



## Evaluation of the Composition and Oxidative Stability of Cold-pressed Sesame Oils in the Market of Zanjan Province, Iran (2019)



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### ARTICLE INFO

#### Article type:

Original article

#### Article history:

Received: 28 June 2020

Revised: 10 November 2020

Accepted: 5 December 2020

DOI: [10.29252/jhehp.6.4.2](https://doi.org/10.29252/jhehp.6.4.2)

#### Keywords:

Sesame oil  
Cold-pressed  
Refined  
Zanjan market

### ABSTRACT

**Background:** Supply centers of cold-pressed sesame oil have recently appeared in the market, while no data are available on the composition and oxidative stability of these oils. The present study aimed to investigate the properties of cold-pressed and refined sesame oil samples in the market of Zanjan province, Iran in 2019.

**Methods:** In total, 10 sesame oil samples were collected, including eight cold-pressed and two refined samples. Fatty acid and sterol compositions were determined by gas chromatography, and tocopherols were measured by high-performance liquid chromatography. In addition, oxidative stability, iodine, and peroxide values were determined.

**Results:** Sesame oil was composed of 82-85% unsaturated fatty acids, with linoleic and oleic acids as the major fatty acids and  $\beta$ -sitosterol as the main sterol. Moreover,  $\gamma$ -tocopherol was the predominant tocopherol. Fatty acid composition significantly differed in various samples ( $P < 0.05$ ), and the total sterol and iodine values were within the standard ranges. Significant differences were observed between the cold-pressed and refined samples in terms of  $\beta$ -sitosterol, campesterol,  $\Delta 7$ -stigmastenol,  $\gamma$ -tocopherol, and oxidative stability ( $P < 0.05$ ). The mean oxidative stability of the pressed samples (8.61 hours) did not meet the standards of edible oils.

**Conclusion:** Due to low oxidative stability, the long-term preservation and use of the cold-pressed sesame oils extracted in shops are not recommended.

## 1. Introduction

Sesame seed (*Sesamum indicum* L.) belongs to the Pedaliaceae family and is the oldest oilseed used for oil production [1]. Approximately 70% of the annual production of sesame seed is in Asia, with the remaining reported in Africa and Latin America [2]. More than 68% of sesame seeds are imported to Iran from India and Pakistan [3]. Sesame oil is considered to be the most

exclusive vegetable oil, which has multiple health benefits for humans.

The sweet smell and good taste of sesame oil make it a natural and desirable salad or cooking oil, especially in Asian countries [4, 5]. The oil content of sesame seed depends on variety, genetics, environment, and ecological factors and has been estimated to be 28 - 60%. In addition, unsaturated fatty acids (UFAs) constitute 80% of the fatty acids in sesame oil, such as oleic acid and linoleic acid.



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The amount of saturated fatty acids (SFAs; e.g., palmitic and stearic acids) in sesame oil has been estimated to be less than 20% of the total fatty acid content [6]. This vegetable oil is also an abundant source of phytosterols, which could reduce serum cholesterol.  $\beta$ -sitosterol is the main phytosterol in sesame oil, which constitutes 60% of the sterol content. In addition,  $\gamma$ -tocopherol constitutes 90 - 95% of the total tocopherol content of sesame oil. Sesame oil also contains significant amounts of sesamol, sesamol, and sesamin, which have antioxidant activities and human health benefits, such as the improvement of the liver function, reduction of serum lipid and cholesterol levels, preventing hypertension, increasing oil stability, and prevention of cancer, ageing disorders, and cardiovascular diseases [1].

Cold-pressing is the most common technique for the extraction of sesame oil, which is used for the grains that contain large amounts of oil. Cold-pressed oils are obtained without altering the composition of the oil through mechanical procedures only, such as expelling or pressing without refining or heat application. These oils may be purified by washing with water, settling, filtration, and centrifugation only [7].

Recently, the pressing of edible oils without chemical refining processes (e.g., degumming, alkali-refining, bleaching, and deodorization) have attracted attention. The supply centers of freshly extracted sesame oil have also emerged in the markets of Zanzan province (Iran), offering the healthiest edible oil. According to Iran Food and Drug Administration (IFDA), these centers are illegal since they cannot eliminate harmful impurities such as gum, wax, free fatty acids, metal compounds, toxins and pesticide residues from crude or unrefined oils. According to Iranian Vegetable Oil Industry association, none of these units have been licensed by the industry [8]. Sesame oil is expensive and may often be adulterated with other low-priced vegetable oils. Therefore, the continuous monitoring of bulk oil supplies and promotion of the authorized plants are recommended [9].

In a study in this regard, Eshaghi *et al.* (2014) investigated the physicochemical properties of sesame, including the micronutrients (antioxidants, tocopherols, and metal ions), essential UFAs, carbohydrates, minerals, vitamins, acids, and nutritional and pharmaceutical properties. Sesame is known as the 'queen of oil grains' [10]. In another research, Ogbonna *et al.* (2013) examined the quality properties of 13 local sesame seeds from Nigeria, reporting that the chemical composition of each seed differed in terms of the oil, moisture, and protein content [11]. Furthermore, Hosseini *et al.* (2017) evaluated the effects of oil extraction methods (solvent, pressure, and water) on the quality of sesame oil, and their findings indicated that the cold extraction of oil yielded the optimal results compared to other oils in terms of the fatty acid profile, antioxidant capacity, and oxidative stability [12].

Considering the increasing tendency of consumers to use freshly extracted vegetable oils in recent years and the scarce data regarding the quality properties of the extracted sesame seed oil supplied in the store centers of Zanzan province, the present study aimed to investigate the composition and oxidative stability of the cold-pressed sesame oils offered in the market of Zanzan province, Iran in 2019 for the first time.

