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Effect of Extraction Method on Organoleptic, Physicochemical Properties and Some Biological Activities of Olive Oil from the Algerian *Chemlal* Variety

Sara Messad[1,2], Souhila Bensmail[2], Omar Salhi[3], Djamila Djouahra-Fahem[2]

[1]Akli Mohand Oulhadj University, Faculty of Natural and Life Sciences and Earth Sciences, Laboratory of Management and Valorization of Natural Resources and Quality Assurance (LGVRNAQ), Bouira, Algeria
[2]Akli Mohand Oulhadj University, Faculty of Natural and Life Sciences and Earth Sciences, Department of Biology, Bouira, Algeria
[3]Biotechnology Laboratory of Animal Reproduction, Institute of Veterinary Sciences, Blida, Algeria

ORCID IDs of the authors: S.M. 0000-0002-9815-0327; S.B. 0000-0002-6366-7537; O.S. 0000-0002-0211-1981; D.D.F. 0000-0002-5442-2319

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ABSTRACT

Objective: The production of olive oil in Algeria is of great importance. The choice of the extraction method is crucial in order to achieve the best yields of high quality oil. This work aims to study the effect of the extraction method on the sensory and physicochemical characteristics and the antioxidant and antimicrobial activities of three types of olive oil derived from the same sample of olive fruits (*Chemlal* variety).

Materials and Methods: Several physicochemical and sensory parameters of olive oil samples were evaluated by referenced methods (ISO, IOC, AFNOR, etc.). The fatty acid composition of oils was determined by gas chromatography. Total phenolic content and antioxidant activity were estimated using the Folin-Ciocalteu assay and FRAP test, respectively. Antimicrobial activity analysis was performed using the agar diffusion technique against five strains.

Results: The analyses revealed that olive oil obtained by manual extraction is classified in the extra virgin category, according to the International Olive Oil Council. The two other oils, traditional and industrial, were of virgin class. Fatty acid composition shows significant differences between the three oils which are characterized by a remarkable richness of phenolic compounds (447-528 mg GAE/kg oil). The best antioxidant activity was noted in the manual olive oil ($PR_{0.5}$=78.60 µg/mL). *Pseudomonas aeruginosa* ATCC 9027 and *Staphylococcus aureus* ATCC 6538 were more sensitive to action of the manual oil compared to other bacterial and fungal strains tested, with inhibition zones of 15.84±0.07 and 15.31±0.20 mm, respectively. This activity was significantly decreased by evaluating antimicrobial effect of the other oils.

Conclusion: The method of extraction significantly affects the properties of olive oil, where the manual method preserves the best quality of oil.

Keywords: Olive oil, extraction process, characterization, antioxidant activity, antimicrobial activity

INTRODUCTION

Olive oil, derived from the fruits of *Olea europaea* L., is one of the most important elements of the Mediterranean diet, not only for its appreciable taste and its usefulness in flavoring of a wide variety of foods, but also for its many beneficial properties due to its chemical composition (1,2). The latter is mainly characterized by a richness in antioxidants, vitamins, phenolic compounds and essential fatty acids for human nutrition, especially polyunsaturated ones. These metabolites are preserved through the consumption of olive oil in crude form, unlike several other common vegetable oils that require a refining step (3-5). For this reason, olive oil production

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has experienced a remarkable economic increase during the last years (2).

The chemical composition, sensory properties and biological activities of olive oil are highly dependent on several factors: fruit ripening, climate, cultivation procedures, harvesting techniques and extraction method (1,5-7).

The key objective of any extraction method is to obtain the highest possible quantity of oil without altering its original quality (3,7). In order to preserve this, it is essential to proceed with extraction directly from fresh fruits of the olive tree and to use mechanical or physical methods, avoiding chemical and enzymatic reactions that could modify the natural composition of oil and its organoleptic characteristics (3,4,6).

Olive oil extraction involves different processes, such as leaf removal, olive washing, crushing, threshing, and oil separation performed either by pressure or centrifugation (4,8). Olives should be processed as soon as possible after harvest to minimize oxidation and preserve low acidity (4). It is worth mentioning, that extra virgin olive oil is the highest quality olive oil and represents only 10% of the total oil produced (6). For this reason, it is very important to select the extraction process that ensures such quality.

