



## A Comparison of the Quality Characteristics of the Virgin and Refined Olive Oils Supplied in Tarom Region, Iran (2019)

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### ABSTRACT

**Background:** Olive oil is one of the healthiest oils in the world with a high nutritional value. Zanjan province has the highest acreage under olive cultivation in Iran. Given the importance of olive oil purity, this study aimed to investigate the quality and authenticity of the olive oils produced in Tarom region, Iran during January-March 2019.

**Methods:** Bulk samples consisting of five virgin olive oils and brand samples consisting of two virgin and three refined olive oils were collected. The fatty acid profile, sterol and squalene contents, and iodine and Cox values of the samples were determined.

**Results:** In terms of fatty acid composition, 30% of the samples were adulterated with other vegetable oils and contained more than 1% linolenic acid. The total sterol in the samples conformed to the standards (>1,000 mg/kg). However, the concentrations of campesterol and delta-7-stigmastenol in two bulk samples were above the national standard limits. The high content of squalene (55.37%) in a bulk sample was attributed to the soybean oil presence.

**Conclusion:** The quality properties of the brand samples complied with the national and Codex standards. Due to possible adulteration with other vegetable oils, the continuous monitoring of bulk olive oil supplies is recommended.

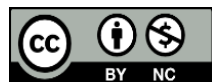
## 1. Introduction

Olive (*Olea europaea* L.) is a plant belonging to the Oleaceae family and the Olea genus. Olive fruit is composed of moisture (50%), protein (1.6%), oil (20-30%), carbohydrates (19%), cellulose (5.8%), and minerals (1.5%). In addition, the fruit contains hydrophilic compounds (phenolic acids, phenolic alcohols, flavonoids) and lipophilic compounds (tocopherols and tocotrienols) with several biological activities, such as antioxidant, anti-carcinogenic, anti-inflammatory, antimicrobial, anti-hypertensive and anti-dyslipidemic properties [1].

Olive oil is an abundant source of valuable nutrients and bioactive compounds with potential therapeutic and medical functions. Triacylglycerols (97-99%) are the main constituents of olive oil [2]. Furthermore, olive oil contains

some minor components, such as free fatty acids, mono- and diglycerides, hydrocarbons, sterols, aliphatic alcohols, tocopherols, pigments, and phenolic compounds. These minor lipid components affect the unique properties and health benefits of olive oil and are removed during the refining process [3].

With respect to the cultivation area of olive trees, Iran is ranked 14<sup>th</sup> worldwide, and olive groves cover approximately 104,000 hectares in this country. As for olive production, Iran is ranked 17<sup>th</sup> with the annual production of 100,000 tons. Zanjan province is one of the most important olive centers in Iran, with the average yield of 45,000 tons. In Tarom region, more than 90% of olive oil is supplied in bulk. The annual consumption of olive oil in Iran has been estimated at 12,000-15,000 tons [4-6].



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Virgin olive oil is extracted from the first pressing, which has a good taste and odor and the maximum free fatty acid content of 2%. Refined olive oil is obtained from virgin olive oil using refining methods, which do not change the first structure of glycerides with the maximum free fatty acid content of 0.3% [7].

Due to the high levels of monounsaturated and valuable minor components with antioxidant activity (e.g., phytosterols, tocopherols, carotenoids, chlorophyll, squalene, and phenolic compounds), virgin olive oil has beneficial health effects and the potential to promote the human health, especially against cardiovascular disorders, colorectal cancer, diabetes, Alzheimer's disease, and inflammatory and autoimmune diseases [8].

The quality of olive oil depends on several factors, such as the variety of the olive, agro-climatic conditions of the cultivation area, maturity and the time of fruit harvesting, storage conditions of the harvested fruits, extraction methods, and packaging [9].

Considering its nutritional value and health benefits, virgin olive oil is a costly product and may be illegally blended with other cheap or low-quality oils so as to be to the profit of the manufactures. Such adulteration is detected by chemical analysis [10].

The type of edible olive oil is determined based on the fatty acid composition [10]. In addition, free and esterified sterols are major constituents of olive oil, which are used to distinguish adulterated olive oil from edible oils, especially sunflower and safflower oils with high oleic acid content [11]. Delta-7-stigmasterol, campesterol, and brassicasterol are also important in detecting the adulteration of olive oil with sunflower, soybean, and rapeseed oils [12].

Previous studies have investigated the quality of the olive oils extracted from different varieties cultivated in Tarom region. For instance, Homapour *et al.* (2014) reported that the quality aspects of the oils obtained from three varieties of olive fruit in Fadak and Gilvan regions complied with the Codex and Iranian national standards [13]. Moreover, Farhang Doost *et al.* (2013) evaluated the properties of the oil extracted from five different olive cultivars in Tarom region and determined the optimal types in terms of the quantity and quality of the extracted oil [14].

According to Zinanlu *et al.* (2016), the formation of oleic acid was significantly affected by the cultivar and temperature of the region. In the mentioned study, the assessment of the fatty acid composition of olive oil indicated that the oleic acid content of the olive oils from Tarom, Gorgan, and Rudbar regions was higher compared to the samples of other regions [15].