## 2. Materials and Methods

### 2.1. Chemicals and Reagents

All the chemicals and reagents used in the present study were of an analytical grade. Acetic acid, chloroform, n-hexane (99.0%), methanol, and potassium hydroxide (85.0%) were provided by Merck KGaA (Darmstadt, Germany). In addition, 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  $\times$  20 cm; thickness: 0.25 mm) was purchased from Merck (Darmstadt, Germany). The HPLC-grade solvents were purchased from Fisher Scientific (NJ, USA).

### 2.2. Sample Collection

Sampling was performed in accordance with the Iranian National Standard No. 493 [13]. In total, 10 samples were assessed in this study, which were obtained randomly from the local markets of Zanzan, Abhar, and Khorramdareh cities, located in Zanzan province. The samples included eight cold-pressed sesame oils (A, B, C, F, G, H, I, and J) and two commercial brands of refined sesame oils (D and E). The collected samples were stored in a cool and dark place and transferred to the Food Control Laboratory of Zanzan University of Medical Sciences for analysis.

### 2.3. Analytical Methods

#### 2.3.1. Fatty Acid Composition

Fatty acids methyl esters were prepared by mixing oil and hexane with seven milliliters of 2N potassium methanol hydroxide at the temperature of 50°C for 15 minutes. The fatty acid composition of the sesame oil samples was determined using a Varian 3800 gas chromatograph (Varian Inc., Middelburg, the Netherlands), equipped with a flame ionization detector (Hewlett-Packard, Palo Alto, CA, USA) and a capillary column (Cp-Sil 88, 50 m  $\times$  0.25 m  $\times$  0.25  $\mu$  m). The column oven temperature was adjusted at 175°C for 30 minutes and slowly increased to 3°C/min and up to 220°C for 60 minutes. The injector and detector temperatures were set at 175°C and 250°C, respectively. Helium was used as the carrier gas with the flow rate of 0.7 ml/min, and one microliter of the sample was injected. The peaks were identified by comparing the retention times with authentic standards and analyzed under the same conditions. The obtained results were expressed as the relative percentage of fatty acids [14].

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### 2.3.2. Sterol Composition

The oil samples (5 g) containing 2.0 milliliters of  $\alpha$ -cholestanol as an internal standard were saponified with 50 milliliters of alcoholic potassium hydroxide solution (0.5 mol/l) and heated for one hour. After the separation of the non-saponifiable material by n-hexane, the sterol fraction was separated using the TLC and injected into a gas chromatograph (6500, Young Lin, South Korea), equipped with a Supelco column (SPBTM-5 24034, Bellefonte, USA; length: 30 m  $\times$  0.25 mm i.d., film thickness: 0.25  $\mu$ m) and a flame ionization detector (Hewlett-Packard, Palo Alto, CA, USA). The injection volume was one microliter, and hydrogen was used as the carrier gas (flow rate: 2 ml/min). The process was run in accordance with an optimized temperature program at the oven temperature of 260°C, injection temperature of 280°C, and detector temperature of 300°C. In addition, the individual sterols were identified based on their relative retention times and the internal standard cholestanol [15].

### 2.3.3. Tocopherol Composition

The tocopherol compounds of the sesame oil samples were measured using HPLC (Waters Alliance e2695 separation Module, USA) in accordance with AOCS, Ce 89-8 with slight modifications. Initially, one gram of the samples was mixed with n-hexane and 12 milliliters of methanol and shaken strongly. Afterwards, 20 milliliters of the sample solutions were injected into the waters scrubbing column (silica particles with diameter of 5  $\mu$ m, column length of 250 mm, and inner diameter of 4.6 mm). The eluent was a 0.5% solution of methanol (v/v) with the flow rate of 1 ml/min, and a fluorescence detector was used with the detection wavelength of 290 nanometers [16]. The concentration of the tocopherol compounds in the samples (W) was measured using the following equation:

$$W = P \times AT \times F \times V / AS \times M \quad (1)$$

where *P* is the tocopherol concentration ( $\mu$ g/ml) in the calibration solution, *AT* shows the average area of the obtained peaks for alpha-tocopherol in the samples, *AS* is the average area of the obtained peaks for the standard alpha-tocopherol solution, *M* is the sample mass (g), *F* shows the dilution factor, and *V* is the volume of the test solution (ml).

### 2.3.4. Iodine Value

Iodine value is a measure of the number of the double bonds in the UFAs. In the present study, the iodine value was calculated based on the percentage of the fatty acids obtained by gas chromatography in accordance with AOCS, cd 1C-85 [17] using the following formula:

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

Where C16:1, C18:1, C18:2, and C18:3 represent the percentage of palmitoleic, oleic, linoleic, and linolenic acids, respectively.

### 2.3.5. Peroxide Value

The peroxide value was measured in accordance with AOCS, Cd 8-53, which is a method to determine the level of all substances in terms of the milliequivalents of peroxide per 1,000 grams of the sample that oxidizes potassium iodide (KI) under test conditions. In the current research, 5.00  $\pm$  0.05 grams of the oil samples and 30 milliliters of an acetic acid-chloroform solution (3:2) were transferred into an Erlenmeyer flask. Following that, 0.5 milliliter of saturated KI solution and 30 milliliters of distilled water were added. Titration was carried out with 0.1 N sodium thiosulfate in the presence of 2.0 milliliters of a starch indicator solution and constant agitation until the blue color disappeared [18].

### 2.3.6. Oxidative Stability

The oxidative stability of the oil samples was measured using the Rancimat 743 apparatus (Metrohm Ltd., Herisau, Switzerland) in accordance with AOCS, CD 12b-92 at the temperature of 110°C. Rancimat test is considered an appropriate method, in which a combination of two factors (temperature and oxygen) is applied simultaneously to the oil sample. In the present study, five grams of the oil samples was placed in a glass test tube and held stationary within a thermostat-controlled block heater ( $\pm$  0.1°C). The test tube was capped with a two-hole rubber stopper to allow a steady stream of clean, dry air with the constant flow rate of 20 l/h through the sample and out the tube.