The aim of our study is to determine the influence of the extraction method on the sensory and physicochemical characteristics as well as the antioxidant and antimicrobial activities of three types of olive oil obtained by different extraction methods.

MATERIALS AND METHODS

Collect and Treatment of Samples

The material used consists of three samples of olive oil from the region of Lakhdaria (Wilaya of Bouira, Algeria) (36°33'58.612"N 3°35'29.018"E). The oil extraction was carried out on olive fruits (*Chemlal* variety) harvested during the end of the harvesting season (January, 2017). Choice of olive fruit was focused on fruits with black pigmentation in order to avoid other early stages (semi-black, green or red fruit), having the possibility to affect the oil extraction yield and its conservation. The harvested fruits were mixed and then divided into three groups used for oil extraction according to three processes:

- The first type of oil was the manual olive oil (MOO), which was obtained in a traditional way: the harvested fruits, free of leaves and branches, were washed with running water to eliminate impurities and then dried in open air in a sunny place. The fruits were then crushed by the feet until a smooth blackish paste was obtained. The drained oil was collected in a glass bottle, and the paste was placed in a clean and inclined container.

- The second type of oil was traditional olive oil (TOO), resulting from a press extraction performed at a traditional oil mill.

- The third sample was represented by industrial olive oil (IOO) obtained following a conventional extraction stage, carried out this time at a modern oil mill (industrial), which ensures higher yields of oil.

The three types of oils were stored in hermetically sealed glass bottles and were kept cool and protected from light to avoid polymerization and oxidation.

Sensory Analysis

The organoleptic evaluation is essential to decide on the classification of an olive oil (category virgin, extra virgin or not), because it allows the detection of possible defects not highlighted by the physicochemical analyses. In our study, this evaluation was carried out based on the principles of IOC/T.20/Doc. nº15/Rev.10 (9), by a panel of 20 tasters made up of former previously trained oil mill workers and old olive oil connoisseurs. The oil samples were kept at about 28°C, while the temperature of the analysis room was about 20°C. The analyses were performed in one run. The attributes were quantified on a continuous scale that allowed the tasters to express in a precise way the intensity with which they perceived each attribute. Once the rating sheets were completed, the method used to rank the oils was based on the calculation of the median, considering that the value of the robust coefficient of variation that defines the ranking (defect perceived with the highest intensity and fruitiness) is 20%.

The principle of tasting emphasizes the absence of defects and the intensity of fruitiness. This means that as soon as the slightest trace of a defect is recognized by the majority of tasters on the panel, the oil is no longer entitled to the "extra virgin" designation (IOC/T.15/NC Nº 3/Rev.14 (10).

Physical and Chemical Analysis

Physical indices including refractive index (11), relative density at 20°C, water and volatile content (12), and ultraviolet (UV) absorbance (13) were studied.

For the chemical characterization of oils, different parameters were determined: acid and peroxide indices (EEC N°. 2568) (14), saponification and iodine indices (15). The estimation of chlorophyll content and carotenoids was carried out according to the method described by Minguez-Mosquera et al. (16).

Fatty Acid Composition

Determination of the fatty acid profile of the three oils was carried out with gas chromatography (GPC) of fatty acid methyl esters (17) using a Chrompack CP 9002 type chromatograph equipped with a capillary column Cp Sil 88 CB (5% phenyl + 95% dimethylpolysiloxane) (30 m length, 0.25 µm thick, 0.32 mm inner diameter), a SPLIT 1/100 injector (250°C), a flame ionization detector (FID) (250°C) and an oven (190°C). Nitrogen was used as the carrier gas injected with a flow rate of 1 mL/min. Fatty acids were identified by their retention time in the column in comparison to given standards. The amount of each fatty acid is given in % of total fatty acid.

Evaluation of the Antioxidant Activity

Extraction of Polyphenols

Before proceeding to evaluation of the antioxidant activity, phenolic compounds of the three olive oils were extracted following the protocol described by Pirisi et al. (18). 20 g of each olive oil were introduced into centrifuge tubes. Subsequently 10 mL of n-hexane and 20 mL of 60% methanol were added. After vortex homogenization for 2 min and centrifugation (3000 rpm, 5 min), the supernatants containing polyphenols were recovered separately. The operation was repeated twice to deplete the oil. The resulting supernatants were then combined and evaporated under vacuum at 40°C until a dry residue was obtained, which was re-suspended in 10 mL of 50% methanol. The yield of the extraction (R%) was calculated by the following formula:

$$R(\%) = \frac{m - m_0}{m_T} \times 100$$

m : Mass of the beaker after evaporation.

m_0 : Mass of the beaker before evaporation.

m_T : Total mass of the test sample.