To the best of our knowledge, data are scarce regarding the quality indicators of the bulk virgin olive oils supplied in Tarom region in recent years. Considering the increased consumer awareness about the health of edible oils, purity of olive oil, and growing number of bulk olive oil products on the market, the present study aimed to evaluate the quality and authenticity of the virgin and refined olive oils produced in Tarom region, Iran in 2019 for the first time.

## 2. Materials and Methods

### 2.1. Chemicals and Reagents

All the chemicals and reagents used in this study were of the analytical grade. Chloroform, n-hexane (99.0%), diethyl

ether (99.7%), methanol, and potassium hydroxide (85.0%) were obtained from Merck KGaA (Darmstadt, Germany). Internal Standard 5  $\alpha$ -cholestanol (95.0%) was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), and the thin-layer chromatography (TLC) plate (Silica gel 60; 20 × 20 cm; thickness: 0.25 mm) was purchased from Merck (Darmstadt, Germany). The other chemicals were of the highest quality available.

### 2.2. Olive Oil Sampling

In total, 10 olive oil samples were assessed in this study, which were obtained randomly from the local stores in Tarom region of Zanjan province during January-March, 2019. Two groups of bulk (non-commercial) and commercial retail olive oils were purchased. The oil samples were transferred to the Food Control Laboratory of Zanjan University of Medical Sciences. The bulk samples included five virgin olive oils (F, G, H, I, and K), and the commercial samples included two virgin olive oils (C and D) and three refined olive oils (A, B, and E).

### 2.3. Analytical Methods

#### 2.3.1. Fatty Acid Composition

The fatty acid composition of the samples was determined via gas chromatography (GC) as the methyl ester, and the fatty acid methyl esters of the samples were prepared by esterification. Approximately 100 milligrams of olive oil was mixed with five milliliters of BF<sub>3</sub> in a methanol solution. The mixture was heated under reflux for two minutes after adding five milliliters of n-hexane. Following that, the mixture was refluxed for one more minute. After cooling, about 15 milliliters of a saturated sodium chloride solution was added and stirred vigorously. After phase separation, the organic phase was collected and dried using anhydrous calcium chloride.

The level of the fatty acid methyl esters was determined using a CG device (model: Varian, 3800, Netherlands) on a Cp-Sill 88 capillary column (50 m × 0.25 mm × 0.25  $\mu$ m; Agilent J&W, Santa Clara, CA, USA), which was equipped with a flame ionization detector (Hewlett-Packard, Palo Alto, CA, USA). Nitrogen was used as the carrier gas with the flow rate of 0.7 ml/min, and the injector temperature was set at 250°C. The initial column temperature was maintained at 60°C for five minutes, increased to 175°C, maintained for 30 minutes, increased to 220°C at the rate of 3°C/min, and maintained for 60 minutes. Afterwards, one microliter of the samples was injected, and the peaks were identified by comparing their retention times with similar analysis standards [16].

#### 2.3.2. Iodine Value

The iodine value is a measure of the number of the double bonds in unsaturated fatty acids. In this study, we used a calculation method to determine the iodine value of the oil samples based on their fatty acid methyl ester composition using the AOCS method Cd 1c-85 and the following formula:

$$\text{Iodine Value} = (\text{C16:1}\%) \times 0.95 + (\text{C18:1}\%) \times 0.86 + (\text{C18:2}\%) \times 1.732 + (\text{C18:3}\%) \times 2.616 \quad (1)$$

where C16: 1, C18: 1, C18:2, and C18:3 represent the

levels of palmitoleic, oleic, linoleic acids, and linolenic acids, respectively [17].

### 2.3.3. Cox Value

The Cox value of the oil samples was determined based on the oxidation index and percentage of the unsaturated fatty acids containing 18 carbons using the following formula:

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

where C18:1, C18:2, and C18:3 show the levels of oleic, linoleic, and linolenic fatty acids, respectively [18, 19].

### 2.3.4. Sterol Measurement

At this stage, two milliliters of the internal standard (0.1% in chloroform) was added to the samples, and the samples were saponified using 2N ethanolic potassium hydroxide solution. The unsaponifiable fraction was eliminated using diethyl ether, and the unsaponifiable sterol fraction was separated using TLC. Following that, the sterol fraction was separated and quantified via CG (6500, YOUNG LIN, South Korea) using a Supelco capillary column (SPBTM-5 24034, Bellefonte, USA; 30 m length  $\times$  0.25 mm i.d.; film thickness: 0.25  $\mu$ m) and a flame ionization detector. The temperature of the column was set at 280°C, and the temperature of the detector and injector was set at 310°C and 300°C, respectively. In this process, helium was employed as the carrier gas with the flow rate of 1.5 ml/min and split ratio of 1:10, and the injection volume was 1.0 microliter. The individual sterols in the oils were also measured based on their relative retention times using the internal standard cholesterol [20, 21].

