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Article

Phenolic Compounds Characterization and Antioxidant Properties of Monocultivar Olive Oils from Northeast Algeria

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Abstract: In Algeria, the olive tree is one of the main fruit species and plays a very important socioeconomic role. The objective of this study was firstly, to identify and quantify the phenolics of some Algerian olive oils, and secondly, to assess the antioxidant activity of the samples. The olive oils used in this study were derived from Algerian cultivars, including Tefahi, Gelb Elfarroudj, Chemlal, and imported cultivar Manzanilla and Zebboudj. For this purpose, gas chromatography—mass spectrometry (GC-MS) was used to identify olive oil fatty acids profile, while the individual phenolic compounds were assessed by ultra-high-performance liquid chromatography—electrospray ionization—high-resolution mass spectrometry (UHPLC-HESI-MS). To verify the antioxidant capacity, five in vitro free radical assays were used. Questionable values of particular physico-chemical parameters, such as the high value of free acidity and the low concentration of monounsaturated fatty acids in oil from the Zebboudj cultivar, indicate that improvements in olive cultivation and oil production practices are needed. Gelb Elfarroudj, Tefahi, and Manzanilla oils contain quantities of monounsaturated fatty acids in accordance with EU regulations. The oil obtained from the Zebboudj cultivar is not usable for food purposes due to the high value in free acidity and the low concentration of monounsaturated fatty acids. Tefahi and Manzanilla cultivars have given oils with the best antioxidant activity as compared to other studied cultivars; this is attributable to their composition in bioactive phenolic compounds, such as secoiridoids, which play an important role in human health as scavengers of free radicals. The results are interesting for producers and consumers to promote the culture of olive oils derived in particular from the Tefahi cultivar. However, in order to improve the health qualities of this oil, the agronomic techniques essentially linked to the time of harvesting of the olives destined for oil production must be improved.

Keywords: Manzanilla; Tefahi; Gelb Elfarroudj cultivar; secoiridoids; radical scavenging; UHPLC-HESI-MS; phenolics

1. Introduction

Olea europaea L., or more commonly olive tree, is largely cultivated for the production of its nutritional and healthy fruits. Extra virgin olive oil (EVOO) is an integral ingredient of the Mediterranean diet and a wide number of analytical techniques were used to identify the chemical composition [1]. These techniques indicated that the fine characteristics, the good health effect, and the biological activity of EVOO are mainly attributed to the presence of the unsaturated fatty acids as major components. They are recognized in olive oils mostly by the presence of the acids: oleic (C18:1), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), linoleic (C18:2), and linolenic (C18:3). The high quality of olive oil is also attributed to the presence in its composition of minor components such as phytosterols, carotenoids, tocopherols and hydrophilic phenols. The major phenolic compounds present in olive oil and conferring it the antioxidant activity belong to the class of secoiridoids mainly represented by oleuropein and ligstroside derivatives, which are strong radical scavengers and are also responsible for bitterness and pungency of EVOO [2–4].

Phenolic compounds are used as quality markers for virgin olive oil, and they are of great interest due to their anticancer, antiviral, and anti-inflammatory properties [5,6]. Their content is an important factor when evaluating the EVOO quality because they have been correlated with the oil oxidative stability and, in particular, its resistance to lipid peroxidation [7,8]. Extra virgin olive oil quality production could be influenced by several factors, for instance: olive cultivar, geographical region, environmental factors (seasonal conditions), irrigation, olive ripeness, harvesting, storage, and extraction procedure [9–11]. Light exposure, elevated temperature, and oxygen are all natural adversaries of EVOO and contribute to its deterioration [12].

The Mediterranean countries head the list of olive oil producers; the International Olive Oil Council classed Algeria as the ninth largest producer country of olive oil in the world, with around 87.5 tons in the 2015/16 season [13–15]. The olive oil production in Algeria is continuously increasing. In fact, the country is following a development policy called “National program for agriculture development” which opens the way for financing and supporting the agricultural sector [16]. Within the framework of this program, the government provides financial aid to farmers who plant olive trees and to the producers of olive oil [17]. Even with this, the commercialization of a high quality of olive oil is still presenting a challenge for the producers in Algeria.

The aim of this study was the chemical characterization of monovarietal oils produced in Algeria and the evaluation of their quality. In particular, the study focused on the comparison of the qualitative characteristics of: (a) Tefahi and Gelb Elfarroudj olive oils (cultivars not known in literature); (b) Chemlal and Zebboudj olive oils (known cultivars); (c) Manzanilla oil (imported cultivar). The authors believe that a quantitative characterization of the bioactive constituents of Algerian olive oils could contribute to their enhancement in a constantly growing international market.

For this, firstly, quality control tests were performed on all olive oils under study. Then, phenolic profile, fatty acid methyl esters pattern, and in vitro antioxidant capacity of the olive oils were determined.

2. Materials and Methods

2.1. Plant Material and Olive Oil Samples

Olive oil from four *Olea europaea* cultivars growing in the northeast of Algeria were used in this study (Setif and Batna provinces) (Figure 1). Three cultivars (Chemlal, Tefahi, and Gelb Elfarroudj) are native to the region, while one is locally grown but it is a foreign cultivar (Manzanilla). Furthermore, Zebboudj olive oil (*Olea oleaster*) was bought from the market and included in this study. The cultivars were growing under the same environmental characteristics in a semiarid region. Drop irrigation was used twice a year: in January (every fortnight) and in August (every 10 days). The method used in this study for olive harvest and extraction was the one used by most of Algerian producers. Healthy olive fruits of all cultivars were hand-picked when the skin of the fruit was black. The oils were extracted

from 200 kg of the collected fruits using a commercial modern mill located in the same region. The mill originated from Jeha Company (Alexandria, Egypt) and was equipped with a two-phase extraction system. The extraction of olive oil started with leaf stripping and olive cleaning, then olives passed into a hammer-crusher to obtain a homogenized olive paste. The malaxation time and temperature of the olive paste were 30 min at 30 °C. After centrifugation, the oil was separated from the paste and water, and then stored in amber glass bottles at room temperature (15–18 °C) in the dark. Soon after the oil extraction, the samples were used in the chemical analytical methods described below.

