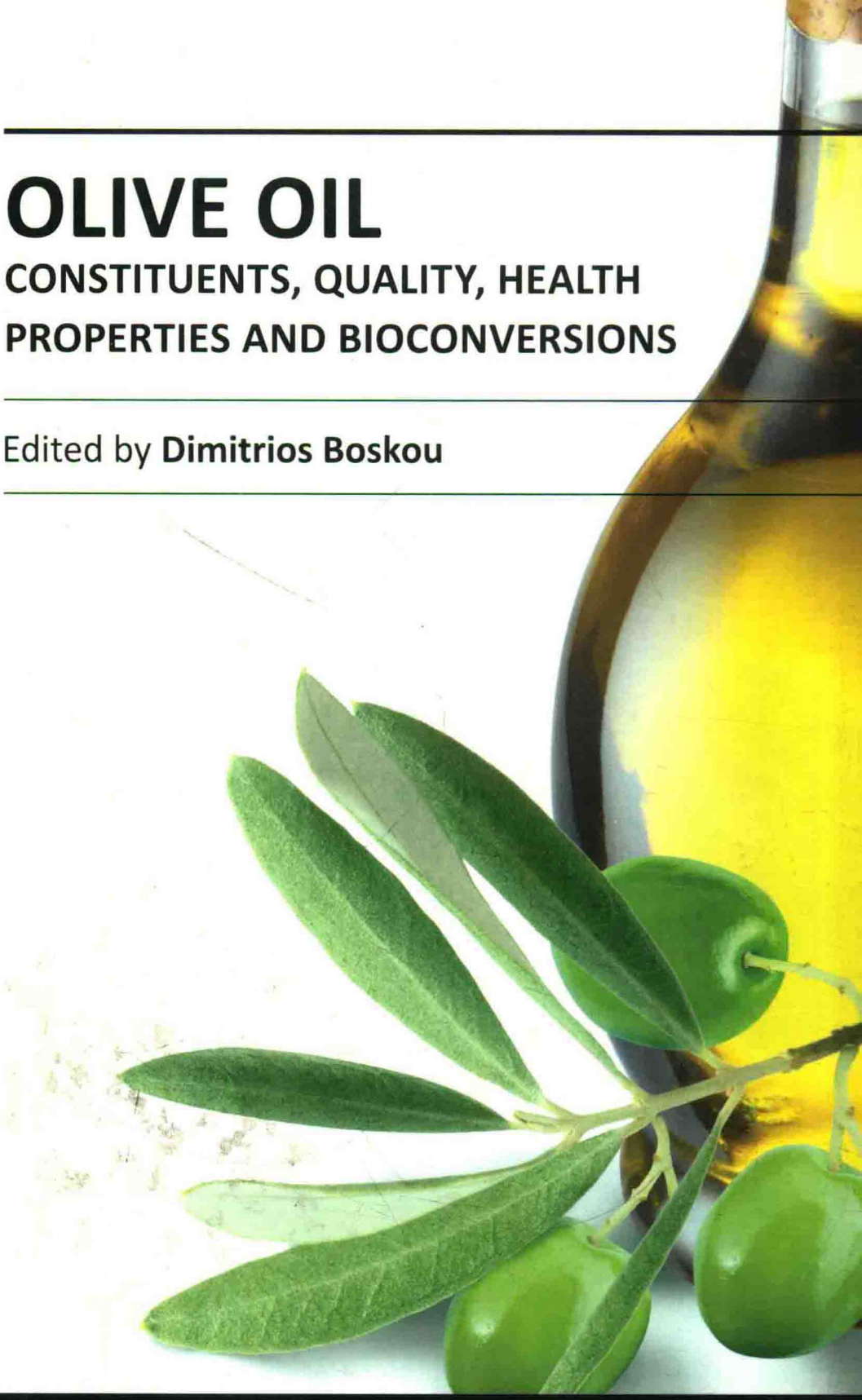
OLIVE OIL

CONSTITUENTS, QUALITY, HEALTH PROPERTIES AND BIOCONVERSIONS

Edited by **Dimitrios Boskou**



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Published by InTech
Janeza Trdine 9, 51000 Rijeka, Croatia

