

The effect of the type of extraction process and different stages of purification on the composition of sesame oil

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ABSTRACT: *The presence of undesirable impurities in the oil reduces the stability of the oil against oxidation, thereby decreasing the quality of the end product. The aim of this study was to investigate the effect of the type of oil extraction process and the different steps of purification on the amount of PAHs (polycyclic aromatic hydrocarbons), lead and arsenic and the oxidative stability of sesame oil. To do so, the cold press method was used for the control sample and the solvent extraction method was used for the experimental samples in different purification steps (neutralization, decolorization and deodorization). The results showed that the highest amounts of PAHs were found for the sesame oil extracted by the cold press method and the lowest values of PAHs were observed after deodorization. The highest concentrations of lead and arsenic were observed for the cold press-extracted sesame oil and the lowest values were found for the sesame oil after deodorization. The highest oxidative stability for the sesame oil after deodorization and the lowest oxidative stability for the sesame oil extracted by the cold press method. Also, the highest peroxide value was found for the sesame oil extracted by solvent and the lowest value for the sesame oil after deodorization. The results revealed that during the solvent purification process, the amount of polycyclic aromatic hydrocarbons and heavy metals (lead and arsenic) decreased, and the oxidation stability increased compared to the oil extracted by the cold press method, and these properties improved in each step. The results of the peroxide test showed that the use of solvent for extraction caused higher oil oxidation as compared to the cold press method and the purification steps improved the oxidative stability.*

KEYWORDS: *Arsenic; Lead; Oxidation stability; polycyclic aromatic hydrocarbons; Purification; Sesame oil.*

INTRODUCTION

Significant investments have been made in oils and fats industry because they are of great importance, not only from a health viewpoint but also from an economic standpoint. Oils and fats supply a significant part of the energy needed by the body, essential fatty acids and fat-soluble vitamins [1]. Edible oils contain high amounts of unsaturated fatty acids (especially polyunsaturated fatty acids) which are very susceptible to oxidation. Oxidation of oils is important because it reduces the quality of food products [2]. Oxidation of lipids during the processing or storage of food not only develops an off-flavor, unpleasant smell and undesirable color, but also reduces their nutritional value and safety. In addition, the compounds resulting from the oxidation of lipids are toxic and cause health problems and diseases such as cancer. One of the oldest oil plants that is well adapted to arid and semi-arid areas like Iran is the sesame plant with the scientific name *Sesamum Indicum*. Sesame is one of the most important oilseed products that is widely used in many parts of the world. Cultivation is placed [3]. About 75% of sesame seeds are protein and fat. The predominant unsaturated fatty acid in sesame oil is oleic acid (4.43%) followed by linoleic acid (3.36%) [4]. Due to the excellent quality of this oil, which has a pleasant smell and a good taste. This seed is called the queen of oil seeds and is highly valued in Iran [5].

The concentration of heavy metals in vegetable oils is a very important criterion for the quality of edible oils for freshness and storage as well as for nutritional and health properties. Heavy metals in vegetable oils also affect the oxidation rate of oils [6]. Memar *et al.* [7] examined the concentration of heavy metals in 58 samples and reported that the amount of lead in 17.5% of the samples exceeded the standard level. Polycyclic aromatic hydrocarbons (PAHs) are a large group of organic compounds composed of two or more aromatic fused rings. According to the EU regulations, the permissible limit of PAHs in oils and fats intended for human consumption is 10 µg/kg [8]. Edible oils may be contaminated with PAHs during drying and/or solvent extraction, and to a lesser extent, by leaking into the plant as well as through soil. Oil is extracted from the most commonly used oil seeds such as soybean and sunflower primarily by the purification process, which significantly reduces the PAHs level. But this decrease is not significant in oils extracted by the cold press method used for the preservation of their bioactive compounds (mustard, safflower, black pepper, borage, etc.). Therefore, these oils can be an important source of PAHs. Studies have shown that the amount of low molecular weight PAHs is often higher than that of high molecular weight PAHs [9]. Rojo Camargo *et al.* [10] studied soybean oil extraction and stated that the purification process including neutralization, decolorization and deodorization resulted in a significant decrease (88%) in the PAHs level. Given the demand for higher quality vegetable oils, the effect of the oil extraction method on the quality of the end product and the lack of studies on sesame oil extraction methods resulting in the highest quality oil, the aim of this study was to investigate the effect of the type of oil extraction process and its different steps on the amount of PAHs (polycyclic aromatic hydrocarbons), lead, arsenic as well as the oxidative stability of sesame oil.

