Hindawi
Journal of Food Processing and Preservation
Volume 2023, Article ID 1197398, 8 pages
https://doi.org/10.1155/2023/1197398

Research Article

Efficacy of Different Sesame Oil Refining Stages in Reduction of Heavy Metals, Antioxidant Activity, and Oxidative Parameters

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Received 23 August 2022; Revised 6 June 2023; Accepted 27 June 2023; Published 11 July 2023

Academic Editor: Adadi Parise

The efficacy of different refining stages on physicochemical parameters, heavy metal, and mineral contents of sesame oil were investigated. The sesame oil from different refining stages (crude oil, neutralization, bleaching, deodorization, and refining stages) was taken from the local oil factory in Yazd, Iran. The fatty acid composition of oil samples was determined by GC-FID. The oxidation indexes including peroxide value, p-Anisidine, free fatty acid, TOTOX, total polar material, as well as antioxidant activity (FRAP and DPPH), and total phenolic content were measured. The mineral and heavy metal content of oil samples were analyzed by ICP-OES. Linoleic acid (41.1-42.4%), oleic acid (41-41.4%), palmitic acid (8.3-9.1%), and stearic acid (6.1-6.3%) were reported as the most dominant fatty acids in all oil samples. The results illustrated that the refining process had no significant effects on fatty acid composition except for slight changes in palmitic acid. It was shown that all oxidation indexes, antioxidant activity, total phenolic content and mineral, and heavy metals were decreased during oil refining. The highest decrease in oxidative indexes was reported in the deodorization stage. The total phenolic content, DPPH, and FRAP ranged from 4.79 to 56.56 mg GAE/100 g, 6852.64 to 867.89 mg/ml, and 54.74 to 127.4 mM $FeSO4.7H_2O$ in RSO and CSO, respectively, which the highest decrease was shown during the neutralizing stage. The order of metals in RSO was shown as P>Ca>K>Fe>Mg>Al>Cr>Pb>Ni>Cd>Cu>Co. Although all metals were decreased by the refining process, the Pb concentration (0.16 mg/kg) of RSO was evaluated as higher than the permissible limit. Considering that the refining process effectively reduces undesirable compounds, the consumption of crude sesame oil is not recommended.

1. Introduction

Vegetable oils are considered an essential part of the human diet which could provide energy, vitamins, and essential fatty acids [1]. Sesame oil is known as a valuable source of antioxidants and bioactive compounds, has high unsaturated fatty acid content, and has high oxidative stability [1, 2]. Sesame oil can be used as cooking oil, salad oil, and seasoning ingredients in food [3]. Crude vegetable oil contains different chemical components including pesticides, aromatic hydrocarbons, heavy metals, aflatoxins, and polycyclic aromatic hydrocarbons [4]. The trace metals could act as pro-oxidants and accelerate the development of free radicals and secondary oxidation metabolites (aldehyde, ketones, alcohols, and volatile compounds) in vegetable oils [5, 6] which deteriorate the quality, taste, and odor of oils.

Therefore, trace metals in vegetable oils are introduced as a quality factor [5]. The refining process of sesame oil could remove some micronutrients and antioxidants as well as undesirable compounds [6] including trace metals, aldehydes, ketones, and free fatty acids [7].

The vegetable oil refining is a common method in the oil industry to produce oil with good quality, although there has been growing interest in the consumption of extracted sesame oil without further refining process (crude vegetable oil) in Iran. In recent years, the shops have been established which extract oil from sesame seed in front of consumers. There is a perception that, due to the in-place production of fresh sesame oil, they are healthier than industrial ones. It could be mentioned that the lack of a refining process in sesame oil could the oxidative indexes such as peroxide, p-Anisidine value, free fatty acid, TOTOX, and total polar material. Also, the presence of heavy metals and minerals in crude vegetable oils along with higher oxidative characteristics could deteriorate the quality of oil and put human health in danger [8]. Although the refining process could decrease the natural antioxidants present in vegetable oils, also, having sufficient knowledge about the changes in physicochemical parameters during each stage of the refining process could be helpful in the preparation of healthy vegetable oil. Therefore, due to growing interest in consumption of crude sesame oil, the current study aimed to investigate the impact of different refining stages on the physicochemical properties of sesame oil (fatty acid composition, oxidative indexes, and antioxidant activity) as well as heavy metals and mineral contents. The result of the current study could give consumers efficient information about the importance of the refining process as well as the producers to understand the effect of different stages of refining on antioxidant activity and heavy metal content. To the best of our knowledge, the literature has not investigated the impact of the refining process on sesame oil characteristics.