Dosage of Phenolic Compounds

Total polyphenols content of the extracts obtained was determined by the Folin-Ciocalteu method (19). To 200 µL of the methanolic extract, 1 mL of Folin-Ciocalteu reagent (diluted 10 times, v/v) and 800 µL of 7.5% sodium carbonate (w/v) were added. After homogenization and incubation for 30 min in the dark at room temperature, the absorbance was measured at 760 nm using a spectrophotometer against a blank prepared under the same conditions, where the extract was replaced by 50% methanol. Gallic acid (1 mg/mL) was used as a standard. The concentration of polyphenols was reported as mg gallic acid equivalent (GAE)/Kg oil.

FRAP Test (Ferric Reducing Antioxidant Power)

The antioxidant activity of phenolic extracts was determined using the FRAP method (20). 250 µL of each extract prepared at different concentrations (62.5; 125; 250 and 500 µg/mL) were mixed with 250 µL of phosphate buffer (0.2 M, pH 6.6) and 250 µL of 1% (w/v) potassium hexacyanoferrate [$K_3Fe(CN)_6$]. After incubation in a water bath at 50°C for 20 min, 250 µL of 10% (w/v) trichloroacetic acid (TCA) was added. Subsequently, the tubes were centrifuged at 3000 rpm for 10 min. 50 µL of the recovered supernatant was added to 200 µL of distilled water and 10 µL of the 0.1% (w/v) $FeCl_3$ solution. The absorbance was measured at 700 nm against a blank. A higher absorbance of the reaction mixture indicated a higher reducing power.

Evaluation of the Antimicrobial Activity

The antibacterial and antifungal activities of olive oils were performed *in vitro* using the agar diffusion technique described by several authors (21,22). The microbial strains used are known to be pathogenic to humans: two Gram$^+$ bacterial strains (*Staphylococcus aureus* ATCC 6538; *Bacillus subtilis* ATCC 6633); two Gram$^-$ bacterial strains (*Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739) and one yeast (*Candida albicans* ATCC 10231). The listed strains were obtained from the collection of the microbiology laboratory "SAIDAL Antibiotical" (Alger, Algeria).

Bacterial inocula were standardized in sterile physiological water, from a pure and fresh culture (18-24 h for bacteria and 48 h for yeast), to obtain an opacity equivalent to 0.5 McFarland (10^7 CFU/mL). Then, a swabbing was performed on the surface of a Muller Hinton Agar. Sterile Whatman paper discs (n°3, 6 mm) were soaked with olive oil (all three types) until impregnation (10 µL), then placed on the surface of the previously inoculated agar and uniformly seeded with the bacterial or fungal suspension to be studied.

Readings were then taken after incubation for 24 h at 37°C for bacterial strains and at 25°C for 48 h in the case of *C. albicans*. Two controls were prepared in parallel: the first did not contain the oil and the second was free of the germ to be tested.

The sensitivity of the target strains towards the different compounds was classified according to the diameters of the inhibition zones obtained: Ø<8 mm: non-sensitive bacteria; 9<Ø<14 mm: sensitive bacteria; 15<Ø<19 mm: highly sensitive bacteria and Ø>20 mm: extremely sensitive bacteria (23,24). The same intervals were respected for the fungal strain.

Statistical Analysis

The variance study was carried out to determine the significant differences (Student's t-test) between the different types of oil by the software "Microsoft Office Excel 2010". Results were represented by the mean with its standard deviation. The differences were considered significant at $p \leq 0.05$ (*), very significant at $p \leq 0.01$ (**) and highly significant if $p \leq 0.001$ (***).