EXPERIMENTAL SECTION

Materials

Sesame oil was obtained from Gohar Daneh Afagh company (Iran), the chemicals including sodium hydroxide, bleaching earth, dichloromethane, acetonitrile, fluoric acid, nitric acid, hydrochloric acid, chloroform, acetic acid, potassium iodide, and sodium thiosulfate from Sigma company (Germany) and the starch solution from Shimaz company (Iran).

Methods

Initially, samples were collected from several factories. To do this, unpurified sesame oil extracted by the cold press method as the control sample (T1), unpurified sesame oil extracted by the solvent extraction method (T2), sesame oil extracted by the solvent extraction method (after neutralization) (T3), sesame oil extracted by the solvent extraction method (after decolorization) (T4) and sesame oil extracted by the solvent extraction method (after deodorization) (T5) as the experimental samples were prepared. All the samples were evaluated for PAHs, lead, arsenic and oxidative stability. It should be noted that all the samples were transferred to the laboratory in dark glass containers and the experiments were conducted in triplicate.

Oil purification steps

Oil purification involved the extraction steps including pressing, neutralization, decolorization and odorization. It was done according to the method of Madihah Abd Hadi *et al.* [11] that with some modification based on preliminary study as briefly described below. In the gumming step, first the oil was mixed with 3% water at 70°C and stirred, and the oil phase was then separated by centrifugation. The gummed oil was treated with an appropriate amount of sodium hydroxide (10% in water) to neutralize free acids, and after separating the soap, the neutralized oil was decolorized with 1% acid-activated bleaching earth. Then it was deodorized under vacuum conditions at 220°C. The obtained product was packed in dark glass bottles.

Tests

Identification of Polycyclic aromatic hydrocarbons

The polycyclic aromatic hydrocarbons were identified using a high performance liquid chromatography (HPLC) (model Younglin Acme 9000, South Korea). To do so, the preparation was done according to the Iranian national standard 8638 No. 10682 [12], the sesame oil samples were injected into the device and the compounds were analyzed. Before injecting the samples into the device, to ensure the absence of PAHs compounds in the glass equipment, all the glass parts were washed several times in an ultrasonic bath with dichloromethane solution [13], and the final solvent used to check the contamination with PAHs was analyzed by chromatography and the results were obtained according to Equation 1.

Equation 1:

$$c_i = \frac{(A_i \times C_{ir} \times V)}{(A_{ir} \times m)}$$

where C_i is the amount of PAH in the sample (ppm), A_i is the area under the curve of PAH in the sample, A_{ir} is the area under the curve of PAH (average of two injections) in the standard solution, C_{ir} is the concentration of each PAH in the standard solution, m is the sample weight (g) and V is the final extraction volume (mL).

Measurement of lead and arsenic

Lead and arsenic were measured by the graphite furnace atomic absorption method according to the Iranian national standard 8636 No. 4098 and No. 6076 [14, 15], respectively. First, 1 mL of the dried sample was weighed and poured into a 50 mL flask to which 5 mL of hydrofluoric acid, 10 mL of nitric acid and 5 mL of hydrochloric acid were added, and it was heated to a temperature of 100°C until the sample became clear. The samples were then brought to the desired volume with the help of 4% nitric acid and prepared for reading by an atomic absorption device.

Measurement of oxidative stability using rancimat

Oxidative stability index (OSI) was measured by a rancimat machine (model 892 Professional Rancimat, Switzerland) according to the Iranian national standard No. 3734 [13]. To do so, dry and clean air was blown at a speed of 20 L/h into a container containing 3 g of oil sample, and the air containing volatile organic acids caused by the oxidation of the sample was blown into a container intended for measuring electrical conductivity (containing 60 mL of distilled water) and the oxidative stability index was automatically measured at 110°C.

Measurement of peroxide value (PV)

The peroxide value was measured according to the Iranian national standard 8636 No. 4179 [16]. To do this, 5 g of oil and 30 mL of chloroform and acetic acid solution (two volumes of acetic acid and one volume of chloroform) were dissolved. The mixture was shaken and then 0.5 mL of saturated potassium iodide solution was added. After keeping in the dark for 1 min, 30 mL of distilled water were added and the solution was titrated with 0.01 normal sodium thiosulfate in the presence of 0.5 mL of 1% starch solution. The peroxide value was calculated in mEq of peroxide per g of sample according to Equation 2.