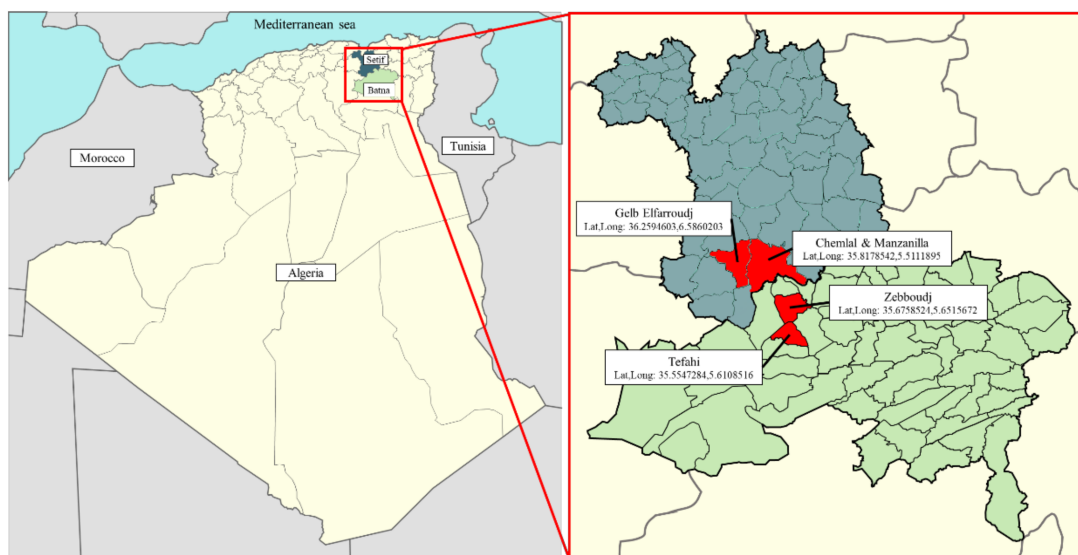


Figure 1. Biogeographical Algerian area where olives are grown.

2.2. Reagents and Standards

Formic acid, water, methanol, acetonitrile (LC-MS grade) were purchased from Biosolve B.V. (Valkenswaard, The Netherlands). All solvents, Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), 2,2-diphenyl,1-picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ), ferric chloride ($\text{FeCl}_3 \times 6\text{H}_2\text{O}$), gallic acid, acetic acid, ferric sulfate ($\text{FeSO}_4 \times 7\text{H}_2\text{O}$), sodium acetate, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), potassium persulfate, phosphate-buffered saline (PBS), tween 40, β -carotene, Trolox, and chemical standards (cinnamic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, ferulic acid, syringic acid, vanillic acid, caffeic acid apigenin, apigenin 7-glucoside, diosmetin, hydroxytyrosol, tyrosol, luteolin, oleuropein, vanillin, pinoresinol), were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Quality Parameters

Free acidity, given as g oleic acid 100 g^{-1} of oil, peroxide value (PV) expressed as $\text{meq O}_2 \text{ kg}^{-1}$ of oil, ΔK and K_{232} , K_{270} extinction coefficients calculated from absorptions at 232 nm and 270 nm were measured as described by the Regulation EC no. 2016/2095 [18].

2.4. FAMES Composition

Fatty acid methyl esters (FAMES) were analyzed using the GC-MS method after extraction and hydrolysis of triacyl glycerols using potassium hydroxide in methanol.

A mass of 0.1 g of oil samples were diluted in 1 mL of *n*-heptane and 0.1 mL of 2N KOH in MeOH solution was added and mixed in a vortex for 2 min. An aliquot of 500 μL of organic phase containing fatty acid methyl esters was diluted with 500 μL *n*-heptane. Immediately, GC-MS analyses were carried out using a DQ II single quadrupole system (Thermo Fisher Scientific, Bremen, Germany). The temperature of ion source and injector were 260 °C and 270 °C, respectively. The capillary column

used was a ZB-WAX (30 m \times 0.25 mm i.d., film thickness 0.25 μ m) (Phenomenex, Italy). The oven program temperature started at 165 $^{\circ}$ C (held for 10 min), it was increased to 200 $^{\circ}$ C at 1.5 $^{\circ}$ C/min, then increased to 250 $^{\circ}$ C at 10 $^{\circ}$ C/min and kept for 20 min at 250 $^{\circ}$ C under isothermal conditions. Ionization energy was 70 eV and the mass range scanned was 35–550 m/z. Helium flow rate of 1 mL min^{−1} was used. Monovarietal olive oil samples of 1 μ L were injected with a split ratio of 1:100 in triplicate. Fatty acid methyl esters identification was carried out using a mass spectrum database and 37-component fatty acid methyl esters mix (Sigma Aldrich Milan, Milan, Italy).

2.5. Individual Phenolic Compounds Characterization by UHPLC-HESI-MS

Liquid–liquid extraction method was used to isolate the phenolic fraction of olive oils. For this purpose, 2 g of each olive oil was mixed with 5 mL of a solution of methanol–water (80:20 v/v); then, the samples were shaken in a vortex for 1 min, placed in an ultrasonic bath for 15 min, and centrifuged at 5000 rpm for 25 min. The resulting aqueous solutions were filtered over PTFE syringe filter 0.45 μ m and quickly analyzed by ultra-high-performance liquid chromatography, using a heated electrospray probe and high-resolution mass spectrometer (UHPLC-HESI-MS) for qualitative determination of phenolic compounds, with three different dilution factors (1:2, 1:10, 1:100 v/v) to encompass the concentration variability. Triplicate samples of monovarietal olive oil were used for phenolic extractions. Experimental conditions used for qualitative determination of the phenolic constituents have been described in literature [19–21].

