Research Article

Changes in chemical and sensory characteristics of Chemlali extra-virgin olive oil as depending on filtration

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The oil extracted from olive paste is a turbid and opalescent juice which contains impurities that can affect its quality. Before olive oil packaging, filtration has recently become a standard processing procedure in order to obtain more brilliant extra-virgin olive oil (EVOO), maintain its quality, and extend its shelf life. In this research work, changes occurring in the concentrations of some minor compounds during industrial filtration of Chemlali EVOO were studied. The chemical parameters with sensorial quality were also determined. After the filtration of oil, a slight increase in quality parameters was noticed, along with a slight influence on the sensory scores. The adopted filtration system reduces the moisture contents. However, non-filtered olive oil might be more stable. Nevertheless, the behavior of phenolic compounds during filtration differed for each family. Indeed, the secoiridoids are the most affected phenolic compounds during EVOO filtration. Their concentrations increase significantly ($p < 0.05$) except the decarboxylated ligstroside aglycon decreases during this process.

Practical applications: This work clearly demonstrates the usefulness of filtration process in the Chemlali EVOO treatment. After industrial-scale filtration process, the analysis of individual concentration of phenolic compounds indicated that the concentrations of secoiridoids increased significantly ($p < 0.05$) mainly 3,4-DHPEA-EA. Besides, olive oil brilliance is achieved by eliminating vegetable water and suspended particles; thus extending final product shelf life.

Keywords: Extra-virgin olive oil / Filtration / Moisture / Phenolic compounds / Secoiridoids

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1 Introduction

Extra-virgin olive oil (EVOO) is the supernatant of the natural juice obtained exclusively by mechanical and physical processes from fresh and healthy olive fruits (*Olea europea* L.), such as washing, pressing, malaxation of olive paste, centrifugation, decantation, and filtration that do not modify its characteristics [1]. It is one of the earliest vegetable oils used by man and the only one that can be consumed without additional refining. Its flavor is characteristic and is markedly different to those of other edible fats and oils. Its excellent organoleptic and nutritive properties, together with the current tendency of consumers to select minimally processed foods, have prompted a re-assessment of its consumption in their regular diet [2]. However, immediately after extraction from olive fruits, EVOO is a naturally turbid and opalescent juice. Turbidity is caused by suspended solid particles of plant-tissue and micro-droplets of vegetation water emulsified in the oil, which can deteriorate the quality by facilitating hydrolysis, fermentation, and can cause the olive oil to become rancid [3]. For this reason, filtration is a special important final step to remove suspended solids and reduce EVOO moisture content. This step maintains EVOO quality by protecting it from chemical degradation, increasing its shelf life before consumption and giving it a more attractive

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Abbreviations: EVOO, extra virgin olive oil; **F,** filtered; **FAEEs,** fatty acids ethyl esters; **FFA,** free fatty acid; **FID,** flame ionization detector; **GC,** gas chromatography; **HPLC,** high phase liquid chromatography; **IOC,** International Olive Council; **NF,** non-filtered; **PV,** peroxide value; **TAG,** triacylglycerol

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brilliant color for consumer acceptance [4]. In the majority of industries, most oil mills store virgin olive oil in tanks without filtering the oil before packaging because it is empirically thought by the producers that filtered virgin olive oil is less stable and therefore more susceptible to alteration [2].

The filtration process has recently become a standard processing procedure with the aim to extend the shelf life of the oil and the consumer acceptance. The conventional filtration process can be carried out with various materials in combination with filtration hardware to improve their performance. These materials, denominated as filter aids, can be produced from a wide variety of raw materials and their use depends on the final purpose. Suspended particles can be removed by using diatomaceous earth, cellulose fibrous materials or pregelatinized starch, as filter aids [5]. There are other more sophisticated systems of filtration: Cross-flow filtration and new patented approaches based on inert gas-flow filtration and filter bags [6, 7]. Olive oil can also be filtered to obtain an impeccable commercial presentation and to remove moisture.

During filtering, quantitative and qualitative changes take place especially on minor components affecting the virgin oil quality [8]. However, the effects of the filtration on shelf life of olive oil are controversial, and different authors have reported both advantages and disadvantages concerning filtration of olive oil [4, 5]. Virgin olive oil filtration was related to the examination of changes of phenol compounds and oil stability, as well as of color, pigments, and sensory characteristics of virgin olive oils [8]. Moreover, filtration with either cotton or paper plus anhydrous sodium sulfate led to an apparent increase in the phenolic content and it has showed a significant decrease in hydroxytyrosol on EVOO after cotton filtration to remove moisture [5, 8].

The aim of this study was to evaluate the impact of filtration process especially on some minor components, and on the organoleptic characteristics of the purified EVOOs. Indeed, each sample of non-filtered and filtered EVOO was examined for acidity, peroxide value, specific extinction coefficient at 232 and 270 nm, total phenol contents and individual phenolic compounds, as well as the composition of the minor components (tocopherols, pigments, fatty acids ethyl esters, and waxes). Finally, the results obtained before and after filtration were compared.

