eISSN 2255-0569

ORIGINAL

Effect of sesame oil extraction by traditional and cold press methods on total aflatoxin content

Efecto de la extracción de aceite de sésamo por métodos tradicionales y de prensado en frío sobre el contenido total de aflatoxinas

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Received: 9 - IV - 2021
Accepted: 5 - V - 2021

doi: 10.3306/AJHS.2021.36.03.22

Abstract

Background: Aflatoxin contamination is an undesirable issue in the oil industry. Methods of extraction have efficient effects on the aflatoxin contents of achieved oil.
Aim: The present survey was performed to assess the effects of traditional and cold press extraction methods on the aflatoxin contents of sesame oil.
Methods: A total of 17 crude sesame, 17 unfiltered oil, and 17 filtered oil samples were collected from different steps of oil production by cold press. Additionally, a total of 9 crude sesame, 9 unfiltered oil, and 9 filtered oil samples were collected from different steps of oil production by the traditional method. All samples were tested for aflatoxins B_1, B_2, G_1, and G_2 . Aflatoxin content was determined by high-performance liquid chromatography.
Results: Dehulling reduced total aflatoxin as 79.79%. Traditional and cold press procedures caused 8.2% and 70.22% transferring of total aflatoxin from crude sesame to achieved oil, respectively.
Conclusion: Traditional method was more effective than cold press in decreasing aflatoxin contents of achieved sesame oil.

Keywords: Sesame, oil extraction, traditional method, cold press, aflatoxin content.

Resumen

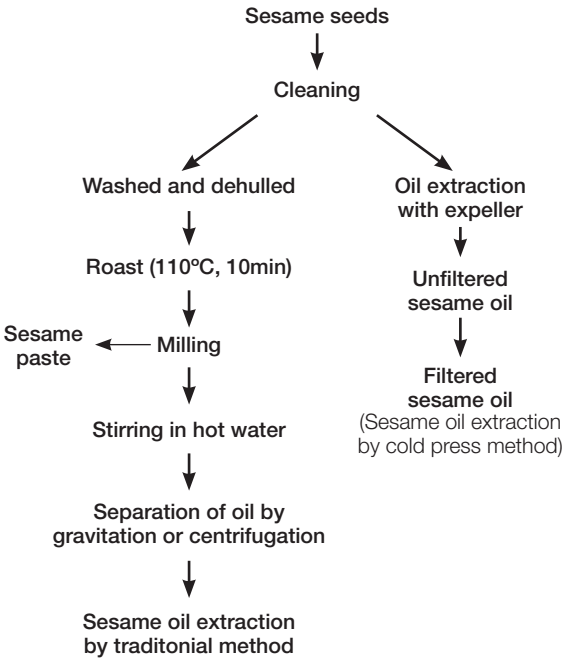
Antecedentes: La contaminación por aflatoxinas es un problema indeseable en la industria del aceite. Los métodos de extracción tienen efectos eficaces sobre el contenido de aflatoxinas del aceite obtenido.
Objetivo: El presente estudio se realizó para evaluar los efectos de los métodos de extracción tradicionales y de prensado en frío sobre el contenido de aflatoxinas del aceite de sésamo.
Metodología: Se recogieron un total de 17 muestras de aceite crudo de sésamo, 17 de aceite sin filtrar y 17 de aceite filtrado, procedentes de diferentes etapas de la producción de aceite por prensado en frío. Además, se recogieron un total de 9 muestras de sésamo crudo, 9 de aceite sin filtrar y 9 de aceite filtrado de diferentes etapas de la producción de aceite por el método tradicional. Todas las muestras fueron analizadas para detectar aflatoxinas B_1, B_2, G_1 y G_2 . El contenido de aflatoxinas se determinó mediante cromatografía líquida de alto rendimiento.
Resultados: El descascarillado redujo el total de aflatoxinas en un 79,79%. Los procedimientos tradicional y de prensado en frío provocaron una transferencia del 8,2% y del 70,22% del total de aflatoxinas del sésamo crudo al aceite obtenido, respectivamente.
Conclusiones: El método tradicional fue más eficaz que el prensado en frío para reducir el contenido de aflatoxinas en el aceite de sésamo obtenido.

Palabras clave: Sésamo, extracción de aceite, método tradicional, prensado en frío, contenido de aflatoxinas.

