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Fatty acid and Antioxidant content of Chemlali Extra Virgin Olive Oil and its Hydrophilic and Lipophilic Fractions

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Abstract

Olive Oil is a fundamental ingredient of the Mediterranean diet for its healthy properties.

The aim of this study was to determine i) the most relevant quality parameters (Free acidity, K232, K270 and PV) of *chemlali* olive oil samples ii) to evaluate fatty acids and antioxidant content of *chemlali* olive oil and its hydrophilic (OOHF) and lipophilic (OOLF) fractions iii) to compare the chemical characterization between olive oil and its fractions. OOHF was extracted from olive oil using water by centrifugation process. OOLF was obtained by filtration process through a hydrophobic composite ceramic membrane. According to quality parameters, all our oil samples were classified in the category "extra virgin olive oil". Our results showed that EVOO and OOLF fatty acid analyses revealed the same amount of MUFA. EVOO and OOHF contained a higher content of unsaponifiable components like polyphenols, which might contribute to olive oil's beneficial effect.

Keywords: Extra Virgin olive oil, hydrophilic fraction, lipophilic fraction, antioxidant content, Fatty acid composition.

1. Introduction

The olive oil sector plays an important role in the Tunisian economy, providing both employment and export revenue. The olive tree (*Olea europaea* L.) is present practically in every region of the country, up to the border of the southern desert. The Tunisian olive grove is dominated by the two major varieties Chetoui in the North and Chemlali in the Centre and the South. Olive oil is composed of two major fractions, the lipophilic one,

accounting for 98-99% of the total triglycerides, and hydrophilic fraction, containing several liposoluble molecules, including tocopherols, phytosterols, coloring pigments and squalene (Bulotta *et al*., 2014; Wu et al., 2016).
The main antioxidants of extra virgin olive oil (EVOO) are carotenoids and phenolic compounds, which are both lipophilic and hydrophilic. Among the glyceride fraction, olive oil shows a high content of fatty acids and particularly, an elevated proportion of monounsaturated fatty acids (MUFA). Unsaturated acids are up to 85% of its composition, due to its high content in oleic acid (C18:1), which might range between 70–85% and other fatty acids as linoleic or palmitoleic acid. Olive oil is considered as a superfood due to its health properties derived from its unique composition, its lipid profile and its bioactive compounds content (Secneler and Galanakis, 2019).
MUFA can modulate the immune response and can be useful in treating certain autoimmune diseases and in general regulation of immunity (Miles and Calder, 2015). Moreover, chronic diseases such as coronary arterial disease, hypertension, diabetes, inflammatory and auto immune disorders can be prevented via olive oil intake due to in part to the high MUFA content (Bermudez *et al*., 2011). The important element that contributes to the accumulation of the intracellular reactive oxygen species (ROS) is the endoplasmic reticulum (ER) stress (Malhi *et al*., 2011). Hydroxytyrosol (3,4-dihydroxyphenylethanol; HT) is mainly responsible for the antioxidant properties of this food, due to an efficient scavenger activity (Del Monaco *et al*., 2015). It has been reported that HT is able both to modulate an adaptive signaling pathway activated after ER stress and to ameliorate ER homeostasis (Giordano *et al*., 2014). In recent years, the interest of scientists has been focused on the preventive effects of phenols against the degenerative diseases mediated by ROS.
Several studies have emphasized the importance of a regular use of olive oil in the benefits of traditional Mediterranean diet on cardiovascular diseases (Sofi *et al*., 2013; Ghorbel *et al*., 2015). The aim of this study was to determine the fatty acid and antioxidant content of chemlali virgin olive oil and its hydrophilic and lipophilic fractions and to evaluate their protective effects on rats intoxicated with dietary contaminants in the second step.

