Evaluation of the Physicochemical Properties and Aflatoxin Levels of Industrial and Non-industrial Sesame Oil

Ramezani, Majid
Department of Nutrition, School of Medicine, Mashhad University of Medical Sciences, Mashhad, I.R. IRAN

Varidi, Mehdi
Department of Food Science and Technology, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, I.R. IRAN

Hashemi, Mohammad; Rezaie, Mitra*[+]
Department of Nutrition, School of Medicine, Mashhad University of Medical Sciences, Mashhad, I.R. IRAN

ABSTRACT: *The present study aimed to evaluate the physicochemical properties (refractive index, acid value, peroxide value, conjugated dienes, Fatty acid composition) and level of aflatoxins (AFB1, AFB2, AFG1, and AFG2) in the sesame oils (cold press) consumed in Iran. In total, 30 sesame oil samples were collected from factories (n=20; industrial) and traditional mills (n=10; non-industrial). No significant differences were observed between the industrial and non-industrial sesame oil samples in terms of the physicochemical properties and AF contamination and therefore, it is not possible to prefer the consumption of one of the oils (industrial or non-industrial) to another. According to the physicochemical examination, the mean peroxide value was 2.93±1.59 and 1.95±1.24 meq/kg, the acid value was 0.86±0.82 and 1.12±0.58 mg KOH/g, the refractive index was 1.4,706±0.0002 and 1.4,705±0.0001 at 28°C, and the conjugated diene value was 12.13±3.25 and 10.02±1.43 µmol/g in the industrial and non-industrial sesame oil, respectively. In addition, the fatty acid profile of the industrial and non-industrial sesame oil indicated high levels of unsaturated fatty acids (84.5% and 83.49%, respectively), with the main fatty acids determined to be oleic acid and linoleic acid. The fatty acid profile of the sesame oil samples indicated no adulteration with other vegetable oils. The mean contamination with AFB1, AFB2, and AFG1 in the non-industrial sesame oil was estimated at 0.06±0.26, 0.02±0.67, and 0.15±0.18 µg/kg, while the mean contamination with AFB1, AFB2, and AFG1 was 0.04±0.84, 0.03±0.61, and 0.17±0.16 µg/kg in the industrial sesame oil. Moreover, the AFB1 and AFs levels in all the sesame oil samples were significantly lower than the Iranian legislation limits (5 and 15 µg/kg, respectively) and the European Union (2 and 4 µg/kg, respectively). Risk assessment based on the margin of exposure revealed the risk of AFB1 and AFG1 exposure through industrial and non-industrial sesame oil consumption and AFB2 exposure through industrial sesame oil consumption.*

KEYWORDS: *Sesame oil; Fatty acid profile; Physicochemical properties; Aflatoxin.*

***** *To whom correspondence should be addressed.*
+ *E-mail: RezaeiGM@mums.ac.ir*
1021-9986/2022/10/3363-3375 13/$/6.03

INTRODUCTION

Sesame belongs to the Pedaliaceae family and genus Sesamum, which contains approximately 36 species, 19 of which are vernacular to Africa [1]. It is believed that the sesame plant originates in Africa [2], while many other countries produce and export sesame seeds, such as China, Japan, India, Egypt, Cameroon, Brasilia, Senegal, and Iran [3].

Sesame oil has therapeutic applications and is widely used for pharmaceutical purposes [4]. Sesame oil contains vitamin E and various important antioxidants, such as sesaminol and sesamolinol, which protect the body tissues against oxidizing agents [5]. Furthermore, sesame seeds have been shown to contain gamma-tocopherol, which enhances the beneficial health effects of sesame seeds by significantly elevating the concentration of this bioactive compound in the human serum. In addition, gamma-tocopherol positively influences vitamin E activity and may prevent cancer and cardiovascular diseases [6].

Sesame is gaining importance as a healthy edible oil and a high-quality source of protein for human nutrition. Sesame seeds are mostly used for oil extraction, with the remnants applied for other consumption purposes [7]. The quality and quantity of the oil content of sesame seeds depend on factors such as the climate, soil, maturity of the plant, and cultivars of the seeds [8].

The physicochemical properties of oils are directly correlated with their lipid and glyceride composition. In the chemical composition of sesame, the seed is the main source of oil (50-60%), protein (18-25%), carbohydrates (13.5%), and ash (5%) [9]. Sesame oil contains high levels of unsaturated fatty acids, which are used in the preparation of margarine and cooking oils. Sesame also contains significant amounts of lignans sesamin and sesamolin, which exert beneficial effects on the serum lipid rate and liver activity, rendering sesame seed oil a potent antioxidant [10]. The main fatty acids in sesame oil include palmitic acid (16:0; 7.0-12.0%), stearic acid (18:0; 6.0-3.5%), oleic acid (18:1; 35-50%), and linoleic acid (18:2; 35-50%) [11, 12].

The refractive index of sesame oil causes the oil to remain stable against oxidation with the presence of multiple natural antioxidants, such as sesamol, sesamolin, sesamin, and tocopherols [13]. Mycotoxin contamination is a severe health threat worldwide, and billions of dollars are lost annually due to this issue [15]. The presence and growth of fungi in food may cause spoilage and reduce the quality and quantity of food [14]. The edible oils that are manufactured from diverse sources (e.g., peanuts, sunflower, and sesame seeds) may be exposed to various pollutants during storage or before harvest, which diminishes the quality of the oil [15]. Mycotoxins are secondary metabolites with low molecular weight and are manufactured by fungi. Approximately 300-400 compounds have been recognized as mycotoxins, most of which are potentially hazardous to humans and animals [16].

Aflatoxins (AFs) are among the most toxic mycotoxins [15], which are classified into four categories B1, B2, G1, and G2. AFs are constructed as secondary metabolites by the fungal strains of Aspergillus (*A. flavus* and *A. parasiticus*) [17]. AFs are also known as variant contaminants, which decrease the quality of edible oils and pose numerous health risks to consumers. These compounds enter oils through primary infected feed, such as cereals, groundnuts, coffee, spices, pistachios, and oilseeds resembling sesame. AFB1 has been identified as the most vigorous and naturally occurring hepatocarcinogen, the risk assessment of which is well recognized [18]. AFB1 has been classified as a group I carcinogen for humans by the International Agency for Research on Cancer (IARC) [19].

The potential risk of foodstuff contamination with AFs has increased in recent years. However, data are scarce on the attendance or levels of AFs in the edible oils consumed in Iran. The maximum permissible limits of AFs vary in different countries. In the United States, the Food and Drug Administration (FDA) has set the maximum permissible level of 20 mg/kg for total AFs in foodstuffs [20], while the Scientific Commission of the European Community has set the maximum admissible level of 2 mg/kg for AFB1 [21]. The maximum tolerance levels set by the Institute of Standards and Industrial Research of Iran (ISIRI) for AFB1 and total AFs are 5 and 15 μg/kg, respectively [23]. Therefore, it is essential to evaluate the levels of AFs in edible oils and determine the feasible contamination level that might represent a health hazard.