2. Material and Methods

2.1. Chemical and Reagents. Isooctane, methanol, 1,1,3,3-tetraethoxypropane, glacial acetic acid, nitric acid, peroxide hydrogen, potassium iodide, sodium thiosulfate, chloroform, starch, hydrochloric acid, p-anisidine, Folin-Ciocalteu reagent, phenol phetalein, 2,4,6-triyridyl-s-triazine, iron (II) sulfate heptahydrate, and sodium hydroxide were purchased from Merck, Germany. The iron (III) chloride was prepared from Chem-Lab NV®, Belgium. Gallic acid and 2,2-diphenyl-1-picrylhydrazyl were purchased from Sigma-Aldrich, USA.

For metal analysis, all reagents were of analytical grade, and glasswares were soaked in diluted nitric acid (10% v/v) overnight and rinsed with distilled deionized water.

2.2. Sample Preparation. Sesame oil from different refining stages, including crude sesame oil (CSO), neutralized sesame oil (NSO), bleached sesame oil (BSO), deodorized sesame oil (DSO), and refined sesame oil which packed and contained synthetic antioxidant (RSO) were taken from a local oil factory in Yazd, Iran, with three replicates. Samples were transferred to the laboratory in dark containers and stored at 4°C until further analysis.

2.3. Fatty Acid Composition. To analyze the fatty acid composition of oil samples, the fatty acid methyl esters (FAME) extraction was done by addition of n-hexane (7 ml) and 2 M potassium hydroxide (2 ml). After incubation at 37°C for 20 min, the upper layer phase was used for further analysis. The fatty acid composition of the samples was analyzed by gas chromatography equipped with flame ionization detection (GC-FID, Yang Lin 6500, South Korea) and capillary column (120 m × 2.5 mm i.d.; 0.25 μm). The detector temperature was held at 90°C for 7 min; ten minutes at 150°C, increased to 200°C for 15 minutes, continued for 20 minutes at 240°C, and then held at 240°C for 50 minutes. Helium gas with a flow rate of 20 ml/min as carrier and hydrogen and air at a ratio of 1 : 30 as an oxidant was used [1]. The FAMEs were identified by comparing the retention times and fragmentation patterns with standards.

2.4. Oxidation Indexes. The peroxide value (PV) and free fatty acid (FFA) were done according to the methods explained by Kheirati Rounizi et al. [1] and Farhoosh et al., respectively [9]. The p-Anisidene value (p-AV) and total polar material (TPM) were measured according to AOCS [10] and Khalili et al. [11], respectively. The TOTOX value was calculated according to the following formula.

$$\text{TOTOX} = 2\text{PV} + \text{p} - \text{AV}. \quad (1)$$

2.5. Sample Extraction for Total Phenolic Content and Antioxidant Activity. The sesame oil extraction was performed by mixing 3 g of oil diluted with methanol (5 ml) and shaking for 30 min at 300 rpm. Then, solutions were stored at -20°C for 30 min. The supernatant solution was separated and stored at 4°C for determination of total phenolic content and antioxidant activity [6].

2.5.1. Total Phenolic Content (TPC). The TPC was determined according to the method described by Chew et al. with slight modifications. Approximately 500 μL of extracted oil samples were combined with 2.5 ml of Folin-Ciocalteu reagent (10 times diluted) and left for 7 min. Then 2 mL of 7.5% Na_2CO_3 was added, and samples were left at dark room temperature for 1 h. The absorbance of samples was recorded at 765 nm by a UV/VIS-spectrometer (HACH, USA). The TPC of samples was expressed as mg GAE (gallic acid equivalents)/100 g oil [12].

2.5.2. DPPH Method. The DPPH method was used according to the research of Gülçin et al. with slight modification. Briefly, 2.5 mL of 0.1 mM DPPH in methanol was mixed with 500 μL of extracted sesame oil samples. The absorbance of samples after 30 min of storage at room temperature was measured at 517 nm by UV/VIS-spectrometer [13]. The radical scavenging activity of sesame oil was evaluated as follows:

Table 1: Experimental scheme for the microwave digestion of oil samples.