2. Materials and methods

2.1. Oil samples

Biologic Extra virgin olive oil (EVOO) samples were obtained from a Chemlali variety cultivar grown in Sfax of Tunisia. The hydrophilic fraction (OOHF) was extracted from EVOO by the method of Montedoro *et al*. (1992) using water instead of methanol to avoid its toxic effect in rats. Briefly, 10 g of EVOO was homogenized with 10 mL of water by a mixer (Ultra-Turrax T25 [IKA Labortechnik, Janke & Kunkel, Staufen, Germany]; 15 000×g/min) and centrifuged at 5000 × g for 10 min. The extraction was performed two times.

2.2. Quality indices

Free acidity (g oleic acid/100 g olive oil), peroxide value (PV) (meq O_2/kg of olive oil) and the UV absorption for the determination of the extinction coefficients of K232 and K270 were measured following the analytical methods described in regulation EEC/2568/91. All parameters were determined in triplicate for each sample.

2.3. Filtration process and membrane analysis

The lipophilic fraction (OOLF) was obtained from EVOO as follows. EVOO was homogenized for 1 min with water (1:1, v/v), and the oil was separated by centrifugation; this procedure was repeated six times. Then, the OOLF was filtered through a hydrophobic composite ceramic membrane prepared totally from the phosphate industry sub product material. The Cross flow experiments were conducted using a pilot plant made in our laboratory using single channel tubular membrane at a temperature of 25◦C (Figure 1).

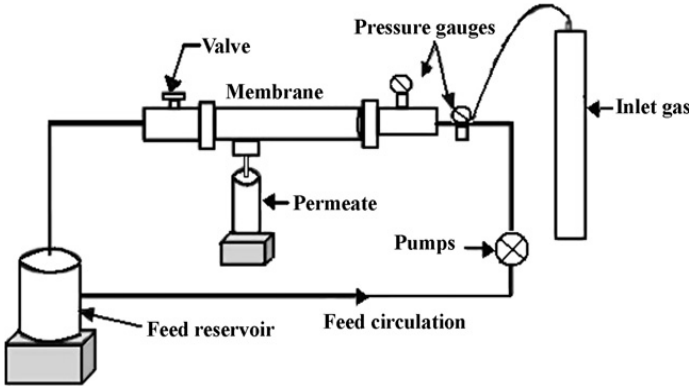

Figure 1. Scheme of the pilot plant

The operating pressure is applied using a nitrogen gas source. The total active area of the membrane is 19.6 cm^2. Before experiments, the elaborated membrane is conditioned by immersion in pure deionized water for at least 24 h, then, the membrane permeability was determined (Table 1). This material was previously used as membrane support (Khemakhem et al., 2011).

Table1. The principle characteristics of the ceramic membrane

Characteristics	Contact angle (°)	Configuration	Surface area (cm^2)	Permeability (L/hm^2bar)
Values	160	Tubular	19.6	24

2.4. Fatty acid Determination

Different constituents of Extra Virgin olive oil and its fractions were analyzed. The fatty acids were converted into fatty acid methyl esters (FAMEs) prepared by dissolving 0.1g of EVOO in methanol and incubated

for 1 hour. Individual FAMES were separated and quantified by gas chromatography using model 5890 series II instrument (Hewlett-Packard Ca Palo Alto, Calif. USA) equipped with a flame ionization detector and a fused silica capillary column HP – INNOWAX (30 m length × 0.25 mm i.d. and 0.25 µm of film thickness). The temperature was programmed to increase from 170 to 270°C at a rate of 5°C/min. Nitrogen ultra was used as carrier gas. The results were expressed as relative area percent of the total FAMEs (Dabbou *et al*., 2009).

2.5. Pigment content

The carotenoids and chlorophylls (mg/kg oil) were determined at 470 and 670 nm, respectively, in cyclohexane using the specific extinction values according to the method of (Minguez Mosquera *et al*., 1992)

2.6. Total Polyphenol and Tocopherol content

The phenolic compounds were extracted, estimated colorimetrically at 765 nm using the Folin-Ciocalteau reagent, and expressed as hydroxytyrosol equivalents as reported by Montedoro *et al*. (1992).