Equation 2:

$$Pv = ((v - v_0) \times N \times 1000) / W$$

where Pv is the peroxide value in mEq of oxygen per kg of the extracted oil, v is the amount of sodium thiosulfate in mL used for sample titration, v_0 is the amount of sodium thiosulfate in mL used for blank titration, N is the normality of sodium thiosulfate used and W is the weight of tested oil in g.

Statistical analysis

First, the normality of the data was checked, and then the results were analyzed by one-way analysis of variance (ANOVA). The means were compared by using Duncan multiple range test. Data were analysed using SPSS 22 software.

RESULTS AND DISCUSSION

Identification of polycyclic aromatic hydrocarbons (ppm)

The results of the measurement of benzo (a) anthracene, chrysene, benzo (b) fluoranthene, benzo (a) pyrene and total polycyclic aromatic hydrocarbons of unpurified sesame oil extracted by the cold press, unpurified sesame oil obtained by the solvent extraction method and purified sesame oil with different steps of purification, including neutralization, decolorization, and deodorization are presented in Table 1. As shown in the Table, the highest amount of PAHs was found for the cold pressed-extracted sesame oil including benzo (a) anthracene (0.93 ppm), chrysene (1.22 ppm), benzo (b) fluoranthene (1.59 ppm), benzo (a) pyrene (1.36 ppm) and total polycyclic aromatic hydrocarbons (5.1 ppm) and the lowest amount of PAHs was observed for sesame oil after deodorization including benzo (a) anthracene (0.5 ppm), chrysan (0.42 ppm), benzo (b) fluoranthene (0.41 ppm), benzo (a) pyrene (0.12 ppm) and total polycyclic aromatic hydrocarbons (1.46 ppm). All treatments were significantly ($p \leq 0.05$) different. This reduction was directly related to each step of purification as it decreased through the purification process. Many researchers stated that the amount of PAHs in crude vegetable oils can be reduced during refining [17, 18]. Moret *et al.* [19] investigated the effect of processing conditions on the content of PAHs in grape oil by using the liquid chromatography method and showed that the oil samples extracted before drying contained relatively high amounts of benzo (a) pyrene (about 2.4-0.9 ppb) which was attributed to the compressing grape pomace by a bulldozer to reduce its volume before storage. Other researchers studied the content of 15 different types of PAHs in soybean, sunflower and extra virgin olive oils and also their changes during the purification process. They showed that extra virgin olive oil had the highest amount of PAHs (26 $\mu\text{g}/\text{kg}$) and the neutralization and especially deodorization steps played a significant role in reducing PAHs in the samples [20]. Yu *et al.* [21] Examined the sources of PAHs in soybean oil and its changes during the purification process and stated that the PAHs level in purified oils was significantly lower than that in unpurified oil. Also, a significant decrease in the PAHs level was observed during the extraction, decolorization and deodorization. Fromberg *et al.* [22] used a semi-automatic method (gel permeation chromatography with solid phase) to investigate polycyclic aromatic hydrocarbons in vegetable oils. This method has been approved by the European Union to determine PAH in vegetable oils. Out of 69 vegetable oil samples (olive oil, rapeseed oil, sunflower oil, grape seed oil) that were obtained from Danish markets, except for one sunflower sample that contained 11 ($\mu\text{g}/\text{kg}$) benzopyrene, the rest of the oils have The amounts of benzopyrene were very low ($<0.2-0.8 \mu\text{g}/\text{kg}$).

Table 1: Results of measurement of benzo (a) anthracene, chrysene, benzo (b) fluoranthene, benzo (a)pyrene and total PAHs in sesame oil under different conditions

Oils	Benzo(a) anthracene (ppm)	Chrysene (ppm)	Benzo (b) fluoranthene (ppm)	Benzo (a) pyrene (ppm)	Total PAHs (ppm)
Unpurified cold press-extracted sesame oil (control)	0.5000 ^a	0.4200 ^a	0.4100 ^a	0.1200 ^a	1.4500 ^a
Unpurified sesame oil obtained by solvent extraction method	0.6600 ^b	0.5400 ^b	0.6700 ^b	0.3700 ^b	2.2400 ^b
Purified sesame oil obtained by solvent extraction method (after neutralization)	0.7000 ^c	0.8700 ^c	0.8600 ^c	0.5700 ^c	3.0000 ^c
Sesame oil after decolorization	0.8700 ^d	0.9200 ^d	1.3300 ^d	1.1100 ^d	4.2300 ^d
Sesame oil after deodorization	0.9300 ^e	1.2200 ^e	1.5900 ^e	1.3600 ^e	5.1000 ^e

Different small letters represent a significant difference ($p \leq 0.05$) per column.