RESULTS

Sensory Analysis

According to the classification made by comparing the value of the median of the majority defect to the reference intervals for each denomination, we concluded that:

- The manual olive oil was in the category "Extra Virgin" (the median of the defects was equal to zero and the median of the fruitiness was greater than zero (m=5) (Figure 1a).
- Traditional olive oil was in the category "Virgin" (the median of defects was equal to: $0 < m \leq 3.5$ (m=1) and the median of fruitiness was greater than zero (m=4) (Figure 1b).
- Industrial olive oil fell into the category "Virgin" (the median of defects was equal to: $0 < m \leq 3.5$ (m=2) and the median of fruitiness was greater than zero (m=3.25) (Figure 1c).

Physicochemical Indices

Acidity is the main criterion of quality and commercial standard of olive oil. However, other criteria of physicochemical and organoleptic quality are also currently associated with these stan-

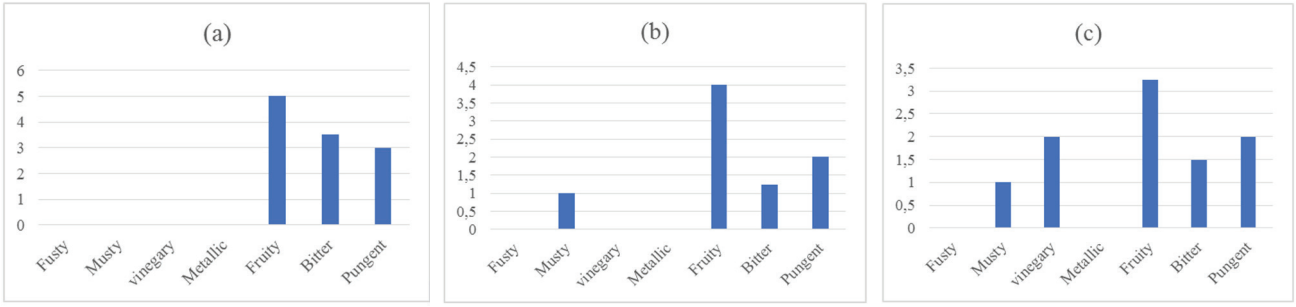

Figure 1. Sensory profile of the studied oil samples: manual (a), traditional (b) and industrial olive oils (c).

Table 1. Physicochemical indices of the three olive oils characterized.

Physicochemical indices - Type of oil	MOO	TOO	IOO
Physical indices			
- Refractive index	1.468±0.00	1.486±0.02	1.468±0.01
- Water and volatile substances content (%)	0.10±0.02	0.095±0.01*	0.067±0.01
- Relative density at 20°C	0.9137±0.01	0.9143±0.00	0.9137±0.00
- $A_{232\ nm}$	2.16±0.02	2.211±0.06	2.211±0.05
- $A_{270\ nm}$	0.158±0.02	0.201±0.03*	0.225±0.03
Chemical indices			
- Acid value (%)	0.80±0.01	0.90±0.01	1.40±0.02
- Peroxide value (meq of O_2/Kg)	15.38±0.03	17.17±0.13	16.66±0.02
- Iodine value	83.93±0.01	83.75±0.04	79.18±0.07
- Saponification index (mg KOH/g oil)	196.7±0.02	192.77±0.09	193.75±0.10
- Chlorophyll content (mg/Kg)	0.24±0.07	0.41±0.02	2.03±0.01
- Carotenoid content (mg/Kg)	7.03±0.05	6.54±0.03	7.62±0.09

MOO: manual olive oil, TOO: traditional olive oil, IOO: industrial olive oil. Significance level: *: p<0.05; Acid index: p<0.001; Peroxide index: p<0.05; Iodine index: p<0.01; Saponification index: p<0.001; Chlorophyll content: p<0.05; Carotenoid content: p<0.001.

dards. The values of the different physical and chemical indices of all samples are listed in Table 1. A significant difference was noted for all chemical indices as well as some physical parameters between the three olive oils.

Fatty Acid Composition

The results of GPC analysis of the fatty acid profile of oils obtained by different extraction processes are summarized in Table 2.

The results obtained indicate a content of fatty acids more or less variable but remaining within the standards of the IOC (10) and Codex Alimentarius (28), except for palmitoleic acid (C16:1ω7), which held a percentage slightly higher than the standard (>3.5%). For all samples, the most intense content was attributed to oleic acid (C18:1ω9), followed by palmitic acid (C16:0), then linoleic acid (C18:2ω6) and palmitoleic acid. Stearic

Table 2. Fatty acid composition (%) of the studied oil samples.