2 Materials and methods

2.1 Chemicals, reagents, and standards

Ethanol (≥99.9%) and *n*-heptane (99.0%) were obtained from Riedel-de Haën (Steinhein, Germany). Cyclohexane (99.5%), *n*-hexane (99.0%), and diethyl ether (≥99.7%) were purchased from Merck KGaA (Darmastadt, Germany). Potassium hydroxide (85.0%) was obtained from CDM (Karlsruhe, Germany) and potassium iodide (>99.0%) was purchased from Chem-Lab (Zedelgem, Belgium). Sodium hydroxide (>99%) was supplied by Scharlau (Chemie, SA, Spain). Acetic acid (100.0%) and chloroform (>99.1%) were obtained from Prolabo (AnalaR NORMAPUR, France). Folin–Ciocalteu reagent was obtained from Fluka (Buchs, Switzerland). Ascorbic acid and BHT were purchased from Sigma–Aldrich (St. Quentin, Fallavier, France). Hydroxytyrosol was provided by SEPROXBIOTECH SL (Madrid, Spain). Double distilled water was used in the HPLC mobile phase.

Lauryl arachidate (>99%) and methyl heptadecanoate (>99%) standards were purchased from Sigma–Aldrich (St. Louis, MO, Germany). α-Tocopherol (>99.9%) was obtained from CALBIOCHEM (Merck KGaA, Darmstadt, Germany).

2.2 Extra-virgin olive oils

Monocultivar EVOO samples were obtained from Chemlali olive trees in the harvesting period at the beginning of December during two crop seasons (2012/2013 and 2013/2014) ($n = 4$; two different samples for each crop season). Chemlali is the main cultivated variety in Tunisia. The maturation index for the used fruits was the same for the all Chemlali olive samples (maturation indices were 4.2). Crude oil used in this work was supplied by CHO Company (Sfax, Tunisia) and obtained by a continuous three phase's system.

2.3 Filtration processes of EVOO

Filtration was carried out with various materials or filter aids in combination with filtration hardware to improve performance. Two different systems were utilized to remove suspended solids and to reduce the water content of EVOO (Fig. 1). The oils were filtered at a temperature below 25°C.

2.3.1 First step of filtration

The filtration system was used in conjunction with different filter aids to remove suspended solids. This step requires a preliminary phase during which the surface of the filtration equipment is recovered with a filter aid, which is deposited by filtration of specially prepared mixes for the formation of the cake layer, called precoat filtration [9]. A small quantity of virgin olive oil and diatomaceous earth (Celite 535 and Celite 545) were used as filter aids (Table 1). Commonly utilized filter aids include diatomite with different particle sizes, and consequently, different permeabilities [5]. Diatomaceous earth is a classic material used as a precoat. It is the fossilized remainder of microscopic algae. It is mainly formed of silica and it presents great chemical stability. During this step, sugars, enzymes, proteins, phospholipids, vegetative water, and waxes were suspended.

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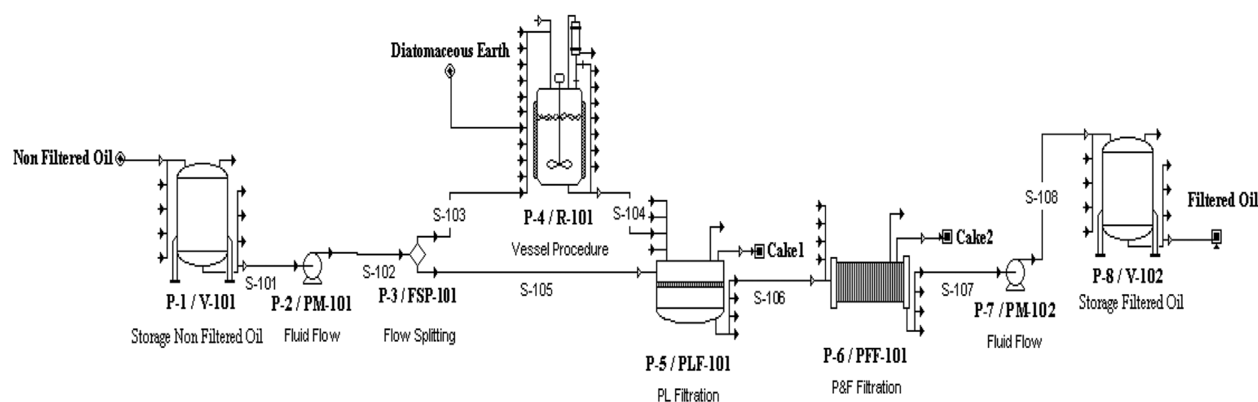

Figure 1. Industrial filtration diagram, P-1/V-101: storage of non-filtered EVOO; P-2/PM-101: pump 1; P-3/R-101: splitting; P-4/R-101: reactor; P-5/PLF-101: pressure leaf filtration (PLF); P-6/PFF-101: plate-frame filtration (PFF); P-7/PM-102: pump 2; P-8/V-102: storage of filtered EVOO; S101, S102, S103, S105: non-filtered EVOO; S104: non-filtered EVOO + adsorbent; S106: filtered EVOO after PLF; S107: filtered EVOO after PLF + PFF; S108: filtered EVOO.

2.3.2 Second step of filtration

Olive oil brilliance was achieved by the elimination of vegetable water using filter presses with cellulose paper (papelera del besós, SA-999, Spain), diatom poly amide-amine, or agents of wet resistance (carboxy-methyl-cellulose). This process was carried out to remove suspended solids and before storage. The technical specifications of the producer are: Base weight 1500–1700 g/m, 3.6–4.1 mm thickness, 12 μm nominal filtration rate, and water flow rate at 1.0 bar, 20°C: 12–29 L/min/m^2.