Briefly, UHPLC used was a Dionex Ultimate 3000 System equipped with an auto sampler controlled by Chromeleon 7.2 Software (Thermo Fisher Scientific, Bremen, Germany and Dionex Softron GmbH, Germering, Germany). A column Luna C18 50 \times 1 mm, 2.5 μ m was used. A flow rate of 50 μ L min^{−1} was set for separation of the selected compounds. The separation was achieved using eluent A (water with 0.1% acetic acid (v/v) pH 3.2) and eluent B (acetonitrile). The gradient elution program was: 0–2 min 5% B; 2–4.5 min linear increase to 10% B; 4.5–16 min linear increase to 25% B; 16–29 min linear increase to 95% B; 29–31 min decrease to 5% B; 31–33 min hold 5% B coming back to the initial conditions and being equilibrated. The column temperature was set at 35 $^{\circ}$ C and the injection volume at 1 μ L. Heated electrospray ion source (HESI) was used for the ionization. HESI parameters were optimized as follows: sheath gas flow rate 35 arbitrary units; auxiliary gas unit flow rate 4 arbitrary units; capillary temperature 250 $^{\circ}$ C; auxiliary gas heater temperature 259 $^{\circ}$ C; spray voltage 3.5 kV; and S lens RF level 30. MS in negative mode was selected for analysis of low-molecular phenolic compounds [19,21]. Detection of phenolic compounds was performed using a quadrupole Orbitrap mass spectrometer (Q Exactive; Thermo Scientific, Bremen, Germany). Full scan (100–800 m/z) acquisition method and a targeted single ion monitoring (SIM) analysis were performed using the mass inclusion list of the target analytes.

Phenolic compounds were quantified using solutions containing all commercial standards at six different concentration levels (5, 2.5, 1, 0.5, 0.25, 0.1 μ g mL^{−1}). Each point of the external calibration graph corresponded to the average of five independent injections.

In the case of secoiridoids derivatives, where commercial standards were not available, their equivalent values were estimated. Monovarietal olive oil contents of 3,4-DHPEA-EA (oleuropein aglycon and stereoisomers), 3,4-DHPEA-EDA (oleacein), p-HPEA-EA (ligstroside aglycon and stereoisomers), deacetoxyl-10-hydroxy oleuropein aglycon (DAc-10-OH Ole Agly), and elenolic acid (EA) were expressed as a p-HPEA-EDA (oleocanthal) equivalent (mg kg^{−1} of oil) [18]. Isolation of p-HPEA-EDA (oleocanthal) from olive oil was carried out according to a reported procedure developed in literature [19–21]. Briefly, 500 mL of olive oil was mixed with 250 mL hexane and 250 mL methanol. The mixture was sonicated for 15 min and after partition, the methanolic phase was centrifuged at 3000 rpm for 10 min. The hexane phase was extracted with 200 mL of methanol again. Combined methanolic phases were evaporated and the oily residue was extracted with 25 mL methanol–water (1:1) and 50 mL of hexane.

The aqueous MeOH layer was used for isolation of oleocanthal. Preparative chromatography was performed on an HPLC Agilent 1100 binary pump equipped with a UV-Vis detector. The chromatographic column was a reverse-phase Supelcosil LC 318 column, 25 cm, 4.6 mm, 3 μ m particle size. HPLC elution was performed at 5 mL/min with a binary gradient system with water (solvent A) and acetonitrile (solvent B). The gradient was: 0–5 min, 30% B; 5–30 min, 100% B; 35–40 min, 30% B. The eluate was monitored at 278 nm and fractions of about 3 mL were collected from the detector. After fractions collection, the solvents were evaporated under reduced pressure using a rotary evaporator, and finally the residue was stored at -20°C . The obtained fractions were analyzed by UHPLC-ESI-MS/MS and those with similar composition were combined for further preparative chromatography in the same conditions. Identity and purity of the oleocanthal were verified through spectroscopic techniques such as ^1H NMR, ^{13}C NMR, and UHPLC-HESI-MS.

Oleocanthal was used as external standard to prepare further calibration solutions (10, 5, 2.5, 1, 0.5, 0.25, 0.1 $\mu\text{g mL}^{-1}$). The deprotonated molecule $[\text{M}-\text{H}]^{-}$ detection was based on calculated exact mass and on retention time of target compounds.

2.6. Olive Oil Extracts for Determination of Total Phenolic Content and for Antioxidant Assays

The oil samples (2 g) were added to 5 mL of a methanol/water (80:20, *v/v*) mixture in a centrifuge tube. After vigorous mixing, they were centrifuged for 25 min at 5000 rpm. The hydroalcoholic phase was collected, washed with 2 mL of n-hexane to remove the residual oil, and then concentrated under reduced pressure using a rotary evaporator at 35°C . The issued phenolic fraction extracts (PFEs) were fully dried using a vacuum desiccator. The oil PFEs were dissolved in the methanol/water mixture to use them in the determination of total phenolic content and in antioxidant assays.

2.7. Total Phenols Content

The total phenol compounds of the oil's phenolic fraction extracts (PFE), was determined by the Folin–Ciocalteu colorimetric assay [22]. Briefly, fifty microliter of the solutions containing different concentrations (5 mg mL^{-1} , 3 mg mL^{-1} , 1 mg mL^{-1} , 0.5 mg mL^{-1} , and 0.25 mg mL^{-1}) oil's PFE to be tested were added to 450 μL of deionized water, 500 μL of Folin–Ciocalteu reagent, and 500 μL of 10% aqueous sodium carbonate solution. Samples were then maintained at room temperature for 1 h and absorbance was measured at 786 nm (UV-Vis Spectrophotometer, Shimadzu Japan) against blank containing 50 μL of the methanol (sample solvent). Gallic acid at different concentrations (300 $\mu\text{g mL}^{-1}$, 150 $\mu\text{g mL}^{-1}$, 75 $\mu\text{g mL}^{-1}$, and 25 $\mu\text{g mL}^{-1}$) was used as standard. The experiment was performed in triplicate and the results were expressed as μg gallic acid equivalents/mg of phenolic fraction extracts ($\mu\text{g GAE mg}^{-1}$ PFE).