Step	Temperature (°C)	Time (min)	Power (w)
1	130	10	600
2	150	15	600
3	180	7	600
4	180	8	600

$$\text{DPPH scavenging\%} = \frac{(Ab_c - Ab_s)}{Ab_c}, \quad (2)$$

where the Ab_c and Ab_s were the absorbance of control and absorbance of sample, respectively. Then, IC_{50} was calculated by calibration curve obtained from the antioxidant activity of each extract at different concentration.

2.5.3. FRAP Method. The FRAP method was used according to the Li et al. method. A total of 150 μL of diluted oil samples was mixed with 3 ml of FRAP reagent containing 2, 4, 6-triyridyl-s-triazine (TPTZ, 10 mM in 40 mM HCl) solution, $FeCl_3.6H_2O$ (20 mM), and acetate buffer (300 mM, pH = 3.6) in a ratio of 1 : 1 : 10, respectively. The absorbance of samples was taken after 5 min at 593 nm against methanol as a blank. A standard curve was prepared using $FeSO_4.7H_2O$ at different concentrations [14].

2.6. Trace and Heavy Metal Analysis. The digestion of oil samples was done in four stages according to Table 1. Briefly, 0.5 g of oil samples were mixed with 7 ml of HNO_3 (65%). After 30 min, samples were treated with 1 ml of H_2O_2 (30%) and put in a microwave digestion system according to Table 1 (Sineo MDS 15, China). Digested samples were filtered through filter paper and a 0.45 μm Millipore filter and diluted to 10 ml with deionized distilled water [1].

To determine the heavy metal concentration, samples were injected into an inductively coupled plasma-optical emission spectrophotometer (Spectro Genesis model, Germany). All analyses were done in triplicate. The limit of detection (LOD) of Ca, Cd, Co, Cu, Fe, Mg, Ni, P, Pb, Cr, K, and Al was determined as 0.00537 mg/kg, 0.665 μg/kg, 0.594 μg/kg, 0.00342 mg/kg, 0.000797 mg/kg, 0.00066 mg/kg, 1.88 μg/kg 0.418 mg/kg, 1.87 ppb, 2.36 μg/kg, 0.00394 mg/kg, and 0.00341 mg/kg, respectively.

2.7. Statistical Analysis. The data were analyzed by the SPSS 18 software. The one-way analysis of variance (ANOVA), followed by the Tukey test, was utilized to analyze the significant differences between the data ($p < 0.05$).

3. Result and Discussion

Vegetable oils are a good source of micronutrients, antioxidants, and vitamins which could be reduced during the refining process. Generally, during the refining process, by reducing the desirable compounds, some undesirable ones, including heavy metals, pesticides, and oxidation products, could be removed. In the current study, variation in the undesirable compound during different refining stages was studied.

3.1. Fatty Acid Composition. Based on the results, approximately ten types of fatty acids were detected by GC-FID (Table 2). Linoleic acid, oleic acid, palmitic acid, and stearic acid were the most dominant fatty acids in all sesame oil samples, which is in line with our previous findings [1]. However, different factors, including variety, environmental conditions, and the refining process, could impact fatty acid composition [15]. The results illustrated that except for slight changes in palmitic acid, the refining process had no significant effects on the fatty acid composition of oil samples which is in accordance with Zhu et al. [16], Sánchez-Machado et al. [17], Liu et al. [18], and Van Hoed et al. [19]. However, except for behenic acid and arachidic acid, all fatty acid profiles were in the standard ranges set by Codex Alimentarius [20]. It was shown that the SFA, MUFA, and PUFA of sesame oil samples varied from 15.9 to 16.7%, 41.8 to 41.4%, and 42.3 to 41.9%, respectively. It could be noted that by improving in the refining stage, SFA was increased and PUFA, MUFA, and COX values were decreased. Therefore, the refining process could increase the oxidative stability of sesame oils. However, COX value (4.86) and PUFA (42.8%) were increased during the deodorization stage, which could negatively affect oil stability. Durmaz and Gökmen indicated that the reduction in oxidative stability during the deodorization stage is owing to a decrease in the tocopherol contents of oils [21].