Publishing Process Manager Alida Lesnjakovic
Technical Editor Teodora Smiljanic
Cover Designer InTech Design Team

First published January, 2012
Printed in Croatia

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Additional hard copies can be obtained from orders@intechweb.org

Olive Oil – Constituents, Quality, Health Properties and Bioconversions,
Edited by Boskou Dimitrios
p. cm.
ISBN 978-953-307-921-9

Preface

Olive oil is an integral component of the dietary pattern known as 'Mediterranean diet', which was first acknowledged almost 40 years ago. Over the years, many investigations (both epidemiological and laboratory) indicated that this diet may be associated with lower levels of systematic inflammation, and lower rates of diseases such as cardiovascular, coronary heart disease, certain types of cancers, diabetes, and others. As a result, olive oil, a staple food for thousands of years for the inhabitants of the Mediterranean region, is now becoming popular among consumers all over the world.

The health effects of olive oil are attributed to its high content of monounsaturated fatty acids and the presence of some minor components, which have become the subject of intensive research over a short period of time. Efforts focus mainly on minor constituents of virgin olive oil with biological importance or on those which affect the organoleptic properties and contribute to its remarkable oxidative stability. Further research is expected to provide new insight into the role of each class of olive oil minor constituents, possible synergism and the magnitude of the contribution of the various bioactive ingredients to the overall positive heath impact in fighting disease.

This book presents some important aspects of the current state of the art in the chemistry, analysis and quality assessment of olive oil and its minor constituents, extraction of olive oil from the fruits, water treatment, and innovative approaches for the production of olive oil based products. It also discusses bioavailabilIty and pharmacological and other properties of bioactive ingredients in the light of new evidence for the composition of olive oil. It also covers also some aspects related to biotechnology and other technologies to retain optimum levels of such bioactive ingredients in the various olive oil forms and to protect the environment from olive mills waste products.

The book, composed of monographic chapters, is organized in five parts.

Part 1 "Olive Oil Composition, Analysis and Quality" discusses broadly non-volatile and volatile components related to flavor (chapters 1and 2), analysis and quality assessment methods (chapters 3-8,10), and traceability of origin (chapter 9). Chapter 11 is an extensive presentation of olive oil produced in Australia - a new country where olive tree was first introduced only two centuries ago and its systematic

cultivation is very recent. Chapter 12 examines olive oil from the point of view of consumers and analyzes the tendencies and preferences in relation to quality and other attributes. Important topics covered in this part are:

Biosynthesis of volatiles
Effect of agronomic and other factors such as storage on quality characteristics
Taste receptors and bitterness perception
Conventional methods of analysis and innovative approaches for the determination of trace metals, organoleptic characteristics, and the detection of sensory defects

Part 2"Olive Oil Extraction and Waste Water Treatment" describes biotechnological and other methods to improve recovery of olive and olive pomace oil and treatment of mill wastes (chapters 13-17). Chapter 13 proposes an improved hydrothermal treatment to obtain a higher level of microcomponents with biological value in olive pomace oil. Chapters 14-16 are presentations related to genetic improvement of olives, microbial biotechnology applications in olive oil industry, enzymatic extraction, green technology and bioremediation. Specific topics analyzed are: treatments of the solid wastes and wastewaters from the two and three phase extraction systems; anaerobic digestion processes ,energy recovery; production of value added products by microorganisms using oil mills waste as substrate.

Part 3 "Bioavailability and Biological Properties of Olive Oil Constituents" presents the chemistry, metabolism, bioavailability (and the different endogenous and exogenous variables involved) and properties of important bioactive compounds such as hydroxytyrosol, oleocanthal, other polar phenols and carotenoids, present in olive oil (chapters 18-21). Emphasis is given to recent research related to anti-inflammatory actions, the role that these compounds may have in the clinical treatment of chronic disease, as well as the possible use of preparations based on olive oil constituents as therapeutic agents. Chapter 22 deals with fatty acids and sensitive neurons involved in the regulation of energy and glucose homeostasis. The research aims at identifying novel pharmacological targets for the prevention and treatment of diabetes and obesity.