At the next stage, the effluent air containing the vaporized products (organic acids) was swept into a second vessel containing deionized water. The conductivity of the deionized water was monitored and recorded automatically. The times required for a drastic increase in water conductivity (maximum of the secondary oxidation products) was also calculated automatically by the software and corresponded to the Oil Stability Index or induction period in hours [19].

## 2.4. Statistical Analysis

Data analysis was performed in SPSS version 23.0 (Statistical Program for Social Sciences, SPSS Corporation, Chicago, IL, USA). All the analyses were performed in triplicate, and the data were expressed as mean and standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan's test were used for the comparison of the data at the significance level of  $P < 0.05$ . In addition, Pearson's correlation-coefficient was employed to evaluate the correlations between the variables.

## 3. Results and Discussion

### 3.1. Fatty Acid Composition

The fatty acid compositions of the sesame oil samples are presented in Table 1. Accordingly, oleic acid and linoleic acid constituted more than 82% of the total fatty acids in the sesame oil samples. In addition, palmitic acid (9-10%) was the main SFA, while palmitic acid and stearic acid constituted approximately 13-15% of the total fatty acids in the samples. Sample B had the highest UFA content due to the higher percentage of linolenic acid (44.69  $\pm$  0.01%), and sample E had the highest SFA value

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**Table 1:** Fatty Acid Composition (%), Iodine Value, Peroxide Value, and Oxidative Stability of Sesame Oil Samples\* (Quantities with same letters in each row not significantly different according to Duncan's test;  $P < 0.05$ ; \*Iranian National Standard Organization [INSO: 13392, INSO: 1752]; \*\*ND: non detectable; \*\*\*refined sesame seed oil)

Fatty acids	Cold- pressed sesame oil								Refined sesame oil		Permitted level*
	A	B	C	F	G	H	I	J	D	E	
Palmitic acid (C16:0)	9.60 ± 0.01 <sup>ab</sup>	9.30 ± 0.01 <sup>a</sup>	9.83 ± 0.01 <sup>ab</sup>	9.49 ± 0.01 <sup>ab</sup>	10.19 ± 0.01 <sup>b</sup>	9.75 ± 0.01 <sup>ab</sup>	9.91 ± 0.01 <sup>ab</sup>	9.11 ± 0.01 <sup>a</sup>	9.79 ± 0.01 <sup>ab</sup>	10.42 ± 0.01 <sup>b</sup>	7.9-12
Palmitoleic acid(16:1)	0.12 ± 0.01 <sup>b</sup>	0.12 ± 0.005 <sup>b</sup>	0.09 ± 0.005 <sup>ab</sup>	0.12 ± 0.01 <sup>b</sup>	0.12 ± 0.01 <sup>b</sup>	0.10 ± 0.01 <sup>b</sup>	0.14 ± 0.01 <sup>b</sup>	0.04 ± 0.08 <sup>a</sup>	0.11 ± 0.004 <sup>b</sup>	0.10 ± 0.002 <sup>b</sup>	ND-0.2
Stearic acid (C18:0)	5.06 ± 0.01 <sup>d</sup>	4.36 ± 0.01 <sup>a</sup>	4.81 ± 0.01 <sup>b</sup>	5.60 ± 0.01 <sup>e</sup>	4.93 ± 0.01 <sup>c</sup>	5.11 ± 0.01 <sup>de</sup>	5.44 ± 0.01 <sup>f</sup>	5.6 ± 0.01 <sup>e</sup>	5.06 ± 0.01 <sup>d</sup>	5.16 ± 0.04 <sup>e</sup>	4.5-6.7
Oleic acid(C18:1)	40.16 ± 0.01 <sup>bc</sup>	40.1 ± 0.01 <sup>bc</sup>	39.6 ± 0.01 <sup>b</sup>	41.88 ± 0.02 <sup>d</sup>	40.16 ± 0.2 <sup>bc</sup>	39.78 ± 0.4 <sup>c</sup>	42.22 ± 0.3 <sup>de</sup>	42.7 ± 0.01 <sup>e</sup>	40.62 ± 0.01 <sup>c</sup>	39.59 ± 0.01 <sup>a</sup>	34.9-45.5
Linoleic acid(C18:2)	43.70 ± 0.01 <sup>b</sup>	44.69 ± 0.01 <sup>c</sup>	44.34 ± 0.01 <sup>c</sup>	41.08 ± 0.01 <sup>a</sup>	43.19 ± 0.01 <sup>b</sup>	43.97 ± 0.03 <sup>bc</sup>	40.7 ± 0.01 <sup>a</sup>	41.15 ± 1.01 <sup>a</sup>	43.07 ± 0.03 <sup>b</sup>	44.46 ± 0.04 <sup>c</sup>	36.9-47.9
Linolenic Acid(C18:3)	0.54 ± 0.03 <sup>b</sup>	0.47 ± 0.01 <sup>c</sup>	0.49 ± 0.01 <sup>f</sup>	0.20 ± 0.01 <sup>a</sup>	0.30 ± 0.01 <sup>c</sup>	0.3 ± 0.01 <sup>c</sup>	0.40 ± 0.01 <sup>d</sup>	0.27 ± 0.01 <sup>b</sup>	0.51 ± 0.01 <sup>e</sup>	0.47 ± 0.01 <sup>i</sup>	0.2-1
Arachidic acid (C20:0)	0.56 ± 0.002 <sup>c</sup>	0.48 ± 0.01 <sup>b</sup>	0.56 ± 0.04 <sup>c</sup>	0.62 ± 0.005 <sup>e</sup>	0.59 ± 0.01 <sup>d</sup>	0.46 ± 0.5 <sup>b</sup>	0.10 ± 0.01 <sup>a</sup>	0.71 ± 0.01 <sup>f</sup>	0.59 ± 0.01 <sup>d</sup>	0.50 ± 0.02 <sup>b</sup>	0.3-0.7
Saturated fatty acid	14.67 ± 0.01 <sup>cd</sup>	13.6 ± 0.01 <sup>a</sup>	14.65 ± 0.05 <sup>cd</sup>	15.11 ± 0.01 <sup>f</sup>	14.20 ± 0.02 <sup>b</sup>	14.86 ± 0.04 <sup>e</sup>	13.35 ± 0.05 <sup>a</sup>	14.71 ± 0.01 <sup>d</sup>	14.60 ± 0.01 <sup>c</sup>	15.58 ± 0.02 <sup>b</sup>	ND
Unsaturated fatty acid	84.41 ± 0.01 <sup>e</sup>	85.26 ± 0.04 <sup>f</sup>	84.5 ± 0.01 <sup>e</sup>	83.16 ± 0.01 <sup>b</sup>	83.67 ± 0.03 <sup>c</sup>	84.05 ± 0.01 <sup>d</sup>	82.93 ± 0.07 <sup>a</sup>	84.2 ± 0.01 <sup>d</sup>	84.2 ± 0.2 <sup>d</sup>	83.04 ± 0.01 <sup>ab</sup>	ND
UFA/ SFA	5.7 ± 0.1 <sup>ab</sup>	6.0 ± 0.1 <sup>b</sup>	5.7 ± 0.1 <sup>ab</sup>	5.5 ± 0.1 <sup>ab</sup>	5.80 ± .01 <sup>ab</sup>	5.6 ± .01 <sup>ab</sup>	5.4 ± .01 <sup>a</sup>	5.7 ± 0.1 <sup>ab</sup>	5.7 ± 0.1 <sup>ab</sup>	5.3 ± 0.1 <sup>a</sup>	5.3-6.2
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>	0.05 ± .002 <sup>a</sup>	0 ± 0.00 <sup>a</sup>	0 ± 0.00 <sup>a</sup>	0.08 ± .001 <sup>ab</sup>	<0.1
Iodine value	111.60 ± 0.01 <sup>f</sup>	113.1 ± 0.01 <sup>b</sup>	112.19 ± 0.01 <sup>e</sup>	107.82 ± 0.01 <sup>b</sup>	110.54 ± 0.5 <sup>d</sup>	111.26 ± 0.01 <sup>ef</sup>	106.9 ± 0.1 <sup>a</sup>	108.75 ± 0.1 <sup>c</sup>	110.85 ± 0.01 <sup>de</sup>	113.60 ± 0.01 <sup>b</sup>	104-120
Peroxide value	5.40 ± 0.1 <sup>d</sup>	4.50 ± 0.1 <sup>bc</sup>	4.90 ± .001 <sup>c</sup>	10.60 ± 0.1 <sup>f</sup>	4.90 ± 0.1 <sup>c</sup>	1.10 ± 0.01 <sup>a</sup>	10.20 ± 0.2 <sup>e</sup>	4.30 ± 0.05 <sup>b</sup>	4.50 ± 0.1 <sup>bc</sup>	4.30 ± 0.1 <sup>b</sup>	<5***
Oxidative stability	8.99 ± 0.07 <sup>e</sup>	9.15 ± 0.1 <sup>f</sup>	7.87 ± 0.1 <sup>b</sup>	8.38 ± 0.01 <sup>d</sup>	8.18 ± 0.05 <sup>e</sup>	9.35 ± 0.03 <sup>g</sup>	7.53 ± 0.1 <sup>a</sup>	9.48 ± 0.1 <sup>b</sup>	20.91 ± 0.01 <sup>j</sup>	18.96 ± 0.26 <sup>i</sup>	>12