Fatty acid	Name	MOO	TOO	IOO
C16:0	Palmitic acid	16.87	17.61	17.68
C16:1ω7	Palmitoleic acid	4.26	3.64	3.80
C17:0	Margaric acid	Trace	0.42	0.33
C18:0	Stearic acid	1.67	2.10	2.11
C18:1ω9	Oleic acid	62.39	61.89	60.87
C18:2ω6	Linoleic acid	14.11	13.43	13.33
C18:3ω3	Linolenic acid	0.20	0.28	0.98
C20:0	Arachidic acid	0.25	0.20	0.20
C20:1ω9	Gondolic acid	0.21	Trace	Trace

MOO: manual olive oil, TOO: traditional olive oil, IOO: industrial olive oil.

acid (C18:0), arachidic acid (C20:0) and linolenic acid (C18:3ω3) represented more or less small amounts.

Yield and Total Polyphenols Content

According to the results shown in Table 3, the best yield of total polyphenols was noted for the olive oil obtained by the manual method. The yields noted for the other two oils were quite close and lower compared to the first type. Total polyphenols content of the three olive oils varied from 447 to 528.45 mg GAE/Kg oil.

Table 3. Yield and polyphenols content of the three olive oil samples.

Type of oil	MOO	TOO	IOO
Yield (%)	11.55	6.45	6.55
Polyphenols content (mg GAE/Kg oil)	528±12.40	496±11.80	447±10.70

MOO: manual olive oil, TOO: traditional olive oil, IOO: industrial olive oil.

Antioxidant Activity

From Figure 2, we can note that polyphenols of the manual olive oil and traditional oil mill had a much higher antioxidant power compared to industrial oil mill.

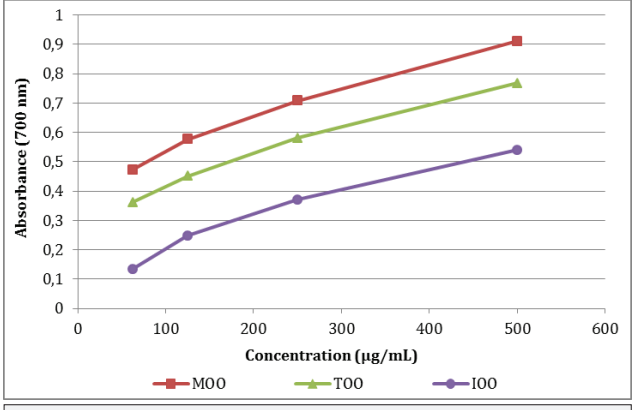

Figure 2. Reducing power of phenolic extracts obtained at different concentrations.

MOO: manual olive oil, TOO: traditional olive oil, IOO: industrial olive oil.

The parameter $PR_{0.5}$ was determined in order to further compare the reducing power. It corresponds to the concentration for which the reduction product gives an absorbance of 0.5 at 700 nm. The OriginPro 8.5 SRI software was used to determine the $PR_{0.5}$ values for each extract with greater accuracy. The values obtained are represented in Table 4.

Table 4. Phenolic extract concentration (µg/mL) of the different oils at A_{700nm}=0.5

Sample	MOO	TOO	IOO
Concentration corresponds to $PR_{0.5}$	78.60	170.00	439.05

MOO: manual olive oil, TOO: traditional olive oil, IOO: industrial olive oil.

Antimicrobial Activity

The results of the inhibition zone diameters of the different olive oils tested on a range of pathogenic bacteria are represented in Table 5. All 3 types of olive oil showed antimicrobial activity on all strains studied with inhibition zones ranging from 12.07 to 15.84 mm. However, modern mill olive oil was found to be inactive against *S. aureus* ATCC 6538 and exhibited lower antimicrobial activity than the traditional olive oil, but without a significant difference only in the case of *C. albicans* ATCC 10231. Activity of the two oils was significantly lowest compared to that provided by the manual extraction.

