

## Physicochemical Properties and Oxidative Stability of Some Virgin and Processed Olive Oils

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### ABSTRACT

This study investigated the fatty acids profile, oxidative stability indices, and bioactive compounds of virgin and processed olive oils (*Leccino* and *Frantoio* cultivars). Results showed that fatty acid profile of the studied oils contained mostly oleic acid followed by palmitic and linoleic acid. Acid values of virgin *Frantoio* and processed *Leccino* were 0.78 and 0.18 mg KOH/g oil, with peroxide values (PV) of 8.74 and 6.03 meq O<sub>2</sub>/kg oil, respectively. Virgin *Leccino* had the highest oxidative stability index with induction period of 18.83 h. The highest contents of phenolics, 321.14 mg/kg, tocopherols, 455.25 mg/kg, and sterols, 2189.1 mg/kg, were recorded in virgin *Leccino* oil. Laboratory processing of virgin olive oil decreased the phenolics to 70%, tocopherols to 50%, and sterols to 25%. Finally, based on the higher content of antioxidant compounds, the virgin *Leccino* oil had higher oxidative stability and bioactivity than *Frantoio* oil.

**Keywords:** Bleaching; Deodorization; Rancimat test; Phenolic compounds.

### INTRODUCTION

Olive oil is an important foodstuff constituting a major part of daily food regimes. This oil is extracted from *Olea europaea* tree belonging to family *Oleaceae*. Virgin olive oil refers to oils that are extracted merely by pressure and other mechanical processes and no other treatments (Preedy and Watson, 2010). Virgin olive oils can control blood cholesterol level due to its sufficient amounts of essential fatty linoleic and oleic acids and anti-oxidative compounds. It also reduces the risk of cardiovascular diseases and cancers by decreasing the penetration rate of fatty acids in to artery walls (Lanteri *et al.*, 2002; Silva *et al.*, 2010a).

Processing remarkably decreases bioactive compounds of olive oil, such as phenols, tocopherols, sterols, and pigments, and also has a great effect on its sensory properties. Tocopherols and phenolic compounds are

effective antioxidants whose initial amounts play a crucial role in inhibiting primary and secondary lipid oxidation (Uyor and Ori-Jesu, 2008). Phenolic compounds are more effective than tocopherols in enhancing the stability of olive oil against oxidation (Silva *et al.*, 2010b).

The unique balanced chemical structure of olive oil may bring about positive effects on human health, a high oxidative stability, appealing taste and odor. Additionally, the favorable effects of olive oil are to a large extent produced by the presence of antioxidants such as phenols, tocopherols, pigments, and fatty acids with one unsaturated bond (Tura *et al.*, 2007; Dabbou *et al.*, 2010).

It seems suitable to use the olive oil in thermal processes since it contains high amounts of mono unsaturated fatty acids (MUFAs), low saturated fatty acids (SFAs) and poly unsaturated fatty acids (PUFAs), very low linolenic acids, and no trans fatty

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acids (Farhoosh *et al.*, 2013). The type and amount of different chemicals in the olive oil are the indicators of its quality, which, in turn, depends to multiple factors including variety, climate, extraction method, and the fruit's ripening stage (Morello *et al.*, 2003). In order to analyze the influence of climatic conditions, Aguilera *et al.* (2005) showed that locations with the high temperature and low altitude could be associated with an increased polyunsaturation and the lowest oleic percentage. This could be attributed to a modification of lipid biosynthesis coinciding with a wet summer. The metabolism and lipid levels of the olive fruit are affected by environmental factors such as light, temperature, and water stress.

Baccouri *et al.* (2008) studied the effect of the olive ripening stage on the quality indices of the two main monovarietal Tunisian virgin olive oils cultivars, namely, Chetoui and Chemlali. They observed a very good correlation between the oxidative stability and the concentrations of total phenols, particularly secoiridoids and  $\alpha$ -tocopherol. Thus, oils from different cultivars and locations have different chemical compounds and, therefore, different oxidative stabilities.

At present, the old olive orchards are located mainly in the north of Iran i.e. the Caspian Coast, having slightly Mediterranean climate. More than 85% of olive production belongs to these areas, especially in Golestan province, one of the northern provinces of Iran (FAO, 2005). From the quality aspect as well, dry land areas are suitable for olive oil cultivation (Wiesman, 2009). There is a plan to increase olive cultivation area in Iran, which requires well-characterized cultivars with well-balanced oil chemical composition in the proper area (Moghaddam, *et al.*, 2012). To this end, an example of activities in the last decade is establishing olive orchards in Qom province, surrounding a sub-desertic zone, which is climatically unique. Here, olive average yield is 10 tons/hectare, which is 300% higher than the country's average, and 40% higher than Europe (Mirhosseini,

2007). Plants that have arid or semi-arid environments as their natural habitat may, therefore, reveal new mechanisms that enable them to resist stressful conditions. So, there is the need to determine the adaptability and quality of different olive cultivars in Qom area ecological conditions before developing plantations for commercial purposes.