due to the higher percentage of palmitic acid (10.42 ± 0.01%).

The analysis of the fatty acid composition showed significant differences in different sesame oil samples ( $P < 0.05$ ). Accordingly, samples E and D had slightly lower contents of stearic acid compared to the standard range. However, the composition of other fatty acids in the samples was satisfactory based on the national and Codex Alimentarius standards [20]. Our findings in this regard are consistent with the study by Benitez *et al.* (2016), which indicated that the most abundant fatty acids in pressed sesame oils were linoleic acid (~43%) and oleic acid (~39%) as UFAs and palmitic acid (~10%) and stearic acid (~5%) as SFAs [21]. Fatty acid composition is an essential indicator of the nutritional value of oils. Sesame seed oil belongs to the oleic-linoleic acid group, and the differences in the fatty acid composition of various sesame seed oils could be due to the diversities in genetics, climate, oil processing methods or harvest conditions [22].

In the current research, Pearson's correlation-coefficient indicated a direct correlation between oleic acid and the UFA content. The UFA/SFA ratio has a marked association with oil stability, and our findings demonstrated that this ratio was within the range of 5.3 ± 0.1 - 6.0 ± 0.1 in the sesame oil samples. The highest ratio

(6.0 ± 0.1) belonged to sample B, and the lowest ratio (5.3 ± 0.1) was observed in sample E. These findings are in line with the previous studies in this regard [22 - 24]. Notably, trace amounts of Trans fatty acids were only detected in samples I and E, the levels of which were below the recommended national and Codex standards limits (< 0.1%) [20].