DISCUSSION

The sensory analysis shows that the three samples of olive oil were of a superior category, which would be due not only to the variety, degree of ripening, climate, cultivation techniques (mainly irrigation) (25), but also because these oils were obtained only by mechanical processes, under conditions that prevented alteration of oil, and which had not undergone any treatment other than washing, decanting, centrifugation and filtration (26). Traditional manual olive oil was extracted from the paste only, without crushing the pit and according to Amirante et al., (27), oils obtained from pitted fruit are more resistant to oxidation than oils obtained by crushing the whole fruit by about 20-25%. The pitting of the olive induces an increase in

Table 5. Diameter (mm) of the inhibition zones obtained with the three olive oils.

Germs - Type of oil	MOO	TOO	IOO
S. aureus ATCC 6538	15.31±0.20	13.59±0.19***	-
B. subtilis ATCC 6633	14.04±0.13	13.00±0.00**	12.07±1.02*
P. aeruginosa ATCC 9027	15.84±0.07	12.81±1.10*	13.07±0.33**
E. coli ATCC 8739	14.23±0.45	13.92±0.26	13.84±0.12
C. albicans ATCC 10231	14.52±0.13	13.37±0.19**	12.42±0.30***

MOO: manual olive oil, TOO: traditional olive oil, IOO: industrial olive oil. -: Absence of inhibition zone. Significance level: *: p<0.05, **: p<0.01, ***: p<0.001.

pleasant aromatic compounds and seems to be responsible for the pronounced fruity aroma.

The results of physicochemical indices indicate that for refractive index, the MOO and IOO samples complied with the Codex Alimentarius (28) standards (1.4677-1.4705) except for the traditional olive oil which slightly exceeded the standards. Its value was influenced by different factors such as free fatty acid content, oxidation level and heat treatment (29).

The values obtained for density were in accordance with the Codex Alimentarius (28) standards (0.910-0.916). This parameter is a function of unsaturation, but also of the state of their oxidation, the more it increases, the more oxidized the oil is (30).

Water and volatile content values were below the maximum limits of IOC (10) (≤0.2%). Cultivar, geographical region, fruit ripening and processing methods are the parameters that influence the volatile composition of olive oil (31).

Regarding the specific extinction coefficients at 232 nm and 270 nm of our samples, they were lower than the maximum limits of IOC (10) (≤2.60 and 0.25, respectively), and confirm the low oxidation and stability of the three oils, especially the MOO sample, which showed the lowest extinction values. Oxidative stability is an important parameter for olive oil quality assessment (32). The specific extinction of a fat is considered a picture of its oxidation state. The higher its extinction at 232 nm, the more peroxidized it is; the higher it is at 270 nm, the richer it is in oxidation by-products (33). Vidal et al. (34) have previously reported the influence of extraction time and temperature on the two extinction coefficients K_{232} and K_{270}.

Free acidity is the main quality factor of olive oil. The values obtained are in accordance with the IOC (10) standards (≤2%). High acidity induces oxidation resulting in rancidity of the oil due to the degradation of unsaturated fatty acids (oleic, linoleic acid) and the production of secondary oxidation compounds (35).

The peroxide value determines the initial oxidation of olive oil, which oxidizes when it enters into contact with air, light, and heat. The values of this index regarding our samples are in accordance with IOC (10) standards (≤20 meq O_2/Kg).

The values of iodine index recorded for the three olive oil samples are in accordance with the Codex Alimentarius (28) standards (75-94).

Determination of the saponification index allows characterization of the molecular weight and the average length of the fatty chain to which it is inversely proportional. The values obtained were in accordance with Codex Alimentarius (28) standards (184-196 mg KOH/g oil).

For chlorophyll and carotenoid contents, a significant difference was noted for the values obtained, as in the case of the other chemical indices. These results would be due to the extraction conditions applied for each type of oil, as demonstrated by the work of Salvador et al. (36) and Psomiadou and Tsimidou (37).

The low levels of chlorophyll obtained during our work (0.24-2.03 mg/Kg) allowed a reduction in the risk of oil oxidation, since chlorophylls are pro-oxidants in the presence of light (38,39). According to the last reference, the chlorophyll concentration can exceed 80 mg/kg for oils obtained from olives in the early stage of ripening and dropping to values of about 2 mg/kg when the fruit is fully ripe. Higher levels of chlorophylls were determined by Baccouri et al. (40) and Sait (41).