2.8. Antioxidant Activity

2.8.1. 2,2-Diphenyl-1-Picrylhydrazyl Test

The free-radical-scavenging capacity of the oil's phenolic fraction extracts (PFEs) was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay as reported in literature [23]. A volume of 1.5 mL of DPPH \bullet solution (100 mM in methanol) was mixed with 37.5 μL of various concentrations (87 mg mL^{-1} , 60 mg mL^{-1} , 50 mg mL^{-1} , 45 mg mL^{-1} , 30 mg mL^{-1} , 25 mg mL^{-1} , 10 mg mL^{-1} , 5 mg mL^{-1}) of oil's PFE, or with methanol as control. After 20 min of incubation at room temperature, the absorbance was recorded at 517 nm with a UV-Vis spectrophotometer (Shimadzu, Japan). Results were expressed as mmol Trolox equivalents mg^{-1} of phenolic fraction extracts (mmol TE mg^{-1} PFE), using the calibration curve prepared with Trolox as standard using different concentrations (1.5 $\mu\text{g mL}^{-1}$, 1 $\mu\text{g mL}^{-1}$, 0.5 $\mu\text{g mL}^{-1}$, 0.25 $\mu\text{g mL}^{-1}$, 0.125 $\mu\text{g mL}^{-1}$). Each determination was performed in triplicate.

2.8.2. Trolox Equivalents Antioxidant Capacity

The Trolox equivalents antioxidant capacity (TEAC), also known as the ability of the oil's PFEs to scavenge the 2,2'-azinobis-(3-ethylbenzothiazine-6-sulfonic acid radical (ABTS⁺), was evaluated as previously described [24]. The ABTS⁺ radical cation was produced by the oxidation of ABTS⁺ (1.7 mM) with potassium persulfate (4.3 mM) in water. The mixture was allowed to stand in the dark at room temperature and the ABTS⁺ solution was diluted with phosphate-buffered saline (PBS) at pH 7.4 to give absorbance of 0.7 ± 0.02 at 734 nm. An aliquot of 50 μmL of a solution containing different concentrations (50–0.5 mg mL^{-1}) of the oil's PFE or methanol (blank) was added to 2 mL of the ABTS⁺ solution, and the absorbance was recorded at 734 nm in a UV/Vis spectrophotometer (Shimadzu, Japan) after allowing the reaction to stand for 6 min in the dark at room temperature. Results were expressed as mmol Trolox equivalents/milligram of phenolic fraction extracts (mmol TEmg⁻¹ PFE) using the calibration curve prepared with Trolox standard at different concentrations (0.215 $\mu\text{g mL}^{-1}$, 0.0625 $\mu\text{g mL}^{-1}$, 0.0312 $\mu\text{g mL}^{-1}$, 0.015 $\mu\text{g mL}^{-1}$). Each determination was repeated at least three times.

2.8.3. Ferric-Reducing/Antioxidant Power

The ferric-reducing/antioxidant power (FRAP) of the phenolic fraction extracts was evaluated as previously described [25]. The fresh working solution ferric-reducing/antioxidant power (FRAP) reagent was prepared by mixing 2.5 mL of 10 mM 2,4,6-tripyridyl-s-triazine solution (prepared in 40 mM HCl) with 25 mL of 0.3 M acetate buffer (pH 3.6) and 2.5 mL of 20 mM FeCl₃·6H₂O solution, and then preheated at 37 °C before use. A volume of 50 μL of a methanolic solution containing different concentrations (25–1 mg mL^{-1}) of the oil's PFE to be tested, or of the solvent, were added to 1 mL of FRAP reagent, and the absorbance was measured at 593 nm in a spectrophotometer (Shimadzu, Japan) after incubation at 20 °C for 4 min. A standard curve was prepared using various concentrations (1 $\mu\text{g mL}^{-1}$, 0.5 $\mu\text{g mL}^{-1}$, 0.25 $\mu\text{g mL}^{-1}$, 0.1 $\mu\text{g mL}^{-1}$) of FeSO₄ × 7H₂O. Each determination was performed in triplicate; the results were expressed as mmol Fe²⁺/milligram of phenolic fraction extracts (mmol Fe²⁺/mg of PFE).

2.8.4. Beta-Carotene Bleaching Assay

The potential of the oil's PFE in the β -carotene bleaching assay (BCB) was determined as described in literature [26]. A mass of 1 mg of β -carotene was dissolved in 10 mL of chloroform. Then, 5 mL of solution was added to 40 μL of linoleic acid and 400 μL of Tween 40. Chloroform was removed using a rotary evaporator and 100 mL of distilled water was added.

A volume of 5 mL was added to 200 μL of a solution containing different concentrations (25–0.5 mg mL^{-1}) of oil's PFE, whereas 5 mL of DMSO was used as blank. The absorbance was measured at 470 nm and results were expressed as 50% mean inhibition concentration (IC 50) with confidence limits (CL) at 95% calculated by Litchfield and Wilcoxon test. Each determination was carried out in triplicate and repeated at least three times.

2.9. Statistical Analysis

The results are expressed as mean values \pm standard deviation (SD) from three separate experiments. The results of total phenolic content and antioxidant activity were analyzed using Student's *t* test. Differences were considered to be statistically significant at $p < 0.05$.