The present study aimed to evaluate the physicochemical properties and AF levels of the industrial and non-industrial sesame oils consumed in Iran.

EXPERIMENTAL SECTION

Sampling and chemicals

In this study, traditional sesame oil samples (n=20) were purchased from local extraction stores in 13 origins

of Mashhad city, Iran (region division of the Municipality of Mashhad), and industrial sesame oil samples (n=10) were purchased from all the commercial brands of sesame oil available in the hypermarkets of Mashhad. Traditional oil samples were taken from sesame seeds available at local extraction shops and mostly imported from Afghanistan and Pakistan. The collected sesame oil samples were packed in a sterile sampl
.e bag and stored at the temperature of 4°C until analysis.

The reagents and standard stock solutions included ammonium thiocyanate, 95% ethanol, hexane, chloroform, 96% methanol, hydrogen chloride, acetonitrile, methanol, sodium chloride, and HPLC-grade water, which were obtained from Merck Chemicals (Darmstadt, Germany). In addition, they were of analytical grade purity, and deionized water was also used for the preparation of the reagents. Aflatoxins B1, B2, G1, and G2 were purchased from Sigma (St. Louis, MO, USA). The laboratory ware (pipette tips and glass tubes) was washed with a detergent solution and rinsed with water before drying in the oven.

Analytical Methods

Physical and chemical properties of sesame oil

Refractive Index (RI): The refractive index (RI) was measured using a refractometer (ATAGO RX-5000) based on the AOCS official method Cc-25 at 28°C, and a few drops of the sample were poured on the prism. The prisms were closed and left standing for 1-2 minutes. Following that, the instrument was adjusted, and lighting was provided to obtain the most distinct reading and determine the RI. The prism was cleaned between the readings by wiping off the oil with a cotton pad moistened with ethyl alcohol and let dry [22].

Peroxide Value (PV): The International Dairy Federation (IDF) standard method 74A:1991 was used to determine the peroxide value (PV) of all the sesame oil samples. To determine the peroxide value, the sample (0.01-0.30 g) was mixed in a glass tube with 9.8 mL chloroform-methanol (7 + 3, v/v) on a vortex mixer for 2-4 s. Ammonium thiocyanate solution (50 µL) was added, and the sample was mixed on a vortex mixer for 2-4 s. Then, 50 (µL iron (II) solution was added, and the sample was mixed on a vortex mixer for 2-4 s. After a 5 min incubation at room temperature, the absorbance of the sample was determined at 500 nm against a blank that contained all the reagents except the sample by using a spectrophotometer. The PV was expressed as the milliequivalents of peroxide per kilogram of the sample and calculated using the following equation [23]:

$$\mathrm{PV} = \frac{(\mathrm{As} - \mathrm{Ab}) \times \mathrm{m}}{55.84 \times \mathrm{w} \times 2} \quad (1)$$

Where, As= Absorbance of the sample; Ab= Absorbance of the blank; m= Slope obtained by standard curve with iron (III) chloride standard solution (in this experiment, m was 0.023); w= Mass in grams of the sample, and 55.84= Atomic weight of iron.

Conjugated Dienes: Conjugated dienes were measured spectrophotometrically (*Cecil* 9005) at 234 nanometers. To do so, the oil samples were diluted with hexane at the ratio of 600:1 [24].

Acidic Value (AV): AOCS methods (AOCS, 2003) were applied for the designation of the acid value (AV), and the results were expressed as mg KOH/g oil. Approximately five grams of the samples was weighed accurately into a 500-milliliter conical flask, and 50 milliliters was added to 100 milliliters of freshly neutralized hot ethyl alcohol, as well as one milliliter of the phenolphthalein indicator solution. After proper shaking, the mixture was gently refluxed if necessary until the substance completely dissolved. In addition, the solution was titrated with sodium hydroxide titrant until pink coloration was observed, persisting for 30 seconds. Finally, the volume of the applied sodium hydroxide titrant was measured, and the AV was calculated using the following equation [25]:

$$\mathrm{AV} = \frac{56.1 \times \mathrm{N} \times \mathrm{V}}{\mathrm{W}} \quad (2)$$

Where *V NaOH* is the volume of the sodium hydroxide titrant (mL), *W* shows the weight of the sample (g), and *N* is the normality of the sodium hydroxide solution.

Fatty acid composition of sesame seed oil: In the present study, the fatty acid composition was determined using the method proposed by *William W. Christie* (1993). Initially, fatty acids were converted into fatty acid methyl esters by shaking a solution containing 0.1 milligram of oil and 1100 microliters of hexane with 100 microliters of methanolic KOH (2 N), followed by analysis via gas chromatography using a Varian 450 (Varian Inc.). The chromatograph was equipped with a flame ionization detector. The utilized column was a CP-Wax 52CB column (30 m×0.25 mm id, 0.2 µm film thickness,

Varian Inc., Middelburg, the Netherlands). The carrier gas was hydrogen, and the total gas flow rate was 1 mL/min. The initial injector temperature was 75°C and the initial auxiliary temperature was 240°C, which was increased by steps of 7°C/min. In addition, the temperature of the injector and detector was 230°C. The obtained results were expressed as the relative percentage of each fatty acid found in the samples [26].

Determination of AFs: The modified AOAC method (970:40) described by *Idris et al*. (1990) and Souleymane *Zio et al*. (2019) [17, 27] was applied for the separation and quantification of the AFs, and Analysis and Purification of AF by High-Performance Liquid Chromatography (HPLC) using Immunoaffinity columns (Neogen Europe, Ayr, UK) and an HPLC apparatus. HPLC analyses were accomplished on a Sykam HPLC system (Eresing, Germany) consisting of an S2100 pump, S7131 reagent organizer, S4011 column thermo controller, S1122 secondary pump, RF-10Axl fluorescence detector, and a Genesis RP C18 analytical reversed-phase column (250×4.6 mm; 4 μm).

Fluorescence detector wavelengths were set at the excitation of 365 and emission of 435 nanometers, respectively. The mobile phase included water, methanol and acetonitrile (4:1:1) and set to run at the flow rate of 1.0 mL/min. The standard stock solutions of AFs (100 μg/L) were produced in benzene-acetonitrile (98:2 v/v) or methanol-water solutions (40:60 v/v), packed in aluminum foil to hamper the gradual breakdown of the AFs, and maintained at a preserved status and temperature of -20°C. The limit of detection (LOD) was also set at the lowest calibration standard of 0.025, 0.05, 0.05, and 0.075 μg/L for AFB1, AFB2, AFG1, and AFG2, respectively. The AF concentrations in the extracted samples were determined and quantified based on the retention time and peak areas, respectively.

A calibration curve (Table 1) was prepared by using the standard solutions at 0.4, 1.2, 2, and 3.6 ng/mL for AFB1and AFG1, and 0.08, 0.24, 0.4 and 0.72 ng/mL for AFB2 and AFG2. The calibration curve was constructed before the analysis of the samples, the linear regression equations were used for AFB1, AFB2, AFG1, and AFG2 measurements in sesame oil samples.