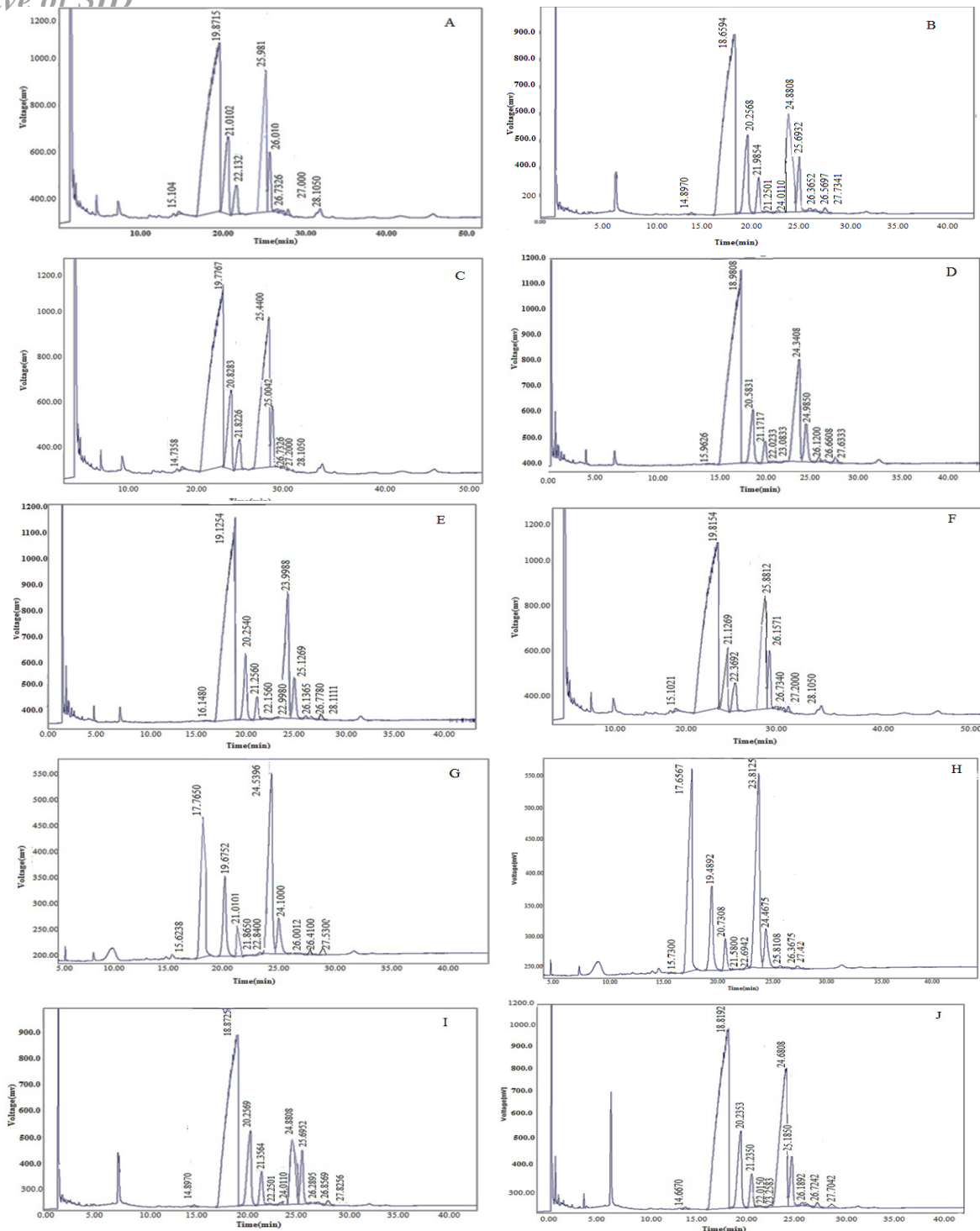
### 3.2. Sterol Composition

Table 2 shows the sterol composition of the sesame oil samples. The sterol chromatograms are also illustrated in Figure 1. The phytosterols profile could be considered a fingerprint for authenticity verification, as well as a reliable index to detect the adulterations of edible oils. Sterols have antioxidant activity and positive health effects [25].

Furthermore, phytosterols (especially beta-sitosterol) have been reported to exert health-promoting effects on serum cholesterol levels and immune function, while they also have anti-inflammatory and anti-carcinogenic properties. Stigmasterol increases in undesirable storage conditions, whereas  $\beta$ -sitosterol decreases [1]. The total sterol content of sesame seed oil has been estimated at 4, 911.31 - 5, 302.6 mg/kg; in the present study, these

**Table 2:** Sterol Composition (%) of Sesame Oil Samples\* (Quantities with same letters in each row not significantly different according to Duncan's test;  $P < 0.05$ ; \*Iranian National Standard Organization [INSO: 13392, INSO: 1752]; \*\*mg/kg)