Introduction

Food security is one of the most critical challenges in the present century[1-5]. Edible oil is among the most commonly used food products playing a crucial factor in human health. Vegetable oils have an essential role in health status. Impurities and contamination of vegetable oils may vary based on the materials, weather, soil, harvesting, storage and processing. Also different oil extraction methods including organic solvent, water (traditional) and press effect on the transferring contamination from materials to the final product. Given consumers' concerns about chemicals used in food products, oil extraction by water and press has been much-paid attention. Sesame (Sesamum indicum L.) is an oilseed cultivated in tropical and temperate regions. Sesame oil is known as the queen of oilseeds due to its high-quality oil[6]. Sesame oil is one of the most widely used products as 4756000 t sesame oil was produced in 2013 worldwide[7]. This kind of oil is extracted by traditional, cold press, and solvent methods. Diagram of sesame oil exaction by traditional and cold press methods are shown in **figure 1**.

Figure 1: Diagram of sesame oil exaction by traditional and cold press methods.



Water flotation is a traditional method in Iran and Sudan for the extraction of sesame oil. Sesame seed is heated to 110°C for 10 min and milled. Enough boiling water is then added to suspend the ground seed on stirring. The mixture is boiled with stirring for 15 min. After cooling, the upper oil layer is separated off and dried by heating. The oil recovery from 0.5 kg seed is 108 ml, equivalent to an oil extraction efficiency of 41%[8]. Cold press is a popular method of sesame oil extraction in Iran where no chemicals, solvent or additives are used, and it is produced in the presence of customers in some shops[9]. However, the product's safety is not assessed in Iran and aflatoxin is produced under improper storage conditions. Sesame oil contamination with aflatoxin has been reported in China, Sudan, and Senegal[10].

Materials for edible oil production are often stored under such conditions for weeks that promote the growth of moulds producing mycotoxin aflatoxins such as *Aspergillus flavus*, *A. parasiticus* and *A. nomius* naturally occurring in foods. Among 18 known aflatoxins, B_1, B_2, G_1 and G_2 are classified in group A of carcinogenic factors by International Agency for Research on Cancer (IARC)[11,12]. Given the harmful effects of aflatoxins on public health, most studies on mycotoxins are presently concentrated on aflatoxin[13]. Furthermore, aflatoxin is continuously monitored in most products by health monitoring systems. Thus, the various national and international standard for the permitted limit of aflatoxin in food products has been developed[14-17]. Aflatoxin produced in oilseeds may be transferred to the final oil product. However, the concentration of these contaminants can be reduced through the processing method (extraction and purification). Traditional and cold press extraction are the two most widely used extraction techniques in Iran. This study aimed to examine the effect of different processes in sesame oil extraction using traditional and cold press methods on total aflatoxin content.

Materials and methods

Sampling

Sufficient amount of sesame seeds were collected from sesame oil extraction shops in Isfahan, Iran. Sesame oil was extracted by traditional method including washing, dehulling, roasting (110ºC, 10 min) and oil extraction steps. The collected sesame seeds were processed by the traditional oil extraction method. In addition to 9 samples of raw sesame seeds, 9 samples were collected from each of the stages of washing, peeling, roasting and oil production. Moreover, 17 raw sesame seeds samples were collected from 17 cold press extraction shops in Isfahan, Iran. First, aflatoxin content in the raw seeds was determined, then the aflatoxin levels of the extracted oil samples were measured, and finally aflatoxin content of the filtered oil was determined. For a complementary assessment, 19 filtered oil samples obtained by the cold press extraction were collected, the aflatoxin content was determined, and the results were reported.

The amounts of aflatoxins B_1, B_2, G_1 and G_2 in the samples were measured according to Iranian national standard No. 6872 (ISIRI, 2004). This analysis included three steps of purification, detection and determination. The toxins were purified by using immunoaffinity columns.

They were detected by Kobra cell and determined by use of florescence detector at 365 nm excitation wavelength and 435nm emission wavelength. The procedure was validated via spiking with different levels of aflatoxins B_1, B_2, G_1, and G_2 and then efficacy parameters such as linearity, accuracy, precision and limit of detection (LOD) were measured. The validity of method was confirmed and it was used for our purpose.

