheim, A., Lerch, K. (1999). Towards a high yield bioconversion of ferulic acid to vanillin. Appl. Microbiol. Biotechnol. 51, 456–461.

[65] Eyal, S., Uzi, R., Yuval, S. (2000). Isolation of Bacillus sp. Capable of transforming isoeugenol to vanillin. J. Biotechnol. 78, 1–9.

[66] Limiroli, R., Consonni, R., Ottolina, G., Marsilio, V., Bianchi, G., Zetta, L. (1995). ^{1}H and ^{13}C NMR characterisation of new oleuropein aglycones. J. Chem. Soc. Perkin Trans. 1, 1519–1523.

[67] Briante, R., La Cara, F., Febbraio, F., Barone, R., Piccialli, G., Carolla, R., Mainolfi, P., De Napoli, L., Patumi, M., Fontanazza, G., Nucci, R. (2000). Hydrolysis of oleuropein by recombinant β-glycosidase from hyperthermophilic archaeon Sulfolobus solfataricus immobilised on chitosan matrix. J. Biotechnol. 77, 275–286.

[68] Brini, F., Saibi, W., Amara, I., Gargouri, A., Masmoudi, K., Hanin, M. (2010). The wheat dehydrin DHN-5 exerts a heat-protective effect on b-glucosidase and glucoseoxidase activities. Biosci. Biotechnol. Biochem. 74, 1050–1054.

[69] Ciafardini, G., Zullo, B. A. (2002). Microbiological activity in stored olive oil. Inter. . Food Microbiol. 75, 111–118.

[70] Walter, W. M. Jr., Fleming, H. P., Etchells, J. L. (1973). Preparation of Antimicrobial Compounds by Hydrolysis of Oleuropein from Green Olives. Appl. Microbiol. 26(5), 773–776.

[71] Briante, R., Patumi, M., Limongelli, S., Febbraio, F., Vaccaro, C., Di Salle, A., La Cara, F., Nucci, R. (2002). Changes in phenolic and enzymatic activities content during fruit ripening in two Italian cultivars of Olea europaea L. Plant Sci. 162, 791–798.

[72] Bianco, A. D., Uccella, N. (2000). Biophenolic components of olives. Food Res. Int. 33, 475–485.

[73] Cheryan, M. (1986). Ultrafiltration Handbook. Technomic Publishing Co., Lancaster, PA. 369.

[74] Pizzichini, M., Russo, C. (2005). Process for recovering the components of olive mill wastewater with membrane technologies. International. Patent WO2005123603.

http://dx.doi.org/10.5772/64774

[75] Leung, S. W., Siddhanti, S., Williams, B., Chan, A. W. K., Minski, M. J., Daniels, C. K., Lai, J. C. K. (2010). Effects of diet intakes on metal and electrolyte distributions in vital organs. Procedia Environ. Sci. 2, 92–97.

[76] Anne, K. (2011). Magnesium and calcium in drinking water and heart diseases. In J. Nriagu (Ed.). Encyclopedia of Environmental Health . 20 Avenue Appia, 1211 Geneva 27, Switzerland, USA: Elsevier. 535–544.

[77] Galanakis, C. M., Tornberg, E., Gekas, V. (2010). Clarification of high-added value products from olive mill wastewater. J. Food Eng. 99, 190–197.

Chapter 15

A Brief Review on Recent Processes for the Treatment of Olive Mill Effluents

Javier Miguel Ochando-Pulido, Rita Fragoso, Antónia Macedo, Elizabeth Duarte and Antonio Martínez Ferez

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64798

Abstract

During the last few decades, olive oil industrial sector has grown as a result of the modernization of olive oil mills, in response to the increasing demand of olive oil worldwide. As an undesired side effect, the amount of olive mill effluents (OME) increased, especially as a result of changing old batch press method for the continuous centrifugation-based olive oil production processes currently used, which ensure higher productivity. This chapter presents the state of the art of OME management, with focus on biological and advanced oxidation processes, either alone or in combination, varying in complexity, ease of operation and costs associated. Up to this moment, there isn't a management strategy that can be adopted in a global scale, feasible in different socio-economic contexts and production scales. The most reasonable approach is to regard OME valorisation as a regional problem, defining decentralized treatment that in some cases can be implemented for a group of olive oil mills in the same geographic area. This aspect is receiving strong attention as European Commission is promoting the transition towards a circular economy, which aims at "closing the production loop" by recycling and reusing resources, bringing benefits for the environment, society and the economy.

Keywords: olive mill wastewater, integral wastewater management, biological processes, advanced oxidation processes

1. Introduction

The production of olive oil employs a very significant number of people and is one of the main industrial activities in countries of the Mediterranean Basin: Italy, Portugal, Greece and Northern African countries—Algeria, Morocco, Tunisia, Libya and Egypt. Other countries such as France, Serbia and Montenegro, the former Yugoslav Republic of Macedonia (FYROM) (Cyprus, Syria, Turkey, Israel and Jordan) also produce a considerable olive oil amount (International Olive Oil Council, IOOC, 2013–2014).

Olive oil production is also rapidly becoming an emergent agro-food industry in China and other countries such as the USA, Australia and the Middle East. It is worth mentioning the case of China, which exhibits favourable edaphoclimatic conditions for the growth of olive trees, and is expected to develop a considerable olive oil production potential in the near future. Hence, the treatment of olive mill effluents (OME), including olive washing wastewater (OWW), olive mill wastewater (OMW, only for press and three phase mills) and wastewater from olive oil washing (OOW), is now a task of global concern.

During the last few decades, a very significant growth of the olive oil industrial sector has been experienced as a result of the modernization of olive oil mills, in response to the increasing demand of olive oil worldwide. Spain is the biggest European Union (EU) producer, with more than 1700 olive mills with licence for operating. Production of olive oil in the Iberian Peninsula accounts for 91,600 tons in Portugal and more than 1,400,000 tons in Spain during the 2013–2014 campaign. In Spain, 70% of the olive oil was obtained in Andalucía where there are 850 olive mills, which yielded a production of 1,022,000 tons of olive oil and 4,778,451 tons of table olives.

The significant boost of this industrial sector in the last years has brought an undesired side effect; the amounts of OME have increased significantly, especially as a result of the change from the older batch press method to the continuous centrifugation-based production processes currently used, which ensure much higher productivity. These continuous systems guarantee a higher yield in recovering olive oil from the olives, up to 21%, but they lead to an increased production of wastewater streams. An average-sized modern olive oil mill currently generates daily several cubic meters of wastewater from the extraction process (OMW), wastewater derived from the washing of the olives (OWW) as well as from olive oil washing process (OOW). These practices have a relevant environmental impact due to water consumption and the production of a huge amount of highly contaminant wastewaters.

The current necessity to maximize the production processes often excludes the planning of the environment protection. Wastewater treatment for ulterior uses in multiple applications contributes to sustainable water consumption and conservation of the water bodies and the ecosystems. In this scenario, the European Directive 2000/60/CE took the lead in establishing the legal framework to confer utmost protection to water, highlighting the reuse of treated wastewater. This strategy can improve the status of the environment both quantitatively, minimizing water abstraction, and qualitatively, preventing pollution; for this reason, it is a top priority in the Strategic Implementation Plan of the European Innovation Partnership (EIP) on Water.

http://dx.doi.org/10.5772/64798

Direct discharge of OME has been reported to cause strong odour nuisance, soil contamination, plants growth inhibition, leaks to the underground, water body pollution and hindrance of self-purification processes, as well as severe impacts to aquatic fauna and to ecological status [1–5]. Discharge of untreated OME is prohibited in Spain, whereas Italian law (L. 574/96) restricts the maximum amount of OME to be disposed on soil to 50 and to 80 m^3ha^{-1}, for wastewater arising from a press or a continuous mill, and in Portugal, irrigation of tree and bush crops with OME is allowed up to 80 m^3 ha^{-1} $year^{-1}$, as long as there has been pH correction (Despacho Conjunto n° 626/2000). Due to the presence of high levels of organic pollutants and refractory compounds, direct disposal of these effluents to the municipal sewage collection systems is also prohibited. Legal limits are established in order to prevent inhibitions of the biological treatment processes that take place in wastewater treatment plants.

OME exhibit several characteristics that make their reclamation by conventional physico-chemical treatments utterly difficult. The presence of phytotoxic recalcitrant pollutants—such as phenolic compounds, long-chain fatty acids, tannins and organohalogenated contaminants—makes these effluents resistant to biological degradation. The physico-chemical composition of OMW is extremely variable as it depends on several factors such as the extraction process, edaphoclimatic and cultivation parameters, as well as the type, quality and maturity of the processed olives. OME typically exhibit intense violet-dark colour, acid pH, strong odour, considerable saline toxicity reflected by high electric conductivity values, and very heavy organic pollutants load.

Process	Effluent	COD (g L^{-1})	BOD_5 (g L^{-1})	TSS (g L^{-1})	pH	EC (mS cm^{-1})	TP (g L^{-1})
Olives washing	OWW	0.8–2.2	0.3–1.5	8–18	5.5–6.6	2.5–3.0	0–0.1
Batch press	OMW-P	30–130	90–100	10–12	4.5–5.0	2.0–5.0	1.0–2.4
Three phase	OMW-3	30–200	5–45	5–35	3.5–5.5	2.0–7.9	0.3–7.5
Olive oil washing	OOW	4–16	0.8–6.0	2–7	4.9–6.1	1.5–2.5	0.1–1.0

COD, chemical oxygen demand; BOD_5, biological oxygen demand; TSS, total suspended solids; EC, electric conductivity; TP, total phenolic compounds.

Table 1. Characteristics of the effluents of batch and continuous olive oil extraction processes.

As it can be seen, OWW is commonly composed of high concentration of suspended solids (mainly peel, pulp, ground, branches and leaves debris) derived from the washing procedure of the olive fruit. The concentration of dissolved organic matter depends on the water flow exchange rate in the washing machines—and usually is below standard limits for discharge on suitable soils (e.g. Guadalquivir Hydrographical Confederation, 2006–2014: total suspended solids (TSS) < 500 mg L^{-1} and chemical oxygen demand (COD) < 1000 mg O_2 L^{-1}).

Table 1 presents the physico-chemical characteristics of the effluents of batch and continuous olive oil extraction processes.

Major organic pollutants load is present in the effluent coming out of the three-phase centrifuge (OMW-3), mostly phytotoxic compounds recalcitrant to biological degradation. Therefore, the presence of these substances would be hardly reflected in biological oxygen demand (BOD_5)

measurements; for this reason, COD seems a more appropriate parameter together with total phenolic (TP) compounds concentration. In the continuous two-phase extraction process, water injection is only performed in the final vertical centrifugation step, therefore the volume of liquid effluent derived from the production process is reduced by one-third on average if compared to the amount required for the three phase.

Much of the organic matter remains in the solid waste, which contains more moisture than the pomace from the three-phase system (60–70% in two-phase system vs. 30–45% in three-phase one). OOW exhibits lower pollutants degree, with COD commonly in the range 4–16 gL^{-1}, when compared with OMW. Inorganic compounds including chloride, sulphate and phosphoric salts of potassium, calcium, iron, magnesium, sodium, copper and traces of other elements are also common traits of OMW and OOW [6, 7].

From this analysis, it is clear that the wastewater streams produced during olive oil production have considerable different characteristics that, along with the final quality requirements, should be taken into account when selecting the most appropriate treatment strategy.

2. Biological treatments

Along the years, several studies have been developed on biological treatment processes for OME, from the simplest lagooning systems to more complex and high technological treatments [8]. This section reviews the aerobic, anaerobic, combined processes and the new trends in recovering added-value compounds from OME.

Aerobic biological treatments have long been proposed for OME treatment using several microorganisms as Pleurotus ostreatus, Bacillus pumilus, Phanerochaete chrysosporium, Aspergillus niger, Aspergillus terreus, Geotrichum candidum, etc. [9–11]. Ehaliotis et al. [12] used Azotobacter vinelandii ability to fix nitrogen to produce a fertilizer from OMW.

Amaral et al. [13] used a strain of Candida oleophila isolated from OMW for its detoxification. Results showed a removal around 50% of the organic load and 83% of total polyphenol content. Germination index increased up to 32% when compared to the values obtained with untreated OMW. Therefore, C. oleophila isolate was able to detoxify OMW and can be used for future application in biological treatments.

Recently, Chiavola et al. [14] investigated the efficiency of a sequencing batch reactor (SBR) in the biological treatment of previously sieved and diluted OMW. Four dilution ratios were tested (OMW/tap water, v/v): 1:25, 1:32, 1:16 and 1:10. Results showed that there was a complete removal of the biodegradable organic content at all the investigated influent loadings (0.08, 0.11, 0.19 and 0.69 mg COD mg $MLVSS^{-1}$ d^{-1}), with average efficiencies around 90 and 60% for COD and TP, respectively. The authors also tested adding a pre- or a post-treatment using membrane technologies: ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO). The introduction of the membrane separation allowed achieving a treated wastewater that complies with Italian legislation limits regarding COD, pH and electrical conductivity. TP concentration did not achieve the limit required for reuse. In view of a full-scale application,

it can be considered the option to mix the OMWs with other liquid streams so as to provide required nutrients along with dilution of the influent loading.

2.1. Bioconversion processes with energy production

Anaerobic digestion technology not only allows the treatment of wastewaters but also produces biogas that can be used as a primary energy resource locally. It is known that for an efficient anaerobic bioconversion process, the wastewater should have a balanced carbon-to-nitrogen-to-phosphorus (C/N/P) ratio and a pH in the range 6.5–7.5. Although OME has unbalanced C/N/P ratio, there are studies of anaerobic digestion of OMW as mono-substrate [15], but its mixture with nutrient-rich streams, co-substrates, significantly enhances process performance. This is not only due to balancing nutrient and alkalinity levels but also because it minimizes the inhibitory effect of phenolic compounds and lipids present in OME. Several studies assess the use of pretreatments for removal of recalcitrant compounds before anaerobic digestion process, for example, advanced oxidation processes (AOPs that will be further presented in this chapter) or coagulation-flocculation.

Gunay et al. [16] reviewed recent developments in OMW anaerobic digestion, addressing co-digestion with different streams (slaughterhouse wastewater, whey, manures, wastewater treatment plant sludge and microalgae waste), focusing on process performance but also including different possible pretreatment technologies.

González-González et al. [17] ran an aerobic pretreatment before anaerobic digestion in order to remove phenolic compounds and decrease COD. They observed a reduction in 78 and 90% of polyphenols and 18 and 21% of COD for aeration periods of 5 and 7 days, respectively. The best methane yield (0.39 m^3methane/kg COD removed) was obtained with OMW aerated for 5 days and was 2.4 times higher than that for untreated OMW.

One of the most studied pretreatments is the use of ultrasound for biomass deconstruction; Oz et al. [18] investigated the applicability of low-frequency ultrasound technology to OMW prior to anaerobic digestion in batch reactors. Results showed that the application of 20 kHz, 0.4 W/mL for 10 min to diluted OMW increased soluble chemical oxygen demand (SCOD)/total chemical oxygen demand (TCOD) ratio from 0.59 to 0.79. This fact led to 20% enhancement in biogas and methane production in trials using pretreated diluted OMW.