Accordingly, this study aimed to compare the physicochemical properties and oxidative stability of virgin and processed olive oils from *Leccino* and *Frantoio* cultivars grown in Qom.

## MATERIALS AND METHODS

### Oil Extraction Process

Olives (*Leccino* and *Frantoio cvs*) with ripening index (RI) of 7 were harvested in October-November, 2011, from Kesht-o Sanat Zeytoon Fadak Saraj in Qom province located at the 15th km of Old Qom-Kashan Road. The oil from olive cultivars was extracted at the shortest time possible using an oil press machine ABENCOR Analyzer (Comercial Abengoa, Seville, Spain). All the olive fruits were deleafed and washed, then, 1.5 kg of olives were crushed with a hummer crusher and slowly mixed for about 30 min at 27 °C. Then, the paste mixed was centrifuged at 3000 rpm for 3 min without addition of water to extract the oil. Finally, the oils were decanted and immediately transferred into dark glass bottles and stored at 4 °C while waiting for the chemical analysis.

### Laboratory Processing

For oils such as palm and olive, in which the main impurities are free fatty acids and have low amounts of phospholipids, the physical refining can be used instead of alkali treatments (Gomes *et al.*, 2012). In this study, for the laboratory processing of the studied cultivars, 1% bleaching earth

(Tonsil, 2% acid-activated) was added to the virgin olive oil inside a flask and was agitated for 30 min at 90 °C with a rotary evaporator under vacuum conditions (40-45 mm Hg). Then, it was filtered using Whatman No. 1 filter paper and deodorized at 220 °C and under vacuum conditions (less than 60 mbar) for 60 min (Gomes *et al.*, 2012). To ensure the full removal of any impurities, the processed oil was centrifuged for 5 min at room temperature and 5000 rpm. It is noteworthy that the objective of this refining process was to investigate its effect on properties of virgin olive oils. However, these oil processing stages are

The external standard method was used in the quantitative analysis of the extracted oils.

The acid and peroxide values were determined according to the AOCS method (Cd 3a-63, 1989; Cd 8-53, 1996). The method prescribed by Endo *et al.* (2001) was used for measuring carbonyl value (CV).

Rancimat (Metrohm, model 743, Switzerland) at 110 °C with air flow rate of 15 L/h was employed for determining the oxidative stability index (Koprivnjak *et al.*, 2008). The oxidisability (Cox) value was calculated based on the amount of 18-carbon unsaturated fatty acids:

$$\text{Cox value} = \frac{[1(\text{C 18:1\%}) + 10.3(\text{C 18:2\%}) + 21.6(\text{C 18:3\%})]}{100} \quad (1)$$

performed under harder conditions in industry.

### Chemicals

Most required chemicals were purchased from Merck (Darmstadt, Germany), Scharlau Chemie (Barcelona, Spain) and Caledon (Ontario, Canada).  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\Delta$ -tocopherol standards were obtained from Sigma (Sigma, St. Louis, USA).

### Experiments

International Olive Council (IOC) Standard #24 was used for derivatization of fatty acids. Fatty acid methyl ester (FAME) profile was determined by GC (Young-Lin, Acme 6000, Korea) equipped with a capillary column (Teknokroma, TR-CN100, 60m  $\times$  0.25 mm i.d.,  $d_f$  = 0.2  $\mu$ m) and FID. H<sub>2</sub> was used as a carrier gas (flow rate = 0.7 mL/min). The temperatures of column, injector, and detector were 175, 250, and 260 °C, respectively. The peaks were identified based on their retention times using authentic standard fatty acids methyl esters and all samples were run in triplicates.

Where

C18:1, C18:2 and C18:3 denote oleic, linoleic and linolenic fatty acids, respectively (Fatemi and Hammond, 1980).

### Analysis of Phenolic Compounds

The determination of phenolic compounds were conducted according to the official method of IOC COI/T.20/Doc No 29. For extraction of phenolic compounds, 2.0 g of olive oil was weighed in a 10 mL screw-cap test tube, syringic acid (1 mL, 0.015 mg/mL) was added to the oil as an internal standard. The mixture was shaken for exactly 30 sec in a vortex apparatus. Then, 5 mL of the methanol/water 80/20 (V/V) was added and shaken for 1 min. The mixture was placed in the ultrasonic bath for 15 min at room temperature, then, the tube was centrifuged at 5000 rpm for 25 min. The methanol layer was separated and the extraction was repeated twice. Samples were filtered through a 5 mL plastic syringe with a 0.45 mm PVDF filter to be used for HPLC analysis as well as for the determination of phenolic compounds.



### HPLC Analysis

20  $\mu\text{L}$  of the internal standard solution (syringic acid 0.015 mg/ml) was injected into the HPLC system (Young-Lin, Acme 9000, South Korea) equipped with Spherisorb ODS-2 column (4.6 x 250 mm, dp= 5  $\mu\text{m}$ ) and UV-Vis detector at 280 nm. All analyses were carried out at room temperature. Ternary linear elution gradient with de-gassed water 0.2 %  $\text{H}_3\text{PO}_4$  (V/V) (A), methanol (B) and acetonitrile (C) was used. Gradient elution was performed as show in Table1.