2.4 Quality indices determinations

The titratable acidity (free fatty acids) was determined according to the method proposed by the International Organization for Standardization (ISO): ISO 660 [10], while peroxides were determined according to the method proposed by ISO 3960 [11]. Ultraviolet (UV) spectrophotometric constants (K_{232}, K_{270}, and ΔK) were carried out following the analytical methods described by the International Olive Council (IOC) [12]. The total phenols were evaluated colorimetrically at 725 nm with the Folin–Ciocalteu reagent [13].

Table 1. Technical specifications of Celite 535 and Celite 545

Characteristics	Celite 535	Celite 545
Retained on 105 μ mesh (%)	–	22
Retained on 45 μ mesh (%)	–	60
Retained on 150 μ mesh (%)	10	–
Median particle size (μm)	42.9	38.9
Permeability (Darcy)	3.00	4.2
Wet density (g/L)	320	336
pH (10% in water)	10.0	10.0
Moisture (%)	<1.0	0.10
Specific gravity	2.3	2.3

2.5 Moisture content

The moisture content was determined following the method proposed by ISO 660 [14]. Briefly, in a capsule, previously dried at 103 ± 2°C and cooled, 10 g of completely homogenized sample were weighed. The samples were placed in an oven (Prolabo, France) at 103 ± 2°C for 1 h, after which they were removed and weighed. Next, they were returned in the oven and the operation was repeated until the weight was constant. The moisture content was calculated as the difference in weights.

2.6 Pigment contents and colour of olive oils

Carotenoid and chlorophyll concentrations (mg/kg of oil) were determined at 470 and 670 nm, respectively, in cyclohexane using the specific extinction values according to the method of Haddada et al. [15]. The analysis of chromatic coordinates (L, a*, b*, and C) were determined by using a Lovibond apparatus according to the previously published protocol described by Cerretani et al. [16].

2.7 Sensory evaluation

The sensory evaluation was determined according to the IOC [17] by the Tunisian National Office of Oil panel. The panel, recognized according to IOC, consisted of selected and well-trained olive oil experts monitored according to their skills in the distinction between similar samples by an experienced panel leader. The oils were then classified according to the median of defects and the median of fruity attribute.

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2.8 Extraction of phenolic fraction from olive oils

The phenolic extracts were obtained following the method described by Gargouri et al. [18]. Briefly, 4 g of each oil sample was added to 2 mL of *n*-hexane and 4 mL of a methanol/water (60:40, v/v) solution in a 20 mL centrifuge tube. After vigorous mixing, they were centrifuged for 3 min. The hydroalcoholic phase was collected and the hexanic phase was re-extracted twice with 4 mL of methanol/water (60:40, v/v) solution each time. Finally, the hydroalcoholic fractions were combined, washed with 4 mL of *n*-hexane to remove the residual oil, then concentrated and dried by evaporative centrifuge in vacuum at 35°C.

2.9 HPLC analysis of phenolic compounds

The extracts analysis of filtered and non-filtered EVOO were performed in an Agilent 1200-HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with a vacuum degasser, an autosampler, a binary pump, and a diode array detector (DAD). The chromatographic separation of these compounds was performed on a 150 × 4.6 mm i.d., 1.8 mm, Zorbax RP-C18 column (Agilent Technologies, Palo Alto, CA, USA). The mobile phases used were water with 0.25% acetic acid as eluent A and methanol as eluent B. The total run time was Eluates were detected at 280 nm. The temperature was maintained at 40°C. The mobile phase used was 0.1% phosphoric acid in water (A) vs. 70% acetonitrile in water (B) for a total running time of 50 min. The elution conditions applied for phenolic compounds were: 0–25 min, 10–25% B; 25–35 min, 25–80% B; 35–37 min, 80–100% B; 37–40 min, 100% B, and finally washing and reconditioning steps of the column were included (40–50 min) linear gradient 100–10% B. The flow rate was 0.6 mL/min and the injection volume was 50 μL. The identification of phenolic compounds and their retention time in comparison with phenolic standards were analyzed within the same condition.

2.10 HPLC analysis of tocopherols

The analysis of tocopherol content was performed with a HPLC method described by Gliszcynska-Swiglo and Sikorska [19]. Briefly, the analysis was executed on an Agilent HP1100 high performance liquid chromatography equipped with Supelco column C18 (250 × 4.6 mm, 5 μm particle size, Spherisorb ODS-2). The optimized separation conditions were carried out by isocratic elution with a 50:50 methanol/acetonitrile mixture; column temperature, 30°C; flow rate, 1 mL/min and injection volume, 20 μL of the sample prepared.

Samples of oils were weighed (0.040–0.200 g) and dissolved in 1 mL of 2-propanol vortex mixed samples were directly injected on to the HPLC column without the use of any additional injector. The eluate was detected using a scanning fluorescence detector set for the emission at 325 nm and excitation at 295 nm. Tocopherols were identified by comparing their retention times with those of the corresponding standards and by spiking the samples with an appropriate standard.

2.11 Evaluation of oxidative stability by the Rancimat apparatus

The oxidative stability was evaluated by a Metrohm Rancimat model 743 (Herisau/Switzerland) following the AOCS Official Method Cd 12b-92 [20]. The oil samples (5.0 g) were heated at 120°C, with a continuous air flow of 20 L/h through the samples. The conductivity cells were filled with 60 mL of deionized water. The time needed for the appearance of a sudden water conductivity rise, caused by the adsorption of volatiles derived from oil oxidation, was registered as the induction time in hours.