Calibration and standard curve drawing

To draw the calibration curve, 1 mg of each aflatoxin pure powder was dissolved in 5 ml toluene: acetonitrile (90: 10) and a standard solution (200 µg/ml) was prepared. After dilution, four standard solutions were obtained from the secondary standard solution and injected into High-Performance Liquid Chromatography (HPLC). To prepare mobile phase solvent, water: acetonitrile: methanol (6:2:3) were mixed, and 300 µL 4M nitric acid and 120 mg potassium bromide were added per L mobile phase. The samples were extracted with 200 mL 80% methanol and 5 g salt. The mixture was filtered and added to 130 mL water to obtain 150 mL diluted extract.

Aflatoxin measurement

The samples were purified using immunoaffinity columns (IAC) with antibodies against aflatoxins B_1, B_2, G_1, and G_2 and a vacuum Manifold system. The first 70 mL of the solution passed through the column. Afterwards, the column was washed with double distilled water. The column was dried, and aflatoxins B_1, B_2, G_1 and G_2 were removed with 1500 µL distilled water as 100 ML were injected into HPLC. To examine the accuracy and precision of the applied method, a control sample was spiked with aflatoxin B1 and was analyzed with the test samples. A calibration curve was drawn every day using 4 standards, and then the samples were injected, and aflatoxin content was determined. Principles of the previous survey were applied[19].

Recovery

To calculate the recovery, a certain amount of standard solution of aflatoxin was added to control the contaminated sample then was injected into HPLC and aflatoxin content was calculated by the following relation:

$$R= (C_{Sample\ spike} - C_{Sample})*100/C_{spike}$$

Where R is recovery value, and C sample spike is aflatoxin concentration in the contaminated sample.

LOD and LOQ

The detection limit (LOD) and limit of quantitation (LOQ) were used to calculate the method's sensitivity. LOD is the lowest concentration in a detectable matrix but cannot be measured precisely, and LOQ is the lowest concentration that is detectable and measurable precisely. These parameters were calculated by using the calibration curve. To do so, calibration gradient (a) and standard deviation of independent variable regression (Sb) were put in LOD=3* Sb/a equation and LOQ=10* Sb/a and LOD and LOQ were calculated.

Statistical analysis

Data were analyzed by SPSS software version 20 (Chicago, IL, version, SPSS Inc). Mean, and standard deviation was presented in frequency distribution tables. Total aflatoxin mean was compared to International and national standard by one-sample t-test[20-22]. Aflatoxin mean in different steps of oil extraction was compared by Wilcoxon signed ranks test.

Results

Standard curve

Aflatoxins B_1, B_2, G_1 and G_2 were analyzed by four standard solutions at 1, 3, 5 and 7 ppm. An example of chromatograms is shown in figure 2. As illustrated in the figure, the peak is in a proper form with a retention time of about 4 min.

Figure 2: Chromatogram using 1ppm by HPLC.

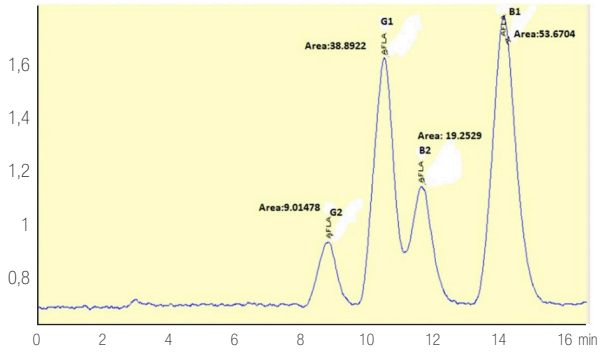
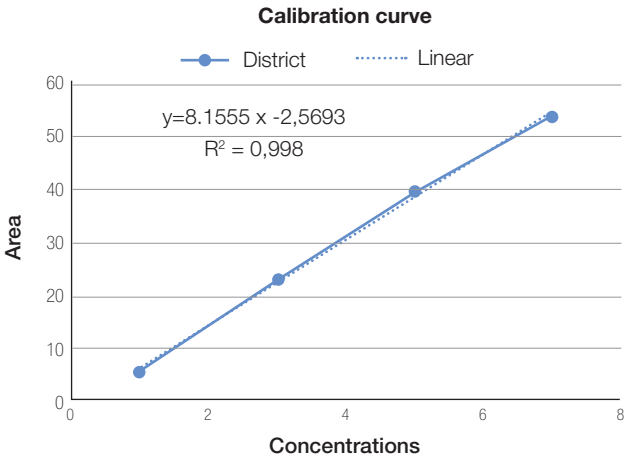


Figure 3: Calibration curve of Aflatoxin B.