The carotenoid contents recorded for our samples were higher compared to those noted by Baccouri et al., (42), Hashempour et al. (43) and Sait (41), who analyzed oils of different varieties (*Chemlal* and *Oleaster*). This difference can be attributed to several factors such as: degree of ripeness of olives, production area, nature of soil, climatic conditions, olive variety and storage time (44,45). These compounds act favorably on oil stability and help protect against oxidative stress, which contributes to make olive oil a healthier product (2,46).

The values of free acidity, peroxide value, specific extinction at 232 nm and 270 nm of our samples, as well as the values of water and volatile matter content, which represent quality criteria according to the IOC and suggest that the manual olive oil is extra virgin olive oil, while traditional oil and industrial one are virgin olive oils. Thus, we can deduce that extraction method also affects several physicochemical properties of olive oil and it is possible de significantly reduce its quality.

Compared to the oils of other oilseeds, olive oil is distinguished by a significant content of monounsaturated oleic acid (more than 60%) and palmitic acid, while linoleic acid is less represented compared to other oilseeds (47). For oleic acid, our results are close to those obtained by Faci et al. (48) who worked on two olive varieties (*Chemlal* and *Azeradj*) from three regions of the wilaya of Tizi-Ouzou (Algeria). They reported contents varying from 61.14% to 67.67% in the *Chemlal* variety crop. Oleic acid behaves differently depending on the stage of maturity. According to Zaringhalami et al. (49), the highest level of oleic acid is observed at early harvest dates and then it gradually decreases as the olives ripen. This behavior is related to changes in the activity of the enzyme oleate desaturase during olive ripening, which converts oleic acid to linoleic acid during ripening (50,51). For palmitic acid and linoleic acid, our results are also comparable to those reported by Faci et al. (48), which vary between 13.12-17.92% for the first fatty acid and 9.12-15.12% for the second. If we consider the percentage of oleic acid (C18:1ω9) alone without taking into account the other quality criteria, our samples can fit into the extra virgin category (55-83%). The ratio of oleic acid/linoleic acid varies according to the degree of maturity. It is used as a stability parameter and several studies have shown that a high ratio indicates a high oxidative stability (52). Our results are close to those reported recently by Douzane et al. (53) and Faci et al. (48) for several olive varieties from central and western Algeria.

The number of phenolic compounds in olive oil is an important factor when evaluating its quality, since natural polyphenols

improve its resistance to oxidation and, in some cases, are responsible for its pronounced bitter taste (51). Yield and total polyphenols content reflect the influence of the treatments applied for each extraction method. These conclusions were confirmed by the determination of phenolic compounds.

Our results are superior to those found by Douzane et al. (54) and Laincer et al. (55), who reported that concentration of polyphenols in the different extra virgin olive oil samples analyzed varies between 109.45-322.18 and 115-420 mg GAE/Kg oil, respectively. Similarly, according to Douzane et al. (53), the concentration of total polyphenols in the different types of virgin olive oil harvested from several regions in Algeria ranges from 46.29-351.45 mg GAE/Kg oil. On the other hand, the levels found in the present study were lower compared to those reported by Medjkouh et al. (56) (710.44-1139.62 mg GAE/Kg oil). According to Marouane et al. (57), the *Chemlal* variety is characterized by moderate polyphenols richness compared to other olive varieties. Therefore, it can be concluded that variation in the phenolic content of olive oil can be attributed to several factors: the influence of climatic and geographical factors, the intra-varietal diversity of olive tree and the stage of maturity, the harvesting period, as well as the conditions of oil extraction (57,58).

Statistical analysis of the polyphenols contents obtained by the Student's t-test did not show a significant difference ($p>0.05$) between the three oils, which reflects that extraction method does not have an effect on the content of these metabolites.

For the antioxidant activity, manual olive oil exhibited the highest $PR_{0.5}$ provided by the lowest polyphenols concentration which was about 78.60 µg/mL, whereas, it was twice to five times higher in the case of the methanolic extract from the traditional (170 µg/mL) and industrial olive oil (439.05 µg/mL), respectively. This reflects a decrease of the antioxidant power of oil induced by the extraction method used.