An important aspect to be taken into account when selecting a pretreatment is the net energy balance; the increase in biogas production should clearly offset the energy input. Ruggeri et al. [19] present an interesting approach, comparing several pretreatment processes based on scoring the biochemical methane potential (BMP) and the Energy Sustainability Index (ESI). ESI considers direct energy use (heat and electricity) and indirect energy use, the energy needed to produce chemicals reagents applied in pretreatments. Results showed that the most effective pretreatment was the addition of $CaCO_3$, with a biogas production of 21.6 NL/L and an ESI of 14 (i.e., the energy obtained in the form of methane is 14 times that of the energy spent).

Regarding the co-substrates studied for OMW co-digestion, manure is one of the most used as it contributes to nutrients balance, has high pH and has high buffering capacity. An example

of a recent study is the research of Khoufi et al. [20] that investigated the co-digestion of OMW with liquid poultry manure (LPM) in batch condition and semi-continuous jet-loop reactor. Authors concluded that the addition of 30% (v/v) LPM gives the best methane yield, and process stability was shown until an organic loading rate (OLR) of 9.5 g COD L^{-1} reactor day^{-1}. Process improvement is probably related to a more balanced nutrients mixture and minimization of the inhibitory effect of ammonia and phenolic compounds. Swine manure has also been used as co-substrate in several anaerobic co-digestion studies. Recently, Kougias et al. [21] carried out batch and semi-continuous mode trials with different mixtures of OMW and swine manure. The best results were obtained using 40% OMW in a semi-continuous reactor, achieving a methane yield of 373 mL CH_4 g^{-1} volatile solids (VS)

Sampaio et al. [22] tested the use of an up-flow anaerobic hybrid digester reactor for OMW digestion. An organic loading rate of 8 kg COD m^{-3} d^{-1} provided 3.7–3.8 m^3 biogas m^{-3} d^{-1} (63–64% CH_4) and 81–82% COD removal. They also tested feeding the reactor with pig slurry and OMW alternately, achieving a biogas production of 3.0–3.4 m^3 m^{-3} d^{-1} (63–69% CH_4).

Another interesting approach for bioconversion of OMW to energy is its use for hydrogen and bioethanol production through anaerobic fermentation. Eroğlu et al. [23] studied Rhodobacter sphaeroides for the photofermentation of OMW under anaerobic conditions, achieving a biohydrogen production of 16 L H_2 L^{-1}. More recently, Battista et al. [24] used a mixture of OMW and olive pomace to produce hydrogen and bioethanol by Saccharomyces cerevisiae anaerobic fermentation. They also tested different pretreatments (ultrasounds, alkaline hydrolysis, and calcium carbonate addition), concluding that ultrasounds and alkaline pretreatment lead to the hydrolysis of the lignin and cellulose. This fact leads to the increase in soluble organic matter (namely sugars) enhancing methane production. Calcium carbonate addition contributed to optimize the process by removing polyphenols, which are inhibitory for the fermentation process.

2.2. Bioconversion into high-added value products

There has been a change of paradigm regarding the economy; the linear vision was replaced by a circular vision, where waste is regarded as a potential source of resources to be reintroduced in the production cycle. Food production chain is one of the main waste producers. Recent studies on food waste valorization have suggested a wide range of possible bioproducts, namely biofuels, enzymes, bioactive compounds, biodegradable plastics and nanoparticles.

Regarding OMW, it has been seen as a source of biologically active phenols (biophenols) due to its high content in phenolic compounds, widely recognized as antioxidants which can be used in several industries, for example, food and pharmaceuticals production. A recent study by Kaleh and Geißen [25] describe the use of acidification, sedimentation and membrane filtration of OMW for biophenols recovery, namely hydroxytyrosol, tyrosol, caffeic acid, oleuropein and luteolin. Synthetic resins and molecularly imprinted polymers were tested as sorbents and results showed that by combining different pretreatments with sorbent options, it is possible to selectively adsorb a specific biophenols.

http://dx.doi.org/10.5772/64798

Goula and Lazarides [26] present an integrated approach aiming at the complete recovering of OMW valuable compounds and reuse of depolluted water stream in the olive mill. Specially designed fermentation, spray drying and encapsulation technologies are addressed producing a number of valuable bioproducts, such as olive paste spread or olive powder (to be included in food formulations) and encapsulated phenols.

Federici et al. [27] discussed several strategies for OMW such as the recovery of antioxidants by chemical methods and the fermentative production of enzymes of commercial interest.

Mateo and Maicas [28] reviewed the most promising microbiological processes for the valorization of by-products from olive oil production. According to these authors', microbiological processes have an interesting potential as they have less environmental impact and, in most cases, can be cost-effective. Relevant to this analysis is the fact that they lead to added value products such as enzymes, biofuels, biopolymers, etc.

In fact, OMW due to its characteristics has been used in several studies as a medium to grow microbial species that consume organic matter and, simultaneously, produce biomass and other bioproducts, for example, enzymes and organic acids.

Laccases are known to efficiently degrade recalcitrant compounds; their production costs are still a drawback to their wider use. Therefore, there have been some experiments to assess the potential of using waste/wastewaters as a growth medium. White-rot fungi have been reported as efficient for phenolic compounds degradation because their extracellular ligninolytic enzymes (e.g. laccase, manganese peroxidase and lignin peroxidase) are able to catalyze lignin-like structures and promote recalcitrant compounds oxidation [29, 30].

More recently, Mann et al. [31] studied the production of laccases from white-rot fungi grown in OMW. The study showed that it was possible to reduce phenols content and phytotoxicity. Furthermore, results point out to the fact that OMW is a source of laccase mediators, since the efficiency of removal of phenols increased when 1% OMW was added to the solution.

Ntougias et al. [32] also focused on microbial depuration of OMW, assessing the use of 49 white-rot fungi strains belonging to 38 species of Basidiomycota. Results showed a reduction in total phenols up to 60% and colour up to 70%.

Nogueira et al. [33] assessed the efficiency of combining photocatalytic oxidation, using two nanomaterials as catalysts (TiO_2 and Fe_2O_3), with biological degradation by fungi (Pleurotus sajor caju and P. chrysosporium). Results showed that biological treatment with fungi after pretreatment with nanomaterials allowing COD, TP and ecotoxicity removal. The highest removal of COD and TP was achieved with the combination of the system Fe_2O_3/UV and Pleurotus sajor caju, respectively, around 60 and 98%, but only a decrease in around 9% in ecotoxicity. The most efficient detoxifying process was the combination of Fe_2O_3/UV with P. chrysosporium, with a reduction around 37%, and 52 and 96% for COD and TP, respectively.

OMW has also been used for production of algal biomass, which accumulates lipids and carbohydrates and therefore can be used for the production of biofuels or recovery of compound. Di Caprio et al. [34] used OMW supplemented with nitrates (to prevent reduction in

the specific growth rate) for the cultivation of Scenedesmus sp. achieving biomass production and depuration of OMW.

3. Physico-chemical and advanced oxidation processes

3.1. Wet oxidation, Fenton advanced oxidation, ozone

Pham Minh et al. [35] studied the catalytic wet air oxidation (CWAO) of OMW with self laboratory-prepared platinum- and ruthenium-supported titanium or zirconium, coupled with anaerobic digestion. The authors reported the effective elimination of the total organic carbon (TOC), up to 97%, and a nearly complete removal of the phenolic content in CWAO at 190°C and 70-bar total air pressure. Moreover, a decrease in the phytotoxicity of the OMW effluent was observed towards Vibrio fischeri. The ruthenium catalysts were proved to be stable over a long operating period. A high mineralization level of the effluent was achieved, and the methane production yield was enhanced in the subsequent anaerobic treatment. However, experiments were conducted on actual diluted OMW (two times dilutions).

Azaboua et al. [36] examined a compact process for the treatment of OMW comprising catalytic oxidation with wet hydrogen peroxide (WHPCO) followed by different biological techniques. WHPCO catalytic processes were performed using a montmorillonite-based aluminium-iron-pillared interlayer clay [(Al-Fe) PILC] as heterogeneous catalyst. The authors examined [(Al-Fe) PILC]/H_2O_2 under ultraviolet irradiations at 25 or 50°C, both under atmospheric pressure. The results obtained revealed that the raw OMW stream was resistant to the photocatalytic process, but a considerable reduction in the COD, colour and total phenolic compounds concentrations was attained throughout the latter process. As a result, a decrease in the inhibition of Vibrio fischeri luminescence of around 70% was reported. Otherwise, a higher methane production was obtained in the biomethanization of OMW when [(Al-Fe) PILC]/H_2O_2 for 2 h was carried out.

Martínez-Nieto et al. [3] studied an advanced oxidation process based on Fenton's reaction for the degradation of the organic matter load present in olive oil mill wastewater from two-phase olive oil extraction process. The authors examined several methods on a laboratory scale in order to use the cheaper Fe^{3+} salts rather than Fe^{2+} salts, examining the performance of several catalysts—Mohr salt [$(NH_4)_2Fe(SO_4)_2$ $6H_2O$, ferric perchlorate and ferric chloride—as well as the best catalyst/oxidant ratio and operating conditions. It was shown that organic matter is efficiently degraded through Fenton-like reaction using $FeCl_3$ as catalyst in the presence of hydrogen peroxide. Organic matter and phenolic compounds removal efficiencies above 95% were attained. Moreover, ferric ions (Fe^{3+}) helped avoid the consumption of the oxidant (H_2O_2) in transforming ferrous ions into ferric ones, which occurs in unproductive parallel reactions. These results revealed Fenton-like reaction as a solution, relatively cheap, for the treatment of these wastewaters. The treated water from this process was ready for irrigation.

In a subsequent research study, Hodaifa et al. [4] optimized the reclamation of OMW by Fenton-like process in a continuous stirred tank reactor (CSTR) at pilot scale. In the start-up

http://dx.doi.org/10.5772/64798

stage, Fenton reaction reached steady state within 3 h. Oxidation of organic matter in OMW was pH dependent. The final values of COD and total phenols at the outlet of the pilot plant were close to 129 and 0.5 mg/L, respectively. Finally, the produced water was apt for irrigation or to be discharged directly into the municipal wastewater system.

Finally, Martínez-Nieto et al. [37] tested Fenton chemical oxidation process using ferric chloride or potassium permanganate as catalysts for the activation of H_2O_2 on an industrial scale. By using potassium permanganate in the system, the final water was transparent with a slight yellow tinge, but odourless with a low total phenol content. The sediments in the decanter were rich in manganese dioxide (MnO_2), which, though non-toxic, would need further management. Finally, the versatile design of the plant offers the possibility to work with both oxidation systems, without the need to make changes in the process. The water produced could be used for irrigation or discharged directly into the municipal wastewater system.

3.2. Combined treatments

Sarika et al. [38] studied the pretreatment of OMW by flocculation with cationic and anionic polyelectrolytes. The majority of the tested flocculants completely removed the TSS and reduced considerably the COD and the BOD_5. The minimum flocculant dosage to attain solid-liquid separation was 2.5–3 g L^{-1}. Authors suggest the post-treatment of the liquid phase by means of high-power ultrasound, advanced oxidation, biological processes or a combination of them, whereas for the solid fraction, they stated that various solid agro-wastes may be composted to yield soil fertilizers (Manios, 2004), as reported in a study by García-Gómez et al. (2003).

Stoller and Chianese [39, 40] studied the purification of OWW to comply with discharge standards in municipal sewers (Italy). The authors proposed a treatment process comprising an initial coagulation-flocculation with aluminium sulphate (AS) or aluminium hydroxide (AH), followed by batch UF and NF in series with composite thin-film spiral-wound membranes. The two pretreatment processes yielded similar COD and BOD_5 rejection efficiencies. However, higher productivity was attained in the subsequent membranes-in-series process after flocculation with AS. Following this, Stoller [41] conducted a deeper study on flocculation as pretreatment of microfiltration (MF), UF, NF and RO membranes in the treatment of three-phase OMW, by examining the particle size distribution in the effluent at the outlet of each stage. Stoller underlined the effect created by a secondary flocculation induced by the AS flocculant-derived salts accumulating near the membrane surface. This fact enhances the particles to be carried away by the tangential flow, thus sensibly reducing fouling. In a following research work, Stoller and Bravi [42] applied the same coagulant-flocculants to pretreat three-phase OMW before batch MF, UF, NF and RO membranes in sequence. In addition, they examined photocatalysis (PC) with nanometric titanium dioxide in anatase form irradiated by UV light and aerobic treatment. All pretreatment processes provided final RO permeate streams complying with irrigation quality standards (COD ranging from 242 to 456 mg/L). However, UV/TiO_2 photocatalysis showed the highest membrane productivity within the shortest residence time (24 h).

As previously described coagulation-flocculation is a common pretreatment, and research works have studied alternatives to conventional chemicals, using biopolymers as chitosan [43] or residues from other industrial activities. For example, Fragoso et al. [44] studied the use of a sludge produced at water treatment plants (drinking-water treatment sludge—DWTS) with similarities to bentonite (namely the presence of aluminium silicate, alkaline pH and particle size), as an alternative to conventional coagulation-flocculation process. Results showed that it was possible to reduce 40–50% of COD, 45–50% of TP, a maximum of nearly 70% TSS and 45% for total solids (TS) and total volatile solids (TVS). This strategy would represent an integrated management of OMW and DWTS, contributing to a decrease in the environmental impact of two industrial activities, olive oil production and drinking water treatment.

In another study, Rizzo et al. [43] addressed the reclamation of OMW by coagulation with a natural organic coagulant, chitosan, followed by advanced oxidation processes: PC, Fenton (F) and photo-Fenton (PF). The maximum organic matter removal efficiencies were achieved after 2.0 and 1.0 h.

El-Gohary et al. [45] studied the integration of wet hydrogen peroxide catalytic oxidation (WHPCO) prior to a two-stage up-flow anaerobic sludge blanket (UASB) for the treatment of OMW. The raw OMW stream was diluted (1:1 v: v) with tap water and pretreated by Fenton's reaction with $FeSO_4$

In a similar line, Walid et al. [46] investigated different combined processes for OMW treatment, including advanced oxidation by UV and/or O_3, and an aerobic biodegradation. Results showed that for both single-stage O_3 treatment and O_3/UV two-stage treatment, the COD remained quite high. The combination of the advanced oxidation by UV/O_3 followed by biodegradation process ensured the highest COD reduction efficiencies, up to 91%.

Martínez-Nieto et al. [47] examined the efficiency of different flocculants—high molecular weight anionic polyelectrolytes—such as commercial QG-2001, QG-2002, DQGALFLOC-130H and Nalco-77171. The optimum dosage of each flocculant, 150, 2.5, 66 and 6 mg L^{-1}, respectively, was determined. The results revealed that 13.5% v/v final sludge separation and 86.5% v/v final clarified water can be obtained

In a recent study, Alver et al. [48] investigated a sequential system comprising coagulation and Fenton reaction. Higher treatment efficiency was achieved by the sequential coagulation and Fenton system. This study demonstrated that the integrated coagulation and Fenton process could be a potential solution for efficient removal of phenolic pollutants from this type of wastewaters.