After recording the chromatogram, the response factor (RF) of the syringic acid was calculated. After that, 20  $\mu\text{L}$  of the sample was injected into the HPLC and chromatograms were recorded at 280 nm. The amounts of phenolic compounds were calculated according to the following formula:

$$\left(\frac{\text{mg}}{\text{kg}}\right) = \frac{(\sum A) \times 1000 \times w_{\text{syr.acid}}}{A_{\text{syr.acid}} \times w} \quad (2)$$

Where,

$\sum A$  is the sum of the peak areas of the phenolic compounds;

$w_{\text{syr.acid}}$  is the weight, in mg, of the syringic acid used as internal standard in 1 mL of the solution added to the sample;

$A_{\text{syr.acid}}$  is the area of the syringic acid internal standard recorded at 280 nm;

$w$  is the weight of the oil used, in grams;

### Tocopherol Analysis

Tocopherols were evaluated according to ISO method (ISO, 9936). Two grams of olive oil were dissolved in 25 mL hexane and injected into the HPLC system (Young-Lin, Acme 9000, South Korea) equipped with LiChrosob SI 60 (4.6 mm x 250 mm, dp= 5  $\mu\text{m}$ ) and UV-Vis detector at 292 nm. The mobile phase was 0.5% isopropanol in n-hexane and its flow rate was 1.0 mL/min. The total run time and column temperature were 40 min and 22  $^{\circ}\text{C}$ , respectively. The injection volume was 20  $\mu\text{L}$ . The detector was a UV-Vis operated at 292 nm. Tocopherols were quantified by an external standard method.

### Sterol Analysis

The analysis of sterols was conducted according to the official method of the IOC COI/ T.20/ Doc. No 30/Rev. 1.

### Preparation of the Unsaponifiable Fraction

500  $\mu\text{L}$  of the 0.2 %  $\alpha$ -cholestanol (Sigma, St. Louis, USA) solution in chloroform were added to 5 g of olive oil, as an internal standard, and were evaporated to dryness under a nitrogen atmosphere. Afterwards, 50 mL of 2 N ethanolic potassium hydroxide solution was added and heated to gentle boiling in a water bath with continuous vigorous stirring until saponification took place, then, 50 mL of distilled water was

**Table 1.** Gradient program applied for separation of phenolic compounds by HPLC.

Time (min)	Flow (ml/min)	A (%)	B (%)	C (%)
0	1.00	96	2	2
40	1.00	50	25	25
45	1.00	40	30	30
60	1.00	0	50	50
70	1.00	0	50	50
72	1.00	96	2	2
82	1.00	96	2	2

added. The extraction of the unsaponifiable fraction was carried out with diethyl ether, and the combined ether extract was purified with water, dried over sodium sulfate and evaporated to dryness under vacuum and nitrogen gas.

### Separation of the Sterol Fraction

An approximately 5 % solution of the unsaponifiables in chloroform was prepared and was loaded on basic TLC plates with silica gel 60 F254 (Merck, Darmstadt, Germany); the solution was then eluted by a mixture of hexane and diethyl ether 65:35 (v/v) to separate the sterol fraction. The plates were sprayed lightly and uniformly with the 2,7-dichlorofluorescein solution and observed under ultraviolet light. The sterol band was identified and scraped off with a spatula, dissolved in chloroform and diethyl ether and evaporated to dryness, then, transformed into trimethylsilyl ethers by adding a silylation reagent containing a 9:3:1 (v/v/v) mixture of pyridine/hexamethyl disilazane/trimethyl chlorosilane (all reagents were supplied by Merck) in the ratio of 50  $\mu$ L for every milligram of sterols. The solution was shaken carefully until the sterols were completely dissolved and then centrifuged.

### Gas Chromatographic Analysis

The mixture was analyzed by a Younglin 6001 series chromatograph (South Korea) equipped with a flame ionization detector and a BPX-70 capillary column (60 m  $\times$  0.25 mm id  $\times$  0.25  $\mu$ m film thickness; SGE, Melbourne, Australia). Helium with a flow rate of 1.27 mL/min was used as the carrier gas. Injector and detector temperatures were 280 and 290  $^{\circ}$ C, respectively, and the column temperature program was 265  $^{\circ}$ C for 45 min, increased at 5  $^{\circ}$ C/min to a final temperature of 300  $^{\circ}$ C. One  $\mu$ L was injected in split mode (1:50). Twelve sterols (cholesterol, campesterol, stigmasterol,  $\Delta$ -7-

campesterol,  $\Delta$ -5-23-stigmastadienol, clerosterol,  $\beta$ -sitosterol, sitostanol,  $\Delta$ -5-avenasterol,  $\Delta$ -5-24-stigmastadienol,  $\Delta$ -7-stigmastenol, and  $\Delta$ -7-avenasterol) in oils were identified according to the IOC method. Sterol concentrations and relative amounts were expressed as mg/100g of oil with respect to the internal standard and proportions (%) of total sterols, respectively. The apparent  $\beta$ -sitosterol was calculated as the sum of  $\beta$ -sitosterol,  $\Delta$ -5-avenasterol, clerosterol, sitostanol, and  $\Delta$ -5, 24-stigmastadienol.