### 2.3.5. Squalene Measurement

The measurement of squalene was performed using an Agilent 7890 B CG instrument coupled with an Agilent MS-5977 inert XL mass selective detector (Agilent Technologies, Little Fall, NY, USA) and a capillary column HP-5MS (5% phenyl methylsiloxane) with the dimension of 60 meters, 0.25 millimeter i.d., and film thickness of 0.25 micrometers (Agilent Technologies, Palo Alto, CA, USA). The injector was isothermal at the temperature of 250°C with the split ratio of 1:20. The flow rate of helium carrier gas was set at 0.8 ml/min, and the sample (1  $\mu$ l) was injected at the temperature of 70°C in the splitless mode. After preserving the sample for five minutes at the temperature of 70°C, the oven temperature was increased from 20°C/min to 200°C, maintained for two minutes, ramped from the rate of 20°C/min to 300°C, and maintained for 15 minutes. Detection was facilitated by electron impact mass spectrometry at 70 eV (ion source: 230°C; transfer line: 270°C), and the selective ion monitoring was performed at the m/z of 57.0, 69.1, 71.1, and 81.1 [22, 23].

### 2.4. Statistical Analysis

Data analysis was performed in SPSS version 23.0 (SPSS Inc., Chicago, IL, USA). All the analyses were carried out in triplicate for each sample, and the obtained results were expressed as mean and standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan's multiple-range

test were also used to determine the significance of the differences ( $P < 0.05$ ).

## 3. Results and Discussion

### 3.1. Fatty Acid Composition

Fatty acid composition is an important indicator of the quality and authenticity of olive oil. Table 1 shows the fatty acid compositions of the olive oil samples in the present study. According to the findings, palmitic (C16:0), stearic (C18:0), arachidic (C20:0), palmitoleic (C16:1), oleic (9c-C18:1), linoleic (9c, 12c-C18:2), and linolenic acids (9c, 12c, 15c-C18:3) were observed in the olive oil samples. In addition, unsaturated fatty acids increased to 85.39% in sample B. All the olive oils samples (except sample K) were rich in monounsaturated fatty acids (MUFAs). Oleic acid (50.53-74.49%) was the main fatty acid found in the olive oil samples (except sample K), followed by linoleic acid (7.94-16.17%). A significant difference was observed in the oleic acid content of the bulk and commercial virgin olive oils ( $P < 0.05$ ). The amount of oleic acid in all the samples (except samples K and F) was in accordance with the Iranian national standard No.1446 (55-83%) [7]. On the other hand, the lowest amount of oleic acid was observed in sample K (29.10%), which could be due to the mixing of this olive oil with other vegetable oils [10]. Saturated fatty acids (SFAs) were also determined to be within the range of 10.61-18.66% in the studied samples.

According to the obtained results, palmitic acid was the major SFA (8.23-15.78%), and the palmitic and stearic acid contents of all the samples were within the acceptable limits of the Iranian national standard No.1446 [7]. However, the level of arachidic acid in sample K exceeded the maximum permissible limit ( $\leq 0.6\%$ ) of the Iranian national standard No.1446. Olive oil has a higher proportion of MUFAs compared to SFAs and polyunsaturated fatty acids. The fatty acid composition of oils is the major determinant of oxidative stability. Oleic acid is the most important MUFA, which plays a key role in the quality and oxidative stability of olive oil [24]. In a study in this regard, Ahangar Banadkahi *et al.* (2016) reported that the level of oleic acid in the olive oil samples produced in Zanjan region was higher than other provinces, such as Guilan, Qazvin, Golestan, Shiraz, and Kermanshah in Iran [25].

In the present study, all the samples (except sample K) were within the standard permissible limit of linoleic acid content (3.5-21%) [7]. In addition, a significant difference was observed between the linolenic acid (6.03%) and linoleic acid contents (46.01%) of sample K, as well as the contents of the other samples ( $P < 0.05$ ). The high level of linolenic acid in sample K was due to the combination of virgin olive oil with other vegetable oils (e.g., soybean and sunflower oils). On the other hand, the level of linolenic acid in samples K, G, and H exceeded the maximum permissible limits of the Iranian national standard ( $\leq 1\%$ ). Linoleic acid (C18:2) is the main fatty acid in soybean oil, while oleic acid (C18:1) is the main fatty acid in olive oil. In addition, soybean oil contains more linolenic acid compared to olive oil. According to the current research, the addition of 4% of canola oil and less than 10% of soybean oil to the olive oil samples could increase the content of linolenic acid (C18:3) to more than 1% [26].

The permissible limit of palmitoleic acid as a MUFA is 0.3–3.5% [27]. In the present study, the palmitoleic content of all the samples was within the permissible range [7]. In general, olive oil is preferred owing to the proven beneficial effects of MUFAs on the serum cholesterol levels. MUFAs and PUFAs are an important indicator of oxidative stability [28]. In the current research, the MUFA/PUFA ratio of the studied olive oil samples varied significantly within the range of 0.55–7.55, and the lowest MUFA/PUFA ratio (0.56) was observed in sample K.