3.3. Electrochemical, solar-driven and heterogeneous photocatalytic treatments

Several electrochemical treatments have already been applied for the treatment of OMW, such as electrocoagulation, electro-Fenton, electrochemical oxidation with polialuminium chloride (PAC), conductive diamond electrochemical oxidation (CDEO), electrooxidation with in situ generated active chlorine, as well as by means of cyclic voltammetry and bulk electrolysis with Ti/RuO_2 or Ti/IrO_2 anodes.

http://dx.doi.org/10.5772/64798

Tezcan et al. [49] applied electrochemical oxidation with PAC in the presence of H_2O_2 on fresh OMW (COD 45,000 mg L^{-1}). The obtained results revealed that the Fe electrode was more effective than the Al electrode. Up to 62–86% COD removal efficiency as well as 100% turbidity and oil and grease abatement could be achieved upon 20–75 mA cm^{-2} current density range. Afterwards, Tezcan et al. [50] investigated the electrochemical oxidation of OMW using Ti/RuO_2 anode on OMW samples from an olive oil mill operating with the three-phase technology. The removal rates of organics increased with the increase in the applied current density, sodium chloride concentration, recirculation rate and temperature. The specific energy consumption (SEC) was found to be in the range 5.35–27.02 kWh kg^{-1}. The treated OMW effluent presented a final COD around 167 mg L^{-1} (99.6% removal efficiency) and almost complete abatement of phenolic compounds. The running costs estimated by the authors were equal to 0.78 €/kg^{-1} COD.

Khoufi et al. [51] studied the reclamation of OMW for agricultural purposes by means of electro-Fenton followed by anaerobic digestion. Up to 65.8% of the total phenolic compounds concentration could be removed by electro-Fenton, and a decrease in the toxicity of 33.1% was ensured. Electrocoagulation of the anaerobically digested effluent provided complete detoxification.

Cañizares et al. [52] evaluated and compared the technical and economic feasibilities of three AOPs: CDEO, ozonation and Fenton oxidation, for wastewaters polluted with different types of organic compounds, including OMW. According to their results, only CDEO could achieve complete organic matter abatement (mineralization) of the pollutants for all the wastes. However, the efficiencies were found to depend on the concentration of the specific pollutants, whereas oxidation with ozone (at pH 12) or by Fenton's reagent was found to depend on the nature of the pollutants. The average estimated operation costs were in the range 2.4–4.0 €/kg^{-1} equivalent O_2 for the CDEO process, 8.5–10.0 €/kg^{-1} equivalent O_2 for ozonation, whereas 0.7–3.0 €/kg equivalent O_2 for Fenton oxidation. Moreover, the expected capital investment for Fenton (approximately 16,000 €) was much lower than that needed for CDEO.

Papastefanakis et al. [53] studied the reclamation of OMW through electrochemical oxidation by means of cyclic voltammetry and bulk electrolysis with Ti/RuO_2 and Ti/IrO_2 anodes. Elimination of the ecotoxicity and up to 86 and 84% colour and phenols removal, as well as 52 and 38% COD and total organic carbon reduction, could be successfully achieved upon oxidation at 28 AhL^{-1} and 50 $mAcm^{-2}$. The authors conclude that Ti/RuO_2 and Ti/IrO_2 stable anode-type electrodes show good activity for the treatment of agroindustrial effluents like OMW, despite the complex effluent composition that could have compromised both the activity and stability of the used anodes.

Moreover, Chatzisymeon et al. [54] studied the photocatalytic treatment of three-phase OMW with TiO_2 in a batch laboratory-scale photoreactor. They found that COD removal was enhanced by the contact time and also affected by the influent COD, whilst all other variables had no significant statistical importance on the COD removal. The energy consumption per unit mass of pollutant removed was noted to be lower for higher influent COD, indicating that TiO_2 photocatalysis can be a promising process for OMW treatment. OMW was almost

completely detoxified at low influent COD, though toxicity was only slightly reduced at major organic loadings.

Justino et al. [55] examined the efficiency of a combined treatment process comprising sequentially fungi with Pleurotus sajor caju and photo-Fenton oxidation or vice versa. The treatment with fungi was carried out on diluted OMW samples, after which the reduction in OMW acute toxicity towards Daphnia longispina was confirmed, providing 72.9% total phenolic compounds removal along with 77% organic matter (COD) abatement. The treatment sequence comprising first photo-Fenton oxidation followed by biological treatment with fungi was found to be more efficient, mainly given by the fact that no dilution of the raw OMW effluent was needed.

Ochando-Pulido et al. [56] studied the photocatalytic degradation of OOW at laboratory scale. The main technical-economical handicap relies on the difficulty in recovering the catalyst. To solve this, a novel nano-photocatalyst with ferromagnetic properties was developed. The photocatalyst offered good results in comparison with other commercial ones. Up to 58.3% COD removal, 27.5% total phenols removal and 25.0% total suspended solids removal were attained. Also, if a pH-temperature flocculation pretreatment was performed, the overall COD removal efficiency increased up to 91%. According to the results obtained in this investigation, the photocatalytic degradation process is an alternative with high possibilities in the treatment of OMW.

Ruzmanova et al. [57] recently examined the treatment of three-phase OMW by photocatalysis with N-doped TiO_2 sol-gel material [57–61]. The adopted doping procedure was validated under visible light, exhibiting higher performances if compared to those obtained with non-doped particles, achieving more than 60% COD removal. The photocatalysis assisted by TiO_2 catalyst sensitive to visible light may represent a very promising solution for the degradation of the organic compounds in OMW and similar effluents.

In a different research work, Papaphilippou et al. [62] proposed an integrated treatment process for OMW consisting sequentially of coagulation-flocculation, extraction of phenolic compounds and post-oxidation by photo-Fenton. After photo-Fenton advanced oxidation, COD removal about 73 ± 2.3% and total phenols of 87 ± 3.1% were, respectively, found. Furthermore, comparative phytotoxicity tests revealed that more biologically potent products were obtained during oxidation.

Michael et al. [63] addressed the depuration of three-phase OMW by means of a solar-driven advanced oxidation process combined with previous coagulation/flocculation, achieving high COD removal (87%) and elimination of the bio-recalcitrant polyphenolic fraction. The overall cost of solar Fenton oxidation was 2.11 €m^{-3}.

4. Conclusions

As it was shown, there are several alternatives for OME treatment, comprising biological and physico-chemical processes, either alone or in combination, varying in complexity, ease of

operation and costs. OME has been a challenge for researchers due to its difficult treatability which motivated the development of new approaches and technologies mainly at laboratory scale but also to a lesser extent at pilot scale. Up to this moment, no strategy is available that can be adopted in a global scale. The most reasonable approach is to regard OME treatment/valorization as a regional problem, defining decentralized treatment that in some cases can be implemented for a group of olive oil mills in the same geographic area. This will lead to an economy of scale, allowing the adoption of more expensive technologies, unaffordable by individual mills, complying with environmental regulations and optimizing resource recovery from OME. This aspect is receiving strong attention as European Commission is promoting the transition towards a circular economy which aims at "closing the loop" of product lifecycle through greater recycling and reuse, bringing benefits for both the environment and the economy. It seems that a stepwise strategy is becoming a new research trend: firstly, promoting the recovery of all valuable compounds from OME; and secondly, treating the partially depurated effluent.

Acknowledgements

The Spanish Ministry of Economy and Competitiveness is gratefully acknowledged for project CTM2014-61105-JIN fund.

Author details

Javier Miguel Ochando-Pulido[1*], Rita Fragoso[2], Antónia Macedo[2], Elizabeth Duarte[2] and Antonio Martínez Ferez[1]

*Address all correspondence to: jmochandop@gmail.com

1 Department of Chemical Engineering, University of Granada, Granada, Spain

2 University of Lisbon, Instituto Superior de Agronomia, LEAF—Linking Landscape, Environment, Agriculture and Food, Tapada da Ajuda, Lisboa, Portugal

References

[1] Karaouzas I., Skoulikidis N. T., Giannakou U., Albanis T. A. Spatial and temporal effects of olive mill wastewaters to stream macroinvertebrates and aquatic ecosystems status. Water Research 45 (19) (2011) 6334–6346.

[2] Danellakis D., Ntaikou I., Kornaros M., Dailianis S. Olive oil mill wastewater toxicity in the marine environment: Alterations of stress indices in tissues of mussel Mytilus galloprovincialis. Aquatic Toxicology 101 (2) (2011) 358–366.

[3] Martínez Nieto L., Hodaifa G., Rodríguez Vives S., Giménez J. A., Ochando J. Degradation of organic matter in olive-oil mill wastewater through homogeneous Fenton-like reaction. Chemical Engineering Journal 173 (2011) 503–510.

[4] Hodaifa G., Ochando J., Rodríguez S., Martínez-Férez A. Optimization of continuous reactor at pilot scale for olive-oil mill wastewater treatment by Fenton-like process. Chemical Engineering Journal 220 (2013) 117–124.

[5] Ntougias S., Gaitis F., Katsaris P., Skoulika S., Iliopoulos N., Zervakis G. I. The effects of olives harvest period and production year on olive mill wastewater properties—Evaluation of Pleurotus strains as bioindicators of the effluent's toxicity. Chemosphere 92 (2013) 399–405.

[6] Niaounakis M., Halvadakis C. P. Olive processing waste management literature review and patent survey, 2nd ed., Elsevier: Waste Management Series, vol. 5 (2006) 23–64.

[7] Paraskeva P., Diamadopoulos E. Technologies for olive mill wastewater (OMW) treatment: A review. Journal of Chemical Technology and Biotechnology 81 (2006) 1475–1485.

[8] Azbar, N., Bayram, A., Filibeli, A., Muezzinoglu, A., Sengul, F., Ozer, A. A review of waste management options in olive oil production. Critical Reviews in Environmental Science and Technology 34 (2004) 209–247.

[9] Aggelis G., Iconomou D., Christou M., Bokas D., Kotzailias S., Christou G., Tsagou V., Papanikolaou S. Phenolic removal in a model olive oil mill wastewater using Pleurotus ostreatus in bioreactor cultures and biological evaluation of the process. Water Research 37 (2003) 3897–904.S.

[10] Garcia I., Jiménez Peña P. R., Bonilla Venceslada J. L., Martín A., Martín Santos M. A., Gómez Ramos E. Removal of phenol compounds from olive mill wastewater using Phanerochaete chrysosporium, Aspergillus niger, Aspergillus terreus and Geotrichum candidum. Process Biochemistry 35 (2000) 751–758.

[11] Tsioulpas A., Dimou D., Iconomou D., Aggelis G. Phenolic removal in olive mill wastewater by strains of Pleurotus spp in respect to their phenol oxidase (laccase) activity. Bioresource Technology 84 (2002) 251–257.

[12] Ehaliotis C., Papadopoulou K., Kotsou M., Mari I., Balis C. Adaptation and population dynamics of Azotobacter vinelandii during aerobic biological treatment of olive-mill wastewater. FEMS Microbiology Ecology 30 (1999) 301–311.

[13] Amaral C., Lucas M. S., Sampaio A., Peres J. A., Dias A. A., Peixoto F., Rosário Anjos M., Pais C. Biodegradation of olive mill wastewaters by a wild isolate of Candida oleophila. International Biodeterioration & Biodegradation 68 (2012) 45–50.

[14] Chiavola A., Farabegoli G., Antonetti F. Biological treatment of olive mill wastewater in a sequencing batch reactor. Biochemical Engineering Journal 85 (2014) 71–78.

[15] Ammary B. Treatment of olive mill wastewater using an anaerobic sequencing batch reactor. Desalination 177 (2005) 157–165.

[16] Gunay A., Karadag D. Recent developments in the anaerobic digestion of olive mill effluents. Process Biochemistry 50 (2015) 1893–1903.

[17] González-González A., Cuadros F. Effect of aerobic pretreatment on anaerobic digestion of olive mill wastewater (OMWW): An ecoefficient treatment. Food and Bioproducts Processing 95 (2015) 339–345.

[18] Oz N. A., Uzun A. C. Ultrasound pretreatment for enhanced biogas production from olive mill wastewater. Ultrasonics Sonochemistry 22 (2015) 565–572.

[19] Ruggeri B., Battista F., Bernardi M., Fino D., Mancini G. The selection of pretreatment options for anaerobic digestion (AD): A case study in olive oil waste production. Chemical Engineering Journal 259 (2015) 630–639.

[20] Khoufi S., Louhichi A., Sayadi S. Optimization of anaerobic co-digestion of olive mill wastewater and liquid poultry manure in batch condition and semi-continuous jet-loop reactor. Bioresource Technology 182 (2015) 67–74.

[21] Kougias P. G., Kotsopoulos T. A., Martzopoulos G. G. Effect of feedstock composition and organic loading rate during the mesophilic co-digestion of olive mill wastewater and swine manure. Renewable Energy 69 (2014) 202–207.

[22] Sampaio M. A., Gonçalves M. R., Marques I. P. Anaerobic digestion challenge of raw olive mill wastewater. Bioresource Technology 102 (23) (2011) 10810–10818.

[23] Eroglu E., Eroglu I., Gunduz U., Yucel M. Effect of clay pretreatment on photofermentative hydrogen production from olive mill wastewater. Bioresource Technology 99 (2008) 6799–6808.

[24] Battista F., Mancini G., Ruggeri B., Fino D. Selection of the best pretreatment for hydrogen and bioethanol production from olive oil waste products. Renewable Energy 88 (2016) 401–407.

[25] Kaleh Z., Geißen S. U. Selective isolation of valuable biophenols from olive mill wastewater. Journal of Environmental Chemical Engineering 4 (2016) 373–384.

[26] Goula A. M., Lazarides H. N. Integrated processes can turn industrial food waste into valuable food by-products and/or ingredients: The cases of olive mill and pomegranate wastes. Journal of Food Engineering 167 (2015) 45–50.

[27] Federici F., Fava F., Kalogerakis N., Mantzavinos D. Valorisation of agroindustrial by-products, effluents and waste: Concept, opportunities and the case of olive mill waste waters. Journal of Chemical Technology and Biotechnology 84 (2009) 895–900.

[28] Mateo J. J., Maicas S. Valorization of winery and oil mill wastes by microbial technologies. Food Research International 73 (2015) 13–25.

[29] Baldrian P. Fungal laccases-occurrence and properties. FEMS Microbiology Reviews 30 (2006) 215–242. doi:10.1111/j.1574-4976.2005.00010.x.

[30] Morillo J., Antizar-Ladislao B., Monteoliva-Sánchez M., Ramos-Cormenzana A., Russell N. Bioremediation and biovalorisation of olive-mill wastes. Applied Microbiology and Biotechnology 82 (2009) 25–39. doi:10.1007/s00253-008-1801-y.

[31] Mann J., Markham J. L., Peiris P., Spooner-Hart R. N., Holford P., Nair N. G. Use of olive mill wastewater as a suitable substrate for the production of laccase by Cerrena consors. International Biodeterioration & Biodegradation 99 (2015) 138–145

[32] Ntougias S., Baldrian P., Ehaliotis C., Nerud F., Merhautova V., Zervakis G. I. Olive mill wastewater biodegradation potential of white-rot fungi—Mode of action of fungal culture extracts and effects of ligninolytic enzymes. Bioresource Technology 189 (2015) 121–130.