Calibration curve

The calibration curve of aflatoxin is shown in **figure 3**. The calibration curve results revealed a linear relationship between different concentrations of aflatoxin B_1, B_2, G_1 and G_2 and area under the curve with the correlation coefficient (r) of all calibration curves being > 0.998.

Results of recovery

Results of validation by spiking sesame and its oil with aflatoxins B_1, B_2, G_1 and G_2 were studied. The amounts of spiking of aflatoxins B_1, B_2, G_1 and G_2 in examined samples were 1, 1, 1, and 1 ppb, respectively. The amounts of aflatoxins B_1, B_2, G_1 and G_2 in examined samples were 133, 1.099, 1.155, and 1.127 ppb, respectively. Additionally, the recovery rates of aflatoxins B_1, B_2, G_1 and G_2 in examined samples were 133%, 109.9%, 155.5% and 112.7%, respectively.

Results of LOD and LOQ

The lowest LOQ for aflatoxins B1 and G1 was<0.5, and aflatoxins B_2 and G_2 were <0/1. The lowest LOD for B1 and G_1 was<0/16, and B_2 and G_2 was <0.03, being the lowest LOD.

Results of aflatoxin measurement in oil extracted by cold press

Minimum, maximum and mean contents of four aflatoxins B_1, B_2, G_1 and G2 are displayed in **table I**. In the present study, 25 samples showed 69.4% aflatoxin contamination with the mean value being 1.337± 0.329 ppb. Total aflatoxin content in all samples ranges < LOQ to 3.1 ppb. Comparison of the result to Iranian national standard for total aflatoxin (15 ppb) suggest that it is lower than the specified amount. Mean aflatoxin B_1 contamination was 0.437±0.33. Aflatoxin B_1 contamination was found in 27.8% of the samples. Aflatoxin B_2 contamination in the samples was 0.106±0.033 ppb. There was no aflatoxin G_1 and G_2 in the samples. In the present study, total aflatoxin, B_1 and B_2 contents were 1.337±0.329 ppb, 0.473± 0.426 ppb and 0.33±0.106, respectively. G_1 and G_2 content was zero. Only one sample showed B_1 content higher than the amount permitted by EU (2 ppb).

Effect of cold press method on aflatoxin content in the extracted sesame oil

Maximum, minimum, mean and standard deviation of four aflatoxins B_1, B_2, G_1, and G_2 in different steps of oil extraction by cold press are presented in **table II**. The mean of total aflatoxin in crude sesame, extracted oil and filtered oil was 1.88±1.15 ppb, 1.32±0.32 ppb and 1.21± 0.032 ppb, respectively. Mean aflatoxin B_1 contents in crude sesame, oil, and filtered oil were 1.31±0.865 ppb, 0.421±0.34 ppb, and 0.192±0.23 ppb, respectively. Mean aflatoxin B_2 in crude sesame and oil was 0.224±0.31 ppb and 0.107±0.032 ppb, respectively. Aflatoxin B_2 in filtered oil was < LOQ. No aflatoxins G_1 and G_2 were found in the samples. Total aflatoxin, B_1 and B_2 in oil samples extracted by cold press were 63.5, 48.68, and 12.06, respectively. These toxins showed 8.33, 54.39 and 10% significant decrease in filtered oil, respectively. As shown in the table, extraction steps and filtration reduced total aflatoxin, B_1 and B_2, as cold press method resulted in 35.63%, 77.80% and 59.83% decrease in total aflatoxin, B_1 and B_2.

Table I: Max., Min., Mean, and SD of four aflatoxins B_1, B_2, G_1 and G_2 in sesame oil produced by cold press.

Aflatoxin type	Min	Max	Mean±SD	Contaminated oil		Non-contaminated oils	
B_1	< LOQ*	2.3	0.473±0.426	69.4	25	30.6	11
B_2	< LOQ	0.2	0.106±0.033	27.8	10	72.2	26
G_1	< LOQ	< LOQ	< LOQ	0	0	0	0
G_2	< LOQ	< LOQ	< LOQ	0	0	0	0
Total aflatoxin	< LOQ	3.1	1.337±0.329	69.4	25	30.6	11

*LOQ: limit of quantitation (B1, G1= 0.5 ppb B2, G2= 0.1 ppb)

Table II: Max., Min., Mean, and SD of four aflatoxins B_1, B_2, G_1 and G_2 in different steps of oil production by cold press.