Measurement of lead and arsenic

Metal content in vegetable oils in terms of toxicity Nutritional effects and also in terms of oil storage life They are of special importance [23]. The results of the measurement of lead and arsenic in unpurified cold press-extracted sesame oil, unpurified sesame oil obtained by the solvent extraction method and purified sesame oil with different purification steps including neutralization, decolorization and deodorization are presented in Table 2. As shown in the Table, the highest (117 ppb) and the lowest (28 ppb) amounts of lead were found for the cold-press extracted sesame oil and the sesame oil after deodorization, respectively. Also, the highest (150 ppb) and the lowest (36 ppb) amounts of arsenic were observed for the cold-press extracted sesame oil and the sesame oil after deodorization, respectively. The results showed significant ($p \leq 0.05$) differences and the reduction was directly related to each step of purification as it decreased through the purification process. Other researchers studied and measured the amount of heavy metals in edible oils. They analyzed the amount of heavy metals in 58 edible oil samples, as one of the most commonly used foods. No lead was observed in 75.86% of the samples, and in 5.17% of the samples, the amount of lead exceeded the permissible limit. Arsenic, copper and iron were not found in 94.12, 24.39 and 6.06% of the samples, respectively [7]. Sedaghat Borujeni *et al.* [24] investigated the effect of the purification process on the amount of heavy metals in soybean oil. The results revealed that the most important step in the arsenic removal process was neutralization, which caused 60% removal of the total arsenic from oil. The highest percentage of mercury removal was observed after adding metal receptors and antioxidants, which was probably related to the citric acid chelation. Most of the lead was removed from the oil in the neutralization step due to the production of insoluble soap during centrifugation. Also, the neutralization step had a significant effect on removing cadmium from oil. Therefore, it could be concluded that neutralization and addition of antioxidants and metal receptors were two important steps in removing heavy metals from oil. Mendalia *et al.* [25], in Research on 4 types of industrial edible oil in Türkiye (olive, hazelnut, sunflower, corn oils) 10 of each collected samples and the content of heavy metals iron, copper, they checked lead and cadmium in them. In all samples the amounts of these metals are lower than the international standard range It was measured

and all in terms of toxicity and the nutritional value was evaluated as appropriate. This is a sign of quality Above the raw materials and extraction process of these oils.

Table 2: Results of measurement of lead and arsenic in sesame oil under different conditions

Oils	Lead (ppb)	Arsenic (ppb)
Unpurified cold press-extracted sesame oil (control)	28.0000 ^a	36.0000 ^a
Unpurified sesame oil obtained by solvent extraction method	44.0000 ^b	51.0000 ^b
Purified sesame oil obtained by solvent extraction method (after neutralization)	68.0000 ^c	72.0000 ^c
Sesame oil after decolorization	99.0000 ^d	123.0000 ^d
Sesame oil after deodorization	117.0000 ^e	150.0000 ^e

Different small letters represent a significant difference ($p \leq 0.05$) per column.