[33] Nogueira V., Lopes I., Freitas A. C., Rocha-Santos T. A. P., Gonçalves F., Duarte A. C., Pereira R. Biological treatment with fungi of olive mill wastewater pre-treated by photocatalytic oxidation with nanomaterials. Ecotoxicology and Environmental Safety 115 (2015) 234–242.

[34] Di Caprio F., Altimari P., Pagnanelli F. Integrated biomass production and biodegradation of olive mill wastewater by cultivation of Scenedesmus sp. Algal Research 9 (2015) 306–311.

[35] Minh D. P., Gallezot P., Azabou S., Sayadi S., Besson M. Catalytic wet air oxidation of olive oil mill effluents 4. Treatment and detoxification of real effluents. Applied Catalysis B: Environmental 84 (2008) 749–757.

[36] Azaboua S., Najjar W., Bouaziz M., Ghorbel A., Sayadi S. A compact process for the treatment of olive mill wastewater by combining wet hydrogen peroxide catalytic oxidation and biological techniques. Journal of Hazardous Materials 183 (2010) 62–69.

[37] Martínez Nieto L., Hodaifa G., Rodríguez S., Giménez J. A. Industrial plant for OMW from two-phase treatment by chemical oxidation. Journal of Environmental Engineering 136 (2010) 1309–1313.

[38] Sarika R., Kalogerakis N., Mantzavinos D. Treatment of olive mill effluents Part II. Complete removal of solids by direct flocculation with poly-electrolytes. Environment International 31 (2005) 297–304.

[39] Stoller M., Chianese A. Optimization of membrane batch processes by means of the critical flux theory. Desalination 191 (2006) 62–70.

[40] Stoller M., Chianese A. Technical optimization of a batch olive wash wastewater treatment membrane plant. Desalination 200 (1–3) (2006) 734–736.

[41] Stoller M. On the effect of flocculation as pretreatment process and particle size distribution for membrane fouling reduction. Desalination 240 (2009) 209–217.

[42] Stoller M., Bravi M. Critical flux analyses on differently pretreated olive vegetation wastewater streams: Some case studies. Desalination 250 (2010) 578–582.

[43] Rizzo L., Lofrano G., Grassi M., Belgiorno V. Pretreatment of olive mill wastewater by chitosan coagulation and advanced oxidation processes. Separation and Purification Technology 63 (3) (2008) 648–653.

[44] Fragoso R., Duarte E. Reuse of drinking water treatment sludge for olive oil wastewater treatment. Water Science and Technology 66 (4) (2012) 887–894.

[45] El-Gohary F. A., Badawy M. I., El-Khateeb M. A., El-Kalliny A. S. Integrated treatment of olive mill wastewater (OMW) by the combination of Fenton's reaction and anaerobic treatment. Journal of Hazardous Materials 162 (2009) 1536–1541.

[46] Lafi W. K., Benbella Shannak B., Al-Shannag M., Al-Anber Z., Al-Hasan M. Treatment of olive mill wastewater by combined advanced oxidation and biodegradation. Separation and Purification Technology 70 (2009) 141–146.

[47] Martínez Nieto L., Hodaifa G., Rodríguez S., Giménez J. A., Ochando J. Flocculation–sedimentation combined with chemical oxidation process. Clean – Soil, Air, Water 39 (10) (2011) 949–955.

[48] Alver A., Bastürk E., Kılıc A., Karatas M. Use of advance oxidation process to improve the biodegradability of olive oil mill effluents. Process Safety and Environmental Protection 98 (2015) 319–324.

[49] Tezcan Un U., Ugur S., Koparal A. S., Bakır Ogutveren U. Electrocoagulation of olive mill wastewaters. Separation and Purification Technology 52 (2006) 136–141.

[50] Tezcan Un U., Altay U., Koparal A. S., Bakir Ogutveren U. Complete treatment of olive mill wastewaters by electrooxidation. Chemical Engineering Journal 139 (2008) 445–452.

[51] Khoufi S., Aloui F., Sayadi S. Treatment of olive oil mill wastewater by combined process electro-Fenton reaction and anaerobic digestion. Water Research 40 (2006) 2007–2016.

[52] Cañizares P., Paz R., Sáez C., Rodrigo M. A. Costs of the electrochemical oxidation of wastewaters: A comparison with ozonation and Fenton oxidation processes. Journal of Environmental Management 90 (2009) 410–420.

[53] Papastefanakis N., Mantzavinos D., Katsaounis A. DSA electrochemical treatment of olive mill wastewater on Ti/RuO_2 anode. Journal of Applied Electrochemistry 40 (2010) 729–737.

[54] Chatzisymeon E., Xekoukoulotakis N. P., Mantzavinos D. Determination of key operating conditions for the photocatalytic treatment of olive mill wastewaters. Catalysis Today 144 (2009) 143–148.

[55] Justino C. I., Duarte K., Loureiro F., Pereira R., Antunes S. C., Marques S. M., Gonçalves F., Rocha-Santos T. A. P., Freitas A. C. Toxicity and organic content characterization of olive oil mill wastewater undergoing a sequential treatment with fungi and photo-Fenton oxidation. Journal of Hazardous Materials 172 (2009) 1560–1572.

[56] Ochando-Pulido J. M., Hodaifa G., Víctor-Ortega M. D., Martínez-Férez A. A novel photocatalyst with ferromagnetic core used for the treatment of olive oil mill effluents from two-phase production process. The Scientific World Journal 2013 (2013) 196470. doi:10.1155/2013/196470.

[57] Ruzmanova Y., Ustundas M., Stoller M., Chianese A. Photocatalytic treatment of olive mill wastewater by N-doped titanium dioxide nanoparticles under visible light. Chemical Engineering Transactions 32 (2013) 2233–2238.

[58] Bangkedphol S., Keenan H. E., Davidson C. M., Sakultantimetha A., Sirisiksoontorn W., Songsasen A. Enhancement of tributyltin degradation under natural light by N-doped TiO_2 photocatalyst. Journal of Hazardous Materials 184 (2010) 533–537.

[59] Chen D., Jiang Z., Geng J., Wang Q., Yang D. Carbon and nitrogen co-doped TiO_2 with enhanced visible-light photocatalytic activity. Industrial and Engineering Chemistry Research 46 (9) (2007) 2741–2746.

[60] Chatterjee D., Dasgupta S. Visible light induced photocatalytic degradation of organic pollutants. Journal of Photochemistry and Photobiology 6 (2–3) (2005) 86–205.

[61] Hu S., Wang A., Li X., Löwe H. Hydrothermal synthesis of well-dispersed ultrafine N-doped TiO_2 nanoparticles with enhanced photocatalytic activity under visible light. Journal of Physics and Chemistry of Solids 71 (2010) 156–162.

[62] Papaphilippou P. C., Yiannapas C., Politi M., Daskalaki V. M., Michael C., Kalogerakis N., Mantzavinos D., Fatta-Kassinos D. Sequential coagulation–flocculation, solvent extraction and photo-Fenton oxidation for the valorization and treatment of olive mill effluent. Chemical Engineering Journal 224 (2013) 82–88.

[63] Michael I., Panagi A., Ioannou L. A., Frontistis Z., Fatta-Kassinos D. Utilizing solar energy for the purification of olive mill wastewater using a pilot-scale photocatalytic reactor after coagulation-flocculation. Water Research 60 (2014) 28–40.

Section 7

Regional Studies

Chapter 16

Olive Oil in Brazil: Economic and Regulatory Control Aspects

Sabria Aued-Pimentel

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64539

Abstract

The oil extracted from olives has characteristics that set it apart from other vegetable oils. Its exceptional sensory and nutritional attributes and its limited production are among the aspects that give it high market value. However, oils of different grades and quality are obtained from the fruit of the olive tree. Thus, producers are interested in improving and disseminating product quality control techniques. Brazil's domestic demand is met by imported olive oils, with Brazil being one of the world's main importers. Recently, the expansions of the market and the commercial production outlook have intensified the work of the Brazilian government in improving the legal requirements to control this product and enable laboratories to monitor quality. Despite government initiatives, the trade of this oil in Brazil has always been, and continues to be, marked by evidence of fraud and adulteration. The present work aims to provide an overview of the economic, regulatory, and inspection aspects involving the olive oil in Brazil, emphasizing the initiatives to improve the control of this important product.

Keywords: Olive oil, Monitoring, Adolfo Lutz Institute, Adulteration, Legislation

1. Introduction

The culture of the olive tree and the production of olive oil are the oldest agricultural activities in human history. Mediterranean countries such as Spain, Italy, and Greece are the main producers of this oil. The knowledge of this culture and its by-products, although ancient, has only recently become more widespread in Brazil. Oils of different grades and quality are obtained from the fruit of the olive tree. The Brazilian domestic demand for olive oil is met by imported oil, with Brazil being one of the world's big importers. This study aims to provide an overview of economic, regulatory, and inspection aspects involving the olive oil in Brazil.

The topics studied deal with legal limitations and practices in Brazil, control and inspection aspects, and the contribution over many years of the Adolfo Lutz Institute as a public health laboratory, monitoring the quality of commercial olive oil in São Paulo city.

2. Economic aspects

2.1. Diffusion of olive trees and olive oil (Brazil and abroad)

Olive cultivation certainly began prior to the sixteenth century BC. This culture spread to the Greek islands through the Phoenicians and was probably brought from Asia Minor and introduced into Greece, Libya, and Carthage.

Later, in the eighth and seventh centuries BC, olive cultivation expanded, and methods for the production and distribution of oil were organized. The climatic conditions of the Mediterranean countries were very favorable to the cultivation of the olive tree, and this culture spread quickly to all the countries of the Mediterranean Sea Basin in what is now a feature of the region [1].

The Phoenicians and Carthaginians were counted as deployers of olives in Spain, and this culture expanded to Portugal, the West Indies, and South America [2, 3]. The cultivation of the olive tree was extended to regions of the American continent where climate conditions are similar to those of the Mediterranean region [1, 3].

In North America, olive growing was brought by Spanish missionaries and deployed initially in California. In South America, this culture was introduced by Mediterranean immigrants, spreading to Argentina, Chile, Peru, Brazil, and other countries [1–3].

Olive tree cultivation was introduced in Brazil many centuries ago. Olive trees were found in several Brazilian states, such as Rio Grande do Sul, Parana, Santa Catarina, Minas Gerais, Espirito Santo, Rio de Janeiro, and São Paulo [2, 3]. The olive groves of the colonial period (sixteenth to nineteenth centuries) were eliminated by order of the King of Portugal who feared competition from Brazilian production [3]. Until recently, the olive tree cultivation and olive oil production were practically nonexistent in Brazil. Some initiatives were taken in the last decade to encourage the production of olive oil in Brazil, but currently, domestic demand is almost met by imported oil, especially from Spain and Portugal (about 90 % of total importation) and Argentina [4–6].

2.2. Current import and export scenario

The production of olive oil is focused on Mediterranean climate countries, which produce around 3.0 million tons of oil per year [7]. The three main olive-producing countries are Spain, Italy, and Greece. In the South American continent, the main production is from Argentina and Chile, both of which produce around 0.7 % of global production [7]. These countries are emerging as promising competitors in this market.

http://dx.doi.org/10.5772/64539

The Brazilian domestic demand is met by imported oils, with Brazil being one of the world's leading importers [7, 8]. Brazilian imports of olive oil are about 73,000 tons [7], and the state of São Paulo is responsible for more than 50 % of the imported volume [6]. The culinary habits of the Brazilian people, especially from the state of São Paulo, had a great influence on the colonizers (from Portugal) and the Italian and Spanish immigrants, and thus the use of olive oil in cooking is greatly appreciated. In the last decade, there was a significant increase in olive oil importation in Brazil (500 %) [8]. The following factors contributed for importation increase: the entry of more affordable products in the domestic market, the increase of the purchasing power of Brazilian social classes, and more information about the health benefits of olive oil in the diet [4]. Brazil is the world's third largest importer of oil, after the United States and the European Union, but the consumption per capita in Brazil is about 0.3 kg/habitant/year. This is very low when compared with countries such as Spain and Italy, where consumption is about 20 kg/habitant/year [7].

3. Regulatory aspects

3.1. Olive oil categories

Oils of different grades and quality are obtained from the fruit of the olive tree [8]. The olive oil of the best quality is known as "extra-virgin" and is obtained from the first cold pressing, from healthy and fresh fruit. Other olive oil flavors and good taste quality, but with more acidity values, are classified as virgin olive oil. Lower quality categories include refined olive oil and olive oil, i.e., a mixture of virgin and refined olive oil. Lampante virgin olive oil is unfit for human consumption as it presents an undesirable flavor and aroma originating from poor-quality olives. Olive pomace oil (*orujo or pomace oil*) is obtained by solvent extraction of the olive-processing residue. Both lampante and olive pomace oil should be refined to become fit for human consumption.

The categories of olive and olive pomace oils are differentiated according to the raw material, the process of obtaining the oil, and other technological procedures applied. Defining each type of oil is a difficult task and often requires the implementation of a wide variety of analytical tests. Differentiating between types was studied and standards established with limits for different identities and quality parameters [9, 10].

The *Codex Alimentarius* presents a standard for olive oil and olive pomace with minimum levels of product purity and quality criteria for each category [11, 12]. Also, hygiene standards are established, along with packaging and labeling, as well as the application of recommendations of certain analytical methods [11].

In addition, olive oil is the only product in the oil and fat sector that has its own international trade agreement. The International Olive Council (IOC) is the intergovernmental organization responsible for the administration of this agreement. Purity criteria stipulated in the IOC trade standard for olive oil and olive pomace oil are constantly being discussed and are heeded, in most cases, in the review of Codex standards for products intended for human consumption [12].

3.2. Identity and quality parameters

The physical and chemical properties of oils and fats, which define the identity of the oil, are mainly related to the predominant molecular structures. A wide range of fatty acids (FA) constitute triglycerides (TAG). The desmethylsterols, which is in the minority fraction of the oil (unsaponifiable), also exhibit profiles themselves for each oil and are an excellent parameter for identifying vegetable oil [9, 10, 13]. The classical indices such as iodine, refraction, and saponification are related to the fatty acid composition ranges and exhibit characteristics for each different oil [9, 10].

The analytical determinations that differentiate olive oil quality categories and olive pomace oil are based on the identification or dosage of chemicals, which may characterize the process of maturation of the olives; extraction, storage, and deterioration of the oil; or other technological processes to which the oil was subjected. Parameters such as acidity index, peroxide value, specific extinction (232 and 270 nm), and impurities (insoluble matter, unsaponifiable matter, moisture) are evaluated to monitor the quality of olive oil. The contents of trans-fatty acids, stigmastadienes, wax, and alkyl esters (fatty acid methyl ester and ethyl ester) are also indicatives of olive oil quality [9, 10].

4. Legislation, control, and inspection in Brazil

Currently, control and inspection of the oil obtained from olives, sold in Brazil, include actions both from the Brazilian Health Ministry (MS) and the Brazilian Agriculture and Livestock Ministry (MAPA). The actions are supported by compatible and complementary laws.