The trans fatty acid contents of samples K (1.28%) and G (0.07%) exceeded the maximum permissible level (0.05%). Trans fatty acids could be observed due to the addition of hydrogenated oils to olive oil [10].

### 3.2. Iodine Value

The iodine value of oil or fat samples is indicative of the number of double bonds. The unsaturation level of olive oil depends on the fatty acid composition. In the present study, a significant difference was observed between the iodine value of sample K and the other samples ( $P < 0.05$ ). Due to the high level of polyunsaturated fatty acids, sample K had the highest iodine value (123.7 g/100 g) and exceeded the maximum permissible limit of the Codex standard (75–94 g/100 g) [27]. These findings are consistent with the previous reports in this regard. According to Haghighat Kharazi *et al.* (2012), the iodine value of Iranian virgin olive oil in Rudbar region was within the range of 86.28–89.74 g/100 g [19]. Furthermore, the iodine value of olive oil samples from various regions of Morocco has been reported to be 79.44–91.38 g/100 g, which does not exceed the authorized limits [29].

### 3.3. Cox Value

Higher Cox value indicates the lower oxidative stability of oils [30]. According to the current research, sample K had the highest oxidation index or Cox value (6.33) compared to the other olive oil samples. Due to the high content of PUFA, the oxidative stability of sample K reduced significantly ( $P < 0.05$ ). Furthermore, the high PUFA/SFA ratio indicated the high degree of unsaturation and tendency of sample K to oxidative reactions. Similar results have been reported by Najafi *et al.* (2015), demonstrating no significant difference between the Cox value of processed and virgin olive, and processing had no significant effects on the PUFA/SFA ratio and Cox value [30].

### 3.4. Sterol Content

The composition of the sterol fraction of olive oil is a reliable index for the detection or verification of adulteration. Table 2 shows the sterol composition of the studied virgin and refined olive oil samples. According to the Iranian national standard No.1446, the total sterol content of virgin olive oil should be 1,000 mg/kg or higher [7,27]. The results of the present study indicated that all the samples were acceptable in terms of the total sterol, cholesterol, and brassicasterol contents. This finding is consistent with the results obtained by Piravi Vanak *et al.* (2012), which denoted that olive oil samples had a higher total sterol than the specified minimum limit of the national standards [31].

Phytosterols (especially beta-sitosterol) has health-

promoting effects and prevents cholesterol absorption, which in turn lowers the plasma cholesterol levels in the human body. In addition, phytosterols have antioxidant properties and prevent various cancers (e.g., colon, prostate, and breast cancer) [31,32]. Sterols constitute the largest proportion of the unsaponifiable fraction of olive oil. These compounds are colorless and neutral, with a high melting point. The amount of phytosterol intake from a normal diet is 150–400 mg/100 g of virgin olive oil per day, which promotes important functional and nutritional properties [31].

Apparent beta-sitosterol is composed of several phytosterols, such as beta-sitosterol, delta-5-avenasterol, delta-5,23-stigmastadienol, clerosterol, sitostanol, and delta-5-24-stigma stadienol, which should exceed 93% of the total sterols [27,33]. In the present study, the highest apparent beta-sitosterol content was observed in sample B (95.19%), and a significant difference was denoted between the sterol content of sample K (56.22%) and the other samples ( $P < 0.05$ ). In this regard, Homapour *et al.* (2014) reported that beta-sitosterol was the most abundant sterol in the olive oil of Iranian varieties grown in Fadak and Gilvan regions (Iran) [31].

According to the Codex Alimentarius Standards and International Council for Olive Oil, the permissible concentration of campesterol is  $\leq 4\%$  [27, 33]. In the current research, the campesterol content of all the olive oil samples (1.96–3.65%), with the exception of sample K (23.31%), was within the standard limit. On the other hand, the campesterol content of sample K was significantly different from the other samples ( $P < 0.05$ ) and exceeded the permissible limits established in the standards. Reports have indicated more than 4% of campesterol in the samples of olive oil mixed with sunflower, rapeseed, palm, soybean, corn, and safflower oils [10].

Exceeding the delta-7-stigmastenol level of 0.5% could be considered a criterion for the mixing of olive oil with sunflower and safflower oils [10]. In the present study, the concentration of delta-7-stigmastenol in sample K was 17.21%, and the statistical analysis indicated a significant difference in the delta-7-stigmastenol levels between sample K and the other samples ( $P < 0.05$ ). Brassicasterol shows the presence of rapeseed oil, which was not detected in the investigated samples in our research. This finding is consistent with the study by Homapour *et al.* (2014) regarding the brassicasterol content of the olive varieties in Fadak region (Iran) [31]. The sterol content depends on the storage time and olive processing conditions. In the current research, the highest stigmasterol content was observed in the olives scattered on the ground [11]. According to the Codex Alimentarius Standards and International Council for Olive Oil, the permissible levels of stigmasterol should be lower than campesterol [27,33]. In the present study, the virgin and refined samples had a significant difference in terms of the stigmasterol content ( $P < 0.05$ ). In addition, the highest stigmasterol content (17.2%) was detected in sample K although the stigmasterol content in all the samples was less than 4%. Similar results have been obtained by Homapour *et al.* (2014). Geographical location also affects the stigmasterol content, and high stigmasterol content leads to sensory defects and high acidity [31].