Statistical analysis

Data analysis was performed in SPSS version 16.0 (Statistical Package of Social Science), and all the chemical analyses were carried out in triplicate. Data were expressed as mean and standard deviation and then Compare Means followed by Independent Samples t-tests. In addition, the industrial and non-industrial sesame oil samples were compared at the significance level of $P<0.05$.

Table 1: Calibration curve plotted for the aflatoxins.

Toxin	Equation of calibration curve	R^2	RSD
AFB1	Y = 251.0089X + 12.68256	0.999	16.81152
AFB2	Y = 642.5432X + 6.72606	0.998	10.36703
AFG1	Y = 71.48837X + 1.57537	0.993	13.10190
AFG2	Y = 211.05586X + 9.31430	0.997	4.70106

R^2= linear correlation coefficient; RSD= residual standard deviation for each

RESULTS AND DISCUSSION

Physical and chemical properties of industrial and non-industrial sesame oil

Quality parameters were regularly applied to measure the physical and chemical properties of the edible oils, and the investigated physicochemical parameters included the PV, AV, conjugated dienes, and RI of the industrial and non-industrial sesame oils (Table 2).

Peroxide Value (PV)

The oxidative state of vegetable oils could be evaluated by analyzing the PV of the oil. The presence of air and metals (e.g., iron and copper) increases the content of peroxide by fatty acid autoxidation. Furthermore, high contents of unsaturated fatty acids in oils and fats have been reported to elevate the hazard of oxidation and free radical production. Free radicals are not only responsible for food corruption, but they also trigger various human diseases, such as cancer, inflammatory disorders, atherosclerosis, tissue injury, and senility [28].

Lipid oxidation plays a key role in food spoilage. The reaction of oxidized fats with proteins and carbohydrates causes significant chemical changes in food, and their oxidation also causes odor, partial/full vitamin deficiency, and other nutritional deficiencies through a chemical intermediary in the variant phase of oxidation [29]. In the present study, the PV of the traditional sesame oil samples was within the range of 0.5209-4.3173 meq/kg oil, while it was estimated at range of 0.9497-5.8473 meq/kg

in the industrial sesame oil samples. In addition, the mean PV was determined to be 1.95±1.124 and 2.93±2.53 mg/kg of oil in the traditional and industrial sesame oil samples, respectively. However, no significant difference was observed in terms of the PV between the industrial and non-industrial sesame oils ($P<0.05$).

Our findings in this regard are consistent with the values reported by *Gadade B. et al.* (2017), which were estimated at 5.55 meq/kg of oil [30]. Furthermore, Ogbonna P. et al. (1997) reported similar PV in the seeds of sesame accessions grown in the Nsukka Plains of South Eastern Nigeria (3.95 meq/kg of oil) [31]. According to the International Codex and the Iranian National Standardization Organization (INSO) No. 13392, the maximum permissible level of PV in oil samples is 15 meq/kg of oil [32, 33]. In the present study, the PV of the oil samples was significantly lower than the standard permissible limit (Fig. 1). Low rancidity indicates high resistance to peroxidation and longer storage life. Moreover, the presence of lignin and tocopherols (natural antioxidants) in sesame seeds has been reported to induce resistance to oxidation, which is a significant index of the constant quality of edible oils.

According to the findings of the current research, the peroxide index of the industrial sesame oil samples was relatively higher compared to the non-industrial sesame oil samples, which could be due to the longer shelf life of the industrial sesame oil samples than the non-industrial samples, which led to the exposure of the samples to light and heat for a longer period. It is also possible that the interval between the oil extraction and packaging of the industrial oil samples was longer than the non-industrial oil samples, which in turn caused the oil to be exposed to ambient oxygen for a longer period and gave rise to oil oxidation. Genotype and growing environment affected the oil quality of sesame seeds. Similarly, *Isam et al.* (2020) reported that the peroxide value varied between Turkey and Sudanese sesame genotypes, and *Ahmed I.A. et al.* (2021) showed that sesame oil from the seeds obtained from a hot dry area like Sudan had a low peroxide value while that obtained from hot, humid conditions, as in Nigeria, possessed a high peroxide value [34, 35]. In addition, *Tenyang et al.* (2017) reported that the peroxide value varied between white and brown sesame seeds [36]. On the other hand, the increased temperature of the apparatus during the oil extraction process may increase the oxidation process of oil. Peroxides are not constant compounds and can show an increase or decrease with the effect of applied temperature. The increase in the peroxide value during the heating process may be due to the accumulation of hydroperoxides that resulted from peroxidation of unsaturated fatty acids by free radicals [34]. Another cause of the higher peroxide content of industrial oil samples than the non-industrial oil samples in the present study could be a purification step in the industrial oil samples, which reduced antioxidants.

Acidic Value (AV)

AV is an indicator of the percentage of free fatty acids in the oil, which expands with the increased oil storage period. Free fatty acid and AV are often evaluated as the rancidity criterion in the quality assessment of oil during storage and heating and increased free fatty acid and AV have been shown to reduce food shelf life during storage.

In the present study, the mean AV of the industrial and non-industrial sesame seed oil samples was estimated at 0.86±0.82 and 1.12±0.58 mg KOH/g of oil, respectively. No significant difference was observed in the AV of the industrial and non-industrial sesame oils. The AV of the industrial sesame oil samples was within the range of 0.22-2.50 mg KOH/g of oil, while it was 0.38-2.20 mg KOH/g of oil in the non-industrial sesame oil samples.

The optimum AV and maximum acceptable AV proposed by FAO are reported to be 2 and 6 mg KOH/g of oil, respectively [37]. The maximum acceptable limit set by INSO No. 8636 and 13392 has been set at 3.5 and 4 mg KOH/g of oil (32, 38). Our findings in this regard are consistent with the recommended values by the FAO and INSO (Fig. 1), as well as the results obtained by *Olasunkanmi G. S. et al.* (2107) and *Ogbonna P. et al.* (2013) [31, 39]. A high AV mostly signals the potent enzymatic hydrolysis of sesame seed during harvesting, handling, or oil processing.