3. Results

3.1. Quality Parameters

The qualitative parameters of the oils studied, obtained using analytical methods described by the International Olive Council [27], highlight some defects of Algerian monovarietal oils.

The results are summarized in Table 1. The oil obtained from the Zebboudj cultivar had a free acidity value ($1.25 \text{ g oleic acid } 100 \text{ g}^{-1}$ of oil) higher than the established limit, whereas the oils obtained from the Manzanilla and Gelb Elfarroudj cultivars had free acidity values 0.80 and 0.75 respectively; the first value was equal to the maximum allowed limit and the second one was very close to it. The peroxide values were also quite high even if below the maximum allowed limit; in particular, they were between $12.75 \text{ meq O}_2 \text{ kg}^{-1}$ and $15.50 \text{ meq O}_2 \text{ kg}^{-1}$. The conjugated trienes K_{270} and dienes K_{232} showed values in the range of 0.12–0.21 and 1.47–2.30, respectively, while ΔK results were in the range of -0.007 – 0.11 .

Table 1. Results of qualitative parameters of monovarietal Algerian olive oils under study.

Cultivars	Free Acidity (%)	PV (meq O ₂ /kg)	K ₂₃₂	K ₂₇₀	ΔK
Tefahi	$0.48 \pm 0.03 \text{ c}$	$12.68 \pm 1.23 \text{ b}$	$1.98 \pm 0.17 \text{ b}$	$0.17 \pm 0.02 \text{ a}$	$-0.002 \pm 0.01 \text{ c}$
Chemlal	$0.50 \pm 0.05 \text{ c}$	$12.75 \pm 1.13 \text{ b}$	$2.29 \pm 0.28 \text{ a}$	$0.19 \pm 0.01 \text{ a}$	$0.001 \pm 0.01 \text{ b}$
Gelb Elfarroudj	$0.75 \pm 0.06 \text{ b}$	$15.50 \pm 1.53 \text{ a}$	$1.97 \pm 0.26 \text{ b}$	$0.21 \pm 0.03 \text{ a}$	$0.003 \pm 0.03 \text{ b}$
Manzanilla	$0.80 \pm 0.04 \text{ b}$	$15.50 \pm 0.24 \text{ a}$	$1.47 \pm 0.24 \text{ c}$	$0.12 \pm 0.01 \text{ b}$	$-0.007 \pm 0.01 \text{ d}$
Zebboudj	$1.25 \pm 0.11 \text{ a}$	$12.51 \pm 0.32 \text{ b}$	$0.39 \pm 0.03 \text{ d}$	$0.11 \pm 0.05 \text{ b}$	$0.11 \pm 0.05 \text{ a}$

Free acidity = g oleic acid 100 g^{-1} of oil, peroxide value (PV) = meq O₂ kg^{-1} of oil. Results were expressed as mean \pm SD. Different letters in columns indicate statistical differences at $p < 0.05$ (Student's *t* test) among cultivars.

3.2. Fatty Acid Composition

Using GC-MS, 12 fatty acids were detected as shown in Table 2. The composition of the samples was a combination of four saturated fatty acids (palmitic acid C16:0, margaric acid C17:0, stearic acid C18:0, and arachidic acid C20:0); six mono-unsaturated fatty acids (palmitoleic acid C16:1 (7-cis) (ω -9), palmitoleic acid C16:1 (9-cis) (ω -7), heptadecenoic acid C17:1, oleic acid C18:1 (ω -7), oleic acid C18:1 (ω -9), and 11-eicosenoic acid C20:1 (ω -9)), and two poly-unsaturated fatty acids (linoleic acid C18:2 (ω -6) and α -linolenic acid C18:3 (ω -3)). The monounsaturated fatty acids were higher in the monovarietal oils of Gelb Elfarroudj, Tefahi, and Manzanilla, due to the high content of oleic acid C18:1 (ω -9). Polyunsaturated fatty acids content was highest in Zebboudj oil mainly due to the high content of linoleic acid. The percentages of fatty acids in our work are very close to those of other Algerian cultivars [28]. Our results in the concentrations of palmitic acid, linoleic acid, and α -linolenic are also in agreement with those reported in other studies about Chemlal Algerian olive oil [29].

Table 2. Fatty acid methyl esters (in percentage) identified by GC-MS in the monovarietal olive oils under study.