### Statistical Analysis

Data were analyzed statistically using analysis of variance (ANOVA) and differences among the means were determined for significance at  $p \leq 0.05$  using least significant differences (LSD) test (by SAS software ver 9.1). The data are presented as mean  $\pm$  standard deviation of the three measurements.

## RESULTS AND DISCUSSION

Table 2 presents fatty acids (FA) profiles of virgin and processed *Leccino* and *Frantoio* oils. The high PUFA/SFA ratio is an indication of unsaturation level of oils and fats, and also indicates the high tendency of oils towards oxidative reactions. Therefore, the lowest oxidative stability, in terms of fatty acid structure, can be attributed to processed *Frantoio* oil. Low oxidation values (Cox value) are an indication of higher oxidative stability of oils. Hence, the processed *Leccino* oil had the lowest Cox value and, therefore, the highest oxidative stability among the olive oil samples. There was no significant difference between the Cox value of processed and virgin *Leccino* oils ( $p < 0.05$ ). High PUFA and low MUFA levels of virgin *Frantoio* oil can explain its low oxidative stability. Processing had no significant effect on the PUFA/SFA ratio and the Cox value

**Table 2.** Fatty acid composition of the studied olive oils (mean  $\pm$  s.d., %) <sup>a</sup>.

Fatty acid	<i>Leccino</i>		<i>Frantoio</i>	
	Virgin	Processed	Virgin	Processed
C16:0	16.49 $\pm$ 0.15 <sup>a</sup>	15.23 $\pm$ 0.12 <sup>b</sup>	15.14 $\pm$ 0.08 <sup>bc</sup>	15.08 $\pm$ 0.02 <sup>c</sup>
C16:1cis9	1.70 $\pm$ 0.13 <sup>b</sup>	1.52 $\pm$ 0.02 <sup>d</sup>	1.80 $\pm$ 0.04 <sup>a</sup>	1.63 $\pm$ 0.05 <sup>c</sup>
C18:0	1.99 $\pm$ 0.14 <sup>b</sup>	2.06 $\pm$ 0.01 <sup>b</sup>	2.30 $\pm$ 0.03 <sup>a</sup>	2.37 $\pm$ 0.03 <sup>a</sup>
C18:1cis9	62.26 $\pm$ 0.22 <sup>b</sup>	64.00 $\pm$ 0.08 <sup>a</sup>	61.04 $\pm$ 0.17 <sup>d</sup>	61.32 $\pm$ 0.02 <sup>c</sup>
C18:1cis11	5.20 $\pm$ 0.16 <sup>c</sup>	5.22 $\pm$ 0.03 <sup>c</sup>	5.53 $\pm$ 0.11 <sup>b</sup>	5.90 $\pm$ 0.06 <sup>a</sup>
C18:2cis9cis12	10.08 $\pm$ 0.04 <sup>c</sup>	9.90 $\pm$ 0.03 <sup>d</sup>	11.75 $\pm$ 0.02 <sup>a</sup>	11.25 $\pm$ 0.05 <sup>b</sup>
C20:0	0.32 $\pm$ 0.00 <sup>c</sup>	0.31 $\pm$ 0.00 <sup>c</sup>	0.42 $\pm$ 0.02 <sup>a</sup>	0.37 $\pm$ 0.00 <sup>b</sup>
C18:3cis9cis12cis15	1.01 $\pm$ 0.00 <sup>b</sup>	0.94 $\pm$ 0.00 <sup>c</sup>	1.07 $\pm$ 0.00 <sup>a</sup>	1.00 $\pm$ 0.05 <sup>b</sup>
C20:1 cis11	0.27 $\pm$ 0.01 <sup>ab</sup>	0.28 $\pm$ 0.00 <sup>a</sup>	0.29 $\pm$ 0.00 <sup>a</sup>	0.26 $\pm$ 0.00 <sup>b</sup>
C22:0	0.10 $\pm$ 0.00 <sup>b</sup>	0.12 $\pm$ 0.00 <sup>a</sup>	0.11 $\pm$ 0.00 <sup>a</sup>	0.12 $\pm$ 0.00 <sup>a</sup>
Others	0.58 $\pm$ 0.01 <sup>b</sup>	0.42 $\pm$ 0.00 <sup>c</sup>	0.55 $\pm$ 0.03 <sup>b</sup>	0.70 $\pm$ 0.03 <sup>a</sup>
SFA	18.90 $\pm$ 0.28 <sup>a</sup>	17.71 $\pm$ 0.16 <sup>c</sup>	17.97 $\pm$ 0.04 <sup>b</sup>	17.94 $\pm$ 0.05 <sup>b</sup>
MUFA	69.43 $\pm$ 0.03 <sup>b</sup>	71.02 $\pm$ 0.02 <sup>a</sup>	68.66 $\pm$ 0.04 <sup>d</sup>	69.11 $\pm$ 0.03 <sup>c</sup>
PUFA	11.09 $\pm$ 0.06 <sup>c</sup>	10.84 $\pm$ 0.03 <sup>d</sup>	12.82 $\pm$ 0.04 <sup>a</sup>	12.25 $\pm$ 0.00 <sup>b</sup>
PUFA/SFA	0.59 $\pm$ 0.00 <sup>b</sup>	0.61 $\pm$ 0.00 <sup>b</sup>	0.71 $\pm$ 0.00 <sup>a</sup>	0.68 $\pm$ 0.00 <sup>a</sup>
Cox value	1.93 $\pm$ 0.00 <sup>b</sup>	1.92 $\pm$ 0.00 <sup>b</sup>	2.11 $\pm$ 0.00 <sup>a</sup>	2.05 $\pm$ 0.00 <sup>a</sup>