Refractive Index (RI)

The RI of oil is correlated with the molecular structure and degree of unsaturation. As the unsaturation degree increases, the RI increases as well [40]. The refractive index varies with variation in the wavelength, temperature, degree and type of unsaturation, the type of succession of component fatty acid. The refractive index is widely used in evaluating quality control, to survey the purity

Table 2. Chemical characteristics of Industrial sesame oils (Ins) and non-industrial sesame oils (n-Ins)

Oil	N	Peroxide value (meq /Kg)	Acid value (mg KOH/g)	Refractive index (at 28o C)	conjugated diene value (µmol/g)
Non-industrial	20	1.9574±1.24[a]	1.1229±0.58[a]	1.4705±0.0001[a]	10.0241±1.43[a]
Industrial	10	2.9313±1.59[a]	0.8645±0.82[a]	1.4706±0.0002[a]	12.1352±3.25[a]

[a] *In column = No significant difference between Industrial and non-industrial sesame oils*

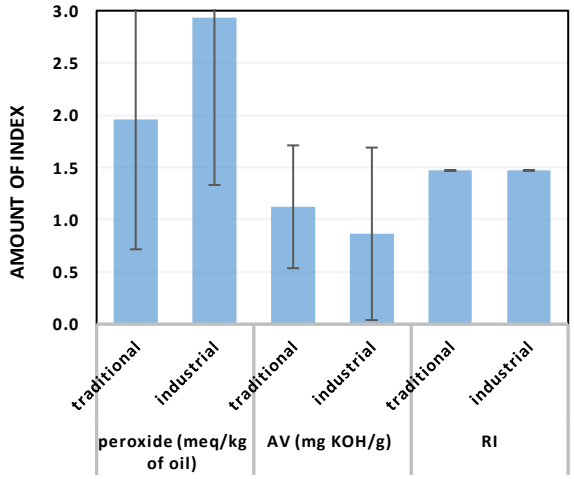

Fig. 1: Comparison of chemical properties with international and national standards. a = Results are consistent with the recommended values by the FAO and INSO

of materials and to follow hydrogenation [41]. Refractive index increases with the increase in the amount of conjugated fatty acids while peroxide values vary with the concentration of primary oxidation products. Refractive index increases with increase in oxidation same as peroxide value [42]. In the current research, the mean RI was estimated at 1.4706±0.0001 at the temperature of 28° C and 1.4705±0.0002 at 28° C in the industrial and non-industrial sesame oil samples, respectively. No significant difference was observed between the industrial and non-industrial sesame oil samples. The observations of the RI of sesame oil in the present study are consistent with the study by *Gharby S. et al.* (2012), which indicated the value to be 1.472 [10], as well as the results was close to 1.471obtained by *Al-Bachir M.et al* (2019) [43]. Furthermore, our findings regarding the RI are in line with the International Codex and INSO No.13392 (1.465-1.469) (Fig. 1) [32, 33].

Conjugated dienes

The presence of conjugated dienes in oils indicates the occurrence of oil oxidation. For the formation of conjugated dienes, fat and oil samples containing unsaturated fatty acids with a minimum of two double bands are required. Linoleic acid is considered to be the most important fatty acid in this group of foods. The oxidation reaction is the most common oil spoilage, which occurs due to the reaction of unsaturated fatty acids with oxygen. Right away after the formation of peroxides as primary oxidation products, rearrangement of non-conjugated double bonds in unsaturated lipids occurs, and conjugated compounds are formed, which absorb UV at 232 nm. In oil oxidation, the increased PV increases conjugated dienes (intermediate oxidation products) [44].

According to the results of the present study, the mean conjugated diene value of the industrial and non-industrial sesame oil samples was 12.13±3.25 and 10.024±1.43 µmol/g, respectively. Similar results were observed by *Sadeghi E et al.* (2019) [45]. No significant difference was observed in terms of the conjugated diene value between the industrial and non-industrial sesame oil samples. Notably, the conjugated diene value was slightly higher in the industrial sesame oil samples compared to the non-industrial, which could be due to the fact that the PV of the industrial sesame oil samples was also slightly higher than the non-industrial samples.

Fatty acid composition of sesame oil

Fatty Acid (FA) composition is an essential indicator of the nutritional value of oils, as well as the persuasive evidence that the consumption of vegetable oils has beneficial effects on human health mainly owing to their FA composition, in which unsaturated FAs are prominent [46, 47]. The FA composition of oils may largely differ depending on the region of origin and plant variety. The fatty acid composition of oils plays a significant task in determining the shelf life, flavor, and nutrition of food products (48). Therefore, determining the FA profile could provide a reliable index of product authenticity. Table 3 shows the detailed FA composition of the examined sesame oils in the present study. Accordingly, the examined oils

had extremely low amounts of Saturated Fatty Acids (SFAs) (14.52% of industrial samples and 15.3% of non-industrial samples). According to our findings, the level of unsaturated FAs was significantly higher and estimated at 83.49% in the non-industrial sesame oil samples and 84.5% in the industrial sesame oil samples. The unsaturated FAs in the non-industrial sesame oils were composed of 41.977% monounsaturated FAs and 41.52% polyunsaturated FAs. The industrial sesame oil contents were also composed of 41.65% monounsaturated FAs and 42.85% polyunsaturated FAs.

In the present study, palmitic acid was the major SFA in the industrial and non-industrial sesame oil samples. On the other hand, Gas chromatographic analysis showed that unsaturated fatty acids present in industrian sesame oil samples were mainly linoleic (42.85%) and oleic (61.64 %), although the contents of linoleic and oleic acid of non-industrial sesame oil samples were 41.52% and 41.96% respectively. The main components of the FA composition of the sesame oil samples in the current research were palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), and linolenic acid (C18:3), which had no significant differences between the industrial and non-industrial sesame oil samples. A sample chromatogram of fatty acids composition in sesame oil is given in Fig. 2.

A slight difference was observed in the FA composition of the sesame oil samples in the current research, which could be due to different cultivars of the sesame seeds. Previous studies have demonstrated that some agricultural parameters could change the FA composition of sesame seed oil [9, 31, 48, 49], the most important of which are cultivar and origin, fruit ripening, harvest period, and climatic conditions. In the present study, the minor FA components of the sesame oil samples, including linolenic acid (C18:3), eicosanoic acid (C20:2), and arachidic acid (C20:0), had no significant differences in the two groups of samples.

Our findings indicated that the FA composition of the sesame oil samples was within the range of the values reported by *Ahmed IA et al.* (2020), *Faez Mohammed et al.* (2018), *Olasunkanmi et al.* (2017), *Borchani et al.* (2010), *and Rahman M.A. et al.* (2007) (8, 13, 39, 48, 50]. Since the examined oil samples contained more oleic and linoleic acids (83.49-84.5% of total FAs), the sesame oil samples could be classified into the oleic and linoleic acid groups. This is consistent with the findings of *Olasunkanmi et al.* (2017). Compared to the national and international Codex standards of sesame oil, our findings regarding the FA composition of sesame oil were in compliance with the recommended ranges of the Codex standards and the Iranian national standards for sesame oil No. 13392. Therefore, the originality of the oils was confirmed, and the likelihood of adulteration with other oils was very low.

AF determination

In the present study, the AF levels were determined using the method described earlier (27). Table 4 shows the AF contamination of the industrial and non-industrial sesame oil samples. For AFB1, AFB2, AFG1 and AFG2, the Limits of Detection (LODs) 0.025, 0.05, 0.05, and 0.075 µg/kg, and the Limits of Quantification (LOQs) were 0.1, 0.05, 0.1 and 0.05 µg/kg, respectively. Relevant data concerning the analytical system for AFs are summarised in Table 5. Among 20 non-industrial sesame oil samples, 13 cases were contaminated with total AFs (65% of total incidence), with the total AF concentration estimated at 0.1-1.2 mg/kg (B1, B2, G1, and no G2). Out of 10 industrial sesame oil samples, seven cases (70% of total incidence) were contaminated with AFs (B1, B2, G1, and no G2), with the range of contamination estimated at 0.1-0.7 mg/kg.