Fatty Acids	Cultivars				
	Tefahi	Chemlal	Gelb Elfarroudj	Manzanilla	Zebboudj
C16:0	$12.27 \pm 0.04 \text{ b}$	$15.75 \pm 0.02 \text{ a}$	$12.70 \pm 0.05 \text{ b}$	$9.97 \pm 0.31 \text{ c}$	$11.59 \pm 0.09 \text{ b}$
C16:1(ω -7)	$0.14 \pm 0.01 \text{ b}$	$0.35 \pm 1.5 \text{ a}$	$0.16 \pm 0.01 \text{ b}$	$0.20 \pm 0.01 \text{ b}$	nf
C16:1 (ω -9)	$1.12 \pm 0.01 \text{ b}$	$1.05 \pm 0.22 \text{ a}$	$1.21 \pm 0.05 \text{ b}$	$0.57 \pm 0.01 \text{ c}$	$0.10 \pm 0.01 \text{ d}$
C17:0	$0.03 \pm 0.01 \text{ b}$	$0.91 \pm 1.56 \text{ a}$	$0.08 \pm 0.02 \text{ b}$	$0.05 \pm 0.04 \text{ b}$	$0.08 \pm 0.01 \text{ b}$
C17:1	$0.05 \pm 0.01 \text{ b}$	$0.15 \pm 0.09 \text{ a}$	$0.10 \pm 0.01 \text{ b}$	$0.08 \pm 0.01 \text{ b}$	$0.05 \pm 0.01 \text{ b}$
C18:0	$2.46 \pm 0.04 \text{ c}$	$4.95 \pm 0.88 \text{ a}$	$2.68 \pm 0.05 \text{ c}$	$2.39 \pm 0.03 \text{ c}$	$4.27 \pm 0.01 \text{ b}$
C18:1(ω -7)	$3.17 \pm 0.11 \text{ a}$	$2.37 \pm 0.55 \text{ b}$	$3.48 \pm 0.39 \text{ a}$	$2.31 \pm 0.11 \text{ b}$	$1.55 \pm 0.03 \text{ c}$
C18:1(ω -9)	$66.93 \pm 0.26 \text{ b}$	$59.97 \pm 1.11 \text{ c}$	$66.03 \pm 0.49 \text{ b}$	$74.9 \pm 0.35 \text{ a}$	$26.65 \pm 0.08 \text{ c}$
C18:2(ω -6)	$12.43 \pm 0.17 \text{ b}$	$9.34 \pm 0.33 \text{ c}$	$12.10 \pm 0.09 \text{ b}$	$8.18 \pm 0.06 \text{ c}$	$48.51 \pm 0.02 \text{ a}$
C18:3(ω -3)	$0.84 \pm 0.09 \text{ b}$	$0.83 \pm 0.01 \text{ b}$	$0.97 \pm 0.10 \text{ b}$	$0.81 \pm 0.01 \text{ b}$	$6.35 \pm 0.01 \text{ a}$
C20:0	$0.26 \pm 0.04 \text{ a}$	$0.32 \pm 0.01 \text{ a}$	$0.33 \pm 0.03 \text{ a}$	$0.27 \pm 0.02 \text{ a}$	$0.33 \pm 0.01 \text{ a}$
C20:1 (ω -9)	$0.27 \pm 0.07 \text{ b}$	$0.25 \pm 0.01 \text{ b}$	$0.24 \pm 0.05 \text{ b}$	$0.31 \pm 0.02 \text{ a}$	$0.16 \pm 0.01 \text{ c}$
$\Sigma \text{SFA}^{\dagger} \%$	15.02 b	21.93 a	15.69 b	12.58 c	16.27 b
$\Sigma \text{MFA}^{\ddagger} \%$	71.7 a	64.14 b	71.2 a	78.4 a	28.5 c
$\Sigma \text{PFA}^{\S} \%$	13.27 b	10.17 c	13.07 b	8.99 c	54.86 a

The results are expressed as mean % \pm SD of total fatty acid methyl esters ($n = 3$). † : saturated fatty acid; ‡ : monounsaturated fatty acid; § : polyunsaturated fatty acid; nf = not found. Different letters for each fatty acid indicate statistical differences at $p < 0.05$ (Student's *t* test) among cultivars.

3.3. Individual Phenolic Compounds Identification

In our study, UHPLC-HESI-MS was used for identification of the phenolic compounds. Table 3 shows the calibration data of reference compounds used as external standards in the UHPLC-HESI-MS characterization.

Table 3. Calibration data of reference compounds used as external standards for identification of phenolic compounds.

Phenolic Compounds	Molecular Formula	Experimental m/z [M-H] [−]	Retention Time	Linear Regression	(r ²)
Tyrosol	C ₈ H ₁₀ O ₂	137.05933	12.05	Y = −124,926 + 8556 × X	0.9937
Hydroxytyrosol	C ₈ H ₁₀ O ₃	153.05438	9.15	Y = −5.29 × 10 ⁶ + 7.31 × 10 ⁷ × X	0.9974
<i>p</i> -Coumaric Acid	C ₉ H ₈ O ₃	163.03847	18.30	Y = −5.83 × 10 ⁶ + 8.72 × 10 ⁷ × X	0.9959
Caffeic Acid	C ₉ H ₈ O ₄	179.03392	14.90	Y = −2.65 × 10 ⁶ + 8.46 × 10 ⁷ × X	0.9750
Ferulic Acid	C ₁₀ H ₁₀ O ₄	193.05026	19.10	Y = −5.99 × 10 ⁶ + 5.15 × 10 ⁷ × X	0.9983
Vanillic Acid	C ₈ H ₈ O ₄	167.03388	14.30	Y = −412,147 + 2.85 × 10 ⁶ × X	0.9812
<i>p</i> -Hydroxybenzoic Acid	C ₇ H ₆ O ₃	137.02304	13.50	Y = 648,298 + 6.49 × 10 ⁷ × X	0.9982
Syringic Acid	C ₉ H ₁₀ O ₅	197.04519	14.56	Y = 746,927 + 5.54 × 10 ⁶ × X	0.9852
Cinnamic Acid	C ₉ H ₈ O ₂	147.04049	3.50	Y = −1.69 × 10 ⁶ + 2.42 × 10 ⁶ × X	0.9888
Gallic Acid	C ₇ H ₆ O ₅	169.01319	7.45	Y = −1.94 × 10 ⁶ + 4.30 × 10 ⁷ × X	0.9968
Protocatechuic Acid	C ₇ H ₆ O ₄	153.01763	10.35	Y = −2.85 × 10 ⁶ + 2.09 × 10 ⁷ × X	0.9929
Oleocanthal	C ₁₇ H ₂₀ O ₅	303.12387	23.50	Y = −3.19 × 10 ⁶ + 3.09 × 10 ⁷ × X	0.9984
Vanillin	C ₈ H ₈ O ₃	151.03867	17.22	Y = −318,221 + 5.02 × 10 ⁶ × X	0.9994
Pinoreosinol	C ₂₀ H ₂₂ O ₆	357.13445	23.05	Y = −61,525.2 + 634911 × X	0.9988
Oleuropein	C ₂₅ H ₃₂ O ₁₃	539.17732	20.60	Y = −3.11 × 10 ⁶ + 3.10 × 10 ⁷ × X	0.9985
Luteolin	C ₁₅ H ₁₀ O ₆	285.04037	23.30	Y = 3.61 × 10 ⁶ + 1.24 × 10 ⁸ × X	0.9992
Diosmetin	C ₁₆ H ₁₂ O ₆	299.05621	24.05	Y = 1.435 × 10 ⁸ × X	0.9853
Apigenin	C ₁₅ H ₁₀ O ₅	269.04565	24.00	Y = 2.37 × 10 ⁷ + 2.18 × 10 ⁸ × X	0.9989
Apigenin-7-glucoside	C ₂₇ H ₃₀ O ₁₀	431.09854	22.90	Y = 9.18 × 10 ⁶ + 7.56 × 10 ⁷ × X	0.9954

The UHPLC-HESI-MS analysis allowed the identification of 13 phenolic compounds and the class of secoiridoids is the most represented as shown in Table 4.