<sup>a</sup> Values with similar letters in each row have no significant differences (LSD test,  $p < 0.05$ ).

( $p < 0.05$ ). That can be due to the relative stability of olive oil's fatty acids against oxidation reactions during the processing (Farhoosh and Moosavi, 2008).

The acid, peroxide and carbonyl values as well as the OSI of the studied olive oil samples are listed in Table 3. Virgin *Frantoio* and processed *Leccino*, with 0.78 and 0.18 mg KOH/g oil (for the AV) and with 8.74 and 6.03 (meq O<sub>2</sub>/kg oil, for the PV), were the minimum and maximum of both parameters, respectively. The acceptable limits of PV and AV for processed oil are 10 meq O<sub>2</sub>/kg oil and 0.4 mg KOH/g oil, respectively, and for oils extracted using the cold press method (or virgin oils) are 15 meq O<sub>2</sub>/kg oil and 0.6 mg

KOH/g oil (Anonymous, 1999). The processing decreased the acid and peroxide values of the studied oils. Bachari Saleh *et al.* (2013) studied the effect of refining process on the conjugated dienes in soybean oil and found that oxidation factors (PV, FFA, p-AV and OSI) of the soybean oil diminished during the refining steps and decreased significantly in deodorization. The PV and AV are associated with total hydroperoxide compounds and the rancid taste induced by hydrolysis of oils and edible fats. Hydroperoxides are the primary product of lipid oxidation, which, due to their low stability, are rapidly decomposed into secondary products like carbonyls. Carbonyl compounds are responsible for the

**Table 3.** Some of oxidative stability indices of the studied olive oils (mean  $\pm$  s.d.) <sup>a</sup>

Index	Oil type			
	Virgin <i>Leccino</i>	Processed <i>Leccino</i>	Virgin <i>Frantoio</i>	Processed <i>Frantoio</i>
AV (mg KOH/ g oil) <sup>b</sup>	0.24 $\pm$ 0.01 <sup>c</sup>	0.18 $\pm$ 0.00 <sup>d</sup>	0.78 $\pm$ 0.04 <sup>a</sup>	0.49 $\pm$ 0.04 <sup>b</sup>
PV (meq O <sub>2</sub> /kg oil) <sup>c</sup>	6.76 $\pm$ 0.43 <sup>b</sup>	6.03 $\pm$ 0.47 <sup>c</sup>	8.74 $\pm$ 0.37 <sup>a</sup>	7.48 $\pm$ 0.22 <sup>b</sup>
CV ( $\mu$ mol/g oil) <sup>d</sup>	5.21 $\pm$ 0.04 <sup>b</sup>	5.56 $\pm$ 0.14 <sup>a</sup>	3.31 $\pm$ 0.03 <sup>d</sup>	4.22 $\pm$ 0.10 <sup>c</sup>
Rancimat test (h)	18.83 $\pm$ 0.12 <sup>a</sup>	17.24 $\pm$ 0.06 <sup>b</sup>	16.61 $\pm$ 0.19 <sup>c</sup>	14.41 $\pm$ 0.31 <sup>d</sup>

<sup>a</sup> Values with different letters in each row have significant differences (LSD test,  $p < 0.05$ ); <sup>b</sup> Acid value; <sup>c</sup> Peroxide value; <sup>d</sup> Carbonyl value.