According to the results of the present study, the highest AFB1 contamination was detected in the non-industrial sesame oils, while the lowest rate was observed in the industrial sesame oils. The relatively low levels of AFs in the industrial sesame oil samples compared to the non-industrial samples could be attributed to the purification process of industrial oils. On the other hand, AFB1, AFB2, and AFG1 were detected in one, two, and 12 non-industrial sesame oil samples, demonstrating 5%, 10%, and 60% of the total non-industrial samples in the research, respectively. In addition, only two, two, and seven out of 10 industrial oil samples (20%, 20%, and 70% of total industrial samples) were contaminated with AFB1, AFB2 and AFG1.

In the current research, AFG1 was most frequently detected in the sesame oil samples, while AFG2 was detected in none of the examined sesame oil samples. The mean contamination level with AFB1, AFB2, and AFG1 in the non-industrial sesame oil samples was estimated at 0.06±0.26, 0.02±0.67, and 0.15±0.18 µg/kg, respectively, while these values were determined to be 0.04±0.84,

Table 3: Percentage of fatty acid components of non-industrial (n-Ins) and Industrial (Ins) sesame oils.

Fatty Acid	Composition (%)				Standard Ranges	
	Industrial sesame oil		Non-industrial sesame oil		INSO 13392B	Codex
	Mean±SD	Range	Mean±SD	Range		
Palmitic acid, C16:0 A	9.15±0.5	8.19-9.61	9.26±0.56	8.23-10.11	7.9-12.0	7.9-12.0
Stearic acid, C18:0 A	5.35±0.18	5.13-5.64	5.49±0.26	5.05-6	4.5-6.7	4.5-6.7
Oleic acid, C18:1 A	41.64±1.58	39.59-43.64	41.96±1.51	39.79-44.58	34.4-45.5	34.4-45.5
Linoleic acid, C18:2 A	42.85±1.59	40.74-45.38	41.52±1.37	39.14-43.72	36.9-47.9	36.9-47.9
linolenic acid (C18:3) A	0.08±0.13	0.00-0.29	0.05±0.11	0.00-0.31	0.2-1.0	0.2-1.0
arachidic acid (C20:0) A	0.4±0.41	0.31-0.53	0.5±0.5	0.00-0.67	0.3-0.7	0.3-0.7
eicosanoic acid (c20:2) A	0.16±0.22	0.00-0.6	0.27±0.22	0.00-0.42	0.3-0.7	0.3-0.7
Saturated fatty acid	14.53	-	15.3	-	-	-
Unsaturated fatty acid	84.5	-	83.49	-	-	-

A: No significant difference between fatty acid profiles of Industrial and non-industrial sesame oils (P>0.05).

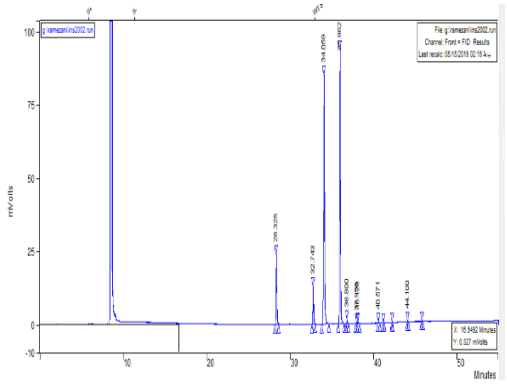

Fig. 2: chromatogram of the fatty acids profile in sesame oil.

0.03±0.61, and 0.17±0.16 µg/kg in the industrial sesame oil samples, respectively. However, no significant differences were observed between AF contamination with AFB1, AFB2, AFG1, and AFG2 between the industrial and non-industrial sesame oil samples.

In a similar study conducted by *Elzupir et al.* (2010) in Khartoum State in Sudan, the AF contamination of edible oils was reported to be lower than the mean level of 187.6 µg/kg of total AFs and the mean levels of 43.6, 0.3, 47.5, and 102.7 µg/kg of AFB1, AFB2, AFG1, and AFG2 [51]. Furthermore, Idris et al. (2010) reported the contamination level of AFB1 in sesame oil samples to be 0.2-0.8 µg/kg, observing no contamination with AFB2, AFG1, and AFG2 in their oil samples.

The Scientific Commission of the European Community has set the maximum permissible levels of 2 and 4 µg/kg for AFB1 and total AFs. The Iranian national Standard No. 5925 has set the legal limit for AFB1 and total AFs in food at 5 and 15 µg/kg, respectively(52). Compared to the rigorous legal levels of the EU and Iranian government, the contamination level of the sesame oil samples in the present study was significantly lower than the legal limits (Fig. 3). High levels of AFs in vegetable oils are mainly due to the use of low-quality oil seeds, which have commonly been stored over prolonged periods at high temperatures and humidity. Furthermore, the management of favorable farming conditions has been emphasized to prevent the appearance of fungi in crops. The training of farmers, reducing moisture, and controlling the storage temperature have also been recommended for the growth control of fungi and prevention of pest damage, while the proper ventilation of the warehouse and sanitary conditions could generally contribute to the improvement of crop storage.

The current status of the mycotoxin contamination of sesame oil in Iran is not considered hazardous to public health. Nonetheless, the frequency of the positive samples in our study highlighted the need for further routine monitoring as an effective food quality control measure. Considering the transfer of AFs from sesame seeds to the oil, the sesame seeds that are to be used for food purposes should be monitored continuously for AF contamination, while the storage conditions should be stringently controlled, and widespread surveillance programs should be initiated. Since the sesame seeds consumed in Iran are mostly imported from Afghanistan and Pakistan, trade barriers should also be imposed on the maximum residue levels at the entry points.

Table 4: Prevalence and levels (µg/kg) of AFs in sesame oil samples (n: total number of samples analyzed, nc: number of contaminated samples).

Toxin	Industrial sesame oil				Non-industrial sesame oil			
	N	nc	Range	mean±SD[A]	n	Nc	Range	mean±SD[A]
AFB1	10	2	0-0.2	0.04±0.843	20	1	0-1.2	0.06±0.268
AFB2		2	0.1-0.2	0.03±0.615		2	0.00-0.2	0.02±0.674
AFG1		7	0.1-0.6	0.17±0.160		12	0.1-0.5	0.15±0.182
AFG2		0	0.00 [n.d]	0.00		0	0 [n.d]	0.00
AFs		7	0.1-0.7	0.24±0.254		13	0.1-1.2	0.23±0.293

[A] *No significant difference between Industrial and Non-industrial sesame oil samples (P>0.05)*

[n.d] *= not detected*

Table 5: Validation of aflatoxin determination in sesame oil by HPLC analysis.