Table 4. Phenolic compounds (mg kg^{−1}) identified in the monovarietal olive oils by UHPLC-ESI-MS.

Phenolic Compound		Cultivars				
		Tefahi	Chemlal	Gelb Elfarroudj	Manzanilla	Zebboudj
Phenolic alcohol	Hydroxytyrosol	0.41 a	nf	nf	0.24a	Nf
	<i>p</i> -Coumaric acid	0.54 b	nf	0.61 a	nf	0.41 b
Phenolic acids	Caffeic acid	0.07 c	0.98 c	1.22 a	0.8 b	0.72 a
	Ferulic acid	0.41 a	0.55 c	0.68 b	nf [†]	nf
	Vanillic acid	nf	nf	0.70 c	nf [†]	1.43 b
	<i>p</i> -Hydroxy benzoic acid	nf	nf	0.42 a	0.32 c	0.45 a
Secoiridoids	Oleacein (3,4-DHPEA-EDA)	8.01 a	2.50 b	2.30 a	25.16 b	nf
	Oleocanthal (p-HPEA-EDA)	2.07 b	3.54 c	1.13 b	4.20 a	nf
	Oleuropein aglycon (3,4-DHPEA-EA)	49.65 a	7.28 a	13.71 a	62.67 a	0.13 a
	Deacetoxy-10-hydroxy oleuropein aglycon (DAc-10-OH Ole Agly);	0.61 a	0.18 a	nf	0.84 b	nf
	Elenolic acid	16.88 b	3.70 b	1.72 a	3.18 b	nf
	Ligstroside aglycon (p-HPEA-EA)	nf	8.09 c	3.46 c	12.86 a	0.22 a
Flavonoids	Luteolin	0.83 b	0.54 a	0.51 b	0.41 a	0.14 b

The results are given by the mean value ($n = 3$) of independent determinations, including extraction and injection. Different letters for each phenolic compound indicate statistical differences at $p < 0.05$. nf = not found.

Monovarietal oil samples showed a very similar phenolic composition. Differences from a quantitative point of view were recorded, in particular for the portion of the secoiridoids where

3,4-DHPEA-EA (oleuropein aglycon) was the main compound found. With regard to flavonoid derivatives, luteolin was detected in all samples, contrary to diosmetin and apigenin-7-glucoside.

The main phenolic compound was 3,4-DHPEA-EA (oleuropein aglycon) with contents varying from 62.67 mg kg⁻¹ to 0.13 mg kg⁻¹, together with p-HPEA-EA (ligstroside aglycon) that ranged from 12.86 mg kg⁻¹ to 0.22 mg kg⁻¹; 3,4-DHPEA-EDA (oleacein) was found in a range of 2.30 mg kg⁻¹ to 25.16 mg kg⁻¹; p-HPEA-EDA (oleocanthal) showed the highest content (3.54 mg kg⁻¹) in Chemlal and the lowest content (2.07 mg kg⁻¹) in Tefahi.

In the group of phenolic acids, the amounts of the compounds were less abundant in olive oils than the amount of the secoiridoids group. *p*-coumaric acid, caffeic acid, ferulic acid, vanillic acid, and *p*-hydroxybenzoic acid were the phenolic acids found in some of the oils with small amounts.

3.4. Antioxidant Activity and Total Content of Phenolic Compounds

The results of the in vitro antioxidant assays performed on the olive oil phenolic fraction extracts are summarized in Table 5. They revealed that the oil PFE issued from the Tefahi cultivar was the most active, while Zebboudj was the least active and did not give any result in 3/5 of the assays (DPPH, FRAP, and TEAC). There was no significant difference observed between the oil PFE of Tefahi and Manzanilla cultivars in the results obtained with FRAP, TEAC, and BCB assays and much closer results were observed in the Folin–Ciocalteu test between the oils of Manzanilla, Chemlal, and Gelb Elfarroudj.

Table 5. Antioxidant activity and total phenolic content of the olive oil phenolic fraction extracts as measured by means of five in vitro assays.

Varieties	Folin–Ciocalteu μg GAE mg ⁻¹ (±SD)	DPPH mmol TEMg ⁻¹ (±SD)	FRAP mmol Fe ²⁺ Emg ⁻¹ (±SD)	TEAC mM TEMg ⁻¹ (±SD)	BCB IC ₅₀ % mgml ⁻¹ (CL95)
Tefahi	237.19 ± 23.70	0.70 ± 0.08	0.84 ± 0.12	0.31 ± 0.07	1.84 (1.52–2.15)
Chemlal	59.24 ± 8.99 **	0.05 ± 0.001 **	0.12 ± 0.02 **	0.008 ± 0.001 **	14.81 (12.74–17.21)*
Gelb Elfarroudj	48.94 ± 5.65 **	0.03 ± 0.004 **	0.044 ± 0.002*	0.020 ± 0.003 **	8.46 (7.41–9.66)*
Manzanilla	46.32 ± 9.10 **	0.22 ± 0.06 **	0.34 ± 0.20	0.37 ± 0.02	6.54 (5.36–7.99)
Zebboudj	37.34 ± 4.71 **	-	-	-	7.44 (5.62–9.84)

Results are expressed as mean ± SD of three experiments for DPPH, FRAP, and TEAC tests, and as 50% mean inhibition concentration (IC₅₀) with confidence limits (CL) at 95% for the BCB assay. GAE: Gallic acid equivalents; TE: Trolox equivalents. * *p* < 0.05; ** *p* < 0.01 versus Tefahi oil phenolic fraction extract.