α-Tocopherol was evaluated according to the method of Gimeno *et al*. (2000). Each oil sample was diluted with n-hexane (1:10), the mixture was vortexed and 200 µl were transferred to a test tube containing 600 µl of methanol and 200 µl of internal standard (300 µg/ml). HPLC separation was carried out on a Hewlett-Packard system (Waldbronn, Germany) equipped with a HP-1100 pump, a Rheodyne model 7725 injector (Cotati, CA, USA, loop volume 20 µl), a HP-1200 M multi-array detector and a Supelcosil ODS- 2 column (150 × 4.5 mm id., film thickness 5 µm).

3. Results

3.1. Quality parameters

The quality criteria, determined at the beginning of this study in order to identify the oil category, are presented in Table 2. According to the measured parameters, all oil samples were classified in the category "extra virgin olive oil" (IOC, 2015).

Table 2. Quality parameters of *Chemlali* olive oil

Free acidity (g oleic acid /100g)	K232	K270	PV (meq O_2/kg of olive oil)
0.5 ± 0.02	2.32 ± 0.02	0.21 ± 0.01	6.0 ± 1.02

3.2. Fatty acid composition of EVOO and its OOLF fraction

Fatty acid composition of EVOO and OOHF are presented in Table 3. Extra virgin olive oil and lipohilic fraction contained respectively 20.27 and 19.81% of saturate (palmitic and stearic acids), 55.09 and 53.50% of monounsaturate (mainly oleic acid), 16.12 and 15.27% of polyunsaturate fatty acids.

Table 3. Fatty acid composition of EVOO and OOLF fractions
PUFA: polyunsaturated fatty acid; MUFA: monounsaturated fatty acid; SFA: saturated fatty acids

Fatty acid (%)	EVOO	OOLF
Palmitic acid (C16:0)	17.60 ± 0.15	17.22 ± 0.12
Palmitoleic acid (C16:1w7)	2.12 ± 0.05	2.03 ± 0.03
Stearic acid (C18:0)	2.23 ± 0.04	2.15 ± 0.01
Oleic acid (C18:1w9)	52.78 ± 0.51	51.69 ± 0.12
Linoleic acid (C18:2w6)	15.44 ± 0.60	14.62 ± 0.08
Linolenic acid (C18:3w3)	0.68 ± 0.02	0.65 ± 0.05
Arachidonic acid (C20:0)	0.44 ± 0.01	0.44 ±0.02
Gadoleic acid (C20:1w-9)	0.19 ± 0.01	0.18 ± 0.02
SFA	20.27 ± 0.11	19.81 ± 0.14
MUFA	55.09 ± 0.59	53.90 ± 0.74
PUFA	16.12 ± 0.63	15.27 ± 0.22
MUFA/PUFA	3.41 ± 0.11	3.53 ± 0.04

3.3. Chlorophyll, carotenoid and antioxidant content of EVOO, OOHF and OOLF

Antioxidant content of EVOO and its fractions are presented in Table 4.

Table 4. Antioxidant content of EVOO and its fractions.
ND: non determined - : absent

Antioxidant content (mg/kg)	EVOO	OOHF	OOLF
Chlorophylls	3.28 ± 0.30	-	3.45 ± 0.12
β-Carotene	5.56 ± 0.48	0.52 ± 0.11	4.66 ± 0.79
Total polyphenols	225.12 ± 5.42	168.59 ± 2.92	55.19 ± 2.51
α-tocopherol	244.66 ± 5.11	ND	235.96 ± 11.52
β-tocopherol	0.05 ± 0.01	ND	_

Chlorophylls are present in olive oils and are the responsible for its greenish coloration. Carotenes are present too in olive oil and are responsible for its yellow coloration. The values of the chlorophylls and carotenes concentrations are 3.28 and 3.45 mg/kg mg/kg for chlorophylls and 5.56 and 4.66 mg/kg for carotenes in EVOO and OOLF respectively.