Toxin	Retention time (min)	Limit of detection (µg/kg)	Limit of quantitation (µg/kg)	Recovery (%)
AFB1	13.46	0.025	0.1	84.5
AFB2	11.21	0.05	0.05	87.6
AFG1	10.07	0.05	0.1	81.6
AFG2	8.55	0.075	0.05	80.4

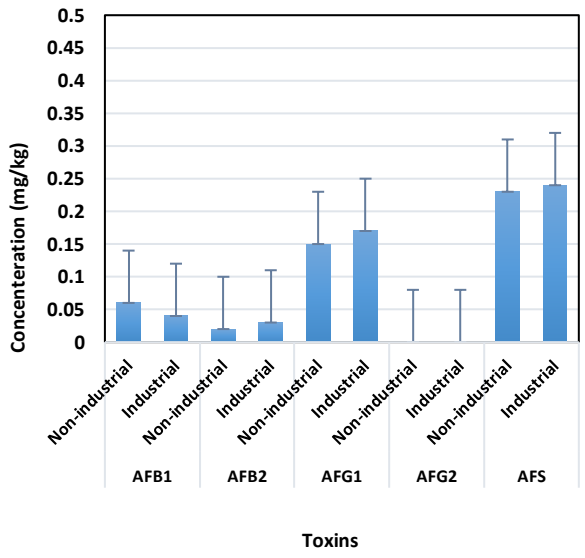

Fig. 3: Comparison of aflatoxins with national and international standards. a = the contamination level of the sesame oil samples was significantly lower than the legal limits.

Health risk assessment

In the present study, the carcinogenic risk of AFs was estimated based on the margin of exposure (MOE) by dividing the benchmark dose lower limit (BMDL) of the AFs by the chronic daily intake (CDI). Initially, the CDI (µg/kg /day) of the AFs was calculated using Equation 3 to estimate the MOE [53, 54].

$$\mathrm{CDI} = (\mathrm{C} \times \mathrm{IRd} \times \mathrm{EF} \times \mathrm{ED}) / \mathrm{BW} \times \mathrm{AT} \qquad (3)$$

In the equation above, *C* is the AF concentration (µg/kg), *IRd* shows the daily intake of edible oil (46 g/day) [55], *ED* is the exposure duration (70 for adults), *EF* represents the frequency of exposure (365 days/year) [54, 56], *BW* is the mean body weight (70 kg for adults) [54, 57], and *AT* (365 days/y×EDi) is the mean exposure time (25,550 days for adults) [54].

Since AFs are potent liver carcinogens, there is no tolerable daily intake (TDI) for AFs; in other words, no safe level could be established as AFs may induce cancer even at extremely low doses [58]. Therefore, most agencies, including the Joint Expert Committee on Food Additives and the US FDA, have not set a specific TDI for AFs. A common method used for the assessment of carcinogenic and genotoxic mycotoxins is the estimation of the MOE [58, 59], and the ratio of BMDL10 and CDI could be considered as the MOE. In the present study, the MOE was calculated using Equation 4 [60], as follows:

$$\mathrm{MOE} = \frac{\mathrm{BMDL}}{\mathrm{CDI}} \qquad (4)$$

Table 6: The probable daily intakes of AFB1, AFB2 and AFG1 from sesame oil.

Oil groups	Mean concentration (µg/kg)			CDI (µg/ kg^{-1} day^{-1})			MOE[a]		
	AFB1	AFB2	AFG1	AFB1	AFB2	AFG1	AFB1	AFB2	AFG1
Industrial	0.04	0.03	0.17	0.026	0.019	0.112	6538	8947	1517
Non-Industrial	0.06	0.02	0.15	0.04	0.013	0.098	4250	>10000	1734

[a]*MOE, Margin of exposure which is calculated as a ratio of benchmark dose lower limit 10% lower bound of AFB1 (170 ng/kg bw/day)*

where *BMDL* is equal to 170 ng/kg^{-1} BW/day^{-1} for the AFs as recommended (58), and *CDI* is the chronic daily intake (µg/kg BW). According to EPA 2010, the exposed population is at a safe range with the MOE value of higher than 10,000 (60).

Table 6 shows the CDI of AFB1, AFB2, and AFG1 *via* the consumption of sesame oil. By using the data on the contamination level and daily intake of edible oils in Iran in the present study, the mean dietary exposure to AFB1, AFB2, and AFG1 through the consumption of industrial sesame oil was estimated at 0.026, 0.019, and 0.0112 µg/kg BW/day, respectively. On the other hand, dietary exposure to AFB1, AFB2, and AFG1 via the consumption of non-industrial sesame oil was determined to be 0.04, 0.013, and 0.098 µg/kg BW/day, respectively. In accordance with EPA 2010, the exposed population is at a secure limit when the MOE value is higher than 10,000. Table 4 shows the MOE in the presence of AFs due to the ingestion of industrial and non-industrial sesame oil.

In line with the mentioned risk estimation, the MOEs of AFB1 and AFG1 of the industrial and non-industrial sesame oil samples in the current research were lower than 10,000, while the MOE value of AFB2 was higher than 10,000 via the consumption of non-industrial sesame oil. Moreover, risk assessment based on the MOE revealed that AFB1 and AFG1 exposure through the consumption of industrial and non-industrial sesame oil and AFB2 exposure through the consumption of industrial sesame oil was significant, which raised concerns in this regard.

CONCLUSIONS

In this study, various properties of industrial and non-industrial sesame oil were evaluated, and no significant differences were observed in the physicochemical properties of the samples, as well as the concentrations of AFB1, AFB2, AFG1, and AFG2 between the industrial and non-industrial sesame oil samples. In all the sesame oil samples, the AFB1 and total AF levels were significantly lower than the permissible limits in Iran (5 and 15 µg/kg, respectively) and the EU (2 and 4 µg/kg, respectively). A low MOE indicates a higher risk than a high MOE. According to the results, the MOE values of AFB1 and AFG1 in the industrial and non-industrial sesame oil samples were lower than 10,000.

In Iran, the consumption of sesame oil may pose the risk of exposure to AFB1 and AFG1 on the consumers. Therefore, the sesame oils produced in traditional mills do not differ in terms of consumption with industrial sesame oils. Regarding AFs although legislation and supervision remain essential in order to ensure the health of the consumers. Effective methods are required to guarantee the safety of consumers against the toxic effects of AFs and maintain public health. For instance, good agricultural practices are aimed at the diminution of fungal growth throughout the processing stages in farms and during storage, as well as the improvement of the quality of the entire process.

Acknowledgements

The Food and Drug Department of Mashhad University of Medical Sciences supported this research financially.

Received : Dec. 11, 2021 ; Accepted : Apr. 4, 2022

REFERENCES

[1] Bankole M., Comparative Studies of the Crude Extracts of Sesame Against Some Common Pathogenic Microorganisms, *Sci. Res. Essays*, **4(6)**: 584-589 (2009).

[2] Ram R., Catlin D., Romero J., Cowley C., Sesame: New Approaches for Crop Improvement, "Advances in New Crops Timber Press", Portland, 225-228 (1990).

[3] Borchani C., Besbes S., Blecker C., Attia H., Chemical Characteristics and Oxidative Stability of Sesame Seed, Sesame Paste, and Olive Oils, *J. Agric. Sci. Technol.*, **12**: 585-596 (2010).