Sterols	Cold- pressed sesame oil								Refined sesame oil		Permitted level*
	A	B	C	F	G	H	I	J	D	E	
Cholesterol	0.48 ± 0.025 <sup>c</sup>	0.4 ± 0.01 <sup>de</sup>	0.44 ± 0.01 <sup>c</sup>	0.18 ± 0.01 <sup>a</sup>	0.25 ± 0.01 <sup>bc</sup>	0.16 ± 0.01 <sup>a</sup>	0.27 ± 0.01 <sup>c</sup>	0.35 ± 0.01 <sup>a</sup>	0.18 ± 0.01 <sup>a</sup>	0.20 ± 0.01 <sup>ab</sup>	0.1 - 0.5
Brassicasterol	0.10 ± 0.01 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.1 - 0.2
Campesterol	19.47 ± 0.01 <sup>ab</sup>	19.40 ± 0.01 <sup>ab</sup>	19.49 ± 0.01 <sup>ab</sup>	19.35 ± 0.01 <sup>a</sup>	19.98 ± 0.01 <sup>c</sup>	19.93 ± 0.01 <sup>c</sup>	19.70 ± 0.1 <sup>b</sup>	19.37 ± 0.1 <sup>a</sup>	20 ± 0.01 <sup>d</sup>	20 ± 0.01 <sup>d</sup>	10.1 - 20
Stigmasterol	6.28 ± 0.01 <sup>a</sup>	6.30 ± 0.1 <sup>ab</sup>	6.20 ± 0.1 <sup>a</sup>	6.70 ± 0.1 <sup>d</sup>	6.40 ± 0.01 <sup>b</sup>	6.28 ± 0.1 <sup>a</sup>	6.50 ± 0.1 <sup>bc</sup>	6.60 ± 0.1 <sup>c</sup>	6.70 ± 0.1 <sup>d</sup>	6.60 ± 0.01 <sup>c</sup>	3.4 - 12
$\beta$ -sitosterol	61.90 ± 0.05 <sup>d</sup>	62.0 ± 0.1 <sup>ef</sup>	62.0 ± 0.1 <sup>ef</sup>	69.94 ± 0.1 <sup>g</sup>	62. ± 0.1 <sup>ef</sup>	60.90 ± 0.1 <sup>c</sup>	60.80 ± 0.1 <sup>c</sup>	60.90 ± 0.1 <sup>c</sup>	55.35 ± 0.1 <sup>a</sup>	59.90 ± 0.1 <sup>b</sup>	57.7 - 61.9
$\Delta$ 5-avenasterol	8.60 ± 0.26 <sup>a</sup>	8.60 ± 0.1 <sup>a</sup>	8.86 ± 0.005 <sup>b</sup>	10.1 ± 0.1 <sup>cd</sup>	10.6 ± 0.1 <sup>e</sup>	10.3 ± 0.1 <sup>d</sup>	10.7 ± 0.1 <sup>ef</sup>	10.0 ± 0.1 <sup>c</sup>	13.5 ± 0.01 <sup>g</sup>	10.9 ± 0.1 <sup>f</sup>	6.2 - 7.8
$\Delta$ 7-avenasterol	0.86 ± 0.01 <sup>c</sup>	0.67 ± 0.01 <sup>b</sup>	0.62 ± 0.01 <sup>a</sup>	0.75 ± 0.01 <sup>c</sup>	0.86 ± 0.01 <sup>c</sup>	0.74 ± 0.01 <sup>c</sup>	0.79 ± 0.01 <sup>d</sup>	1.00 ± 0.01 <sup>f</sup>	1.01 ± 0.01 <sup>f</sup>	1.01 ± 0.01 <sup>f</sup>	1.2 - 5.6
$\Delta$ 7-stigmasterol	0.70 ± 0.1 <sup>c</sup>	0.65 ± 0.01 <sup>d</sup>	0.70 ± 0.01 <sup>c</sup>	0.33 ± 0.01 <sup>a</sup>	0.50 ± 0.1 <sup>c</sup>	0.46 ± 0.1 <sup>b</sup>	0.51 ± 0.01 <sup>c</sup>	0.38 ± 0.01 <sup>a</sup>	0.80 ± 0.1 <sup>f</sup>	0.81 ± 0.01 <sup>f</sup>	0.5 - 7.6
Other sterols	0.81 ± 0.17 <sup>b</sup>	1.28 ± 0.01 <sup>c</sup>	0.37 ± 0.01 <sup>a</sup>	1.00 ± 0.01 <sup>bc</sup>	0.87 ± 0.01 <sup>b</sup>	0.69 ± 0.5 <sup>b</sup>	0.83 ± 0.01 <sup>b</sup>	1.25 ± 0.1 <sup>c</sup>	2.30 ± 0.1 <sup>d</sup>	1.01 ± 0.01 <sup>bc</sup>	0.7 - 9.2
Total sterol**	5201.10	5204.30	5302.60	5030.1	5107.11	5031.11	5023.0	5108.30	4911.31	4965.10	4500-19000



**Figure 1:** GC Chromatograms of sterol fraction of sesame oil samples (A, B, C, F, G, H, I, J): oils extracted with cold-press; D and E: commercially refined oils

values were within the Codex standard range (4,500-19,000 mg/kg) and consistent with the findings of Gharby *et al.* (2017) [1].

In the sesame oil samples in the present study, the levels of brassicasterol, campesterol, and stigmasterol were entirely compatible with the Codex standards. The standard levels of these compounds are estimated to be 10-20% for campesterol and 3.4-12 % for stigmasterol [20].  $\beta$ -sitosterol is the predominant sterol in sesame oil, which was observed to be slightly higher than the

standard level (57.7-61.9 mg/kg) in some of our samples (B, C, F, and G). The next major component was campesterol, the level of which reached approximately 19-20% of the total sterols. In addition,  $\Delta 5$ -avenasterol constituted about  $8.6 \pm 0.1$ - $10.7 \pm 0.1\%$  and  $10.9 \pm 0.1$ - $13.5 \pm 0.05\%$  of the sesame oil composition in the cold-pressed and refined samples, respectively.

The minor sterols detected in the samples of the present study were  $\Delta 7$ -stigmasterol and  $\Delta 7$ -avenasterol. In sample D, the  $\beta$ -sitosterol value was slightly lower

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than the standard level. On the other hand, the  $\Delta 5$ -avenasterol level in all the samples was higher than the permissible limit (6.2-7.8 mg/kg). The  $\Delta 7$ -avenasterol levels were lower than the permissible range specified in the national standard (1.2-5.6 mg/kg), and the level of  $\Delta 7$ -stigmastanol in some samples (F, H, and J) was below standard (0.5-7.6 mg/kg) [26]. Significant differences were observed between the cold-pressed and refined sesame oil samples in terms of the  $\beta$ -sitosterol, campesterol, and  $\Delta 7$ -stigmastanol contents. In addition, the statistical analysis indicated an inverse correlation between  $\beta$ -sitosterol and stigmastanol. These findings are in line with the results obtained by Unal and Yalcin (2008) [23], as well as the studies performed in Sudan and Morocco. According to the literature,  $\beta$ -sitosterol is the major sterol that constitutes approximately 60% of the total sterols of sesame seed oil, and geographical origin has an insignificant impact on sterol composition [27, 28].