3.2. Oxidation Indexes. Vegetable oil's oxidative stability could be affected by different factors including antioxidant activity, fatty acid composition, and minor compounds [21]. To investigate the influence of the refining process on the oxidation stability of sesame oil, the peroxide value, p-Anisidine value, TOTOX, free fatty acid, and total polar material were determined (Figure 1). Vegetable oil's oxidative stability is related to different factors, including oil type, antioxidant content, fatty acid composition, and the presence of metals [21]. The PV of CSO was 11.24 meq O_2/kg and, after refining, reached 1.04 meq O_2/kg, which is within the permissible limits set by Codex Alimentarius [20]. The p-AV was 6.43 in CSO and reached 1.08 in refined oil. According to the results, there was no significant decrease in PV and p-AV values of CSO and NSO. There are reports that during oil degumming and neutralizing, adding an aqueous or buffer solution would increase the moisture content of the oil [22]. The FFA was evaluated at 0.17% in CSO samples which reached 0.05% in RSO. It was shown that FFA was increased during the bleaching stage, which could be related to the acidity and moisture of bleaching clay, bleaching temperature, and ester hydrolysis by bleaching clay [15, 16]. The increase in FFA of the deodorized oil was reported by Zhu et al. [16]. Despite the FFA, the PV, p-AV, TPM, and TOTOX values were constantly reduced by the progress of the refining process. There is some evidence that the type and amount of bleaching clay are related to an increase or decrease in PV [16]. In the deodorization

Table 2: Fatty acid composition of sesame oil during refining process.

Fatty acid composition	CSO	NSO	BSO	DSO	RSO
Palmitic acid	8.3 ± 0.01^{b}	8.7 ± 0.02^{b}	8.6 ± 0.02^{b}	8.6 ± 0.02^{b}	9.1 ± 0.01^{a}
Palmitoleic acid	0.1 ± 0.001^{a}	0.1 ± 0.001^{a}	0.1 ± 0.001^{a}	0.1 ± 0.001^{a}	0.1 ± 0.001^{a}
Stearic acid	6.1 ± 0.001^{a}	6.1 ± 0.001^{a}	6.3 ± 0.001^{a}	6.3 ± 0.001^{a}	6.1 ± 0.001^{a}
Oleic acid	41.4 ± 0.05^{a}	41.3 ± 0.04^{a}	41.2 ± 0.4^{a}	41.3 ± 0.04^{a}	41 ± 0.02^{a}
Linoleic acid	41.9 ± 0.06^{b}	41.6 ± 0.05^{b}	41.6 ± 0.05^{b}	42.4 ± 0.05^{a}	41.1 ± 0.02^{b}
α Linolenic acid	0.4 ± 0.01^{a}	0.4 ± 0.01^{a}	0.4 ± 0.01^{a}	0.4 ± 0.01^{a}	0.4 ± 0.01^{a}
Arachidic acid	0.9 ± 0.01^{a}	0.9 ± 0.01^{a}	0.9 ± 0.01^{a}	0.9 ± 0.01^{a}	0.9 ± 0.01^{a}
Gondoic acid	0.3 ± 0.01^{a}	0.3 ± 0.01^{a}	0.3 ± 0.01^{a}	0.3 ± 0.01^{a}	0.3 ± 0.01^{a}
Behenic acid	0.4 ± 0.01^{a}	0.4 ± 0.01^{a}	0.4 ± 0.01^{a}	0.4 ± 0.01^{a}	0.4 ± 0.01^{a}
Lingoceric acid	0.2 ± 0.01^{a}	0.2 ± 0.01^{a}	0.2 ± 0.01^{a}	0.3 ± 0.01^{a}	0.2 ± 0.01^{a}
SFA	15.9 ± 0.04^{b}	16.3 ± 0.04^{a}	16.4 ± 0.02^{a}	16.5 ± 0.02^{a}	16.7 ± 0.03^{a}
MUFA	41.8 ± 0.01^{a}	41.7 ± 0.01^{a}	41.6 ± 0.02^{a}	41.7 ± 0.03^{a}	41.4 ± 0.02^{a}
PUFA	42.3 ± 0.01^{ab}	42 ± 0.02^{b}	42 ± 0.02^{b}	42.8 ± 0.02^{a}	41.9 ± 0.01^{b}
PUFA/SFA	2.66 ± 0.01^{a}	2.57 ± 0.02^{b}	2.56 ± 0.01^{b}	2.59 ± 0.02^{b}	2.5 ± 0.01^{b}
MUFA/PUFA	0.98 ± 0.01^{a}	0.99 ± 0.02^{a}	0.99 ± 0.02^{a}	0.97 ± 0.01^{a}	0.98 ± 0.01^{a}
COX value	4.82 ± 0.02^{ab}	4.78 ± 0.01^{b}	4.78 ± 0.02^{b}	4.86 ± 0.01^{a}	4.77 ± 0.01^{b}