By the late 1990s, before the creation of the Brazilian National Health Surveillance System and the National Health Surveillance Agency (ANVISA), the purity and quality standards of oils and fats were established under the MS. From the creation of the vegetable classification law of MAPA [14], it has the legal obligation to inspect and supervise the entire production chain, besides performing supervision techniques to ensure compliance with the requirements of official classification standards. In light of this legal requirement, the official standard classification of olive oil and olive pomace oil was elaborated. The preparation of the official standard for olive oil and olive pomace oil had the participation of researchers and technicians from the Brazilian MS and MAPA, universities, representatives of the productive sector, and consumer protection organizations. This joint work culminated in the publication of Normative Instruction no. 1, on 30/01/2012 [15]. This is the Brazilian Technical Regulation of Olive Oil and Pomace Olive Oil with the tolerance limits for various parameters of purity and quality. This document is based on trade standards of IOC and *Codex Alimentarius* standards. The official classification standard of the olive oil and olive pomace oil sold in Brazil was also established. **Tables 1–5** (Appendix) present the classification (group and type), quality requirements, sensory characteristics, and other complementary parameters of olive oil and olive pomace oil [15].

Currently, the MAPA challenge is the establishment of a collaborative network for analyzing oil coming into the country. On the other hand, under the Brazilian Health Ministry, the actions

http://dx.doi.org/10.5772/64539

of sanitary supervision together with the public health network laboratory are already a well-established practice. Resolution 270/05 of the National Health Surveillance Agency (ANVISA) [16] is a technical sanitary regulation for vegetable oils, which must be met in health inspections. It came into effect before the Normative Instruction No. 1 of MAPA and emphasizes the health risk aspect. Currently, the Normative Instruction No. 1 of the MAPA as the resolution 270/05 must be met in enforcement actions in Brazil.

Considering the limits of some parameters, such as alpha-linolenic acid (18:3 n3) and campesterol, adopted in the standards for olive oil and pomace oil, some observations must be made. Several studies have demonstrated that limitations established in IOC standards on the alpha-linolenic acid (18:3 n3) and campesterol contents are restrictive and do not reflect the variability among cultivars grown in other areas outside the Mediterranean region [13]. Mailer [17] published a study showing that Australian olive oil can present the range for alpha-linolenic acid from 0.42 to 1.91 %. For this component, oils exceeded the maximum linolenic acid level of 1.0 % recommended by the IOC. In current revision of *Codex Alimentarius* standard of olive oil and olive pomace oil, the limit for alpha-linolenic acid (18:3 n3) was not established due the aspects discussed above [11].

Campesterol content is a valuable tool to detect adulteration with commodity oil. The concentration of this desmethylsterol in many commodity oils, such as sunflower or soybean oils, is higher than in olive oil [18]. The IOC trade standard includes a decision tree for evaluating the authenticity of samples which show campesterol concentrations between 4.0 and 4.5% of total sterols. This includes the following conditions to consider the authenticity of an olive oil: stigmasterol contents ≤ 1.4 % and delta-7 stigmastenol < 0.3 % [12].

Brazilian law adopted the limits of the IOC trade standard of 1 % for alpha-linolenic acid content (18:3 n3) and ≤4.0 % for campesterol to prevent practices of adulteration with, for example, soybean oil, which is a commodity with low commercial value in Brazil [15].

Some discrepancies may be noted for some parameters in Brazilian olive oil and olive pomace standard **(Tables 1–5 - Appendix)**, in comparison with IOC provisions [12]. For example, the limit of stigmastadiene content in virgin and extra-virgin olive oil is 0.15 (mg/kg) established in Brazil. In current IOC standard, it is different and more restrict, i.e., in extra-virgin olive oil and virgin olive oil, the contents must be less than 0.05 and in ordinary virgin olive oil less than 0.10 [12, 15]. These differences will likely be resolved in the future with the improvement of the Brazilian technical regulation for olive oil and olive pomace oil.

4.1. Monitoring of commercial olive oil at the Adolfo Lutz Institute

The Adolfo Lutz Institute was founded in 1940 and has operated since its foundation as a research government institute and also as a public health laboratory in the state of São Paulo. It is part of the Brazilian Public Health Laboratory Network, working in epidemiological surveillance in the study and diagnosis of diseases such as dengue, Zika, AIDS, hepatitis, and meningitis, among others. The laboratory also gives support to health surveillance, monitoring the quality of food samples including commercial olive oils.

Olive oil, among the food products analyzed at the Adolfo Lutz Institute, is one most frequently subjected to allegations of fraud and adulteration. According to the rules of the Brazilian Ministry of Agriculture and Livestock, the olive oil to be marketed in the country, either in bulk or bottled in the country of origin, must obtain a classification document [15]. The inspection for correct classification is done by MAPA inspectors. The inspection in retail is performed by inspectors either from the Brazilian Health Ministry or the Brazilian Ministry of Agriculture and Livestock to verify the adequacy of identity and quality. According to Brazilian Federal Law 986/69 [19] and Resolution No. 22 of 03/15/00, ANVISA [20], the producer or importer of olive oil is obliged to inform the municipal health surveillance at the beginning of importation or marketing and arrange the collection of the sample for analysis. The analyses are carried out in official laboratories. The Adolfo Lutz Institute is the health laboratory of the state of São Paulo. Companies that manufacture, sell, offer for sale, or otherwise deliver to the consumer corrupted, adulterated, counterfeit, altered, or damaged food will incur in a sanitary infraction subject to the penalties of the law [19].

The exceptional sensory and nutritional attributes of olive oil and its limited production are among the aspects that give it high market value. Olive oil trade in Brazil has always been, and continues to be, marked by evidence of fraud and adulteration. So it has long been of interest to researchers of the Adolfo Lutz Institute to increase the scientific knowledge of this product to improve quality control.

Some studies were published in the 1970s and 1980s either by researchers of the Adolfo Lutz Institute, universities, or other scientific institutions [21–23]. These studies indicated frauds in olive oil, by adding commodity oils such as soybean, coconut, and babassu. The classic index and the fatty acid profile detected the adulteration. At this time the gas chromatographic technique to analyze fatty acid methyl esters began to be implemented in institutions. However, only in the early 1990s, at the Adolfo Lutz Institute, a more comprehensive study was developed focusing in detail on the different categories, the characteristics of identity and quality of olive oil, and the wide range of analytical tests designed to differentiate them. The physical-chemical, analytical, and regulatory aspects were addressed to differentiate the various types of oils from olives. A special emphasis was given to spectrophotometric techniques and derivation of the ultraviolet spectra obtained from vegetable oils [9].

At the beginning of the 1980s, the Brazilian trade market was opened with the Mercosur countries (Southern Cone Market), which intensified the import of Argentinian olive oil to the local market. A study carried out at the end of the 1990s, in the Adolfo Lutz Institute laboratories, evaluated the quality and identity of 23 oil brands in the trade of São Paulo, 13 from Europe and bottled in the country of origin, and 10 Argentine oils, with 5 bottled in Brazil. Five samples were adulterated with other vegetable oils, and two of them (bottled in Brazil) were probably adulterated with partially hydrogenated oil. This finding illustrated the importance of the details of the composition of fatty acids of vegetable oil, taking the geometric isomers into consideration, to help detect an uncommon type of adulteration [24].

http://dx.doi.org/10.5772/64539

In the period 2001–2014, the proportion of frauds or adulterations verified in samples declared as olive oil and analyzed at the Adolfo Lutz Institute was higher than in the period before (**Figure 1**). An improvement in controlling analysis in Adolfo Lutz Institute, with the implementation of new analytical methodologies, was observed in this period. A study published in 2008 with 15 samples showed that although the samples presented the fatty acid profile of authentic olive oil, the analysis of the difference of ECN 42 showed adulteration of samples with edible commodity vegetable oils. The oils were added in low quantity (less than 3 %) and were rich in linoleic acid (18:2 9,12 *cc*), such as soybean, maize, or sunflower oil [25]. In addition, in another study more elaborate fraud was evidenced, i.e., the replacement of virgin olive oil by probably pomace oil. The evidence was generated by a combination of the analysis of trans-fatty acids, stigmatadiene content, specific extinction at 270 nm, and the difference of ECN 42 [26].

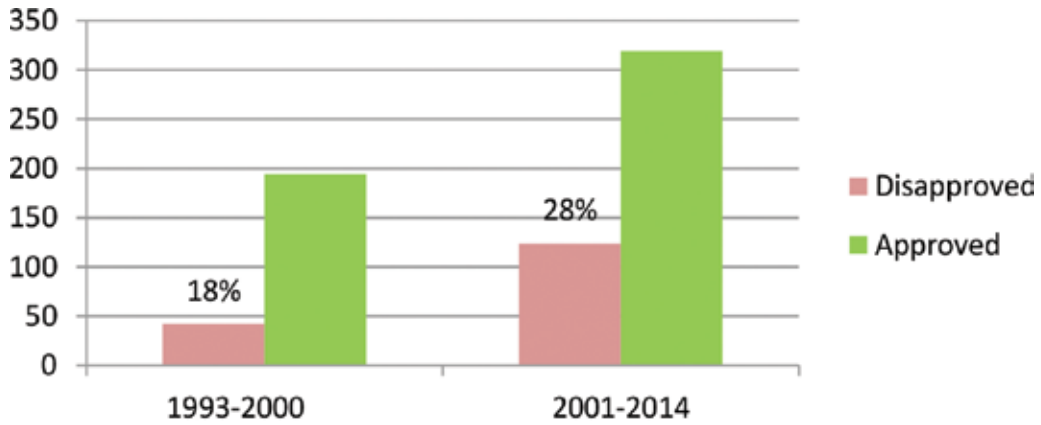


Figure 1. Commercial olive oil samples analyzed at Adolfo Lutz Institute.

The latest study of monitoring carried out at the Adolfo Lutz Institute took place between the years 2012 and 2014. Fifty-four samples of 14 different brands were analyzed. Twenty-five samples were sent by sanitary surveillance of the state of São Paulo, to attend a program of the National Health Surveillance Agency (ANVISA). Of the total, 38 were declared as extra-virgin olive oil and the others as olive oil. The parameters studied were composition of fatty acids, acid value, peroxide index, and specific extinction at 270 nm. The adequacy of nutrition labeling was also verified. Since 2003, Brazil has adopted mandatory nutrition information on the label of packaged foods as a strategy to prevent chronic disease [27]. Nutrients required on the label of edible vegetable oils are total fat, saturated and trans-fatty acids, but the producers have also declared monounsaturated and polyunsaturated fatty acids. Twenty-eight samples (52 %) of 11 brands showed no characteristic olive oil profile of fatty acids. Twenty-four samples that were adulterated probably with soybean oil were declared as extra-virgin olive oil. Thirty-one samples (57 %) had monounsaturated fatty acid and/or polyunsaturated fatty acid contents varying by more than 20 % from the declaration on the label. All the adulterated samples were bottled in Brazil, highlighting the need for tighter control in the trade

and distribution of the product with a view to a more secure product which is increasingly consumed by the population

5. Technical support of the International Olive Council for analytical improvement

In 2000, during a technical visit by IOC representatives to Brazil and, in particular, to the Adolfo Lutz Institute, a technical cooperation agreement was made that enabled the inclusion of the laboratory in the annual proficiency-testing scheme organized by the IOC. Since 2002, the institute has received annually samples of the rounds for vegetable oils, which include several determinations that help in ensuring the analytical quality of various tests performed by the laboratory. During this period it was possible to implement some methods in the laboratory such as stigmatadiene content and the difference of ECN 42. In addition, it was possible to monitor the quality of the results generated in these trials and routine testing such as the composition of fatty acids, including trans-fatty acids, acidity index, peroxides, humidity, impurities, absorbency in ultraviolet at 232 and 270 nm and Delta K, tocopherol contents, unsaponifiable matter, and others.

6. Final considerations

Edible olive oil is greatly appreciated by the Brazilian population. Knowledge of Brazilians about the health benefits for olive oil consumption and about the different levels of quality of this product is increasing. The Brazilian position as the world's third largest importer of olive oil, combined with a history of fraudulent practices in this product, has led the Brazilian government to implement its legislation bringing an increase perspective to improve the product inspection. However, there are many technical challenges to be overcome, including the structuring of a national network with effective provisions for inspection and supervision, supported by qualified laboratories in the control of this product.

Appendix

Normative Instruction No. 1, 30/01/2012

Brazilian Ministry of Agriculture and Livestock—MAPA

Classification, quality requirements, sensory characteristics, and other complementary parameters of olive oil and olive pomace oil (**Tables 1–5**)

http://dx.doi.org/10.5772/64539

Group	Virgin olive oil			Olive oil	Refined olive oil	Pomace olive oil	Refined pomace olive oil
Type	**Extra-virgin**	**Virgin**	**Lampante**	**Unique**	**Unique**	**Unique**	**Unique**
Acid value (%)	≤0.80	≤2.00	<2.00	≤1.00	≤0.30	≤1.00	≤0.30
Peroxide value (mEqv/kg)	≤20		(*)	≤15	≤5	≤15	≤5
E							
270	≤0.22	≤0.25	(*)	≤0.90	≤1.10	≤1.70	≤2.00
DK	≤0.01		(*)	(*)	≤0.16	≤0.18	≤0.20
232	≤2.5	≤2.6	(*)	(*)	(*)	(*)	(*)

(*) Not applied
E: specific extinction in the ultraviolet

Table 1. Limits of olive oil and olive pomace oil quality parameters.

Group	Virgin olive oil			Olive oil	Refined olive oil	Pomace olive oil	Refined pomace olive oil
Type	**Extra-virgin**	**Virgin**	**Lampante(**)**	**Unique**	**Unique**	**Unique**	**Unique**
Median of the defect (Md)	0	>0 and ≤3.5	>3.5	(*)	(*)	(*)	(*)
Median of the fruity (Mf)	>0	>0	0	(*)	(*)	(*)	(*)

(*) Not applied
(**) Lampante virgin olive oil when obtained median defect (Md) of less than 3.5 and median fruity (Mf) zero

Table 2. Limits of the sensory characteristics of virgin olive oi.l

Group	Virgin olive oil			Olive oil	Refined olive oil	Pomace olive oil	Refined pomace oil
Type	**Extra-virgin**	**Virgin**	**Lampante**	**Unique**	**Unique**	**Unique**	**Unique**
Stigmastadiene (mg/kg)	≤0.15		≤0.5			(*)	
Wax (mg/kg)	≤250		≤300	≤350		>350	
ECN 42 difference	≤0.2		≤0.3	≤ 0.3		≤0.5	
Fatty acid composition (%) (**)							
C18:1t	≤0.05		≤0.10	≤0.20		≤0.40	
C18:2t + C18:3t	≤0.05		≤0.10	≤0.30		≤0.35	
C14:0				≤0.05			

Group	Virgin olive oil			Olive oil	Refined olive oil	Pomace olive oil	Refined pomace oil
Type	**Extra-virgin**	**Virgin**	**Lampante**	**Unique**	**Unique**	**Unique**	**Unique**
C16:0			7.50–20.0				
C16:1			0.3–3.5				
C17:0			≤0.3				
C17:1			≤0.3				
C18:0			0.5–5.0				
C18:1			55.0–83.0				
C18:2			3.5–21.0				
C18:3			≤1.0				
C20:0			≤0.6				
C20:1			≤0.4				
C22:0			≤0.2			≤0.3	
C24:0			≤0.2				

(*) Not applied; (**) percentage of total fatty acids

Table 3. Limits of olive oil and olive pomace oil complementary parameters.