Five-ring D-L-triterpenes (erythrodiol and uvaol) are found abundantly in the olive fruit skin. Physical extraction

**Table 1:** Fatty acid composition (%), iodine value, and Cox value of olive oil samples\*

| Fatty acids          | Brand refined oil          |                                      |                            | Brand virgin oil          |                           | Bulk virgin oil           |                           |                            |                           |                           | Permitted level* |
|----------------------|----------------------------|--------------------------------------|----------------------------|---------------------------|---------------------------|---------------------------|---------------------------|----------------------------|---------------------------|---------------------------|------------------|
|                      | A                          | B                                    | E                          | C                         | D                         | F                         | G                         | H                          | I                         | K                         |                  |
| Palmitic acid        | 12.29 ± 0.95 <sup>cd</sup> | 11.26± 0.95 <sup>bc</sup>            | 15.78± 1.1 <sup>e</sup>    | 14.42± 0.98 <sup>de</sup> | 13. 2± 0.97 <sup>d</sup>  | 8. 23± 1.04 <sup>a</sup>  | 14.30± 0.97 <sup>de</sup> | 13. 65± 0.97 <sup>d</sup>  | 13.80± 0.97 <sup>d</sup>  | 10.82± 0.95 <sup>b</sup>  | 7.5 – 20         |
| Palmitoleic acid     | 0.86 ± 0.05 <sup>d</sup>   | 0.75± 0.07 <sup>c</sup>              | 1.72± 0.08 <sup>f</sup>    | 1.13± 0.03 <sup>e</sup>   | 0.85± 0.07 <sup>d</sup>   | 0.45± 0.05 <sup>a</sup>   | 0.74± 0.10 <sup>c</sup>   | 0.69± 0.06 <sup>b</sup>    | 0.64± 0.05 <sup>b</sup>   | 0.82± 0.03 <sup>d</sup>   | 0.3 – 3.5        |
| Stearic acid         | 2.68 ± 0.65 <sup>a</sup>   | 3.21± 0.67 <sup>a</sup> <sup>b</sup> | 2. 05±0.65 <sup>a</sup>    | 3.02 ±0.66 <sup>ab</sup>  | 2.77± 0.65 <sup>a</sup>   | 2.13± 0.86 <sup>a</sup>   | 2.76± 0.65 <sup>a</sup>   | 2.88± 0.65 <sup>a</sup>    | 2.62± 0.65 <sup>a</sup>   | 4.18± 0.75 <sup>b</sup>   | 0.5 – 5          |
| Oleic acid           | 69.27 ±.065 <sup>g</sup>   | 74.49± 0.67 <sup>i</sup>             | 63.16± 0.65 <sup>d</sup>   | 61.49± 0.65 <sup>c</sup>  | 63.01± 0.66 <sup>d</sup>  | 50.53± 0.27 <sup>b</sup>  | 66.97± 0.90 <sup>f</sup>  | 64.71± 0.73 <sup>e</sup>   | 70.81± 0.59 <sup>h</sup>  | 29.10± 0.65 <sup>a</sup>  | 55 – 83          |
| Linoleic acid        | 10.90 ± 1.01 <sup>b</sup>  | 8.72± 1.15 <sup>a</sup>              | 14.87± 0.95 <sup>de</sup>  | 16.17± 0.95 <sup>e</sup>  | 13.21± 0.95 <sup>cd</sup> | 7.94± 1.32 <sup>a</sup>   | 12.89± 0.96 <sup>c</sup>  | 14.72± 0.84 <sup>cde</sup> | 9.66± 0.63 <sup>ab</sup>  | 46.01± 1.04 <sup>f</sup>  | 3.5 – 21         |
| Linolenic acid       | 0.94 ± 0.015 <sup>d</sup>  | 0.89± 0.02 <sup>c</sup>              | 0.84± 0.2 <sup>b</sup>     | 0.88± 0.15 <sup>bc</sup>  | 0.87± 0.2 <sup>bc</sup>   | 0.57± 0.25 <sup>a</sup>   | 1.28± 0.40 <sup>e</sup>   | 1.58± 0.4 <sup>f</sup>     | 0.94± 0.25 <sup>d</sup>   | 6.03± 0.25 <sup>g</sup>   | ≤ 1              |