2.12 Antioxidant activity

Antioxidant activity was evaluated by measuring the radical-scavenging effect of olive oil methanolic extract toward the synthetic radical DPPH (1,1-diphenyl-2-picrylhydrazyl), as reported previously [13]. In succinct terms, aliquots (50 mL) of various concentrations of the compounds tested in methanol were added to 5 mL of a 0.004% methanol solution of DPPH. After 30 min of incubation, the absorbance was read against a blank at 517 nm. The inhibition of free radicals DPPH in percentage ($I\%$) was calculated in the following way: $I\% = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$, where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. The tested compound concentration, which provided 50% inhibition (IC_{50}, expressed in μg/mL) was calculated from the graph plotted inhibition percentage against the extract concentration. The synthetic antioxidants BHT and ascorbic acid were used as positive controls and all tests were carried out in quadruplicate.

2.13 Determination of fatty acids ethyl esters (FAEEs) and waxes

Fatty acids alkyl esters and waxes were determined by gas chromatography (GC-FID) according to the method reported in IOC [21] "Determination of the content of waxes, fatty acid methyl esters and fatty acid ethyl esters by capillary gas chromatography." The operating conditions were as follows: Oven temperature, 80°C for 1 min and then increased from 20°C/min up to 140°C, then increased from 5°C up to 335°C and maintained for 20 min; the injector was programmed from 70 to 300°C; detector temperature was 350°C. Helium was used as the carrier gas, with a flow through the column of 1 mL/min and 1:50 Split ratio. The

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fatty acid ethyl esters were eluted in the following order: Ethyl palmitate, methyl hepatdecanoate (internal standard), ethyl linoleate, ethyl oleate, and ethyl stearate. The waxes were eluted in the second half of the chromatogram starting with the diterpene esters [22].

2.14 Statistical analysis

The results were expressed as mean ± standard deviation (SD) of four measurements for the analytical determination. Significant differences between the values of all parameters were determined at $p < 0.05$ according to the one-way ANOVA: Post Hoc Comparisons (Student Newman–Keuls test). This analysis was performed using SPSS Statistics 17.0 for Windows.

3 Results and discussion

3.1 Extra-virgin olive oil quality

Lipid hydrolysis generates free fatty acids by chemical or enzymatic reaction. This phenomenon is of particular interest in water-containing lipid matrices. The hydrolytic degree of the samples was evaluated by the analysis of the free acidity.

Table 2 shows that the free fatty acid content (FFA) (% C18:1) of non-filtered oils (below 0.8% [0.27%]), remained almost the same after filtration (0.28%) and maintained the classification of olive oil as extra-virgin. Similar results reported by Mailer and Graham [23], showed that free fatty acids are influenced by endogenous enzymes within the olive fruit. The filtered olive oil was clear and free of water and solids. Therefore, it is free of lipase enzyme; and thus, free fatty acids were expected to remain constant.

Table 2. Characteristics of non-filtered (NF) and filtered (F) EVOO samples obtained from Chemlali cultivar

	NF-EVOO	F-EVOO
Acidity (% oleic acid)	0.27 ± 0.00^a	0.28 ± 0.00^a
Peroxide value (meq O_2/kg)	8 ± 0.20^a	9 ± 0.18^b
K_{232}	1.90 ± 0.03^a	1.99 ± 0.02^b
K_{270}	0.13 ± 0.00^a	0.13 ± 0.00^a
ΔK	0.00 ± 0.00^a	0.00 ± 0.00^a
Induction time (h)	7.30 ± 0.11^a	7.20 ± 0.13^a
Total phenols (mg/kg)	204 ± 4.00^a	198 ± 4.00^a
Moisture and volatile substances at 105°C (%)	0.09 ± 0.00^a	0.00 ± 0.00^b
Total tocopherols (mg/kg)	164 ± 3.00^a	172 ± 2.00^b
IC_{50} (μg/mL)	19.95 ± 0.30^a	20.10 ± 0.40^a

NF: non-filtered; F: filtered; EVOO: extra-virgin olive oil; K_{232} and K_{270}: spectrophotometric indices; ΔK: specific extinction variation; IC_{50}: median inhibitory concentration (concentration at which an antagonist exerts its half-maximal effect).
Each value represents the mean of four determinations of two successive crop seasons ($n = 4$; two different samples for each crop season) ± standard deviation. Different lower case letters (a and b) within the same row indicate significant differences ($p < 0.05$) between the non-filtered (NF) and filtered (F) EVOO samples.

PV of oil is the second parameter of quality. It is a measure of primary oxidation, and considered as an important indicator of the oxidation level. The filtration of oil affects slightly the level of the peroxide index. Indeed, PV increased significantly ($p < 0.05$) from 8 to 9 meq O_2/kg in non-filtered and filtered oil, respectively. In a related study, Fregapane et al. [2] affirmed that filtration caused an increase in the rate of peroxide formation.

UV spectrophotometry was a good index to measure oxidative alterations. The specific absorption coefficients in the ultraviolet region are necessary to determine the oxidation stage of oil. The absorption at specified wavelengths at 232 and 270 nm in the UV region is related to the formation of conjugated diene and triene in the olive oil. Indeed, the experimental data showed a slight decrease in K_{232} following filtration. It was on average 1.90 for non-filtered olive oil and 1.99 after the filtration process. However, similar K_{270} values were obtained on crude and filtered olive oils. These results are in good agreement with those reported by Tsimidou et al. [24]. In fact, the evaluation of conjugated dienes and trienes revealed higher K_{232} and an almost equal value of K_{270} after membrane cross-flow filtration and paper filtration of olive oil.