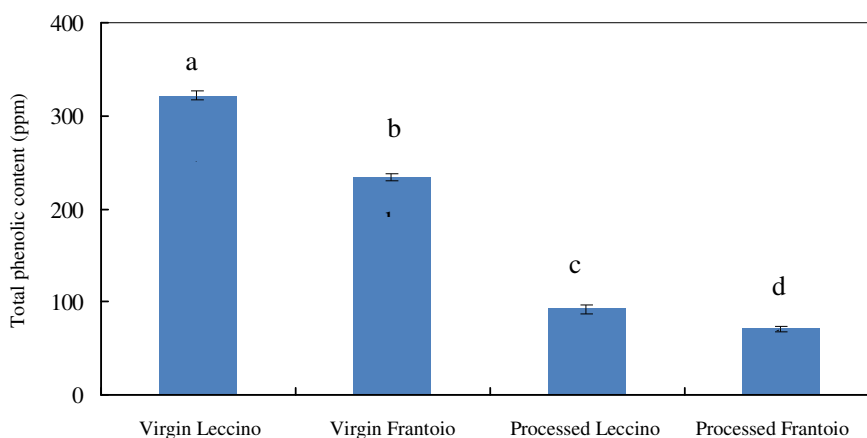
well-known rancid tastes of oils and oxidized fats (Endo *et al.*, 2001).

The laboratory processing of the studied olive oils enhanced their carbonyl values. This could be explained by the decomposition of hydroperoxides during the processing and their conversion into carbonyl compounds. Farhoosh *et al.* (2009) studied the effect of refining on soybean and canola oils and found that bleaching and deodorizing decreased both AV and PV and increased the carbonyl value. There are similar reports indicating that deodorizing decreases the AV as low as possible (Alhassan Suliman *et al.*, 2013).

The oxidative stability index (OSI) (determined by Rancimat test) is the required time for the development of measurable rancidity in oils and fats. By Iranian standards, the proper OSI for household cooking oil is 15 h at 110°C (Anonymous, 2011). Therefore, virgin *Leccino*, processed *Leccino*, and virgin *Frantoio* olive oils can be suggested for household cooking with respect to their OSIs values of 18.83, 17.24, and 16.61 h, respectively. Ceci and Carelli (2007) determined the OSI of 37 oils of different olives grown in different climatic conditions throughout Argentina. The OSIs of studied cultivars ranged from 6.7 to 19.2 h at 110°C.

The decreased OSI in processed oils can be associated with the destructive effect of refining on olive oil natural antioxidants. Aparicio *et al.* (1999) used the Rancimat to determine the oil stability and found a close relationship between virgin olive oil stability, phenolic and tocopherolic contents, and the oleic acid to linoleic acid ratio. Results obtained by Alhassan Suliman *et al.* (2013) confirmed the destructive effect of refining on antioxidants such as tocopherolic and phenolic compounds of olive oil.

The levels of phenolic compounds are presented in Figure 1. Phenolic compounds are mostly studied in terms of antioxidant activities. According to the literature, polyphenols, especially ortho-diphenols, hold the largest contribution to olive oil's stability (Borchani *et al.*, 2010; Kellie *et al.*, 2002). Maximum and minimum phenolic compounds belonged to virgin *Leccino* (321.14 mg/kg) and processed *Frantoio* (70.90 mg/kg). The most effective factors on the amount of phenolic compounds include variety, extraction method, processing, and storage conditions. Kiralan *et al.* (2009) reported that phenolic content of virgin olive oils in some important cultivars grown in Turkey ranged from 38.31 to 495.42 mg/kg. Blekas *et al.* (2002), in an attempt to study the effect of total phenolic compounds on



**Figure 1.** Total phenolics content of the studied olive oils. Columns with different letters have significant differences (LSD test,  $p < 0.05$ ).



Greek virgin olive oils stabilities, identified a good correlation between total phenolic compounds and olive oil stability. Therefore, the studied olive oils can be considered as a valuable source in terms of their considerable amounts of phenolic compounds. Farhoosh *et al.* (2008) performed a study on canola, soybean, sunflower, maize, and olive oils and reported their total phenolic compounds as 48.2, 45.8, 45.3, 30.8 and 15.3 mg/kg, respectively.

Based on the detailed analysis of olive oil components, the phenolic compounds and tocopherols are almost depleted after a short heating period (Santos *et al.*, 2013). There is a particular worry regarding olive oil since its bioactive compounds may be lost during the thermal process. Some authors have reported that phenolic compounds are eliminated during refining. In fact, these substances are affected by thermal processing, but their loss is dependent on their chemical structure and, perhaps, on their antioxidant activity (Gómez-Alonso *et al.*, 2003). The main components of olive oil phenolic fraction are hydroxytyrosol, tyrosol and their derivatives (secoiridoids). It has been reported that hydroxytyrosol and their derivatives are extensively lost, with a 50% reduction in EVOO after frying for only 10 min at 180 °C.