| Arachidic acid       | 0.47 ± 0.15 <sup>ef</sup>  | 0.43± 0.17 <sup>d</sup>              | 0.36± 0.17 <sup>c</sup>    | 0.43± 0.17 <sup>d</sup>   | 0.50± 0.17 <sup>f</sup>   | 0.31± 0.17 <sup>b</sup>   | 0.07± 0.17 <sup>a</sup>   | 0.48± 0.17 <sup>ef</sup>   | 0.46± 0.17 <sup>de</sup>  | 0.78± 0.17 <sup>g</sup>   | ≤ 0.6            |
| Saturated fatty acid | 15.32 ± 0.15 <sup>bc</sup> | 15.01± 1.10 <sup>b</sup>             | 18.66± 1.10 <sup>d</sup>   | 17.71± 1.00 <sup>cd</sup> | 17.57± 1.01 <sup>cd</sup> | 10.61± 1.14 <sup>a</sup>  | 17.24± 1.10 <sup>cd</sup> | 17.11± 1.00 <sup>cd</sup>  | 16.65± 1.00 <sup>bc</sup> | 15.69± 1.00 <sup>bc</sup> | -                |
| Mono-unsaturated Fat | 70.34 ± 1.04 <sup>f</sup>  | 75.23± 1.08 <sup>g</sup>             | 64.95± 1.01 <sup>d</sup>   | 62.77± 0.99 <sup>c</sup>  | 63.84± 1.10 <sup>c</sup>  | 51.14± 0.95 <sup>b</sup>  | 67.79 ± 1.03 <sup>e</sup> | 65.41± 1.01 <sup>d</sup>   | 71.33± 1.05 <sup>f</sup>  | 29.07± 1.1 <sup>a</sup>   | -                |
| poly-unsaturated fat | 12.32 ±1.5 <sup>bc</sup>   | 10.16±1.8 <sup>ab</sup>              | 16.15± 1.21 <sup>de</sup>  | 17.48± 1.14 <sup>e</sup>  | 14.54± 1.13 <sup>cd</sup> | 9.14± 1.10 <sup>a</sup>   | 14.62± 1.13 <sup>cd</sup> | 16.78± 1.17 <sup>de</sup>  | 11.36± 1.56 <sup>ab</sup> | 52.18± 1.14 <sup>f</sup>  | -                |
| MUFA/PUFA            | 5.75 ± 0.60 <sup>cd</sup>  | 7.55± 1.17 <sup>e</sup>              | 4.03± 0.24 <sup>b</sup>    | 3.59± 0.18 <sup>b</sup>   | 4.40± 0.32 <sup>b</sup>   | 5.76± 1.09 <sup>cd</sup>  | 4.60± 0.34 <sup>bc</sup>  | 3.90± 0.21 <sup>b</sup>    | 6.50± 0.78 <sup>d</sup>   | 0.55± 0.01 <sup>a</sup>   | -                |
| C18:2/C18:3          | 11.24± 1.60 <sup>bc</sup>  | 9.68± 1.40 <sup>ab</sup>             | 17.47± 1.40 <sup>e</sup>   | 18.36± 1.40 <sup>e</sup>  | 14.96± 1.30 <sup>d</sup>  | 13.67± 1.40 <sup>cd</sup> | 9.91± 0.85 <sup>ab</sup>  | 9.25± 0.62 <sup>ab</sup>   | 10.38± 0.85 <sup>b</sup>  | 7.62± 0.62 <sup>a</sup>   | -                |
| Trans fatty acid     | 0±0.00 <sup>a</sup>        | 0±0.00 <sup>a</sup>                  | 0±0.00 <sup>a</sup>        | 0±0.00 <sup>a</sup>       | 0±0.00 <sup>a</sup>       | 0±0.00 <sup>a</sup>       | 0.07±0.002 <sup>b</sup>   | 0±0.00 <sup>a</sup>        | 0±0.00 <sup>a</sup>       | 1.28± 0.05 <sup>c</sup>   | 0.05             |
| Iodine value         | 85.13 ± 4.08 <sup>b</sup>  | 85.14± 3.60 <sup>b</sup>             | 90.9±1 4.20 <sup>b</sup>   | 87.10± 4.80 <sup>b</sup>  | 88.02± 3.80 <sup>b</sup>  | 77.56± 3.60 <sup>a</sup>  | 87.99± 4.9 <sup>b</sup>   | 89.77± 4.7 <sup>b</sup>    | 84.53± 5.30 <sup>b</sup>  | 123.79± 2.70 <sup>c</sup> | 75 – 94          |
| Cox value            | 2.01 ± 0.001 <sup>b</sup>  | 1.84± 0.001 <sup>b</sup>             | 2.34± 0.001 <sup>def</sup> | 2.47± 0.01 <sup>ef</sup>  | 2.18± 0.01 <sup>bc</sup>  | 1.45± 0.001 <sup>a</sup>  | 2.28± 0.01 <sup>de</sup>  | 2.50± 0.01 <sup>f</sup>    | 1.90± 0.01 <sup>b</sup>   | 6.33± 0.001 <sup>g</sup>  | -                |

Quantities with same letters in each row were not statistically different according to Duncan's test ( $P < 0.05$ )

MUFA, Monounsaturated fatty acid; PUFA, Polyunsaturated fatty acid

\*Iran National standard (INSO: 1446)