Group	Virgin olive oil			Olive oil	Refined olive oil	Pomace olive oil	Refined pomace oil
Type	**Extra-virgin**	**Virgin**	**Lampante**	**Unique**	**Unique**	**Unique**	**Unique**
Desmethylsterols composition (%) (*)							
Cholesterol			≤0.5				
Campesterol			≤4.0				
Stigmasterol			Less than campesterol				
Brassicasterol			≤0.1				≤0.2
Beta-sitosterol + delta-5,23-estigmastadienol + clerosterol + beta-Sitostanol + delta-5-Avenasterol + delta-5,24-Estigmastadienol.			≥93.0				
Δ-7-Stigmastenol			≤0.5				
Erythrodiol and uvaol (**)			≤4.5				>4.5
Total sterols (mg/kg)			≥1.000			≥1,600	≥1,800

(*) Percentage of total desmethylsterols
(**) Olive oil with a wax content between 300 and 350 mg/kg is considered lampante virgin olive oil if the total aliphatic alcohol content is less than or equal to 350 mg/kg or the percentage of erythrodiol and uvaol is less than or equal to 3.5.

Table 4. Limits of olive oil and olive pomace oil complementary parameters.

Group	Virgin olive oil			Olive oil	Refined olive oil	Pomace olive oil	Refined pomace olive oil
Type	**Extra-virgin**	**Virgin**	**Lampante**	**Unique**	**Unique**	**Unique**	**Unique**
FAME + FAEE < 75 mg/kg or 75 mg/kg < FAME + FAEE < 150 mg/kg and FAEE/FAME < 1,5		(*)	(*)	(*)	(*)	(*)	(*)
Refractive index (20 °C)		1.4677–1.4705				1.4680–1.4707	
Saponification index (mg KOH/g)		184–196				182–193	
Moisture and volatile matter (%)		≤0.2				≤0.1	
Unsaponifiable matter (g/kg)		≤15				≤30	
Iodine index (Wijs)		75–94				75–92	
Arsenium (mg/kg)				<0.1			
Lead (mg/kg)				<0.1			
Iron (mg/kg)				≤3			
Copper (mg/kg)				≤0.1			

(*) Not applied; FAME, fatty acid methyl ester; FAEE, fatty acid ethyl ester

Table 5. Limits of olive oil and olive pomace oil other parameters.

Author details

Sabria Aued-Pimentel

Address all correspondence to: spimente@ial.sp.gov.br

Adolfo Lutz Institute, São Paulo, Brazil

References

[1] Gracian J. The chemistry and analysis of olive oil. In: Boekenoogen HA. Analysis and characterization of oils, fats and fat products; London: Interciense Publ 1968. v.2, p. 317-591.

[2] Peixoto AR. Olive tree. In: Oleaginous plants tree. São Paulo: Nobel; 1973. p. 9–54.

[3] Pimentel Gomes. The culture of olive in Brazil. São Paulo: Melhoramentos; 1979. 208 p

[4] Bertoncini EI. Cultivation of olive trees in the state of São Paulo [Internet]. 2015. Available from: http://www.aptaregional.sp.gov.br/acesse-os-artigos-pesquisa-e-

tecnologia/edicao-2012/julho-dezembro-2/1214-cultivo-de-oliveiras-no-estado-de-sao-paulo/file.html. [Accessed in: 2015.11 15]. http://ainfo.cnptia.embrapa.br/digital/bitstream/item/46574/1/sistema-16.pdf. [Accessed in: 2016 02 12].

[5] Silva LFO, Oliveira AF, PioI R, Alves TC, Zambon CQ. Quality variation of olive oil in olive cultivars. Bragantia 2012; 71:202-09 DOI: 10.1590/S0006-87052012000200008

[6] Empresa Brasileira de Pesquisa Agropecuária. Olive culture (Olea europaea L.). [Internet]. 2009. Available from: http://ainfo.cnptia.embrapa.br/digital/bitstream/item/46574/1/sistema-16.pdf. [Accessed in: 2016 02 12].

[7] Key-figures on the world market for olive oils. Data adopted at the 102nd session of the IOOC (Madrid, Spain), 24–26 November 2014. Available from: http://www.mercacei.com/pdf/Balance.Olive.Oil.2014.pdf. [Accessed in: 2015.11.15].

[8] Barjol JL. Olive oil: trends in quality and product category shares. In: Workshop Authentication of olive oil [Internet]. 2013. Available from: http://ec.europa.eu/agriculture/events/2013/olive-oil-workshop/proceedings_en.pdf. [Accessed in: 2014.01.07].

[9] Aparicio R, Harwood J. Olive oil manual. 1nd Ed. In: Aparicio R. Authentication olive oil 14, 475–496. Madri: Ediciones y Mundi-prensa; 2003.

[10] Boskou D. Olive oil: chemistry and technology. 2nd ed Champaign: AOCS Press; 2006.

[11] Codex Alimentarius (FAO/WHO). Codex standards for olive oils, and olive pomace oils, Codex Stan 33, Adopted in 1981. Revision: 1989, 2003, 2015. Amendment: 2009, 2013 [Internet]. 2015. Available from: file:///C:/Users/Celso/Downloads/CXS_033e_2015.pdf [Accessed in: 2016.02.07].

[12] International Olive Oil Council. Trade Standard Applying to Olive Oil and Olive-Pomace Oil [Internet]. 2015. Available from: http://www.internationaloliveoil.org/estaticos/view/222-standards. [Accessed in: 2016.02.07].

[13] Srigley CT, Oles CJ, Kia ARF, Mossoba MM. Authenticity assessment of extra virgin olive oil: evaluation of desmethylsterols and triterpene dialcohols. Journal of the American Oil Chemists' Society 2016; 93: 171-181. DOI 10.1007/s11746-015-2759-4.

[14] Ministério da Agricultura e Abastecimento. Lei 9,972 de 25 de maio de 2000.[Internet]. Available from: http://www.planalto.gov.br/ccivil_03/leis/L9972.htm. [Accessed in: 2016.02.07].

[15] Ministry of Agriculture, Livestock and Supply, Normative Instruction MAPA n° 1 30/01/2012 [internet]. Available from: http://www.azeiteonline.com.br/wp-content/uploads/2012/02/INSTRU%C3%87%C3%83O-NORMATIVA-N%C2%BA-1-DE-30-DE-JANEIRO-DE-2012_MAPA.pdf. [Accessed in: 2016 02 07].

[16] Ministério da Saúde. Resolução n° 270/05 da Diretoria Colegiada da Agência Nacional de Vigilância Sanitária [internet]. Available from: http://portal.anvisa.gov.br/wps/wcm/

connect/82d8d2804a9b68849647d64600696f00/RDC_n_270.pdf?MOD=AJPERES. [Accessed in 2016.02.07].

[17] Mailer, R J. Variation in oil quality and fatty acid composition in Australian olive oil. Aust J Exp Agric 2005; 45:115–119. DOI 10.1071/EA04045.

[18] Aparicio R, Morales M, Aparicio-Ruiz R, Tena N, Garcia-Gonzalez D. Authenticity of olive oil: mapping and comparing official methods and promising alternatives. Food Res Int 2013; 54:2025–2038. DOI: 10.1016/j.foodres.2013.07.039

[19] Ministry of the Navy. Department of the Army. Ministry of Military Aeronautics. Decree-Law 986 of October 21, 1969 [Internet]. Available from: http://www.planalto.go.br/ccivil_03/Decreto-Lei/Del0986.htm. [Accessed in 2016 02 07].

[20] Ministério da Saúde. Resolução n° 22 de 15 de março de 2000. ANVISA [Internet]. Available from: http://portal.anvisa.gov.br/wps/wcm/connect/b6059200474581528da7dd3fbc4c6735/RESOLUCAO_22_2000.pdf?MOD=AJPERES. [Accessed in: 2016.02.07].

[21] Badolato ESG, Durante F, Almeida MEW, Silveira NV. Óleo de oliva – avaliação de sua qualidade. Rev Inst Adolfo Lutz 1981; 41:63-70.

[22] Szpiz RR, Pereira DA, Jablonka FH Avaliação de óleos comestíveis comercializados no Rio de Janeiro. Rio de Janeiro, EMBRAPA-CTAA, 1985. (EMBRAPA-CTAA Boletim de Pesquisa, 13).

[23] Soares LV, Amaya DA. Identificação e quantificação de adulterantes em óleo de oliva por cromatográfia gasosa. Bol SBCTA 1981; 15:1-17.

[24] Aued-Pimentel et al. Multivariate analysis applied to quality assessment of olive oil commercialized in São Paulo city, Brazil. In: Koseoglu SS, Rhee KC, Wilson RF. Eds. Proceedings of the World Conference on Oilseed and Edible Oils Processing. 205-211, 1996. Champaign: AOCS Press.

[25] Aued-Pimentel S, Takemoto E, Kumagai EE, Cano CB. Determinação da diferença entre o valor real e o teórico do triglicerídeo ecn 42 para a detecção de adulteração em azeites de oliva comercializados no Brasil. Quim Nova 2008; 31: 31-34. DOI: 10.1590/S0100-40422008000100006.

[26] Aued-Pimentel S, Silva AS, Takemoto E, Cano CB. Stigmastadiene and specific extinction (270 nm) to evaluate the presence of refined oils in virgin olive oil commercialized in Brazil. Food Sci Technol 2013; 33: 479-84. DOI: 10.1590/S0101-20612013005000067.

[27] Ministério da Saúde. Resolução RDC n° 360, de 23 de dezembro de 2003 [internet]. Availible from: http://portal.anvisa.gov.br/wps/wcm/connect/ec3966804ac02cf1962abfa337abae9d/Resolucao_RDC_n_360de_23_de_dezembro_de_2003.pdf?MOD=AJPERES. [Accessed in: 2015.04.15].

Chapter 17

Tocopherols: Chemical Structure, Bioactivity, and Variability in Croatian Virgin Olive Oils

Maja Jukić Špika , Klara Kraljić and Dubravka Škevin

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64658

Abstract

Virgin olive oil (VOO) represents a rich source of natural antioxidants, with tocopherols as the most effective group of lipophilic, phenolic antioxidants. α-Tocopherol represents more than 95% of the total tocopherols in virgin olive oil, and it possesses the highest biological activity among members of the vitamin E family. Content and composition of the tocopherols of virgin olive oil depend on several agronomic factors, as well as olive processing and oil storage conditions.

In this chapter, the tocopherol homologue activity in virgin olive oil and the biological importance are discussed. Research work is reported on the tocopherol content and composition variability in virgin olive oils of the most widespread Croatian cultivar "Oblica" and Italian cultivar "Leccino." Factors studied such as year, growing area and olive fruit ripening and their influence on the tocopherol content and composition of virgin olive oils are discussed. The effect of filtration of the oil and storage conditions on tocopherols are also examined.

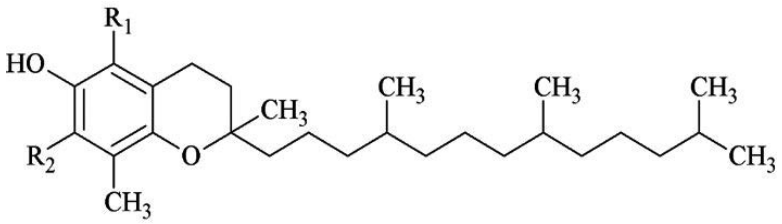
Keywords: tocopherols, structure, antioxidant activity, concentration variability, virgin olive oil

1. Introduction

1.1. Structure variation and biological activity of virgin olive oil tocopherols

Tocopherols are the natural antioxidants synthesized at various levels and in different combinations by all plant tissues. They are amphipathic molecules with the polar chromanol ring and hydrophobic saturated side chain. The general structure of tocopherols is shown in

Figure 1. The four homologues, α-, β-, γ-, and δ-tocopherol, differ in the number and position of methyl groups in the aromatic ring [1].



Tocopherol	R_1	R_2
α-	CH_3	CH_3
β-	CH_3	H
γ-	H	CH_3
δ-	H	H

Figure 1. Tocopherol structures. The table indicates the number and position of methyl groups on the aromatic ring.

Tocopherols act as antioxidants by scavenging peroxy radicals of polyunsaturated fatty acids or by reacting with singlet oxygen and other reactive oxygen species (ROS). ROS quenching occurs by charge transfer mechanism. Termination of polyunsaturated fatty acids oxidation chain reactions is achieved by donation of a hydrogen atom from the hydroxyl group on the chromanol ring resulting in a "tocopherol radical" formation. The tocopherol radicals are resonance stabilized within the chromanol ring and do not propagate the chain reactions or are rapidly recycled back to the corresponding tocopherol, allowing each tocopherol to participate in many peroxidation chain-breaking events. One tocopherol molecule can protect about 10^3–10^8 polyunsaturated fatty acids at low peroxide values [1, 2]. Tocopherol homologues possess different antioxidant activity as a result of their structural differences. The antioxidant activity of tocopherol homologues decreases in the order δ > β > γ > α *in vitro*, while *in vivo*, that is vitamin E activity, decreased in order α > β > γ > δ [3]. Although there is no significant difference in the absorption of tocopherols from the gastrointestinal tract, the highest vitamin E activity of α-tocopherol can be explained by its preferential retention and incorporation into lipoproteins by the hepatic α-tocopherol transfer protein, occurring in higher plasma and tissue level [1, 4].

The main role of tocopherols is the protection of lipids from peroxidation. Therefore, they are abundantly found in plant-based food, but vegetable oils are considered to be the best source of tocopherol in nutrition [3]. Their content and composition mostly depend on the type of oil [2, 5]. Tocopherol content of virgin olive oils (VOOs) varies from 97 to 785 mg/kg. Despite differences in the concentration of total tocopherols that can be attributed to agronomical,

http://dx.doi.org/10.5772/64658

geographical, and technological factors, α-tocopherol is the dominant homologue in virgin olive oils making more than 90% of total tocopherols. γ-Tocopherol is in virgin olive oils present in low amounts (<10%), and β-tocopherol is present only in trace amounts [6–8].

Tocopherols possess high antioxidative activity; this makes them important components in cardiovascular disease and cancer prevention [9, 10, 11]. Furthermore, tocopherols appear to act synergistically with other antioxidants [12]. This indicates that intake of tocopherol in the form of food like virgin olive oil, which is rich in other natural antioxidants such as biophenols and carotenes, might improve their efficiency.

Considering the importance of tocopherols in daily nutrition and disease prevention, it is important to know and examine the influence of certain factors on their content and composition.

2. Varieties grown in Croatia

2.1. Plant material and growing areas selected

Cultivars "Oblica" and "Leccino," in three consecutive years (2010, 2011, and 2012), grown at two different locations in Dalmatia (Kaštela and Šestanovac), Croatia, were studied. The fruit samples were hand-harvested from the olive trees, at biweekly intervals, from the end of September till mid-November. An aliquot of 100 fruits was taken from each fruit sample to determine ripening index which is based on evaluation of the olive skin and pulp color [13]. The olive fruits were processed by centrifugal extraction using an Abencor laboratory oil mill (mc2, Ingenierias y Sistemas, Seville, Spain) within 24 h after the harvesting. Tocopherol content and composition were determined in all produced virgin olive oils using standard method (ISO 9936:2006) with normal-phase HPLC analysis. Total tocopherols were calculated as a sum of the concentration of the individual tocopherols. Results are given in milligrams of tocopherol per kg of oil.