Measurement of oxidative stability using rancimat system

The results of the measurement of the oxidation stability of unpurified sesame oil extracted by the cold press method, unpurified sesame oil obtained by the solvent extraction method and purified sesame oil with different purification steps including neutralization, decolorization and deodorization are presented in Table 3. As shown in the Table, the highest (11.17 h/110°C) and the lowest (7.61 h/110°C) oxidative stability indexes were found for the sesame oil after deodorization and the unpurified sesame oil obtained by the cold press method, respectively. All treatments were significantly ($p \leq 0.05$) different and this stability was directly related to each step of purification as it increased through the purification process. The refining consists in a set of operations used to obtain an edible product including degumming, neutralization, bleaching and deodorization. The first degumming is carried out to remove phospholipids. Neutralization and bleaching are used to eliminate free fatty acids and pigments that can promote fat oxidation and lead to undesirable colures in the final product. The neutralized oil is treated with bleaching agents such as activated carbon. Finally, the deodorization removes volatile compounds and decomposes peroxides to improve the oil flavor quality and stability. The resulting product is referred as refined oil [26]. Hoseini *et al.* [27] evaluated the type of extraction method on the oxidative stability of sesame oil. They stated that oils extracted by the cold press method, shop press and traditional press and refined oils had the highest oxidation resistance for 20, 17.6, 13.5 and 10.01 h, respectively. Farmani *et al.* [23] collected 9 samples of sesame oil from different oil shops in Mazandaran province and investigated its oxidative induction period. The results showed that the oxidative stability of 9 samples at 110 °C in the range of 5.9 to 10.2 hours and samples No. 4 and 6 showed less resistance than other samples, which was due to the higher amount of metals in these samples. Hojjati [28] stated that the oxidative stability index of purified sunflower oil samples was within the standard limit, while the sunflower oil prepared by the cold press method was 4.96-28.11 h, which was not within the standard range and suitable for household use.

Table 3: Results of measurement of oxidative stability of sesame oil under different conditions

Oils	Oxidative stability (h/110-°C)
Unpurified cold press-extracted sesame oil (control)	7.6100 ^a
Unpurified sesame oil obtained by solvent extraction method	7.6400 ^b
Purified sesame oil obtained by solvent extraction method (after neutralization)	8.6700 ^c
Sesame oil after decolorization	10.5400 ^d
Sesame oil after deodorization	11.1700 ^e

Different small letters represent a significant difference ($p \leq 0.05$) per column.

Measurement of peroxide value (PV)

The results of the measurement of peroxide value of unpurified sesame oil extracted by the cold press method, unpurified sesame oil obtained by the solvent extraction method and purified sesame oil with different purification steps including neutralization, decolorization and deodorization are presented in Table 4. As shown in the Table, the highest (16.95 mEq/kg oil) and the lowest (mEq 2.22 mEq/kg oil) peroxide values were found for the unpurified sesame oil obtained by the solvent extraction method and the sesame oil after deodorization, respectively. The results indicated that all treatment were significantly ($p \leq 0.05$) different. Pourabdollah *et al.* [15] studied the peroxide index of oil extracted from the bleaching earth used by Iran Oil Products Company. The results revealed that the highest and the lowest amounts of peroxide were observed for the oil extraction with hexane for 6 and 2 h, respectively. When using bleaching earth for purification, a significant decrease in the peroxide index occurred. Naji *et al.* [29] investigated the effect of different color removal soils (Iranian, Pakistani and German bentonite) on the amount of peroxide index of soybean, sunflower, rapeseed and palm oil. The results showed that decolorization reduces the peroxide index in all oils and there was no significant relationship between the type of decolorizing soil used and the changes in the peroxide index. Therefore, the decolorizing soil that is cheaper and more accessible in our country (Iranian bentonite soil) was selected. Also, the best quality after decolorization was seen in soybean oil. Hojjati [28] compared the peroxide index of sesame oil extracted by pressing and refined within 60 d. He reported that the amount of peroxide when using the press extraction method was initially lower than the purification method, however the changes in the peroxide value of the purified sesame oil were far less than that of the cold press-extracted sample during 60 d. Therefore, the food industry is always looking for new separation technologies to use natural compounds with higher purity and, as a result, to produce healthy and high quality products [30].

Table 4: Results of measurement of peroxide value of sesame oil under different conditions

Oils	Peroxide Value (mEq 02/kg oil)
Unpurified cold press-extracted sesame oil (control)	12.5000 ^d
Unpurified sesame oil obtained by solvent extraction method	16.9500 ^e
Purified sesame oil obtained by solvent extraction method (after neutralization)	10.6800 ^c
Sesame oil after deodorization	6.1200 ^b
Sesame oil after deodorization	2.2200 ^a

Different small letters represent a significant difference ($p \leq 0.05$) per column.

CONCLUSIONS

The results of this study showed that the type of extraction process and purification steps have a significant effect on reducing PAHs and heavy metals such as lead and arsenic decreased during the solvent purification as well as cold press method. Also, its oxidative stability increased compared to oil extracted by the cold press method, and the properties were improved in each step of purification. However, the results of the peroxide test showed that the use of solvent for extraction caused higher oxidation of the oil as compared to the cold press method and the purification steps (neutralization, decolorization and deodorization) improved the oxidative stability.

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