In fact, EVOO and OOHF contained high amounts of phenols (225.12 and 168. 59 mg/ kg, respectively) while OOLF contain less quantity (55.19 mg/

Kg). EVOO and OOLF presented the same amount of α-tocopherol while OOHF was deprived from this component.

4. Discussion

The virgin olive oils are classified into EVOO, virgin, and lampante, according to the degree of acidity (ratio of free fatty acids to total oleic acid): ≤0.8%, ≤2%, and >2%, respectively (Mezghani Larousi *et al*., 2016). Lower acidity values guarantee high quality oil obtained from healthy olives and under ideal conditions. Regarding oxygen, EVOO must have a peroxide index (meq O2/kg of olive oil) ≤20. Thus, a rapid hydroperoxide formation demonstrates the initiation of the oxidative reactions that precede rancidity (Elez- Martinez *et al*., 2007). According to quality parameters, all our oil samples were classified in the category "extra virgin olive oil". All these characteristics are key factors to EVOO quality, thus the chemical composition of many health-promoting compounds, such as unsaturated fatty acids (especially oleic acid), as well as minor components such as tocopherols or phenolic compounds must be preserved. Fatty acids can be classified in saturated or unsaturated relying on the absence or presence of double bonds in their hydrocarbon chain. Dietary fatty acids upon ingestion are incorporated into biological membranes, including the mitochondrial membrane, contributing to cell structure and at the same time modulating many of its properties (Desnoyers *et al*., 2018). Our results showed that EVOO and OOLF fatty acid analyses revealed the same amount of MUFA. Triacylglycerols constitute a big part of olive oil and a high percentage of the saponifiable fraction is constituted by MUFA (Cavahim *et al*., 2019). The principal triacylglycerol detected in olive oil is oleic acid (52.78%, 51.69%) respectively in EVOO and OOLF, representing about half of the total triacylglycerol portion found in EVOO. Other triacyclglycerols also present are palmitic acid, linoleic acid and stearic acid (Boskou *et al*., 2006; Arnada *et al*., 2004).

The unsaponifiable fraction contains more than 200 compounds; among them, phenolic compounds account for 3% of the total oil composition (Lastra Romero, 2011). This fraction contributes to the specific characteristics of olive oil, such as aroma, taste, color and oxidative stability (Frankel *et al*., 2003). EVOO is known for having a high content of antioxidant compounds with protective properties against free radicals. In our previous study, we have demonstrated that *chetoui* olive oil and its fractions reduced biomarkers of oxidative stress in the heart of rats treated with acrylamide and aluminium via their strong antioxidant activity and thereby restored the DNA integrity of myocardial cells (Ghorbel *et al*., 2015). The main antioxidants of EVOO are carotenoids and phenolic compounds, which are both lipophilic and hydrophilic. The lipophilics include tocopherols, while the hydrophilics include flavonoids, phenolic alcohols and acids, secoiridoids and their metabolites. Various studies indicate that EVOO phenolic compounds have

antioxidant, anti-inflammatory, antimicrobial activity, by modulating gene expression of proteins involved in the inflammation process, the oxidative stress resistance and in lipid metabolism (Covas *et al*., 2006; Konstantinidou *et al*., 2010). Our results showed a higher content of unsaponifiable components like polyphenols in EVOO and OOHF which might contribute to olive oil's beneficial effect.

This approach leads to the next step, testing the beneficial effects of EVOO, OOHF and OOLF against toxicity induced by dietary contaminants in rat models.

5. Conclusion

Quality parameters revealed that our oil samples were classified in the category "extra virgin olive oil". Our results showed that EVOO and OOLF fatty acid analyses revealed the same amount of MUFA. However, a higher content of unsaponifiable components like polyphenols was found in EVOO and OOHF which might contribute to olive oil's beneficial effect. Olive oil is considered as a superfood due to its health properties derived from its unique composition, its lipid profile and its bioactive compounds content.

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