Sample Type	Sample number	Aflatoxin type	Min	Max	Mean±SD
Crude Sesame	17	B_1 B_2 G_1 G_2 Total	< LOQ* < LOQ < LOQ < LOQ <1/2	4.57 1.35 < LOQ < LOQ 5.67	0.865±1.31 0.224±0.31 < LOQ < LOQ 1.88±1.15
Unfiltered Oil	17	B_1 B_2 G_1 G_2 Total	< LOQ < LOQ < LOQ < LOQ <1/2	1.04 0.19 < LOQ < LOQ 1.75	0.421±0.34 0.107±0.032 < LOQ < LOQ 1.32±0.15
Filtered Oil	17	B_1 B^2 G_1 G_2 Total	< LOQ < LOQ < LOQ < LOQ <1/2	0.63 < LOQ < LOQ < LOQ 1.33	0.192±0.23 < LOQ < LOQ < LOQ 1.21±0.032

*LOQ: Limit Of Quantitation (B_1, G_1= 0.5 ppb B_2, G_2= 0.1 ppb.

Effect of traditional water method on aflatoxin content in the extracted sesame oil

Maximum, minimum, mean and standard deviation of four aflatoxins B_1, B_2, G_1 and G_2 in different steps of the oil extraction by traditional method are presented in **table III**. Mean total aflatoxin in crude sesame, dehulled sesame, roasted sesame, and oil was 17.46±13.35, 3.53±2.17, 2.86±1.33 and 1.43±0.628 ppb, respectively. Mean aflatoxin B1 in crude sesame, dehulled sesame, roasted sesame and oil was 16.1±12.946, 2.71±2.04, 2.07±1.26 and 0.36±0.74 ppb, respectively, and mean aflatoxin B_2 was 0.77±0.605, 0.21±0.135, 0.18±0.09 and 0.11 ±0.48 ppb, respectively. No G_1 and G_2 were found in the samples. Dehulling resulted in 79.79%, 86.95% and 72.72% in total aflatoxin, B_1 and B_2, respectively. However roasting at 110ºC had no significant ($p>0.05$) effect on aflatoxin decrease. Traditional (water) method resulted in 91.80%, 97.76%, and 85.71% decrease in total aflatoxin, B_1 and B^2, respectively. Comparative results of total aflatoxin, B_1 and B_2 measurement in different steps of oil extraction by traditional method.

Effect of cold press and traditional method on aflatoxin content in the extracted sesame oil

The method of edible oil extraction may affect aflatoxin decrease depending on the type of oil, extraction and purification methods. There were no studies on the effect of the sesame oil extraction process, so studies on similar oilseeds were used. Filtration resulted in 8.33, 54.39 and 100% decrease in total aflatoxin, B_1 and B_2 in sesame oil produced by the cold press method. Cold press and filtration decreased total aflatoxin, B_1 and B_2.

Discussion

Aflatoxin contamination is an undesirable issue in the oil industry. Consumption of oil contained diverse aflatoxin levels caused severe health-related complications, including cancer, neoplasia, mutations, hepatic toxicities, renal failure, abortion and even fetal malformation[23]. Thus, it is essential to control the aflatoxin content of edible oils. The oil extraction stage from seeds is one of the most significant steps in controlling aflatoxin in achieved oils[10]. Several reports disclosed that the oil extraction method caused significant effects on the aflatoxin contents of oils[10,24].

The present survey was performed to assess sesame oil extraction by traditional and cold press methods on total aflatoxin contents of achieved oil. The present research findings disclosed that the traditional method of sesame oil extraction was more effective in reducing aflatoxin contents than the cold-press technique. One of the main reasons for the high aflatoxin contamination in sesame oil samples may be attributed to unhygienic conditions of the sesame oil extraction in producing units. Thus, good hygiene, safety and supervision in the sesame oil-producing units may be crucial to decrease the aflatoxin content of sesame oil. Additionally, using high-quality sesame is another way to decrease the risk of aflatoxin residues in achieved oil. Aflatoxin content found in the present investigation was within the range permitted by the Iranian national standard. However, regarding the daily consumption of contaminated sesame oil, this aflatoxin content may have some health threats to the consumers. In a study conducted in Sudan, aflatoxins B_1, B_2, G_1, and G_2 contents were measured, and the amount of G_1 and G_2 at present study were inconsistent with their findings, with our found amounts being lower[25]. A study on aflatoxin content in edible oils (sesame, peanut, and cottonseed) using HPLC was found that aflatoxin content in all samples was within the permitted limit[26].In contrast to our results, no aflatoxin B_2 was found in samples. Elzupir et al. (2010)[27] determined aflatoxin content in edible oils (unpurified sesame oil, cottonseed oil and peanut oil) by HPLC. In