The oxidative stability of the oil was determined by Rancimat apparatus with conductivity measurement carried out to evaluate induction periods. Filtration of Chemlali olive oils had no important influence on the oxidative stability (Table 2). Indeed, the induction time decreased not significantly ($p > 0.05$) from 7.30 h in crude virgin olive oil to 7.20 h in filtered oil. The slight changes were explained by the direct relationship between the content in phenolic compounds in virgin olive oil and its oxidative stability. Similar results of oxidative stability have been obtained by different authors for non-filtered and filtered olive oils determined at the moment of filtration, prior to storage [2, 24].

Virgin olive oil contains phenolic compounds responsible for its fragrance and peculiar flavor [18]. Polyphenols are important antioxidants that protect biological systems against oxygen radicals [22]. Table 2 shows the changes in the total phenol content (mg of gallic acid/kg of oil) of filtered and non-filtered virgin olive oils.

The total amount of phenol was reduced by filtration as described by Koidis and Boskou [25]. This behavior can be explained by the variation in water content of EVOO. In fact, it is assumed that the majority of phenolic compounds, having amphiphilic characteristics, are located around water droplets in olive oil. The filtration process removes moisture, and the water content is reduced together with a proportion of the phenolic compounds [8].

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Water is one of the main components of the virgin olive oil turbidity and it is well-known that the presence of this phase in oil accelerates the deterioration of the product. The water content was reduced by filtration from 0.09 to 0% in filtered olive oil because the filter cake was used to retain this fraction of EVOO. These results are in good agreement with previous studies [8, 26].

Moreover, the orientation of phenolic compounds in the oil–water interface and the active surface of water droplets influenced the protection against the oxidation of oil. Recently, some researchers [8, 27] determined that EVOO contains a low quantity of water (ranging from 450 mg/k^g to 3000 mg/kg depending on the extraction technology), that increases when samples were not filtered. Part of the total water content present in virgin olive oil is free and available for chemical and enzymatic reactions. It also keeps hydrophilic phenols dissolved. This can explain the hydrolytic process that occurs both to phenols (by esterases) and triacylglycerols (by lipase) during prolonged EVOO storage. Thus, more rapid oxidation of the non-filtered oil could be expected. Instead, according to Gomez-Caravaca et al. [8], the stability of non-filtered samples, when measured in terms of resistance to accelerated oxidation (value by OSI or Rancimat instruments), was in all cases higher than that of the corresponding filtered oils. This coincided with a higher total phenolic content in non-filtered EVOO. In fact, total phenols decreased slightly ($p > 0.05$) with filtration due to the not significant diminution ($p > 0.05$) of the oxidative stability measured by Rancimat apparatus.

The most important lipophilic antioxidants quantified by HPLC-FD in EVOO samples were tocopherols, and nearly 95% of the total ones is α-tocopherol. Tocopherols, with a hydroxyl group that can donate a hydrogen atom to reduce free radicals, are powerful antioxidants and contribute to the increase of the olive oil shelf life. After Chemlali oil filtration through cellulose paper, experimental data showed slightly higher values of total tocopherols ranging from 164 mg/kg to 172 mg/kg in non-filtered and filtered samples, respectively. Indeed, the tocopherols revealed a tendency toward loss of these molecules in their non-clarified oils. These results agree with the study performed by Bendini et al. [28], which confirmed the effect of the filtration on the lipophilic antioxidant compounds of the cloudy and clarified EVOOs.

3.2 Color and pigment contents

Color variations in olive oil are related to differences in pigment composition, which are mostly carotenoids and chlorophylls. The color can range from pale yellow to deep green. Olive oil color was expressed numerically as the lightness L*, chromatic coordinates a*, b*, and the chroma C according to the method described by Escolar et al. [29].

Table 3 shows the color and the pigment contents of non-filtered and filtered EVOO samples obtained from Chemlali

Table 3. Color (expressed as lightness L*, chromatic coordinates a*, b*, and chroma C) and pigment contents of non-filtered (NF) and filtered (F) EVOO samples obtained from Chemlali cultivar

	NF-EVOO	F-EVOO
L*	91.14	91.77
a*	−5.32	−5.60
b*	121.40	121.58
C	124.11	125.00
Chlorophylls (mg/kg)	7.00 ± 0.11^a	5.80 ± 0.10^b
Carotenoids (mg/kg)	2.44 ± 0.02^a	2.33 ± 0.03^b

NF: non-filtered; F: filtered; EVOO: extra-virgin olive oil.
Each value represents the mean of four determinations of two successive crop seasons ($n = 4$; two different samples for each crop season) ± standard deviation. Different lower case letters (a and b) within the same row indicate significant differences ($p < 0.05$) between the non-filtered (NF) and filtered (F) EVOO samples.

cultivar. Results presented in Table 3 indicate that filtration influenced slightly the color, chlorophyll, and carotenoid concentrations of the studied olive oil. These results are in accordance with the results obtained by Bottino et al. [7]. The lightness (L*) of the oil increased after filtration, which is probably the consequence of the removal of a significant part of most suspended solids and vegetable water. Furthermore, when the sample of cloudy oil had a deep green colour, the a* value decreased after filtration and the intensity of green color was minimized. The b* value had a tendency to increase because the yellow color was more evident when the olive oil had been filtered [8].