CSO: crude sesame oil; NSO: neutralized sesame oil; BSO: bleached sesame oil; DSO: deodorized sesame oil; RSO: refined sesame oil; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyuinsaturated fatty acids; COX value: calculated oxidizability value = $[1 \times (C18 : 1\%) + 10.3 \times (C18 : 2\%) + 21.6 \times (C18 : 3)]/100$. Different letters in each row shows significant differences at level of $p < 0.05$.

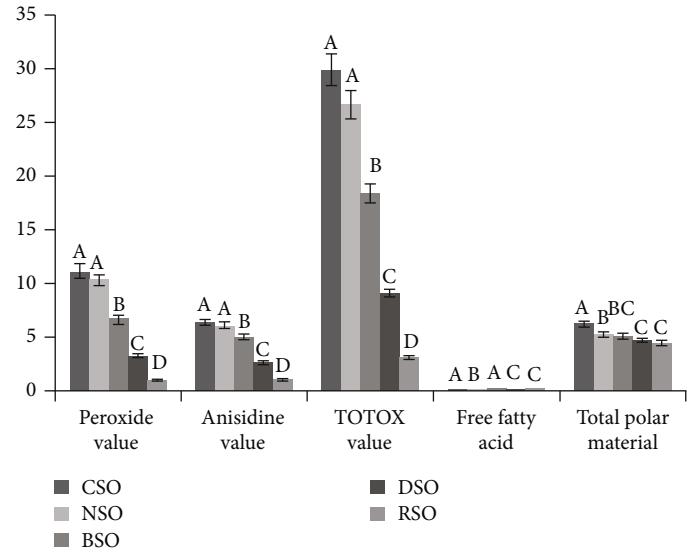

Figure 1: Oxidation indexes of sesame oil during refining process. CSO: crude sesame oil: NSO: neutralized sesame oil; BSO: bleached sesame oil; DSO: deodorized sesame oil; RSO: refined sesame oil. Different letters shows significant differences at level of $p < 0.05$.

stage, many undesirable compounds, FFA, and oxidation products are removed by the high temperature of the steam distillation process owing to their volatility [12, 15]. As reported, during the deodorization stage, FFA formed by triglycerides is removed [9]. So, PV, p-AV, TOTOX, and FFA of DSO samples were significantly decreased ($p < 0.05$), which is in accordance with the result of Chew et al. [12]. Having investigated that the temperature, time of heating, and steam rate of the deodorization stage could affect PV [16], the highest reduction in TPM was shown during the neutralization stage, which is consistent with the findings of Farhoosh et al. [9]. Generally, all oxidative indexes were reduced in RSO and reported at the acceptable level set by Codex Alimentarius [20]. Therefore, factors such as initial oil quality, fatty acid composition, and used technology could change the oxidative indexes [9]. However, it would be better to mention that during the refining process, trace elements, including Cu, Fe, and Ni, were reduced; thereafter, the oxidative stability of oil was increased.

3.3. Total Phenolic Content and Antioxidant Activity. The results of FRAP, DPPH, and TPC of sesame oil samples at various refining stages are given in Table 3. As can be seen, a sharp decrease in TPC was observed during the

Table 3: Antioxidant activity and phenolic content of sesame oil during refining process.