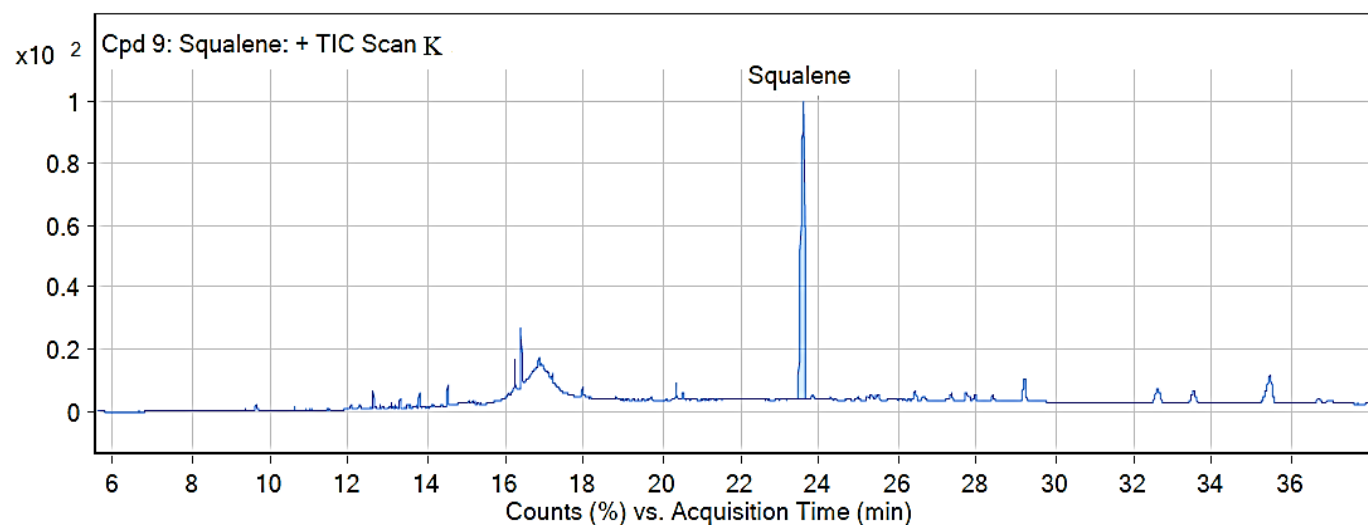
**Table 2:** Sterol and squalene contents of olive oil samples (%)

| Sterols                  | Brand refined oil         |                          |                          | Brand virgin oil         |                          |                          | Bulk virgin oil          |                          |                         |                          | Permitted level* |
|--------------------------|---------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|-------------------------|--------------------------|------------------|
|                          | A                         | B                        | E                        | C                        | D                        | F                        | G                        | H                        | I                       | K                        |                  |
| Cholesterol              | 0.23±0.025 <sup>a</sup>   | 0.23±0.02 <sup>a</sup>   | 0.36±0.025 <sup>b</sup>  | 0.24±0.025 <sup>a</sup>  | 0.25±0.17 <sup>a</sup>   | 0.36±0.035 <sup>b</sup>  | 0.22±0.015 <sup>a</sup>  | 0.37±0.015 <sup>b</sup>  | 0.32±0.025 <sup>b</sup> | 0.24±0.025 <sup>a</sup>  | ≤ 0.5            |
| Brassicasterol           | ND*                       | ND                       | ND                       | ND                       | ND                       | ND                       | ND                       | ND                       | ND                      | ND                       | ≤ 0.1            |
| Campesterol              | 3.26±0.13 <sup>a</sup>    | 2.64±0.50 <sup>a</sup>   | 2.07±0.16 <sup>a</sup>   | 1.96±0.02 <sup>a</sup>   | 3.22±0.04 <sup>a</sup>   | 3.38±0.09 <sup>a</sup>   | 3.65±0.12 <sup>a</sup>   | 2.62±0.23 <sup>a</sup>   | 3.51±0.03 <sup>a</sup>  | 23.31±0.45 <sup>b</sup>  | ≤ 4              |
| Stigmasterol             | 1.23±0.04 <sup>c</sup>    | 0.96±0.03 <sup>b</sup>   | 0.26±0.17 <sup>a</sup>   | 2.0±0.017 <sup>g</sup>   | 1.59±0.017 <sup>e</sup>  | 2.80±0.017 <sup>h</sup>  | 1.31±0.017 <sup>d</sup>  | 1.69±0.017 <sup>f</sup>  | 0.28±0.017 <sup>a</sup> | 17.21±0.017 <sup>h</sup> | ≤ Campesterol    |
| delta-7-Stigmastenol     | 0.42 ±0.032 <sup>cd</sup> | 0.48 ±0.027 <sup>d</sup> | 0.36 ±0.035 <sup>c</sup> | 0.51±0.0015 <sup>f</sup> | 0.25±0.025 <sup>ab</sup> | 0.39±0.017 <sup>cd</sup> | 0.33±0.027 <sup>c</sup>  | 0.76±0.032 <sup>e</sup>  | 0.23±0.04 <sup>a</sup>  | 1.23±0.2 <sup>g</sup>    | ≤ 0.5            |
| Apparent beta-sitosterol | 92.16±2.9 <sup>a</sup>    | 95.19±0.86 <sup>a</sup>  | 94.78±0.5 <sup>a</sup>   | 93.70±0.95 <sup>a</sup>  | 94.09±0.77 <sup>a</sup>  | 92.55±1.08 <sup>a</sup>  | 93.52±1.08 <sup>a</sup>  | 93.89±1.29 <sup>a</sup>  | 92.45±1.03 <sup>a</sup> | 56.22±2.01 <sup>b</sup>  | ≥ 93             |
| Erythrodiol and uvaol    | 0.37±0.45 <sup>bc</sup>   | 0.49±0.04 <sup>c</sup>   | 0.66±0.07 <sup>d</sup>   | 1.70±0.07 <sup>e</sup>   | 0.74±0.45 <sup>d</sup>   | 2.80±0.52 <sup>f</sup>   | 0.32±0.025 <sup>bc</sup> | 0.30±0.02 <sup>b</sup>   | 0.43±0.04 <sup>bc</sup> | 0.14±0.015 <sup>a</sup>  | ≤ 4.5            |
| Total sterol             | 1021.4±5.4 <sup>a</sup>   | 1018.5±14.7 <sup>a</sup> | 1025.6±3.6 <sup>a</sup>  | 1351.8±7.02 <sup>d</sup> | 1028.1±4.2 <sup>a</sup>  | 1775.1±4.0 <sup>e</sup>  | 1261.5±2.5 <sup>c</sup>  | 1249.1±2 <sup>c</sup>    | 1064.2±7.5 <sup>b</sup> | 3724.7±7 <sup>f</sup>    | ≥ 1000 ppm       |
| Squalene                 | 5.90±0.15 <sup>b</sup>    | 3.30±0.3 <sup>a</sup>    | 4.20±0.37 <sup>a</sup>   | 13.8±0.42 <sup>c</sup>   | 17.9±0.43 <sup>d</sup>   | 28.69±0.26 <sup>e</sup>  | 30.64±0.17 <sup>f</sup>  | 29.51±1.06 <sup>ef</sup> | 30.17±1.7 <sup>ef</sup> | 55.37±2.02 <sup>g</sup>  | -                |