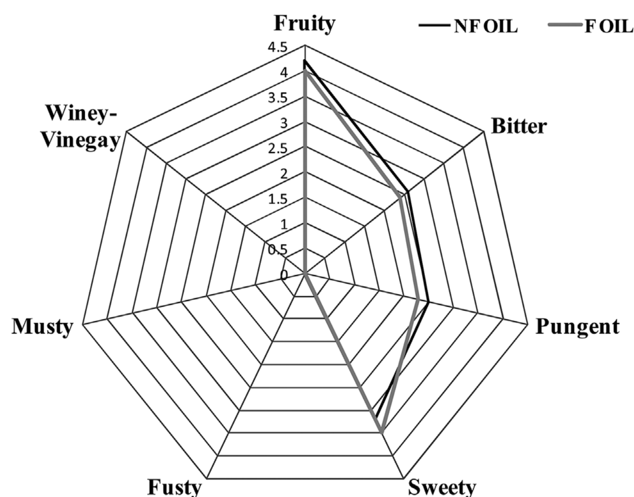

Figure 2. Sensory profiles of non-filtered (NF) and filtered (F) EVOO samples obtained from Chemlali cultivar. Results are expressed as medians of eight assessments for each taster in organoleptic tests.

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3.3 Sensory assessment

Sensory analysis is an essential technique to characterize olive oil and investigate consumer preferences. A slight decrease was seen in fruitiness, pungency, and bitterness attributes after filtration of Chemlali EVOO samples were detected (Fig. 2), probably because these characteristics primarily depend on the concentration and composition of phenolic compounds in olive oils as was previously described [30, 31]. The reason could be due to the hydrophilic phenolic compounds affecting pungency and bitterness of olive oils, along with olive tissue solid particles and vegetable water droplets. These results are in accordance with those obtained by Bubola et al. [3]. Indeed, the filtration of Buza virgin oil samples caused a slight change, concerning individual ketones, connected to sensory characteristics: Bitter and pungent.

3.4 Antioxidant activity

The DPPH free radical method determines the antiradical power of antioxidants. The DPPH radical scavenging activity of filtered and non-filtered EVOO were measured and compared to that of commercial synthetic antioxidants such as ascorbic acid ($IC_{50} = 5.00$ μg/mL) and BHT ($IC_{50} = 7.94$ μg/mL). The lowest IC_{50} values indicated the highest free radical scavenging activity of the sample. No significant differences ($p > 0.05$) related to the filtration method were