### 3.3. Tocopherol Composition

Tocopherols are important natural lipophilic antioxidants in vegetable oils, which have protective effects against the oxidative deterioration of PFAs. According to the information in Table 3,  $\gamma$ - and  $\delta$ -tocopherols were detected in the sesame oil samples (Table 3). The tocopherol contents of the cold-pressed and refined sesame oil samples were within the ranges of  $302.3 \pm 0.1$ - $687.99 \pm 0.2$  and  $411 \pm 0.1$ - $545.8 \pm 0.1$  mg/kg, respectively. The main tocopherol (i.e.,  $\gamma$ -tocopherol content) ranged from  $298.50 \pm 0.1$  to  $680.4 \pm 0.1$  mg/kg, and a significant difference was observed in the  $\gamma$ -tocopherol content between the cold-pressed and refined sesame oil samples ( $P < 0.05$ ). In addition, the  $\gamma$ - and  $\delta$ -tocopherols of sample G were lower than the national standard limits [26].

Our findings in this regard are consistent with the previous studies. For instance, Gharby *et al.* (2011) reported that the tocopherol content of Moroccan sesame seed oil was 446 mg/kg, and  $\gamma$ -tocopherol as the main component constituted about 90.5% of the total tocopherols [29]. The antioxidant activity of  $\gamma$ -tocopherol has been reported to be higher than  $\alpha$ -tocopherol, which also has a higher biological activity compared to other tocopherols [29, 30]. Furthermore, Jalili *et al.* (2019) have reported that the  $\gamma$ -tocopherol content of cold-pressed sesame oil samples was within the range of 273-666 mg/kg [31]. The high stability of sesame oil could be attributed to the tocopherol content. Sesame has the highest volume of  $\gamma$ -tocopherol among oilseeds [31]. The tocopherol content of oils depends on the storage conditions after harvesting and interval between oil extraction and tocopherol determination [32].

The longer interval between oil extraction and tocopherol determination is associated with the decreased tocopherol content. In addition, genetic variation is effective in the tocopherol content of oils [30].

The lower content of  $\gamma$ -tocopherol in some of the sesame oil samples (G and E) in the current research could be due to heating during oil extraction by the press. The refined oils also lost some of their biologically active compounds in this process, including phenolic compounds, tocopherols, phytosterols, and carotenoids, which are more susceptible to oxidative deterioration [33].

### 3.4. Iodine Value

Iodine value is a quality parameter that is regularly used to measure the physicochemical properties of edible oils. Moreover, iodine value is an index of the total number of the double bonds in lipids and oils; the higher number of double bonds is associated with the increased iodine value and lower oxidative stability [1, 34]. According to the information in Table 1, the iodine value of the cold-pressed sesame oil samples was within the range of  $106.9 \pm 0.1$ - $113.1 \pm 0.1$  g/100 g, and the iodine value of the refined sesame oils was within the range of  $110.85 \pm 0.01$ - $113.6 \pm 0.1$  g/100 g. The overall ranges of the iodine value in the present study were consistent with the Iranian national standards, while lower than the iodine value of the sesame oils in Morocco and Congo (117 g/100 g) [1]. In the current research, sample I had the lowest iodine value ( $106.9 \pm 0.1$  g/100 g), while the highest iodine value ( $113.6 \pm 0.1$  g/100 g) was observed in sample E. The high iodine value of the sesame oil samples confirmed the high levels of UFAs in their fatty acid profile, which is desirable from a nutritional perspective [34, 35].

### 3.5. Peroxide Value

Autoxidation of oil results in initial products and odorless molecules (e.g., hydroperoxides). According to the information in Table 1, the peroxide value of the cold-pressed and refined sesame oil samples was  $1.1 \pm 0.1$ - $10.6 \pm 0.1$  and  $4.3 \pm 0.1$ - $4.5 \pm 0.01$  meq O<sub>2</sub>/kg, respectively. The peroxide value of all the samples was below the maximum allowable standard limit (10 meq O<sub>2</sub>/kg), with the exception of the pressed samples I and F. On the other hand, a significant difference was observed in the peroxide value of the cold-pressed samples (A, F, H, and I) and refined sesame oil samples ( $P < 0.05$ ).

In the current research, the lowest peroxide value ( $1.1 \pm 0.01$  meq O<sub>2</sub>/kg) was observed in sample H, while the highest peroxide value ( $10.6 \pm 0.1$  meq O<sub>2</sub>/kg) was detected in sample F. Furthermore, an inverse correlation was denoted between the peroxide value and  $\gamma$ -tocopherol content of the samples (tables 1 and 3). The peroxide value of the sesame seed oil of Morocco and Sudan has been reported to be 2.7 and 6.9 meq O<sub>2</sub>/kg, respectively [1, 26]. In a study, Ogbonna *et al.* (2013) also examined the quality properties of 13 local sesame seeds from Nigeria, reporting that the ranges of iodine and peroxide values were 76.1-130 g/100g and 2.2-10 meq/kg, respectively and within the Codex standard range [11].

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**Table 3:** Tocopherol composition (mg/kg) of sesame oil samples\* (\*quantities with same letters in each row not significantly different according to Duncan's test;  $P < 0.05$ ; \*\*Iranian National Standard Organization [INSO: 13392, INSO: 1752])