Sesame oil samples	DPPH IC_{50} mg/ml	FRAP mM FeSO4.7H2O	Phenolic content Mg GAE/100 g
CSO	867.89 ± 14.24^{a}	127.48 ± 1.25^{a}	56.56 ± 1.4^{a}
NSO	1310.65 ± 13.33^{b}	76.5 ± 1.57^{b}	30.20 ± 0.46^{b}
BSO	2517.33 ± 12.98^{b}	72.87 ± 1.95^{b}	26.55 ± 0.22^{c}
DSO	4523.58 ± 24.32^{c}	64.35 ± 1.29^{c}	14.36 ± 1.51^{d}
RSO	6852.64 ± 31.01^{d}	54.74 ± 1.94^{d}	4.79 ± 0.94^{e}

CSO: crude sesame oil; NSO: neutralized sesame oil; BSO: bleached sesame oil; DSO: deodorized sesame oil; RSO: refined sesame oil. Different letters in each column shows significant differences at level of $p < 0.05$.

neutralization stage which is in line with Szydłowska-Czerniak et al. [23] and Pan et al. [7]. It was shown that oil washing with water and soap formation during the neutralizing stage account for phenolic compound removal [21]. On the other hand, during the neutralization process with sodium hydroxide, polar compounds with weak acids, including phenolics, could be easily removed by aqueous solutions and entrapped in the sodium soap [23]. In Chew et al.'s research, the highest decrease in TPC was shown in deodorized kenaf seed oil [12]. A continuous decrease in total phenolic content was reported in the deodorizing and refining stages which decreased 91.53% of the phenolic content by the end of the refining process.

The antioxidant activity of CSO was determined at 127.48 mM $FeSO_4.7H_2O$ and 867.89 mg/ml for the FRAP and DPPH (IC_{50}) methods, respectively. In the current study, the IC_{50} was calculated in DPPH method which indicated the concentration of samples that scavenge 50% of DPPH free radicals. Therefore, a higher IC_{50} will show lower antioxidant activity. According to the results, the IC_{50} of oil samples was increased by the refining process which indicated a reduction in antioxidant activity. The reduction patterns of antioxidant activity by the FRAP method and the increase by the DPPH method were similar to total phenolic content, which is in line with Liu et al. [18]. It can be observed that the antioxidant activity of sesame oil by two analytical methods have differed which is related to their different mechanisms [6]. As demonstrated, the FRAP assay, a hydrophilic antioxidant method, could not detect well the lipophilic antioxidant and antioxidant with functional groups whose reduction potential is half a time lower than the Fe^{3+}/Fe^{2+} reaction [6]. The FRAP is determined by the antioxidant ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) by donating an electron [7]. On the contrary, the DPPH assay had a high affinity toward hydrophilic and lipophilic antioxidants [6]. In addition, the DPPH assay was done in a methanol solution which is a strong hydrogen-band-accepting solvent and caused the reaction to occur very slowly [18]. Also, it is shown that the variation in the antioxidant activity of vegetable oils is due to genetics and agronomics as well as environmental and technological factors [6, 24]. The result of the current study indicated that antioxidant activity decreased during refining, which confirms the results of Durmaz and Gökmen [21], Szydłowska-Czerniak et al. [23], and Szydłowska-Czerniak et al. [24]. The highest reduction in antioxidant activity by the FRAP method is apparent in the neutralizing stage which is quite similar to the result of total phenolic content consistent with Szydłowska-Czerniak et al.'s results [23] and Liu et al. [18]. It may well be that washing the oil during the neutralization step could lead to the degradation of phenolic compounds [7]. Szydłowska-Czerniak et al. reported that the highest reduction of antioxidant activity and phenolic content happened in the bleaching of palm oil [24]. It is noteworthy that at the end of the refining process, the FRAP value had about 57.05% reduction. Therefore, CSO had significantly higher antioxidant activity and phenolic content compared to refined oil.