Part 4 "Innovative techniques for the production of olive oil based products" covers topics such as replacement of animal in meat products by olive oil to obtain products rich in monounsaturated fatty acids (a healthier fatty acid profile, enzymatic production of structured olive oil triacylglycerols and applications in the cocoa butter equivalents and neutraceuticals industry (chapters 23-25). Chapter 26 focuses on the role olive oil may play as an inducer of lipase production. Chapter 27 deals with the incorporation of olive oil into phospholipid membranes of liposomes carrying active cytotoxic agents, in particular, photosensitizers. It reports also on the use of these totally natural and biocompatible olive oil-containing liposomes in ointments and creams for application on skin areas contaminated with bacteria.

Part 5 "Regional studies" contains chapter 28 that discusses olive cultivation and olive oil production in Albania. The chapter is an agro-economic study analyzing the structural and constitutional reforms, which followed the transition from a centrally planned to a market economy, and the impact on the growth and perspective of olive oil sector in this country.

It is hoped that this book will serve as useful source of knowledge recently accumulated and as a comprehensive reference for a broad audience, mainly food scientists, biotechnologists, nutritionists, pharmacologists, researchers in Biosciences, olive growers, olive oil producers, but also members of the general public and consumers who are looking to extract health benefits from the diet of the people living in the countries surrounding the Mediterranean Sea.

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Contents

Part 1

Olive Oil Composition, Analysis and Quality

1

Volatile and Non-Volatile Compounds of Single Cultivar Virgin Olive Oils Produced in Italy and Tunisia with Regard to Different Extraction Systems and Storage Conditions

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1. Introduction

Virgin olive oil has a fundamental role in the markets of alimentary oils because of its unique aroma, its stability and its healthy benefits. In this chapter the attention will be focused on Tunisian and Italian single cultivar olive oils.

The oils under investigation were produced by different extraction systems and characterised for their volatile and non-volatile compounds (Benincasa et al., 2003; Cerretani et al., 2005; Garcia et al., 1996). It is well known that volatile and non-volatile components of products of plant origin are dependent on genetic, agronomic and environmental factors. There are few reports (Angerosa et al., 1996, 1998a, 1998b, 1999; Morales et al., 1995; Solinas et al., 1998) on the evaluation of the relationships between the aroma components of virgin olive oil with the metabolic pathways and varietal factors. Olive ripening process and, to some extent, the fruit growing environment, affect also the composition of the volatile compounds of the oil (Aparicio & Morales, 1998; De Nino et al., 2000; Guth & Grosh, 1993; Montedoro & Garofalo, 1984; Morales et al., 1996). Volatile and non-volatile compounds are retained by virgin olive oils during their mechanical extraction process from olive fruits (*Olea europaea* L.). Non-volatile compounds such as phenolic compounds stimulate the tasting receptors such as the bitterness perception, the pungency, astringency and metallic attributes. Instead volatile compounds, stimulating the olfactive receptors, are responsible for the whole aroma of the virgin olive oil. The chromatograms of volatile compounds of olive oils were obtained by solid phase micro extraction-gas chromatography/mass spectrometry (SPME-GC/MS) (Hatanaka, 1993; Kataoka et al., 2000; Steffen & Pawliszyn, 1996). The method is based on the assay of the terminal species of the "lipoxygenase pathway" which are present in the volatile fraction of the sampled compounds (Hatanaka, 1993).

2. Materials and methods

2.1 Extraction of olive oil and storage

The olive oils investigated (60 Italian and 60 Tunisian) were single cultivar virgin olive oils (SCVOOs) produced in different regions of Tunisia (Chamlali Cv.) and Italy (Coratina Cv.). Olives were handpicked at the optimal olive ripening degree. Immediately after harvest, olive fruits were transported and cleaned, each fruit sample was divided into three portions of 20 Kg. One portion was extracted using pressure system (see paragraph 2.1.1), the second and the third were extracted by centrifugation systems, three and two phases, respectively (see paragraph 2.1.2 and 2.1.3). The oils obtained were stored in three types of packaging (opaque glass, transparent glass and polyethylene terephtalate PET) and monitored for six months.