Taking into account that the content of total polyphenols did not vary significantly between the three oils, it can be deduced that there are some kind of phytochemical (flavonoids, tannins,...) or other metabolites (responsible or participated in the antioxidant activity) which are affected during the extraction process and require specific and additional assays to be identified.

Iron reduction is a rapid, reproducible, and easy to perform antioxidant activity assay. The reducing power is probably due to the presence of hydroxyl groups in the phenolic compounds which can serve as electron donors. Several works have proved the presence of high antioxidant activity exerted by phenolic extracts from different olive oil samples, comparable to reference antioxidants such as BHT, using different assay methods (DPPH Test, β-carotene bleaching test, ABTS test, ...) (55-57). Virgin olive oil is a rich source of natural antioxidants (carotenoids, tocopherols, phenolic compounds) that can act, through different mechanisms, to confer an effective defense system against free radical attack (59). The phenolic compounds participate with about 30% in the oxidative stability of olive oil among other constituents (α-tocopherol, fatty acids, and carotenoids). They are able to give a hydrogen atom to the lipid radical formed during the propagation phase of lipid oxidation (59,60). While the interest of phenolic compounds is mainly related to their antioxidant activity that can last up to 6 days, they also present several other important biological activities *in vivo* and that can be beneficial in the fight against several diseases related to excessive free radical formation (59,61).

Several studies have demonstrated the presence of antibacterial activity of olive oil (21,62,63). In this study, a significant difference was observed in the sensitivity of the tested strains towards the antimicrobial effect of the studied oil samples. Our results clearly illustrate the influence of the extraction method on the antimicrobial activity of olive oil samples. Practices followed during large-scale extraction processes of olive oil reduce such activity compared to the manual one, closely related to their composition.

Comparing our work to those reported by Djedioui (64), who worked on oil of the variety *Rougette de la Mitidja*, the diameters of inhibition were higher with respect to Gram-positive bacteria. However, Laribi (65) reported that *E. coli, P. aeruginosa* and *K. pneumoniae* were the least sensitive species towards the different olive oil extracts. This was in contrast to *S. aureus* species which was sensitive to all the extracts tested. The antibacterial activity may be related to the presence of phenolic compounds such as tannins and flavonoids, which are important antibacterial substances (21,66-68). Olive oil is mainly composed of monounsaturated oleic acid and linoleic acid. Carvalho and Caramujo (69) and Dilika et al. (70) observed a high antibacterial activity of oleic and linoleic acids especially towards Gram-positive strains compared to Gram-negative ones. Bisignano et al. (71), Tuck and Hayball (72) and Cicerale et al. (73) have confirmed the inhibitory effect of hydroxytyrosol towards both Gram-positive and Gram-negative strains. Aldehydes present in olive oil also show antibacterial activity. A study by Carvalho and Caramujo (69) showed that saturated and unsaturated aldehydes in olive oil are effective against both Gram strains. The antibacterial activities tested may also result from a synergy between the compounds present in oil. It is likely that the decrease in activity is due to a change in the properties of the substance responsible for the activity in the presence of other oil compounds, resulting in a combination of two active components (major or minor) acting in synergy, or minor oil components that are also active at low concentrations (52).

CONCLUSION

In the present study, the effect of three extraction methods (manual, traditional and industrial) on the quality and biological properties of olive oil (*Chemlal* variety) was investigated. According to findings related to the sensory and physicochemical parameters, and fatty acid content, oils were classified into two groups-extra virgin and virgin category. The oleic acid content of the manual oil (62.39%) remained higher than the others, which affirms that the oils obtained from pitted fruits and with-

out addition of ingredients have the best quality criteria. Analysis of the polyphenols composition of olive oil samples revealed a higher content (528±12.40 mg GAE/Kg oil) for oil obtained by the manual extraction than the other types, which is characterized also by the best $PR_{0.5}$ provided by the lowest concentration of polyphenols (78.60 µg/mL). In addition, the manual olive oil showed more significant antimicrobial activity on all bacterial and fungal strains studied with inhibition zones greater than 14.04 mm, mainly against *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 9027 and *C. albicans* ATCC 10231. All the properties of the other olive oils studied, and which were obtained using modern extraction processes, were reduced when compared to the manual oil.

As a conclusion, the manual method of extraction preserves the best quality of oil on the sensory and physicochemical level with more interesting nutritional and biological effects.

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