In each row, the mean in same letters shows no significant difference at the Duncan's 5% level of probability.

ND: Not detected

\*Iran National standard (INSO: 1446)

**Figure 1:** Chromatogram of Squalene Content of Sample K

could reduce the erythrodiol and uvaol contents. According to the Codex Alimentarius Standards, virgin and refined olive oils contain less than 4.5% of these compounds [10]. According to the information in Table 2, the erythrodiol and uvaol values of all the samples in the current research conformed to the Iranian national standards despite the significant differences between the samples. These findings are in line with the study by Temime *et al.* (2008) [34].

### 3.5. Squalene Content

Squalene is an unsaturated terpenoid hydrocarbon, which is an important constituent of the unsaponifiable compounds of edible vegetable oils. Squalene plays a pivotal role in the synthesis of steroids [35]. The extracted squalene from plant sources (e.g., olive and palm fruits, soybeans, and amaranth seeds) is used for numerous medical purposes owing to its antioxidant, antibacterial, and antifungal properties [36]. Table 2 shows the squalene content of the olive oil samples in the present study. Accordingly, the squalene content of the refined samples was lower in the virgin samples, and a significant difference was observed between the samples in this regard ( $P < 0.05$ ). The highest level of squalene (55.37%) was observed in sample K (Figure 1), which could be attributed to the presence of soybean oil in this sample. In this regard, Popa *et al.* (2015) reported that the squalene content in the distilled phase of olive oil was 5.6% (w/w) [23]. Similarly, Ambrosewicz *et al.* (2012) claimed that refined olive oil has a lower squalene content compared to virgin olive oil [37].

## 4. Conclusion

The results of this study were indicative of the adulteration of bulk olive oil with other vegetable oils. In addition, the quality characteristics of the commercial samples were consistent with the Codex and national standards. The composition of fatty acids, along with sterol profiles, could be considered an effective tool for discriminating the purity and quality of olive oil. Iran is one of the leading importers of edible oils in the world. Considering the health-promoting properties and special flavor of olive oil, proper policies must be adopted for the optimal production of olive oil in Iran. Given the position and high potential of Zanzan province in olive oil production and the increased interest in the purity of olive oil, the continuous quality control of bulk olive oil supply centers, promotion of the authorized plants, and use of modern methods for the rapid detection of the olive oils adulterated with lower-priced vegetable oils are recommended.

## Authors' Contributions

P.G.A., and M.A.M., designed the study; S.M.M.F., performed the experiments; S.M.M.F., P.G.A., and M.A.M. drafted the manuscript; P.G.A., supervised data analysis and

edited the manuscript. All the authors read and approved the final manuscript.

## Conflict of Interest

The Authors declare that there is no conflict of interest.

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