[4] Bedigian D., History and Lore of Sesame in Southwest AsiaL'Histoire Et Le Savoir Du Sésame Dans Le SudOuest Asia, *Economic Botany*, **58(3)**: 329-353 (2004).

[5] Morris J.B., Food, Industrial, Nutraceutical, and Pharmaceutical Uses of Sesame Genetic Resources. "Trends in New Crops and New Uses", 153-156 (2002).

[6] Cooney R.V., Custer L.J., Okinaka L., Franke A.A., Effects of Dietary Sesame Seeds on Plasma Tocopherol Levels, *Nutr Cancer.*, **39(1)**: 66-71 (2001).

[7] Abou-Gharbia H.A., Shehata A.A.Y., Shahidi F., Effect of Processing on Oxidative Stability and Lipid Classes of Sesame Oil, *Food Res. Int.*, **33(5)**: 331-340 (2000).

[8] Mohammed F., Abdulwali N., Guillaume D., Tenyang N., Ponka R., Al-Gadabi K., Bchitou R., Abdullah A.H., Mohammed Naji Kh., Chemical Composition and Mineralogical Residence of Sesame Oil from Plants Grown in Different Yemeni Environments, *Microchem. J.*, **140**: 269-277 (2018).

[9] El Khier M.K.S., Ishag K.E.A., Yagoub A., Chemical Composition and Oil Characteristics of Sesame Seed Cultivars Grown in Sudan, *Res. J.* Agric. *& Biol. Sci.*, **4(6)**: 761-766 (2008).

[10] Gharby S., Harhar H., Bouzoubaa Z., Asdadi A., El Yadini A., Charrouf Z., Chemical Characterization and Oxidative Stability of Seeds And Oil of Sesame Grown in Morocco, *J. Saudi Soc. Agric.*, **16(2)**: 105-111 (2017).

[11] Hilali M., Charrouf Z., Aziz Soulhi A.E., Hachimi L., Guillaume D., Influence of Origin and Extraction Method on Argan Oil Physico-Chemical Characteristics and Composition, *J. Agric. Food Chem. J.*, **53(6)**: 2081-2087 (2005).

[12] Diwakar B.T., Dutta P.K., Lokesh B.R., Naidu KA., Physicochemical Properties of Garden Cress (Lepidium sativum L.) Seed Oil, *Journal of the American Oil Chemists' Society*, **87(5)**: 539-548 (2010).

[13] Rahman M., Hossain M., Ahmed G., Uddin M., Studies on the Characterization, Lipids and Glyceride Compositions of Sesame (Sesamum indicum linn.) Seed Oil, *Bangladesh J. Sci. Ind. Res.*, **42(1)**: 67-74 (2007).

[14] Moricz A.M., Fater Z., Otta K.H., Tyihak E., Mincsovics E., Overpressured Layer Chromatographic Determination Of Aflatoxin B1, B2, G1 and G2 in Red Paprika, *Microchemical Journal.*, **85(1)**: 140-144 (2007).

[15] Mohammed S., Munissi J.J.E., Nyandoro S.S., Aflatoxins in Sunflower Seeds and Unrefined Sunflower Oils From Singida, Tanzania, *Food Addit Contam: Part B.*, **11(3)**: 161-166 (2018).

[16] Rai MK, Bonde SR, Ingle AP, Gade AK., Mycotoxin: Rapid Detection, Differentiation and Safety, *J. Adv. Pharm. Educ. Res.*, **3(1)**: (2012).

[17] Magzoub R., Yassin A., Abdel-Rahim A., Gubartallah E., Miskam M., Saad B., Sabar S., Photocatalytic Detoxification of Aflatoxins in Sudanese Peanut Oil Using Immobilized Titanium Dioxide, *Food Control.*, **95**: 206-214 (2019).

[18] Sweeney M.J., Dobson A.D., Mycotoxin Production by Aspergillus, Fusarium and Penicillium Species, *Int. J. Food Microbiol.*, **43(3)**:141-158 (1998).

[19] Anttila A., Bhat R.V., Bond J.A., Borghoff S.J., Bosch F.X., Carlson G.P., et al. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 82 (2002).

[20] Wu F., Mycotoxin Risk Assessment for the Purpose of Setting International Regulatory Standards, ACS Publications; (2004).

[21] Elbashir A.A., Ali S.E.A., Mycotoxin Risk Assessment for the Purpose of Setting International Regulatory Standards, *Food Addit Contam: Part B.*, **7(2)**: 135-140 (2014).

[22] Society A.O.C., Firestone D., Official Methods and Recommended Practices of the American Oil Chemists' Society: AOCS press; (1994).

[23] International IDF Standards International Dairy Federation., IDF-Square Vergote 41. Brussels SA (1991).

[24] Saguy I., Shani A., Weinberg P., Garti N., Utilization of Jojoba Oil for Deep-Fat Frying of Foods, *LWT-Food Science and Technology.*, **29(5-6)**:573-577 (1996).