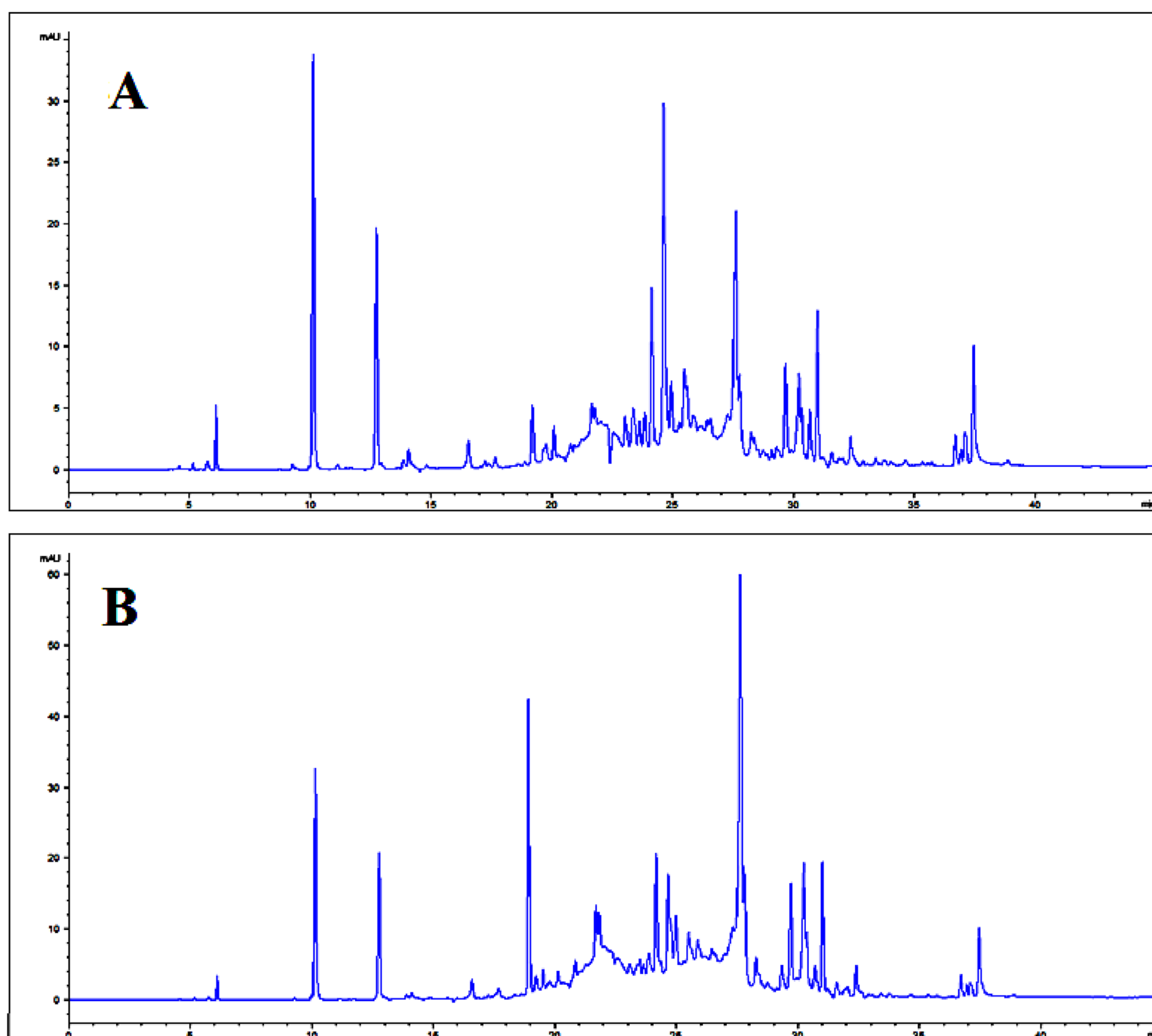

Figure 3. Phenolic extract chromatograms of non-filtered (A) and filtered (B) Chemlali EVOO samples obtained by HPLC.

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observed (Table 2). These results agree with the study performed by Fregapane et al. [2], which confirmed that no difference related to the filtration method was observed.

3.5 Behavior of phenolic compounds during filtration process

Phenolic compounds are a group of polar components, which contain one or more aromatic hydroxylated rings, including functional derivatives and are part of non-glycerine components in virgin olive oil [31]. Polyphenols are hydrophilic compounds that can be found in the olive fruit; however, many of these compounds are modified or lost during the production process of virgin olive oil [8]. In general, the phenols follow a typical partitioning model, where the main fruit secoiridoids (oleuropein, demethyloleuropein, and ligstroside) are degraded during the crushing/malaxation operation, forming several secoiridoid aglycon derivatives according to the previously proposed mechanism [32]. The final quantity of phenols is also influenced by the cultivar, climatic conditions during growth, and degree of ripening [33].

The filtration effect on the phenolic profile by using HPLC was also investigated. Figure 3 depicts representative HPLC chromatograms of phenolic extracts from filtered and non-filtered virgin olive oils of the Chemlali variety. The analysis of the individual concentrations indicated that within the same family, the filtration effect was different for each compound (Table 4). The identified phenolic compounds belong to different phenolic classes of phenolic alcohols, secoiridoids, lignans, flavonoids, and phenolic acids. Among the phenolic alcohols, hydroxytyrosol (3,4-DHEPA) and tyrosol (*p*-HPEA) were characterized, corresponding to the retention time 10.10 and 12.72 min, respectively.

Representative complex phenols were identified in Chemlali EVOO such as oleuropein aglycon, ligstroside aglycon, and their derivatives, which belong to the secoiridoids group. They showed a same trend during filtration expect the decarboxylated ligstroside aglycon. Indeed, the concentrations of decarboxylated oleuropein aglycon, oleuropein aglycon, and ligstroside aglycon increased significantly ($p < 0.05$) after the filtration step (Table 4). This trend has been reported in previous studies at the laboratory scale using cotton as the filter medium [8], and gas-flow filtration as filter aids at the pilot plant scale [6].

With regard to lignans, two compounds were detected, namely pinoresinol and acetoxypinoresinol, which had retention times of 24.11 and 24.61 min, respectively. Another phenolic group detected was composed of flavonoids. The most noteworthy compounds identified in this group were luteolin and apigenin. These results are similar to those reported by several authors for other olive oil varieties [34, 35]. Indeed, the concentration of luteolin diminished significantly ($p < 0.05$) during filtration. Whereas, apigenin augmented slightly but significantly ($p < 0.05$) in the filtered oil sample (Table 4). These results are similar to those reported by Bakhouche et al. [4], who showed that phenolic alcohols and flavonoids progressively decreased after all filtration steps, in particular after the second step of filtration.

Table 4. Phenolic composition (mg/kg) of non-filtered (NF) and filtered (F) EVOO samples obtained from Chemlali cultivar

Phenolic compound	Retention time (min)	NF-EVOO	F-EVOO
3,4-DHPEA	10.10	20.50 ± 0.15^a	20.40 ± 0.13^a
p-HPEA	12.72	16.00 ± 0.12^a	17.00 ± 0.11^b
p-coumaric	16.57	0.78 ± 0.00^a	0.83 ± 0.00^b
Luteolin	22.39	11.10 ± 0.08^a	7.90 ± 0.05^b
Pinoresinol	24.11	8.30 ± 0.06^a	9.10 ± 0.06^b
Acetoxypinoresinol	24.61	19.58 ± 0.14^a	8.89 ± 0.06^b
Apigenin	25.82	2.98 ± 0.02^a	4.91 ± 0.03^b
Decarboxylated oleuropein aglycon	27.24	11.31 ± 0.08^a	14.70 ± 0.10^b
3,4-DHPEA-EA	27.58	36.19 ± 0.27^a	72.30 ± 0.47^b
3,4-DHPEA-EDA	27.75	10.20 ± 0.08^a	13.26 ± 0.09^b
p-HPEA-EA	30.20	21.89 ± 0.16^a	28.45 ± 0.18^b
Decarboxylated ligstroside aglycon	30.32	8.77 ± 0.06^a	6.20 ± 0.04^b