3.4. Trace and Heavy Metal Analysis. Metal cations could be found in vegetable oils as suspended solid impurities or attached by phospholipids, lipids, proteins, and nonlipid carriers [8]. As seen in Table 4, the heavy metal and mineral content of sesame oil at different stages of the refining process was significantly decreased. As it is obvious, heavy metal and mineral concentrations significantly differ in different technological refining processes. Accordingly, CSO had the highest concentration of heavy metal and mineral contents. The concentration of heavy metals including Pb, Cd, Ni, and Cu ranged 0.92-0.16, 0.21-0.05, 1.08-0.13, and 0.76-0.03 mg/kg, respectively. The order of cations in the refined sesame oil is P>Ca>K>Fe>Mg>Al>Cr>Pb>Ni>Cd>Cu>Co. The Pb concentration of CSO was determined to be 0.92 mg/kg decreasing to 0.16 mg/kg, during the refining process. Even in refined samples, the amount of Pb was estimated to be higher than the permitted limit (0.1 mg/kg). In investigations by Kheirati Rounizi et al. [1], Ramezani et al. [25], and Gebrekidan and Desta [26], the concentrations of Pb in sesame oil samples were reported higher than the standard limit set by Codex Alimentarius which could be resulted from environmental conditions including rain drainage, soil erosion, and geology [8]. The Cd concentration was decreased during the refining process and reached 0.05 mg/kg, which is in the range of the standard limit set by Codex Alimentarius and estimated higher than the results of Kheirati Rounizi et al. [1] and Ramezani et al. [25]. According to the results, all studied were decreased during the neutralization stage. It was indicated that the formation and removal of insoluble sodium soap in the reaction of sodium hydroxide by phospholipids could lead to the reduction of metals present in phospholipid and protein structure during the neutralization stage [15]. Therefore, the decrease

Table 4: Mineral and heavy metal content of sesame oil during refining process.

Metal (mg/kg)	CSO	NSO	BSO	DSO	RSO	Maximum level
Al	12.11 ± 0.62^{a}	7.66 ± 0.20^{b}	6.16 ± 0.38^{b}	3.95 ± 0.07^{c}	0.42 ± 0.09^{d}	ND
Mg	8.81 ± 0.01^{a}	5.81 ± 0.53^{b}	7.02 ± 0.09^{a}	2.96 ± 0.03^{c}	0.72 ± 0.18^{d}	ND
Ca	47.59 ± 0.04^{a}	29.05 ± 4.92^{b}	30.52 ± 0.43^{b}	19.01 ± 2.19^{c}	8.17 ± 0.27^{d}	ND
K	17.72 ± 0.59^{a}	13.84 ± 1.18^{b}	10.37 ± 0.02^{c}	4.07 ± 3.45^{d}	3.04 ± 0.72^{d}	ND
P	26.01 ± 0.24^{a}	16.10 ± 0.62^{b}	18.35 ± 1.48^{c}	18.27 ± 0.60^{c}	14.29 ± 0.80^{d}	ND
Fe	7.84 ± 0.11^{a}	5.13 ± 0.16^{b}	2.72 ± 0.02^{c}	2.05 ± 0.37^{c}	2.95 ± 0.50^{c}	R: 1.5 V: 5
Pb	0.92 ± 0.01^{a}	0.65 ± 0.09^{b}	0.68 ± 0.03^{b}	0.54 ± 0.02^{c}	0.16 ± 0.03^{d}	0.1
Cd	0.21 ± 0.0006^{a}	0.17 ± 0.007^{b}	0.12 ± 0.008^{c}	0.03 ± 0.001^{c}	0.05 ± 0.001^{c}	0.05
Ni	1.08 ± 0.01^{a}	0.45 ± 0.06^{b}	0.27 ± 0.007^{c}	0.94 ± 0.02^{a}	0.13 ± 0.01^{d}	0.2
Cu	0.76 ± 0.05^{a}	0.23 ± 0.05^{b}	0.22 ± 0.003^{b}	0.23 ± 0.02^{b}	0.03 ± 0.003^{c}	R: 0.1 V: 0.4
Cr	1.10 ± 0.003^{a}	0.65 ± 0.07^{b}	0.56 ± 0.02^{b}	0.62 ± 0.01^{b}	0.17 ± 0.01^{c}	ND
Co	0.04 ± 0.004^{a}	0.01 ± 0.003^{b}	0.01 ± 0.001^{b}	0.01 ± 0.004^{b}	0.02 ± 0.001^{b}	ND

CSO: crude sesame oil; NSO: neutralized sesame oil; BSO: bleached sesame oil; DSO: deodorized sesame oil; RSO: refined sesame oil; R: refined oil; V: virgin oil; ND: not determined. Different letters in each row shows significant differences at level of $p < 0.05$.

in all heavy metals and minerals during the neutralization stage is attributed to the settling down of the suspended impurities in sesame oil, which is consistent with the results of Rakmi et al. on palm oil [27]. Also, the reduction of phosphorus content of sesame oil samples in the neutralization stage can be due to the use of citric acid or malic acid in the degumming process.