[25] Society AOC. Official Methods and recommended Practices of the American Oil Chemists of Analysis: 7 th ed., AOCS-Pr.Champaign IL; (2020).
[26] Christie WW., Preparation of Ester Derivatives of Fatty Acids for Chromatographic Analysis, *Advances in Lipid Methodology.*, **2(69)**: e111 (1993).
[27] Zio S., Dembele I., Bazié R.S.B., Tankoano A., Tapsoba F., Hama-Ba F., et al. Aflatoxins and Moisture Levels in Edible Oils Produced in Burkina Faso, *E European J. Nutr. Food Saf.*, 1-12 (2020).
[28] Gotoh N., Wada S., The Importance of Peroxide Value in Assessing Food Quality and Food Safety, JAOCS, *Journal of the American Oil Chemists' Society*, **83(5)**: 473 (2006).
[29] Birjandi M., Sepahvand R., Hassanzadazar H., Hatamikia M., Kazemi-Vardanjani A., A Survey on Peroxide Content and Acidity Level of Persian Doughnuts in Lorestan Province, West of Iran, *J. Glob. Pharma Technol.*, **10(8)**: 32-35 (2016).
[30] Gadade B., Kachare D., Satbhai R., Naik R., Nutritional Composition and Oil Quality Parameters Of Sesame (Sesamum indicum L.) Genotype, *Int. Res. J. Multidiscip Stud.*, **3(7)**: 2454-8499 (2017).
[31] Ogbonna P., Ukaan S., Chemical Composition and Oil Quality of Seeds of Sesame Accessions Grown in the Nsukka Plains of South Eastern Nigeria, *Afr. J. Agric. Res.*, **8(9)**: 797-803 (2013).
[32] Institute of Standards and Industrial Research of Iran. E"dible Cold Pressed Oils - Specifications & Test Methods. 13392": 1st. Revision,; (2015).
[33] Alimentarius C., Codex Standard for Named Vegetable Oils, Codex Stan 210 2005.
[34] Mohamed Ahmed I.A., Musa Özcan M., Uslu N., Juhaimi F.A., Osman M.A., Alqah H.A., et al. Effect of Microwave Roasting on Color, Total Phenol, Antioxidant Activity, Fatty Acid Composition, Tocopherol, And Chemical Composition of Sesame Seed and Oils Obtained From Different Countries, *J. Food Process. Preserv.*, **44(10)**: e14807 (2020).
[35] Ahmed I.A.M., Uslu N., Özcan M.M., Juhaimi F.A., Ghafoor K., Babiker E.E., et al. Effect of Conventional oven Roasting Treatment on the Physicochemical Quality Attributes of Sesame Seeds Obtained From Different Locations, *Food Chemistry*, **338**: 128109 (2021).
[36] Tenyang N., Ponka R., Tiencheu B., Djikeng F.T., Azmeera T., Karuna M.S., et al. Effects of Boiling and Roasting on Proximate Composition, Lipid Oxidation, Fatty Acid Profile and Mineral Content of Two Sesame Varieties Commercialized and Consumed in Far-North Region of Cameroon, *Food Chem.*, **221**: 1308-1316 (2017).
[37] Joint Fao/Who Food Standards Programme, Recommended International Standards for Edible Sesame Seeed Oil, Codex Alimentarius Commission; (1969).
[38] Institute of Standards and Industrial Research of Iran. "Crude Sesame Oil -Specifications and Test Methods, No 8636". 1st ed, 1st Revision, (2014).
[39] Olasunkanmi G.S., Omolayo F.T., Olusegun O.T., Fatty Acid Profile, Physico-Chemical And Functional Properties of Oil and Protein Isolate Simultaneously Extracted from Sesame (Sesamum Indicum) Seed. *Food Sci. Technol.*, **18(1)**: 1-10 (2017).
[40] Pandurangan M., Murugesan S., Gajivaradhan P., Physico-Chemical Properties of Groundnut Oil and their Blends with Other Vegetable Oils, *J. Chem. Pharm. Res.*, **6(8)**: 60-66 (2014).
[41] Olaleye A.A., Adamu Y.A., Lawan U., Effects of Temperature Change on the Physico-Chemical Properties of Sesame Seed Oil, *Sci. J. anal. Chem.*, **7(1)**: 13 (2019).
[42] Godswill A.C., Amagwula I.O., Victory I.S., Gonzaga A.I., "Effects of Repeated Deep Frying on Refractive Index and Peroxide Value of Selected Vegetable Oils". (2018).
[43] Al-Bachir M., Othman Y., Comparative Studies on Some Physicochemical Properties of Oil Extracted from Gamma Irradiated Sesame (Sesamum Indicum L.) Seeds, *Journal of Food Chem and Nanotechnology*, **5(2)**: 36-42 (2019).
[44] Aparicio R., Roda L., Albi M.A., Gutiérrez F., Effect of Various Compounds on Virgin Olive Oil Stability Measured by Rancimat, *J. Agric. Food Chem. J.*, **47(10)**: 4150-4155 (1999).
[45] Sadeghi E., Mahtabani A., Karami F., "Considering the Oxidative Stability of Cold-Pressed Sesame, Sunflower, and Olive Oils under Different Storage Conditions", The Annals of the University Dunarea de Jos of Galati Fascicle VI-Food Technology, **43(2)**:70-83 (2019).

[46] Bialek A., Bialek M., Jelinska M., Tokarz A., Fatty Acid Composition and Oxidative Characteristics of Novel Edible Oils in Poland, *CyTA-Journal of Food*, **15(1)**: 1-8 (2017).

[47] Foster R.H., Hardy G., Alany R.G., Borage Oil in the Treatment of Atopic Dermatitis, *Nutrition*. **26(7-8)**: 708-718 (2010).

[48] Ahmed I.A.M., AlJuhaimi F., Özcan M.M., Ghafoor K., Şimşek Ş., Babiker E.E., et al. Evaluation of Chemical Properties, Amino Acid Contents and Fatty Acid Compositions of Sesame Seed Provided from Different Locations, *J. Oleo Sci.* 2020:ess20041.

[49] Hassan M.A., Studies on Egyptian Sesame Seeds (Sesamum Indicum L.) and its Products 1-physicochemical Analysis and Phenolic Acids of Roasted Egyptian Sesame Seeds (Sesamum indicum L.), *World J. Dairy Food Sci.*, **7(2)**: 195-201 (2012).

[50] Borchani C., Besbes S., Blecker C., Attia H., Chemical Characteristics and Oxidative Stability of Sesame Seed, Sesame Paste, and Olive Oils, *J. Agric. Sci. Technol*., (2010).

[51] Elzupir A.O., Suliman M.A., Ibrahim I.A., Fadul M.H., Elhussein AM., Aflatoxins Levels in Vegetable Oils in Khartoum State, Sudan, *Mycotoxin Res.*, **26(2)**: 69-73 (2010).

[52] Iranian National Standardization Organization. "Maximum Tolerated limits of Mycotoxins in Foods and Feeds, No. 5925", Tehran, Iran: Institute of Standard and Industrial Research of I.R. Iran; (2002). (Persian).

[53] Adel M., Dadar M., Fakhri Y., Oliveri Conti G., Ferrante M. Heavy Metal Concentration in Muscle of Pike (Esox lucius Linnaeus, 1758) from Anzali International Wetland, Southwest of the Caspian Sea and Their Consumption Risk Assessment. T**oxin Reviews**. **35(3-4)**:217-23 (2016).

[54] Environmental Protection Agency (EPA), "Exposure Factors Handbook": 2011 Edition. EPA/600/R-09/052F, US EPA, Office of Research and Development Washington, DC; (2011).

[55] Institute of Standards and Industrial Research of Iran. Food & Feed-Maximum Limit of Heavy Metals 12968, 1st ed.

[56] Ghasemidehkordi B., Malekirad A.A., Nazem H., Fazilati M., Salavati H., Shariatifar N., et al. Concentration of Lead and Mercury in Collected Vegetables And Herbs from Markazi province, Iran: a Non-Carcinogenic Risk Assessment, *Food Chem. Toxicol*., **113**: 204-210 (2018).

[57] Environmental Protection Agency (EPA). Risk Assessment Guidance for Superfund. EPA/540/1-89/002; 2004.

[58] European Food Safety Authority (EFSA), Opinion of the scientific Panel on Contaminants in the Food Chain on a Request from the Commission Related to the Potential Increase of Consumer Health Risk by a Possible Increase of the Existing Maximum Level for Aflatoxins in Almonds, Hazelnuts and Pistachios and Derived Products, *EFSA J.*, **446**: 1e127 (2007).

[59] Joint F.A.O., WHO Expert Committee on Food Additives, "Evaluation of Certain Food Additives and Contaminants: Sixty-Eighth Report of the Joint FAO/WHO Expert Committee on Food Additive", Technical. Report. Series., No 947. WHO, Geneva, Switzerland, pp. 169e180; (2007).

[60] Environmental Protection Agency (EPA), "Application of the Margin of Exposure (MoE) Approach to Substances in Food That Are Genotoxic and Carcinogenic Example: Benzo[A]Pyrene and Polycyclic Aromatic Hydrocarbons", (2010).