2.1.1 Pressure system (PS)

Olives are ground into an olive paste using large millstones. In general, the olive paste stays under the stones for 45–50 minutes. After grinding, the olive paste is spread onto fibre disks, that are easier to clean and maintain, stacked on top of each other and then placed into the press. Afterwards, this pile of disks are put on a hydraulic piston where a pressure of about 400 atm is applied. By the action of this pressure, a olive paste and a liquid phase is produced.

Finally, the liquid phase containing oil and vegetation water is separated by a standard process of decantation.

2.1.2 Two-phase centrifugation (2P)

This system does not need water addition and produces a liquid phase (oil) and a solid waste-water-dampened phase (pomace). The olive paste is kneaded for 60 minutes at 27°C and the oil is extracted with a horizontal centrifugation decanter and separated by means of an automated discharge vertical centrifuge.

2.1.3 Three-phase centrifugation (3P)

This system allows the crushing of olives into a fine paste. This paste is then malaxed for 60 minutes in order to achieve the coalescence of small oil droplets. The aromas are created during these two steps through the action of enzymes. Then, the paste is pumped into an industrial decanter where the phases are separated. Water (500 liters per ton) is added to facilitate the extraction process with the paste. The high centrifugal force created into the decanter separates the phases readily according to their different densities (solid phase pomace, vegetation water, oil). The solid materials is pushed out of the system by the action of a conical drum that rotates with a lower speed. The separated oil and vegetation water are then rerun through a vertical centrifuge, which separates the small quantity of vegetation water still contained in the oil.

2.2 Analytical methods

The physic-chemical and organoleptic analysis of VOO were carried out according to the methods described by the European Union Regulations (UE 61/2011).

In particular, analysis of fatty acid methyl esters, total phenols, free acidity, peroxide number, conjugated dienes and trienes, sensory analysis and volatile compounds were conducted as described in the following paragraphs.

2.2.1 Fatty acid methyl ester analysis (FAMEs)

FAMEs analysis were carried out after performing alkaline treatment obtained by dissolving the oil (0.05 g) in n-hexane (1 mL) and adding a solution of potassium hydroxide (1 mL; 2 N) in methanol (Christie, 1998). FAMEs were analyzed by gas chromatography by mean of a Shimadzu 17A chromatograph equipped with detector flame ionization and a capillary column. Peaks were identified by comparing their retention times with those of authentic reference compounds.

The fatty acid composition was expressed as relative percentages of each fatty acid calculated considering the internal normalization of the chromatographic peak area.

2.2.2 Total phenols analysis

Total phenols content was determined according to the method developed by Gutfinger (1981). Briefly, an amount of olive oil (2.5 g) was dissolved with hexane (5 mL) and extracted with a solution of methanol and water (5 mL; 60/40). The mixture was then vigorously agitated for 2 minutes. Folin-Ciocalteu reagent (0.5 mL) and bi-distilled water (4.8 mL) were added to the phenolic fraction. The absorbance of the mixture was measured at 725 nm and results were given as mg of caffeic acid per Kg of oil.

2.2.3 Free fatty acids, peroxides, ultra-violet light absorption

Acidity value, peroxide value (PV) and ultra-violet light absorption, conjugated diene (K232) and conjugated trienes (K270), were determined according to the Regulation EEC/ 2568/91 of the European Union Commission (EEC, 1991).

2.2.4 Sensory analysis

Olive oils were evaluated by a panel according to the official method for the Organoleptic assessment of virgin olive oil referenced COI/T.20/Doc. No 15/Rev. 2.