In order to study the effect of cultivar, year, growing area location, and fruit ripening index on the tocopherol content in olive oil samples, factorial ANOVA was performed (Fisher's F-test), followed by Tukey"s honest significant difference (HSD) test. A significance level of $p \leq 0.05$ was applied. The correlation coefficients (r) determined by Pearson correlation matrix were used to define the influence of climatic conditions during the year on the VOOs tocopherol content. The obtained data were analyzed using Statistica software version 11.0 (StatSoft, Inc., USA, 2012).

Croatia is a fringe growing area of olive trees cultivation. Selected growing area locations are from different olive growing subregions. Kaštela is located at 28 m above sea level and influenced by Mediterranean climate, while Šestanovac is located at 358 m influenced by continental climate in winter and the Mediterranean climate in summer period.

The monthly mean values of temperature and rainfalls registered for studied years at selected locations were obtained from weather stations (Meteorological and Hydrological Service of Croatia) (**Figures 2** and **3**).

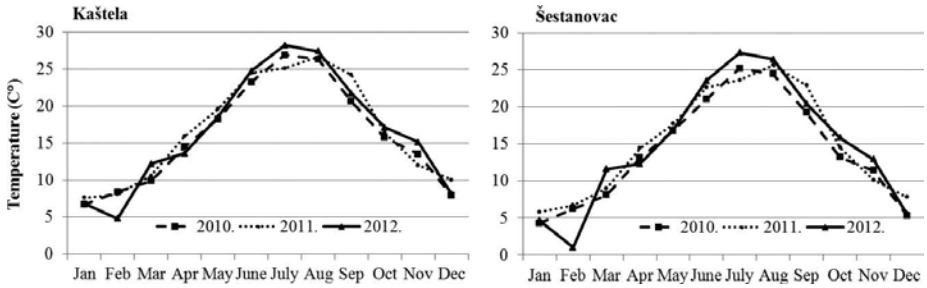


Figure 2. Microclimate temperature parameters (°C) measured for two olive growing areas in the year 2010, 2011, and 2012.

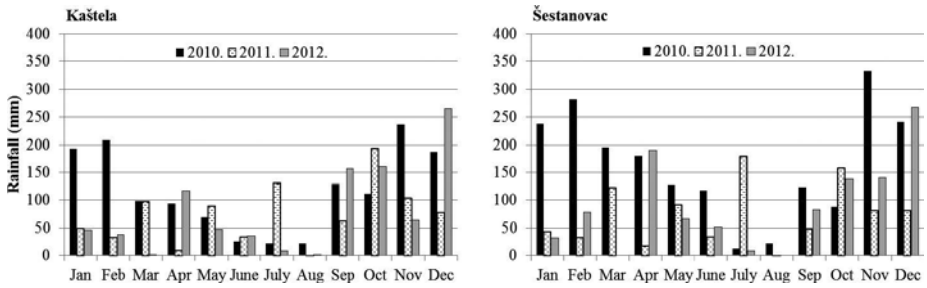


Figure 3. Monthly rainfall (mm) for two olive growing areas in the year 2010, 2011, and 2012.

Year 2010 was recorded as the highest rainfall year and with lowest average daily air temperature (**Figures 1** and **2**). During olives intense growth and ripening (July-November), higher mean daily temperatures were recorded in 2012, compared to overall 3 years of research, while 2011 is the year with the lowest rainfall. Kaštela could be considered as the more drought affected and warmer growing area.

2.2. The influence of the cultivar on tocopherol content and composition

The quality of virgin olive oil is influenced by several factors, but the olive cultivar stands out as the most important one [14]. The content of tocopherols in the virgin olive oils varies from 97 to 785 mg/kg [6–8], from 163 to 510 mg/kg in the Spanish oils [8], 98–370 mg/kg in the Greek oils [14], 97–403 mg/kg in oils from Turkey [6], 120–478 mg/kg in oils from Tunisia [15], and 138–298 mg/kg in the Portuguese oils [16].

Tocopherol content of studied cultivars is presented in **Figures 4–6**. Cultivar shows a significant impact on the content of the α-, γ-, and total tocopherols. "Oblica" VOOs have a modest total tocopherol content, ranging from 186 to 442 mg/kg. Significantly higher total tocopherol content had varietal oils from "Leccino" with average value of 510 mg/kg (337–784 mg/kg). Higher total tocopherol content in "Leccino" VOOs also was recorded for several different cultivars in the study reported by Tura et al. [17] and Koprivnjak et al. [18]. In "Oblica" and "Leccino" VOO samples δ-tocopherol was not detected.

http://dx.doi.org/10.5772/64658

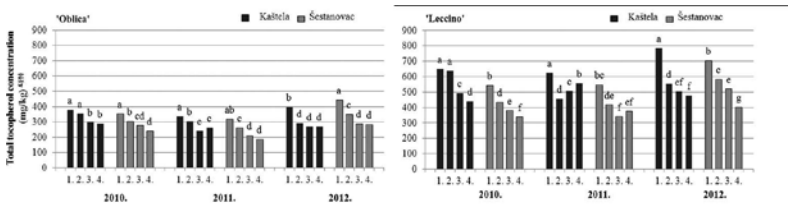


Figure 4. Total tocopherol content (mg/kg) of "Oblica" and "Leccino" virgin olive oils during ripening at two different growing locations in three successive crop years. ¢Cultivar has a significant effect ($p < 0.05$); §year has a significant effect ($p < 0.05$); †growing location has a significant effect ($p < 0.05$); harvest period has a significant effect ($p < 0.05$). The means marked with different letters (within the same cultivation year), labeled with different letters, are significantly different (Tukey's test, $p < 0.05$).

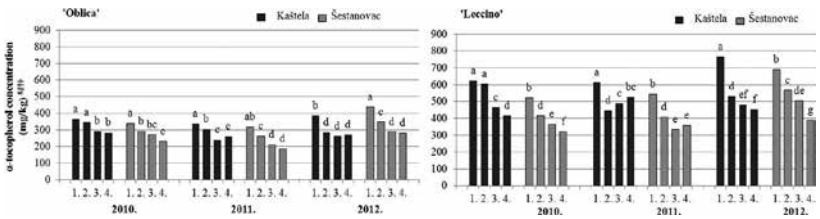


Figure 5. α-Tocopherol content (mg/kg) of "Oblica" and "Leccino" virgin olive oils during ripening at two different growing locations in three successive crop years. ¢Cultivar has a significant effect ($p < 0.05$); §year has a significant effect ($p < 0.05$); †growing location has a significant effect ($p < 0.05$); harvest period has a significant effect ($p < 0.05$). The means marked with different letters (within the same cultivation year), labeled with different letters, are significantly different (Tukey's test, $p < 0.05$).

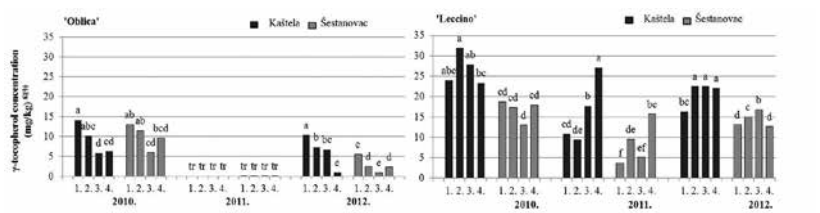


Figure 6. γ-Tocopherol content (mg/kg) of "Oblica" and "Leccino" virgin olive oils during ripening at two different growing locations in three successive crop years. ¢Cultivar has a significant effect ($p < 0.05$); §year has a significant effect ($p < 0.05$); †growing location has a significant effect ($p < 0.05$); harvest period has a significant effect ($p < 0.05$). The means marked with different letters (within the same cultivation year), labeled with different letters, are significantly different (Tukey"s test, $p < 0.05$).

α-Tocopherol comprises more than 97% in all analyzed VOO samples (**Figure 5**). The γ-tocopherol content in "Leccino" oils ranged from 4 to 32 mg/kg (average 17 mg/kg), while the content in "Oblica" oils was significantly lower (**Figure 6**). Average concentration in "Oblica" oils was 7 mg/kg, and values ranged from traces to 14 mg/kg. The γ-tocopherol concentrations obtained in this study for both varieties are within the concentrations reported in different varietal virgin olive oils studies [16, 17, 19].

2.3. Environmental factors and tocopherols of Croatian olive oils

Production geographic area, marked by soil factors, altitude and latitude, and climatic conditions during the year, has a significant impact on the properties and chemical composition of virgin olive oils [7, 17, 20–23]. Studies of environmental factors impact on VOOs chemical composition have quite different results, due to the fact that all these factors together interact and as result varieties behave differently in different agroclimatic conditions.

Mean temperature (°C)					
Parameter					
	July	**August**	**September**	**October**	**November**
α-Tocopherol conc.	0,287*	0,252*	−0.021	0,391*	0,302
γ-Tocopherol conc.	0,059	0,044	0,055	0,114	−0,068
Total tocopherol conc.	0,295*	0,244*	−0,037	0,376*	0,293
Rainfall (mm)					
α-Tocopherol conc.	−0,190	−0,079	0,015	−0,217	0,009
γ-Tocopherol conc.	−0,067	0,252*	0,048	−0,010	−0,329
Total tocopherol conc.	−0,206*	−0,053	0,030	−0,206	−0,002

* Significant difference at $p < 0.05$.

Table 1. Correlation factors of virgin olive oil tocopherol content and a microclimate parameters (mean temperature and rainfall; **Figures 1** and **2**) in the period of olives' intensive growth and ripening.

Year and geographical production area significantly influenced the content of tocopherol in "Oblica" and "Leccino" VOOs (**Figures 4 – 6**). The correlation coefficients of rainfall and mean daily temperature during intense growth and ripening of olive fruit (July-November) with α-, γ-, and total tocopherol content were calculated and presented in **Table 1**. It is evident that the mean daily air temperatures have a significant impact on the α- and total tocopherol content. The highest average α- and total tocopherol content in "Oblica" and "Leccino" VOOs was recorded in year 2012, while in oils from year 2011, concentrations were the lowest of three studied years (α-tocopherol: "Oblica": 302 mg/kg in year 2010, 264 mg/kg in year 2011, 319 mg/kg in year 2012; "Leccino": 467 mg/kg in year 2010, 464 mg/kg in year 2011, 547 mg/kg in year 2012.) (**Figure 5**). A Spanish group of scientists [24] recorded lower tocopherol content in the year with the lowest average air temperature. For the lower α- and total tocopherol content in year 2011, a possible explanation lies in rainfall, due to the fact that tocopherols negatively correlate with rainfall in July (**Figure 2** and **Table 1**). Results are in line with a Beltrán et al. [8] publication according to which the year has a very significant impact on the α- and total tocopherol content in "Picual", "Frantoio", and "Hojiblanca" VOOs, and the highest content was recorded in the year with the lowest rainfall. In the same study, the effect of the year on the γ-tocopherol was not recorded, contrary to our results on the "Oblica" and "Leccino" VOOs (**Table 1**). The γ-tocopherol positively correlates with the rainfall in August. In the year

http://dx.doi.org/10.5772/64658

2010, more rainfall and, as mentioned previously, the highest γ-tocopherol content in oils of the two investigated cultivars were recorded (**Table 1** and **Figure 6**).

Higher α-, γ-, and total tocopherol content had "Oblica" and "Leccino" VOOs obtained from fruits harvested at the growing area of lower altitude (**Figures 4 – 6**). The correlation coefficient results are in accordance with the observation (**Table 1**) that oils from growing area of higher average temperatures synthesize more tocopherols. Similar results were published by Arslan et al. [6] and Aguilera et al. [22]. On the other hand, Tura et al. [17] report that the growing area had no effect on α-, β- and total tocopherol content and that only the γ-tocopherol content was influenced, with the highest content in oils of the highest altitude area.

2.4. Ripening and tocopherols of Croatian olive oils

The fruit ripening is one of the most important factors that lead to changes in the chemical composition of virgin olive oil [25]. The knowledge of varietal oil characteristics, as well as changes that take place through fruit maturation, contributes to the higher quality of VOOs. The genetic composition directly affects the ripening; thus, in each cultivar, different changes in composition of the olive fruit and its virgin olive oils are confirmed [25].

A wide range of α-, γ-, and total tocopherol content was perceived during ripening of "Oblica" and "Leccino" VOOs (**Figures 4 – 6**). Analysis of variance showed a difference in tocopherol content of VOOs derived from olives harvested at different ripening stages.

The total tocopherol content in VOOs decrease with increase in the fruit ripening index from which the oil is produced (**Figure 4**). The average total tocopherol content reduction of about 30% in VOOs from unripe to ripe olives has the same trend as the α-tocopherol; this is expected since the α-tocopherol abudance in VOOs is around 95%. The results are in line with research reported by Matos et al. [16], Bengana et al. [25] and Beltrán et al. [8]. Depending on the olive fruit pigmentation, variations in the total tocopherol content in the "Chetoui" (138–496 mg/kg) and "Chemlali" (224–350 mg/kg) were also noted [15].

In general, the α-tocopherol content decreases during ripening, although a decrease rate of the studied cultivars was not quite equal (**Figure 5**). Lower α-tocopherol content for 25% (Kaštela) and 35% (Šestanovac) in "Oblica" VOOs obtained from ripe olive fruits was recorded in comparison with initial content in oils from the unripe olive fruits. The loss of α-tocopherol is more pronounced in the "Leccino" VOOs of both locations. Decrease of the α-tocopherol through the ripening period has also been reported in other studies [8, 26–28].

The γ-tocopherol content in "Oblica" VOOs decreases as the fruit ripening index increases, although decrease rate was not the same at both studied locations (**Figure 6**). A significant decrease of 70% as average value was recorded in the oils from the Kaštela, while in oils from Šestanovac, an increase in the γ-tocopherol content in oils from ripe olive fruits was observed. This is consistent with Beltrán et al. [8] who have also reported a similar trend for "Frantoio," "Hojiblanca," and "Picual" VOOs. γ-Tocopherol in "Leccino" VOOs was not clearly associated with the increase in olive fruit ripening index (**Figure 6**), what was also reported for virgin olive oils of several different cultivars [15, 16, 19].

3. Filtration and storage: a short literature review

3.1. Filtration

Virgin olive oil after processing is a metastable mixture that can be consumed without refining [29]. From the commercial point of view, apart from major manufacturers and industrial producers who prefer filtered oils, there is an increasing interest of consumers for unfiltered oils which they consider as minimally processed [30]. Suspended substances (cellulose, hemicelluloses, pectin, proteins) can affect the quality by increasing triacylglycerols hydrolysis causing a free fatty acids increase. Therefore, filtration is the process of clarification aimed at a faster process during which the qualitative and quantitative changes in the composition of virgin olive oils may take place. In fact, there is a controversy and there are some confusing comments in relation to "cloudy" and filtered oils.

In the study of six different Spanish and Italian varietal VOOs by Fregapane et al. [31], filtration was not found to cause significant differences in the α-tocopherol content. A new filtration method based on the flow of an inert gas developed and patented by the University of Bologna and Sapio [32] had also no effect on the lipophilic phenols level [33].