Tocopherols are natural antioxidants that can protect oils and fats against damages by reacting with free radicals and driving the oxidative reactions towards their final stages. The amount and type of tocopherolic compounds of the studied oils are listed in Table 4. The predominant tocopherolic compound was  $\alpha$ -tocopherol and its amount

was more than 85% of total tocopherolic compounds. Virgin *Leccino* and processed *Frantoio* oils had the maximum and minimum contents of tocopherolic compounds with 455.25 and 114.81 mg/kg, respectively. Dabbou *et al.* (2010) determined the total tocopherol and phenols of *Leccino* cultivar planted in Tunisia and reported their amounts as 369.04 mg/kg (for tocopherol) and 176.16 mg/kg (for phenol). Rossi *et al.* (2007) studied the effect of tocopherols and tocotrienols on the stability of processed vegetable oils and reported a good correlation between tocopherols and tocotrienols of olive oil and its stability. In our study, the virgin *Leccino* oil had higher oxidative stability due to its higher amount of tocopherolic and phenolic compounds than the *Frantoio* oil. This confirms the influence of olive oil bioactive compounds on its thermal stability. Aguilera *et al.* (2005) measured phenolic and tocopherolic contents of virgin *Leccino* and *Frantoio* olives grown in Andalusia and found that their amounts were 718 and 635 mg/kg (for phenols) and 334 and 286 mg/kg (for tocopherols). Therefore, climatic conditions have an important effect on the phenolic and tocopherolic contents of extracted oils. Additionally, the oxidative stability of virgin olive oil is influenced by SFA/UFA ratio and tocopherolic compounds.

As shown in Table 5, the sterolic compounds of the studied oils is mostly composed of  $\beta$ -sitosterol,  $\Delta$ -5-avenasterol and campesterol.  $\beta$ -sitosterol comprises about 78-85 % of total sterols of the studied oils. Virgin *Leccino* and processed *Frantoio* had the maximum (2189.1 mg/kg) and

**Table 4.** Tocopherols composition of the studied oils (mean  $\pm$  s. d., mg/kg).<sup>a</sup>

Tocopherols	Oil type			
	Virgin <i>Leccino</i>	Processed <i>Leccino</i>	Virgin <i>Frantoio</i>	Processed <i>Frantoio</i>
$\alpha$ -tocopherol	405.6 $\pm$ 4.6 <sup>a</sup>	194.0 $\pm$ 1.6 <sup>c</sup>	230.0 $\pm$ 1.6 <sup>b</sup>	96.5 $\pm$ 0.0 <sup>d</sup>
$\beta$ and $\gamma$ -tocopherols	48.2 $\pm$ 0.0 <sup>a</sup>	29.6 $\pm$ 0.3 <sup>c</sup>	39.1 $\pm$ 0.1 <sup>b</sup>	17.9 $\pm$ 0.2 <sup>d</sup>
$\Delta$ -tocopherols	1.4 $\pm$ 0.2 <sup>a</sup>	0.9 $\pm$ 0.0 <sup>c</sup>	1.6 $\pm$ 0.0 <sup>a</sup>	1.1 $\pm$ 0.1 <sup>b</sup>
Total tocopherols	455.3 $\pm$ 4.4 <sup>a</sup>	225.5 $\pm$ 0.4 <sup>c</sup>	270.7 $\pm$ 1.7 <sup>b</sup>	114.8 $\pm$ 0.2 <sup>d</sup>

<sup>a</sup> Values with different letters in each row have significant differences (LSD test,  $p < 0.05$ ).



**Table 5.** Sterolic contents of the studied olive oils (mean $\pm$  s.d., mg/kg)<sup>a</sup>.

Sterols	Oil type			
	Virgin <i>Leccino</i>	Processed <i>Leccino</i>	Virgin <i>Frantoio</i>	Processed <i>Frantoio</i>
Cholesterol	1.95 $\pm$ 0.19 <sup>b</sup>	1.43 $\pm$ 0.03 <sup>c</sup>	2.59 $\pm$ 0.00 <sup>a</sup>	1.91 $\pm$ 0.44 <sup>b</sup>
Brassicasterol	19.07 $\pm$ 1.85 <sup>a</sup>	ND	11.06 $\pm$ 0.1 <sup>b</sup>	ND
24-Methylencholesterol	ND	ND	3.10 $\pm$ 0.03 <sup>a</sup>	ND
Campesterol	71.65 $\pm$ 5.89 <sup>b</sup>	61.70 $\pm$ 0.36 <sup>c</sup>	96.88 $\pm$ 0.66 <sup>a</sup>	53.54 $\pm$ 0.08 <sup>d</sup>
Campestanol	ND	ND	15.81 $\pm$ 0.80	ND
Stigmasterol	29.81 $\pm$ 1.66 <sup>a</sup>	11.49 $\pm$ 0.17 <sup>c</sup>	21.68 $\pm$ 0.21 <sup>b</sup>	22.62 $\pm$ 0.12 <sup>b</sup>
Stigmastanol	ND	ND	4.32 $\pm$ 0.08	ND
Clerosterol	2.57 $\pm$ 0.14 <sup>b</sup>	2.10 $\pm$ 0.06 <sup>c</sup>	4.23 $\pm$ 0.00 <sup>a</sup>	2.23 $\pm$ 0.13 <sup>bc</sup>
$\beta$ -Sitosterol	1713.30 $\pm$ 3.03 <sup>a</sup>	1320 $\pm$ 21.14 <sup>c</sup>	1477.42 $\pm$ 32.21 <sup>b</sup>	1219.07 $\pm$ 3.40 <sup>d</sup>
Avenasterol	153.42 $\pm$ 2.33 <sup>a</sup>	71.16 $\pm$ 1.28 <sup>c</sup>	105.46 $\pm$ 12.23 <sup>b</sup>	88.04 $\pm$ 1.43 <sup>d</sup>
5,24-Stigmastadienol	15.50 $\pm$ 0.05 <sup>a</sup>	13.54 $\pm$ 0.31 <sup>b</sup>	12.57 $\pm$ 0.21 <sup>b</sup>	10.80 $\pm$ 0.05 <sup>c</sup>
$\beta$ -Stigmastinol	17.70 $\pm$ 0.93 <sup>a</sup>	12.64 $\pm$ 0.13 <sup>b</sup>	9.85 $\pm$ 0.02 <sup>c</sup>	8.93 $\pm$ 0.06 <sup>d</sup>
7-Avenasterol	25.00 $\pm$ 4.79 <sup>a</sup>	13.50 $\pm$ 0.00 <sup>c</sup>	17.06 $\pm$ 0.47 <sup>b</sup>	7.38 $\pm$ 0.35 <sup>d</sup>
Others	139.14 $\pm$ 15.72 <sup>a</sup>	23.00 $\pm$ 5.01 <sup>c</sup>	29.76 $\pm$ 6.78 <sup>bc</sup>	32.51 $\pm$ 1.57 <sup>b</sup>
Total	2189.10 $\pm$ 25.37 <sup>a</sup>	1530.97 $\pm$ 17.60 <sup>c</sup>	1809.36 $\pm$ 6.37 <sup>b</sup>	1447.11 $\pm$ 5.22 <sup>d</sup>