4. Discussion

The qualitative parameters of the studied oils highlight very high values which bring into question their quality. In particular, Zebboudj, Manzanilla, and Gelb Elfarroudj oils had high values of free acidity (0.75–1.25 g of oleic acid 100 g⁻¹ of oil), which could be connected with the prolonged ripening before the olive harvest.

The amount of phenolic compounds in EVOO is an important factor when evaluating its quality, given that the natural phenols improve its resistance to oxidation, and to a certain extent, are responsible for its sharp bitter taste.

In this study, for the first time, the composition of secoiridoids in olive oils of some Algerian cultivars was analyzed with UHPLC-HESI-MS. The most abundant secoiridoids of the olive oil analyzed were the aglycones of oleuropein and ligstroside, and these results agree with previous data found for Algerian Chemlal olive oils [30,31]. In general, all the samples showed lower concentrations of oleocanthal, if compared to the other cultivars of the countries of the Mediterranean basin [19,20].

We suggest that the composition in phenolic compounds of the oils under study could be higher if the harvesting time is done earlier; previous studies demonstrated that olives have the highest phenolic compound content at the phase between green and darker skin [13,32]. The extraction of olive oil from black olive fruits was justified by the Algerian producers in that it allows them to get better olive oil yield [33].

The phenolic compounds are known to contribute to antioxidant/radical scavenging activity; their concentrations have a relationship with the percentage of radical inhibition [34]. Consequently, the lack of antioxidant activity in some experiments when testing Zebboudj oil PFE could be explained by the low concentration of phenolic compounds. However, Zebboudj oil PFE demonstrated good BCB results, probably due to the presence of vanillic acid, since previous studies reported its antioxidant and protective effects on the peroxidation of lipids [35].

The antioxidant activity of Tefahi oil PFE may be related to its composition in secoiridoids mainly due to oleuropein aglycon (3,4-DHPEA-EA) and elenolic acid. The antioxidant activity of Manzanilla oil PFE is probably due to the presence also of high amounts of secoiridoids, mostly oleuropein aglycon (3,4-DHPEA-EA) and ligstroside aglycon (p-HPEA-EA). We noticed that in both Tefahi and Manzanilla, there were high amounts of oleuropein aglycon (3,4-DHPEA-EA), a hydrophilic secoiridoid that demonstrated in other works a metal-chelation and a free-radical-scavenging action [36]. Some other phenolics may contribute to the antioxidant activity with their remarkable presence such as the phenolic acid oleacein (3,4-DHPEA-EDA) in both Tefahi and Manzanilla oil PFE.

In another study about the Manzanilla cultivar growing in Australia, the authors reported amounts of caffeic acid (0.46 mg kg^{-1}) less than the amounts found in this study (0.8 mg kg^{-1}), whereas they detected other compounds that we could not find such as vanillic and ferulic acids. The same study reported that a significant gradual decrease was noted in major polyphenolic compounds in the later harvest stage [37].

Other authors tested the antioxidant activity of Algerian olive oils (cultivar Chemlal and *Olea europaea* L. subsp. Oleaster) extracted using laboratory technics instead of extraction in the mill, and the results of antioxidant activity were higher with respect to our results [30,38]. Unfortunately, there are no studies on the composition and antioxidant activity of olive oils from Algerian cultivars growing in the same region of our study (Setif and Batna); for this reason, it is difficult to compare the results. Certainly the region of cultivation of the various cultivars plays a decisive role in the chemical characteristics of the oils produced [39].

The technics used in the commercial mills for the extraction of olive oil may play also a crucial role in the olive oil quality such as the malaxation temperature. In literature it was reported that a significant increment of total phenols concentration was found with a maximum at 27°C , whereas for higher temperatures ($30\text{--}36^\circ\text{C}$), a progressive decrement was observed [40]. The malaxation temperature used in the commercial mill in this study was 30°C ; we suggest that reducing this temperature may give better olive oil quality.

In our study, the olive oils were obtained with the method used from local producers in Algeria. This method did not give us a very high olive oil quality. In general, to produce EVOOs with high quality which reinforces its enhancement in the international market, a better knowledge of the local cultivars and the best agronomic techniques are necessary.

5. Conclusions

Antioxidant activity as well as fatty acids composition, phenolic acids, and secoiridoids content of some Algerian monovarietal olive oils were investigated. The results indicate that the quality parameters are acceptable for all oils, except for Zebboudj oil. The most important results from the chemical point of view were the phenolic portion. Manzanilla reported the greatest quantity of secoiridoids in particular as regards oleuropein aglycon, ligstroside aglycon, and oleacein (62.67 mg kg^{-1} , 12.86 mg kg^{-1} , and 25.16 mg kg^{-1} , respectively). Tefahi oil showed quality parameters in line with international regulations, an appreciable content of monounsaturated fatty acids (66.93%) but modest levels of oleuropein aglycon (49.65 mg kg^{-1}) and other secoiridoids if compared to those of other oils of the Mediterranean areas. The antioxidant activity of Tefahi oil was found to be the best among the cultivars used. This study constitutes a starting point for the diffusion and marketing of Tefahi monovarietal oils due to their phenolic content and antioxidant capacity.

This is just a preliminary report since only a single sample from each cultivar was investigated. However, a more in-depth study must be done on agronomic techniques and ripening stage before the olive harvest takes place, which has been shown to have great influence on the quality of the oils produced.

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