2.2.5 SPME-GC/MS analysis

Aroma components of products of plant origin are dependent on genetic, agronomic and environmental factors (Benincasa et al., 2003). The complexity of the mass-chromatograms in terms of number of components might represent a drawback when different samples are to be matched. Therefore, in order to consider the minimum set of components that mostly reflect the biogenesis of an oil (Aparicio & Morales, 1998), hexanal (1), 1-hexanol (2), (E)-2-hexenal (3), (E)-2-hexen-1-ol (4) and (Z)-3-hexenyl acetate (5) were chosen as markers of linoleic and linolenic acids specific lipoxygenase oxidation [(path A and B), Fig. 1].

2.2.5.1 Preparation of samples and standard solutions

A solution (200 mg/Kg) was prepared by dissolving 0.04 g of each analytes (see paragraph 2.2.5) in 200 g of commercial seeds oil. In the same manner a solution containing the internal standard (ethyl isobutyrate) was prepared.

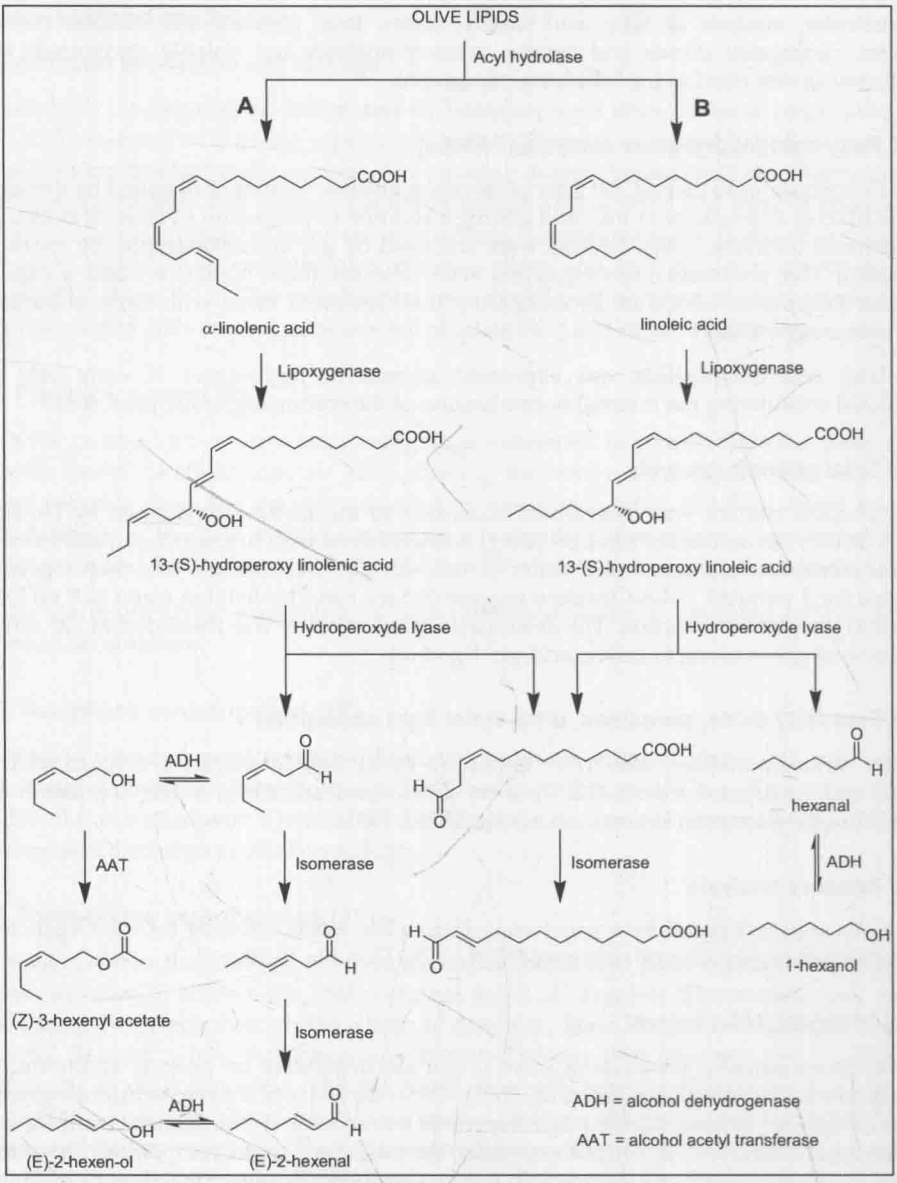

Fig. 1. Linoleic and linolenic acids specific lipoxygenase oxidation.