In the bleaching stage, color pigments, traces of soaps, phospholipids, metal ions, and oxidation products could be removed by activated bleaching clay [15]. Although it was shown that bleaching clay could chelate some trace metals [15], in the current study, some cations, including Mg, Ca, Pb, and P, were increased during the bleaching stage. Although the Ca and Pb increases were considered insignificant, this increase may be due to the water used for washing and the adsorbents used in the cleaning process [8]. Also, it is possible that inappropriate removal of magnesium or calcium soap produced during the neutralization stage leads to increased Ca and Mg concentrations in the bleaching stage. The dramatic increase in P of bleached oil could be ascribed to a lack of phospholipids catalyzed which is related to the degumming process [22].

The increase in Ni, Cu, and Cr during the deodorization stage can be explained by metallic equipment contaminations [22]. This increase in Fe content of rapeseed during the deodorization step was described by Szydłowska-Czerniak and Łaszewska [6]. The insignificant increase of Fe, Pb, and Co in refined sesame oil could have resulted from probable contamination of storage conditions and equipment used in the refining process [28].

The Fe and Cu concentrations in sesame oil samples ranged from 2.95 to 7.84 and 0.03 to 0.76 mg/kg, respectively. Except for Cu of refined oil, the Cu and Fe concentrations of samples were reported to be higher than the standard limits set by Codex Alimentarius [20]. The presence of these metals in vegetable oil as prooxidants could trigger catalytic oxidation [28]. Also, it has been seen that essential elements such as Cu, Fe, Mn, and Ni can reduce the oxidation stability of the oil, while Ca, Mg, and Na reduce the efficiency of oil the refining process [8].

4. Conclusion

Vegetable oil refining is considered a critical process to make the oil suitable for human consumption, especially in terms of heavy metals. In recent years, with the establishment of shops that extract sesame oil in place, the importance of investigating about the effects of the refining process has been highlighted. Therefore, in the current study, the effect of different stages of sesame oil refining on antioxidant activity and heavy metal contents was studied. According to the results, there has been a slight change in the fatty acid composition of sesame oil during oil refining. Overall, all oxidation indexes, antioxidant activity, total phenolic content, mineral, and heavy metals were reduced. It was indicated that the deodorization stage had more effective in the reduction of FFA, PV, p-AV, TOTOX, and TPM. Although, the neutralizing stage showed the highest reduction in antioxidant activity (DPPH and FRAP) which is quite similar to the result of total phenolic content, according to the results, the Pb content in sesame oil samples was reduced during the refining process. Although it was shown that the refined oil had a higher Pb concentration than permissible limits, it is important to reduce the Pb concentration of vegetable oils by quality evaluation of the sesame seed before importing, lowering the probable sources of metal contamination, and improving the refining process. Also, the awareness of public attention about the probable hazards of crude sesame oil (without any refining) consumption could be raised.

Abbreviations

CSO: Crude sesame oil
NSO: Neutralized sesame oil

BSO:	Bleached sesame oil
DSO:	Deodorized sesame oil
RSO:	Refined sesame oil
GC-FID:	Gas chromatography–flame ionization detector
PV:	Peroxide value
p-AV:	p-Anisidine value
TOTOX value:	Total oxidation value
TPM:	Total polar material
FFA:	Free fatty acid
TPC:	Total phenolic content
DPPH:	2,2-diphenyl-1-picrylhydrazyl
FRAP:	Ferric reducing antioxidant power.

Data Availability

Data are available on request from the authors.

Conflicts of Interest

All authors declare no conflict of interest.

Authors' Contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Mohammad Sarafraz Ardakani, Fateme Akrami Mohajeri, Elham Khalili Sadrabad, Fatemeh Pourramezani, Elaheh Askari, Gholamali Javdan, and Hossein Fallahzadeh. The first draft of the manuscript was written by Mohammad Sarafraz Ardakani and Elham Khalili Sadrabad. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors are thankful to the Department of Food Hygiene and Safety, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

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