3.2. Storage

The overall quality of virgin olive oil decreases over the time as a consequence of oxidative and hydrolytic degradations which also cause the partial loss of other minor constituents having health-promoting effects. Consequently, VOOs is generally consumed within one year from its production [34–36]. As it was mentioned formerly, the main role of tocopherols is the protection of lipids from peroxidation, and according to Aparicio and Luna [37], their contribution accounts for around 11%. Thus, monitoring of tocopherol levels during its shelf life, it is needed.

The degradation rate of the α-tocopherol content during storage under the store shelves conditions was reported by Psomiadou and Tsimidou [38]. Keepability test carried out on five VOOs in conventional storage at room temperature resulted in insignificant α-tocopherol losses in samples kept sealed [39]. On the other hand, in the same study, considerable α-tocopherol losses were observed in samples opened periodically within the period of two years storage as a result of renewal of oxygen supply. Rastrelli et al. [40] investigated storage conditions regarding availability of oxygen in the oil headspace and lighting. These authors reported the loss of tocopherols in VOO samples, stored 12 months in completely filled dark glass bottle, in range from 20 to 25%. Similarly rate of α-tocopherol losses was also reported in storage condition study [7], which is contrariwise almost total loss of α-tocopherol in "Arbequina" oils in same storage conditions (in darkness and at ambient temperature for 12 months) [41]. Under medium temperature at accelerated storage conditions (50°C, 36 weeks), the α-tocopherol loss was much more rapidly in oils stored in open bottles than in close bottles [42]. The research of VOOs storage conditions (room temperature, +4 and −20°C) influences on tocopherol content evidenced α-tocopherol content decreasing trend with storage time [7]. In same study, after 12 months

http://dx.doi.org/10.5772/64658

storage, the highest loss of α-tocopherol was recorded in oils stored at room temperature, among which the oils obtained from unripe olives showed the greatest α-tocopherol stability. Storage at lower temperatures did not always delayed α-tocopherol consumption compared to room temperature.

As it can be seen, different effect of storage conditions on tocopherol content and α-tocopherol degradation rate is shown through literature affecting the amount of tocopherol in oils within the period in which positive chemical and organoleptic properties remain preserved and VOOs are desirable to consume.

4. Conclusions

Research work on the tocopherol content and composition variability in virgin olive oils of Croatian cultivar "Oblica" and Italian cultivar "Leccino" showed that the cultivar has a major influence on the tocopherol content and composition. Location also has an impact, which can be associated with a microclimate characteristic of the growing area.

The cultivation year, climate characteristics of temperature and rainfall, has significant impact on the variation of the tocopherol content. The amount of rainfall in July correlated negatively with the total tocopherol content, and the highest content was recorded in the year with the lowest rainfall. Both cultivars have gained an average higher α-, γ-, and total tocopherol content at a warmer and of low altitude location. The fruit ripening stage has a significant impact on the composition of the tocopherols and primarily α-tocopherol. The clear decrease in α-tocopherol content as ripening progresses was observed.

Acknowledgements

The authors acknowledge the Ministry of Science, Education and Sports of the Republic of Croatia (Project No: 091-091 0468 0364) for financial support.

Author details

Maja Jukić Špika[1*], Klara Kraljić[2] and Dubravka Škevin[2]

*Address all correspondence to: maja@krs.hr

1 Institute for Adriatic Crops and Karst Reclamation, Split, Croatia

2 Faculty of Food Technology and Biotechnology, Zagreb, Croatia

References

[1] DellaPenna D, Pogson BJ. Vitamin synthesis in plants: tocopherols and carotenoids. Annu Rev Plant Biol. 2006;57:711–738. doi:10.1146/annurev.arplant.56.032604.144301

[2] Kamal-Eldin A, Appelqvist L-A. The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids. 1996;31(7):671–701. doi:10.1007/BF02522884

[3] Wanasundara PKJPD, Shahidi F. Antioxidants: science, technology, and applications. In: Shahidi, F., editors. Bailey"s Industrial Oil and Fat Products. 6th ed. Hoboken: Wiley, Hoboken, New Jersey, SAD; 2005. p. 464–466. doi:10.1002/047167849X.bio002

[4] Schwartz H, Ollilainen V, Piironen V, Lampi A-M. Tocopherol, tocotrienol and plant sterol contents of vegetable oils and industrial fats. J. Food Compos Anal. 2008;21:152–161

[5] Codex stan 210. Codex standard for named vegetable oils. 1999; FAO/WHO, Rome.

[6] Arslan D, Karabekir Y, Schreiner M. Variations of phenolic compounds, fatty acids and some qualitative characteristics of Sarıulak olive oil as induced by growing area. Food Res Int. 2013;54(2):1897–1906. doi:10.1016/j.foodres.2013.06.016

[7] Jukić Špika M, Kraljić K, Koprivnjak O, Škevin D, Žanetić M, Katalinić M. Effect of agronomical factors and storage conditions on the tocopherol content of Oblica and Leccino virgin olive oils. J Am Oil Chem Soc. 2015;92(9):1293–1301. doi:10.1007/s11746-015-2688-2

[8] Beltrán G, Jiménez A, del Rio C, Sánchez S, Martínez L, Uceda M, Aguilera MP. Variability of vitamin E in virgin olive oil by agronomical and genetic factors. J Food Compos Anal. 2010;23(6):633–639. doi:10.1016/j.jfca.2010.03.003

[9] Saldeen K, Saldeen T. Importance of tocopherols beyond α-tocopherol: evidence from animal and human studies. Nutr Res. 2005;25(10):877–889. doi:10.1016/j.nutres.2005.09.019

[10] Bostick RM, Potter JD, McKenzie DR, Sellers TA, Kushi LH, Steinmetz KA, Folsom AR. Reduced risk of colon cancer with high intake of vitamin E: the IOWA women's health study1. Cancer Res. 1993;53:4230–4237

[11] Suzukawa M, Ayaori M, Shige H, Hisada T, Ishikawa T, Nakamura H. Effect of supplementation with vitamin E on LDL oxidizability and prevention of atherosclerosis. Biofactors. 1998;7(1, 2):51–54

[12] Pryor WA. Vitamin E and heart disease: basic science to clinical intervention trials. Free Radic Biol Med. 2000;28(1):141–164. doi:10.1016/S0891-5849(99)00224-5

[13] Uceda M, Frias L. Harvest dates: evolution of the fruit oil content, oil composition and oil quality. Proc II Seminario Oleicola Internacional. 1975; Córdoba, Spain: 6–17.

[14] Boskou D. Olive oil, Chemistry and Technology. 2nd ed. Champaign, SAD: AOCS Press; 2006.

[15] Baccouri O, Guerfel M, Baccouri B, Cerretani L, Bendini A, Lercker G, Zarrouk M, Daoud B, Miled D. Chemical composition and oxidative stability of Tunisian Monovarietal virgin olive oils with regard to fruit ripening. Food Chem. 2008;109(4):743–754. doi:10.1016/j.foodchem.2008.01.034

[16] Matos LC, Cunha SC, Amaral JS, Pereira JA, Andrade PB, Seabra RM, Oliveira BP. Chemometric characterization of three varietal olive oils (Cvs. Cobrançosa, Madural and Verdeal Transmontana) extracted from olives with different maturation indices. Food Chem. 2007;102(1):406–414. doi:10.1016/j.foodchem.2005.12.031

[17] Tura D, Gigliotti C, Pedò S, Failla O, Bassi D, Serraiocco A. Influence of cultivar and site of cultivation on levels of lipophilic and hydrophilic antioxidants in virgin olive oils (Olea europea L.) and correlations with oxidative stability. Sci Hort. 2007;112(1):108–119. doi:10.1016/j.scienta.2006.12.036

[18] Koprivnjak O, Vrhovnik I, Hladnik T, Prgomet Ž, Hlevnjak B, Majetić Germek V. Characteristics of nutritive value of virgin olive oils from Buža, Istarska bjelica, Leccino and Rosulja cultivars. Croat J Food Technol Biotechnol Nutr. 2012;7(3, 4):172–178

[19] Beltrán G, Aguilera MP, del Rio C, Sanchez S, Martinez L. Influence of fruit ripening process on the natural antioxidant content of Hojiblanca virgin olive oils. Food Chem. 2005;89(2):207–215. doi:10.1016/j.foodchem.2004.02.027

[20] Dag A, Ben-Gal A, Yermiyahu U, Basheer L, Nir Y, Kerem Z. The effect of irrigation level and harvest mechanization on virgin olive oil quality in a traditional rain-fed "Souri" olive orchard converted to irrigation. J Sci Food Agric. 2008;88(9):1524–1528. doi:10.1002/jsfa.3243

[21] Rotondi A, Bendini A, Cerretani L, Mari M, Lercker G, Toschi TG. Effect of olive ripening degree on the oxidative stability and organoleptic properties of cv. Nostrana di Brisighella extra virgin olive oil. J Agric Food Chem. 2004;52(11):3649–3654. doi: 10.1021/jf049845a

[22] Aguilera MP, Beltrán G, Ortega D, Fernández A, Jiménez A, Uceda M. Characterisation of virgin olive oil of Italian olive cultivars: "Frantoio" and "Leccino", grown in Andalusia. Food Chem. 2005;89(3):387–391. doi:10.1016/j.foodchem.2004.02.046

[23] Mousa MY, Gerasopoulos D, Metzidakis I, Kiritsakis A. Effect of altitude on fruit and oil quality characteristics of "Mastoides" olives. J Sci Food Agr. 1996;71(3):345–350. doi: 10.1002/(SICI)1097-0010(199607)71:3<345::AID-JSFA590>3.0.CO;2-T

[24] Romero MP, Tovar MJ, Ramo T, Motilva MJ. Effect of crop season on the composition of virgin olive oil with protected designation of origin "Les Garrigues". J Am Oil Chem Soc. 2003;80(5):423–430. doi:10.1007/s11746-003-0715-z

[25] Bengana M, Bakhouche A, Lozano-Sánchez J, Amir Y, Youyou A, Segura-Carretero A, Fernández-Gutiérrez A. Influence of olive ripeness on chemical properties and phenolic

composition of Chemlal extra-virgin olive oil. Food Res Int. 2013;54(2):1868–1875. doi: 10.1016/j.foodres.2013.08.037

[26] Salvador MD, Aranda F, Fregapane G. Influence of fruit ripening on "Cornicabra" virgin olive oil quality a study of four successive crop seasons. Food Chem. 2001;73(1): 45–53. doi:10.1016/S0308-8146(00)00276-4

[27] Gutiérrez F, Jimenez B, Ruiz A, Albi MA. Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties Picual and Hojiblanca and on the different components involved. J Agric Food Chem. 1999;47(1):121–127. doi: 10.1021/jf980684i

[28] Benito M, Lasa JM, Gracia P, Oria R, Abenoza M, Varona L, Sachez-Gimeno AC. Olive oil quality and ripening in super high density Arbequina orchard. J Sci Food Agr. 2013;93(9):2207–2220. doi:10.1002/jsfa.6028

[29] Brkić Bubola K, Koprivnjak O, Sladonja B. Influence of filtration on volatile compounds and sensory profile of virgin olive oils. Food Chem. 2012;132(1):98–103. doi:10.1016/ j.foodchem.2011.10.038

[30] Gordillo B, Ciaccheri L, Mignani AG, Gonzalez-Miret ML, Heredia FJ. Influence of turbidity grade on color and appearance of virgin olive oil. J Am Oil Chem Soc. 2011;88(9):1317–1327. doi:10.1007/s11746-011-1787-y

[31] Fregapane G, Lavelli V, León S, Kapuralin J, Desamparados Salvador M. Effect of filtration on virgin olive oil stability during storage. Eur J Lipid Sci Technol. 2006;108(2): 134–142. doi:10.1002/ejlt.200501175

[32] Cerretani L, Rocculi P, Bendini A, Romani S, Bacci A. Oil clarifying process and apparatus for implementing the process. World Patent Application WO, 107096. 2009.

[33] Lozano-Sánchez J, Cerretani L, Bendini A, Gallina-Toschi T, Segura-Carretero A, Fernández-Gutiérrez A. New filtration systems for extra-virgin olive oil: effect on antioxidant compounds, oxidative stability, and physicochemical and sensory properties. J Agric Food Chem. 2012;60(14):3754–3762. doi:10.1021/jf205353b

[34] Gómez-Alonso S, Mancebo-Campos V, Salvador MD, Fregapane G. Evolution of major and minor components and oxidation indices of virgin olive oil during 21 months storage at room temperature. Food Chem. 2007;100(1):36–42. doi:10.1016/j.foodchem. 2005.09.006

[35] Tsimidou MZ, Georgiou A, Koidis A, Boskou D. Loss of stability of "veiled" (cloudy) virgin olive oils in storage. Food Chem. 2005;93(3):377–383. doi:10.1016/j.foodchem. 2004.09.033

[36] Kalua CM, Bedgood DR, Bishop AG, Prenzler PD. Discrimination of storage conditions and freshness in virgin olive oil. J Agric Food Chem. 2006;54(19):7144–7151. doi: 10.1021/jf061038j

[37] Aparicio R, Luna G. Characterisation of Monovarietal virgin olive oils. Eur J Lipids Sci Technol. 2002;104:614–627

[38] Psomiadou E, Tsimidou M. On the role of squalene in olive oil stability. J Agric Food Chem. 1999;47(10):4025–4032. doi:10.1021/jf990173b

[39] Psomiadou E., Tsimidou M, Boskou D. α-Tocopherol content of Greek virgin olive oils. J Agric Food Chem. 2000;48(5):1770–1775. doi:10.1021/jf990993o

[40] Rastrelli L, Passi S, Ippolito F, Vacca G, De Simone F. Rate of degradation of α-tocopherol, squalene, phenolics, and polyunsaturated fatty acids in olive oil during different storage conditions. J Agric Food Chem. 2002;50(20):5566–5570. doi:10.1021/jf011063j

[41] Morelló JR, Motilva MJ, Tovar MJ, Romero MP. Changes in commercial virgin olive oil (cv Arbequina) during storage, with special emphasis on the phenolic fraction. Food Chem. 2004;85(3):357–364. doi:10.1016/j.foodchem.2003.07.012

[42] Krichene D, Allalout A, Mancebo-Campos V, Salvador MD, Zarrouk M, Fregapane G. Stability of virgin olive oil and behaviour of its natural antioxidants under medium temperature accelerated storage conditions. Food Chem. 2010;121(1):171–177. doi:10.1016/j.foodchem.2009.12.026

Edited by Dimitrios Boskou and Maria Lisa Clodoveo

Olive tree products provide a number of documented presentations of the production and quality of the two most important olive tree products: virgin olive oil and table olives. It is a source that familiarizes readers with recent approaches and innovations that can be introduced in the virgin olive oil extraction and stabilization technology and the preparation of table olives with emphasis on the presence of bioactive constituents. It also describes advances in the methods of checking authenticity and in the evaluation of attributes that may influence consumers' perceptions and preferences. Other topics discussed are squalene, a trove of metabolic actions, pigments, geographical indication, biotechnology in table olive preparation, and recovery of hydroxytyrosol from olive-milling wastes.

ISBN 978-953-51-4164-8

Photo by loloalvarez / iStock

IntechOpen