Tocopherols	Cold-pressed sesame oil								Refined sesame oil		Permitted level**
	A	B	C	F	G	H	I	J	D	E	
$\gamma$ -Tocopherol	538 $\pm$ 0.2 <sup>d</sup>	524.30 $\pm$ 0.1 <sup>c</sup>	547.00 $\pm$ 0.2 <sup>f</sup>	549.30 $\pm$ 0.1 <sup>g</sup>	298.50 $\pm$ 0.1 <sup>a</sup>	570.40 $\pm$ 0.2 <sup>h</sup>	547.60 $\pm$ 0.1 <sup>f</sup>	680.40 $\pm$ 0.1 <sup>i</sup>	539.60 $\pm$ 0.1 <sup>e</sup>	402.60 $\pm$ 0.5 <sup>b</sup>	521-983
$\delta$ -Tocopherol	4.05 $\pm$ 0.06 <sup>c</sup>	4.03 $\pm$ 0.07 <sup>c</sup>	4.0 $\pm$ 0.06 <sup>c</sup>	6.40 $\pm$ 0.1 <sup>e</sup>	1.54 $\pm$ 0.07 <sup>b</sup>	5.97 $\pm$ 0.07 <sup>de</sup>	0.78 $\pm$ 0.07 <sup>a</sup>	6.63 $\pm$ 0.06 <sup>e</sup>	5.60 $\pm$ 0.5 <sup>d</sup>	4.00 $\pm$ 0.1 <sup>c</sup>	4-21
Tocopherol Content	524.0 $\pm$ 0.07 <sup>c</sup>	528.0 $\pm$ 0.07 <sup>c</sup>	551.0 $\pm$ 0.07 <sup>c</sup>	557.7 $\pm$ 0.1 <sup>cd</sup>	302.3 $\pm$ 0.1 <sup>a</sup>	578.4 $\pm$ 0.2 <sup>d</sup>	554.1 $\pm$ 0.2 <sup>cd</sup>	687.99 $\pm$ 0.2 <sup>e</sup>	545.8 $\pm$ 0.1 <sup>c</sup>	411 $\pm$ 0.1 <sup>b</sup>	330-1010

### 3.6. Oxidative Stability

Lipid oxidation reduces the quality of edible oils by altering their chemical, sensory, and nutritional properties. The primary products of oxidation are unstable while heating and evolve to yield secondary oxidation products in the subsequent stages. The Rancimat method is considered to be a direct measure of the changes in oil quality and oxidative stability. Oxidative stability refers to the ability of oils and lipids to resist oxidative deterioration during processing and storage periods, which affect shelf life and suitability for consumption [28].

The oxidative stability of cold-pressed sesame oils extracted in shops has not been investigated in previous studies. According to the information in Table 1, the induction time evaluated by the Rancimat method was within the range of 7.53  $\pm$  0.1 - 9.48  $\pm$  0.1 hours in the cold-pressed sesame oil samples and 18.96  $\pm$  0.26-20.91  $\pm$  0.01 hours in the refined samples. The difference in the oxidative stability of the cold-pressed and refined samples was considered statistically significant ( $P < 0.05$ ). In the current research, Pearson's correlation-coefficient indicated a direct correlation between the  $\delta$ -tocopherol content and oxidative stability ( $P < 0.05$ ). Accordingly, the refined sesame oil samples (D and E) had superior oxidative stability despite their high level of unsaturation; this quality could be associated with the presence of efficient antioxidants, such as lignans (sesamol, sesamol, and sesamin), phenolic compounds, tocopherols (especially  $\gamma$ -tocopherol), and sterols [1, 27]. To date, the standard range of the oxidative stability of sesame oil has not been defined. In our research, the mean oxidative stability values of the cold-pressed samples (8.61 hours) did not meet the minimum standard level of frying (13 hours) and mixed oils (12 hours) at the temperature of 110°C [36, 37].

According to the results of the present study, the highest oxidative stability among the cold-pressed samples (9.48  $\pm$  0.1 hours) was observed in sample J, which could be attributed to the presence of the highest amount of tocopherols (687.99  $\pm$  0.2 mg/kg). According to the literature,  $\gamma$ -tocopherol plays a pivotal role in oxidative stability. In the current research, sample G had a low oxidative stability (8.18 hours), and its  $\gamma$ -tocopherol content was significantly lower than the standard limit. In addition, a negative correlation was denoted between the UFA/SFA ratio and oxidative stability.

At the same temperature (110°C), the Rancimat induction time of the cold-pressed sesame oil in Morocco has been reported to be 28.5 hours. In similar studies, the mean oxidative stability of sesame oils has been

estimated at 9.6 and 8.3 hours [38]. According to Mohammadi *et al.* (2014), the oxidative stability of sesame oil is 19.94 hours at the temperature of 110°C [39]. Previous findings in this regard have also emphasized the key role of seed variety and processing techniques in oxidative stability [40].

### 4. Conclusion

Considering properties such as the fatty acid profile and iodine value, sesame oil is highly unsaturated. According to the results, the refined sesame oil samples had significantly better quality properties compared to the cold-pressed sesame oils, especially in terms of oxidative stability. Due to low oxidative stability, the long-term preservation and use of cold-pressed sesame oils extracted in shops are not recommended for cooking and frying. The storage condition of these oils is also important since exposure to light, temperature, and oxygen availability may significantly affect their oxidative stability. Therefore, it is essential to ensure the authenticity and safety of crude sesame oils using proper and advanced methods by supervisory organizations such as the IFDA and the Standards Organization. In addition, further monitoring is required to reduce consumer health risks regarding aflatoxins and toxic metals, prevent unfair trading practices, and the problems associated with mislabeling. Since the sesame seeds used in Iran are mainly imported from Pakistan and India, it is recommended that the current standards of sesame oil be revised, especially in terms of shelf life, oxidative stability, peroxide value, and sterol content (e.g.,  $\Delta^5$ -avenasterol,  $\Delta^7$ -avenastagimastanol, and  $\Delta^7$ -avenasterol).

### Authors' Contributions

P.G.H.A., M.A.M., and N.D.Z., designed the study; N.D.Z., performed the experiments; N.D.Z., P.G.H.A., and M.A.M., drafted the manuscript; P.G.H.A., and M.A.M., 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.

### Acknowledgments

Hereby, we extend our gratitude to the Food Control Laboratory of Zanjan University of Medical Sciences for the financial support of this research project (Project No.: A-12-1345-2; ethics code: IR. ZUMS. REC.1398.217). We

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would also like to thank the Department of Food Science and Technology of the Islamic Azad University of Hidaj Branch, Hidaj, Iran for their assistance in this study.

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