<sup>a</sup> Values with different letters in each row have significant differences (LSD test,  $p < 0.05$ ).

minimum (1447.1 mg/kg) contents of total sterolic compounds. Our research shows that a part of sterols is reduced and removed during the oil processing (deodorizing and bleaching) due to a series of dehydration reactions that take place in the sterol structure. The reduced sterolic compounds were about 20% and 30% for *Frantoio* and *Leccino* in the laboratory processing, respectively. Phytosterols inhibit cholesterol absorption and, therefore, decrease the serum cholesterol level in human body. Additionally, phytosterols have antioxidant activities (Wang *et al.*, 2002). Singh (2013) showed that sitosterols enhanced the stability of vegetable oils and decreased the formation of polymerized triglycerides at high temperatures. Therefore, the higher oxidative stability of virgin *Leccino* oils can be explained by its higher content of sterolic compounds compared to the other studied oils.

### CONCLUSIONS

In sum, results showed that laboratory processing of virgin olive oil significantly decreases phenolic, tocopherolic, and

sterolic compounds by 70%, 50% and 25 %, respectively. Virgin olive oil has higher oxidative stability than the processed counterparts, which shows the key role of its inherent antioxidant compounds. Therefore, it is suggested that olive oil should be consumed without refining and deodorizing, as far as possible. Moreover, *Leccino* olive oil had higher oxidative stability and bioactive compounds than *Frantoio* oil, making the former more suitable for frying processes and as a salad dressing.

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## خواص فیزیکوشیمیایی و پایداری اکسایشی برخی از روغن های زیتون بکر و فرآوری شده

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### چکیده

در پژوهش حاضر، ساختار اسید چرب، برخی شاخص های پایداری اکسایشی و ترکیبات زیست فعال روغن بکر و فرآوری شده زیتون (ارقام لچینو و فرانتویو) مورد مطالعه قرار گرفت. نتایج نشان داد که روغن های مورد مطالعه عمدتاً حاوی به ترتیب اولئیک، (C18:1)، پالمیتیک (C16:0) و لینولئیک (C18:2) اسید است. روغن فرانتویو بکر و لچینو فرآوری شده به ترتیب دارای اعداد اسیدی ۰/۷۸ و ۰/۱۸ میلی گرم پتاس بر گرم روغن و اعداد پراکسید ۸/۷۴ و ۶/۰۳ میلی اکی والان گرم بر کیلوگرم روغن بودند. روغن لچینو بکر با ۱۸/۸۳ ساعت دارای بالاترین شاخص پایداری اکسایشی (آزمون رنسیمت) بود. بیشترین میزان ترکیبات فنلی، توکوفرولی و استرولی در بین روغن های مورد مطالعه به ترتیب ۳۲۱/۱۴، ۴۵۵/۲۵ و ۲۱۸۹/۱ پی پی ام متعلق به روغن لچینو بکر بود. فرآوری آزمایشگاهی روغن های زیتون بکر باعث کاهش چشمگیر ترکیبات فنلی تا ۷۰٪، توکوفرول ها تا ۵۰٪ و استرول ها تا ۲۵٪ گردید. در آخر این که روغن لچینو بکر پایداری اکسایشی و ترکیبات زیست فعال بیشتری نسبت به سایر روغن ها داشت.