2.2.5.2 Experimental procedure and instrumentation

The assay of secoiridoid glycosides, such as oleuropein, in virgin olive oil has been proposed as a marker of quality (De Nino et al., 1999, 2005; Perri et al., 1999). With reference to the works previously mentioned, the chromatogram of volatile compounds was considered a useful target. Only the peaks with a certain threshold value (S/N equal to five)

were taken into account and integrated. Identification of analytes was made by comparison of their mass spectra and retention times with those of authentic reference compounds.

The experimental work was carried out using a Varian 4000 Ion Trap GC/MS system (Varian, Inc. Corporate Headquarters, U.S.A.) equipped with a CP 3800 GC. Volatile components were adsorbed by means of a divilbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber and separation was obtained by means of a capillary column FactorFOUR (Varian VF-5ms). The ion trap temperature was set at 210 °C with an ionization time of 2 ms, reaction time at 50 ms and scan rate at 1000 ms. The transfer line temperature was set at 230 °C. The column was a 30 m Chrompack CP-Sil 8 CB low bleed/MS (0.25 mm i.d., 0.25 μm film thickness). The GC oven temperature was initially held at 40 °C for 3 min, then ramped at 1 °C/min to 70 °C and finally ramped at 20 °C/min to 250 °C and held for 8 min. The carrier gas was helium at 1 mL/min. Analyses were performed in splitless mode. Mass spectra were collected in EI in positive mode.

2.2.5.3 Quantitative analysis

The calibration curves were obtained by covering two concentration range: 0.4-4 mg/Kg with six steps at 0.4, 0.8, 1.5, 3, 4 mg/Kg for each analyte, with 1.5 mg/Kg of internal standard and 5-150 mg/Kg with six steps at 5, 10, 25, 50, 100, 150 mg/Kg for each analyte, with 40 mg/kg of internal standard. Each experimental value corresponds to the average of three replicates.

The quantitative assay was performed by selecting the area of the ionic species as follows: m/z 41, 56, 67, 72, 82 for hexanal; m/z 55, 56, 69 for 1-hexanol; m/z 55, 69, 83, 97 for (E)-2-hexenal; m/z 57, 67, 82 for (E)-2-hexen-1-ol; m/z 67, 82 for (Z)-3-hexenyl acetate, respectively and m/z 71, 88, 116 for the internal standard.

2.2.5.4 Statistical analysis

The data obtained for each compound were subjected to statistical analysis. Statistical treatment was performed by STATGRAPHICS Plus Version 5.1 (Statistical Graphics Corporation , Professional Edition - Copyrigth 1994-2001). The approach chosen to analyse the set of data obtained was Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA). Also, in order to check possible differences between the oils, two-way analysis of variance (ANOVA) was performed considering, as main factors, the nationality of the sample and the type of storage container. Moreover, to evaluate significant differences between averages, Tukey test was performed on the oil quality parameters. Differences were considered statistically significant for $P \geq 0.01$ and $P \geq 0.05$. The values obtained for free acidity and FAMEs were analyzed after arcsine transformation in order to meet assumptions for ANOVA.

3. Results and discussions

3.1 FAMEs analysis

VOOs under investigation showed the typical profile of fatty acids of the areas of production. In general, the oils were dominated by palmitic acid (C16: 0), stearic acid (C18: 0), oleic acid (C18: 1) and linoleic acid (C18: 2). The observed values do not show a particular pattern that can indicate the mode of extraction and the type of packing. It is well known, in fact, that fatty acids are dependent on genetic factors, soil and climate (Christie, 1998; Dabbou, et al., 2010; Gharsallaoui, et al., 2011; Manai, et al., 2007).