NF: non-filtered; F: filtered; EVOO: extra-virgin olive oil. 3,4-DHPEA: hydroxytyrosol; *p*-HPEA: tyrosol; 3,4-DHPEA-EA: oleuropein aglycon; 3,4-DHPEA-EDA: dialdehydic form of elenolic acid linked to hydroxytyrosol; *p*-HPEA-EA: ligstroside aglycon.
Each value represents the mean of four determinations of two successive crop seasons ($n = 4$; two different samples for each crop season) ± standard deviation. Different lower case letters (a and b) within the same row indicate significant differences ($p < 0.05$) between the non-filtered (NF) and filtered (F) EVOO samples.

3.6 Effect of filtration system on waxes and FAEEs

Waxes are a group of esters of fatty acids and long-chain aliphatic alcohols known as wax esters. These compounds with a high melting point and low solubility in the oils contribute to the development of cloudiness when stored at low temperatures. Therefore, the distribution and amount of waxes in oil is a good indicator of its quality and authenticity. The sum of C40, C42, C44, and C46 waxes has been established as $\leq$250 mg/kg for edible virgin olive oils [36].

The C40–C46 wax contents of non-filtered and filtered EVOOs using IOC method [21] are shown in Table 5. The wax content is related to the turbidity of EVOO because the suspended solid is also a derivate from waxes. It is known that the turbidity of olive oils can be caused by crystallization of waxes due to their low solubility in oil, and is generally facilitated by low temperature [5].

The filtration process affects the amount and nature of waxes in olive oil. Filtered EVOO showed a decrease

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Table 5. Contents of wax esters and fatty acids ethyl esters (FAEEs) in non-filtered (NF) and filtered (F) EVOO samples obtained from Chemlali cultivar

	NF-EVOO	F-EVOO
Wax esters		
C40 (mg/kg)	33.35 ± 0.23^a	33.25 ± 0.26^a
C42 (mg/kg)	22.50 ± 0.16^a	21.42 ± 0.17^b
C44 (mg/kg)	12.17 ± 0.08^a	10.05 ± 0.09^b
C46 (mg/kg)	4.75 ± 0.03^a	3.90 ± 0.03^b
C40-C46 (mg/kg)	72.77 ± 0.51^a	68.62 ± 0.55^b
FAEEs		
Et C16:0 (mg/kg)	3.63 ± 0.03^a	3.65 ± 0.03^a
Et C18:2 (mg/kg)	3.65 ± 0.03^a	3.62 ± 0.03^a
Et C18:1 (mg/kg)	10.10 ± 0.07^a	10.00 ± 0.08^a
Et C18:0 (mg/kg)	0.78 ± 0.00^a	0.80 ± 0.00^b
FAEEs (mg/kg)	18.16 ± 0.13^a	18.07 ± 0.14^a

NF: non-filtered; F: filtered; EVOO: extra-virgin olive oil; Et: ethyl; FAEEs: fatty acids ethyl esters.
Each value represents the mean of four determinations of two successive crop seasons ($n = 4$; two different samples for each crop season) ± standard deviation. Different lower case letters (a and b) within the same row indicate significant differences ($p < 0.05$) between the non-filtered (NF) and filtered (F) EVOO samples.

(between 5 and 10%) in the C40–C46 wax contents. This behavior can be explained by the reduction in suspended solid of EVOO. Slight changes in C40 and C42 but an important decrease in C44 was noticed in Chemlali EVOO because waxes with chain lengths lower than 40 carbon atoms (C40) are called soluble waxes, those with lengths between C40 and C43 are called partially soluble waxes and those with 44 or more carbon atoms are called crystallizable waxes [37]. These results are in accordance with the results obtained by Lin et al. [38]. They also detected a decrease of the total wax concentration in seed oils after a membrane filtration process.

The determination of the fatty acids alkyl esters (FAAEs) content mainly ethyl (FAEEs) and methyl esters (FAMEs) was proposed since it was demonstrated that they were present at a certain concentration when olive fruits with fermentative alterations had been used for oil extraction. They are formed by the esterification of free fatty acids (FFAs) with low molecular alcohols, such as methanol and ethanol. Filtration of Chemlali EVOOs had no important influence on total FAEEs (Table 5).

4 Conclusions

According to the results of this research work, industrial filtration could make EVOO more brilliant and offer an impeccable commercial presentation. During filtration, quantitative and qualitative changes take place, especially on minor components. After Chemlali oil filtration, a decrease in moisture content was noticed. The loss of water was accompanied by a slight reduction of phenols. In fact, the majority of phenol compounds located around water droplets remained in non-filtered olive oil. The analysis of the individual phenolic compounds indicated that within the same family, the filtration effect was different for each compound. A significant increase ($p < 0.05$) of secoiridoids was recorded for the filtered olive oil mainly 3,4-DHPEA-EA. Furthermore, this oil sample had a higher component of yellow color, luminosity, and a lower intensity of green color. With regard to waxes, slight changes in C40 and C42 but an important decrease in C44 was noticed in filtered Chemlali EVOO. The loss of waxes could be a result of the rise of these compounds in non-filtered oil, which was highly correlated with the turbidity classes. Finally, filtration of Chemlali EVOOs had no considerable influence on the FAEEs contents.

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