Table III: Max., Min., Mean, and SD of four aflatoxins B_1, B_2, G_1 and G_2 in different steps of oil production by the traditional method.

Sample Type	Sample number	Aflatoxin type	Min (ppb)	Max (ppb)	Mean±SD (ppb)
Crude Sesame	9	B_1 B_2 G_1 G_2 Total	1.8 0.1 < LOQ* < LOQ 2.4	40.5 1.9 < LOQ < LOQ 41.9	16.1±12.94 0.77±0.605 < LOQ < LOQ 17.46±13.35
Washed and dehulled sesame	9	B_1 B_2 G_1 G_2 Total	0.65 < LOQ < LOQ < LOQ 1.38	6.4 0.5 < LOQ < LOQ 7.3	2.71±2.04 0.21±0.135 < LOQ < LOQ 3.53±2.17
Roasted sesame	9	B_1 B^2 G_1 G_2 Total	0.5 < LOQ < LOQ < LOQ 1.2	4 0.4 < LOQ < LOQ 4.8	2.07±1.26 0.18±0.09 < LOQ < LOQ 2.86±1.33
Oil	9	B_1 B_2 G_1 G_2 Total	< LOQ < LOQ < LOQ < LOQ 1.2	2.3 0.2 < LOQ < LOQ 3.1	0.36±0.74 0.11±0.048 < LOQ < LOQ 1.43±0.628

*LOQ: Limit Of Quantitation (B_1, G_1= 0.5 ppb B_2, G_2= 0.1 ppb)

contrast to our results, total aflatoxin in all sesame oil was above the level specified by FDA (20 µg/kg). In their study, aflatoxins G_1 and G_2 were found in all samples, while in our study, no G1 and G2 were observed in the samples[25]. Asadi et al. (2011)[28] measured aflatoxin content in 182 sesame samples in Iran. 18/1% of the samples showed B_1 contamination. Also, 8 samples had aflatoxin B_2, and one sample had G_1 and G_2. Abalaka (1984)[29] studied aflatoxins in purified and unpurified peanut oil and found that purified oil had no aflatoxins.

A study on aflatoxin content in peanut was conducted in India, and it was found that the cold-press method resulted in 85% decrease in total aflatoxin[10]. Our results are not consistent with the results of the study mentioned above. In the present study, washing and dehulling decreased total aflatoxin, B_1 and B_2 significantly, while roasting at 90-100ºC had no significant effect on aflatoxin. Aflatoxin is resistant to heating as it is degraded at 237-306ºC[30]. Jalili (2016)[30] studied the effect of roasting at different temperatures on aflatoxin content in peanut and concluded that roasting at 150ºC resulted in 60.8-79 decrease in B_1 and G_1 and roasting at 140ºC resulted in 5.8-64.58 decrease in B_1 and G_1 and roasting at 100ºC for 30ºC did not affect aflatoxin decrease. Our finding was consistent with their results. Arzandeh and Jinap (2011)[31] examined the effect of roasting on aflatoxin content in peanut. The results showed that roasting at 90-150ºC resulted in 78.4, 57.3, 73.9 and 25.2% decrease in B_1, B_2, G_1, and G_2. In another study, Hussain et al. (2011)[32] studied peanut roasting at 150ºC for 120 min and observed a noticeable decrease (95%) in aflatoxin content. Our results are not in agreement with their finding. The roasting procedure also had significant effects on the aflatoxin degradation among the Nigerian peanut seeds[33]. One of the limitation of our study was the lack of similar national or international studies on sesame oil extraction by water (traditional) and cold press.

Conclusion

In the present study, the traditional method was more effective than the cold press in decreasing aflatoxin. One of the main reasons for high aflatoxin contamination in sesame oil samples is inappropriate materials storage conditions in sesame oil extraction. Thus proper hygiene, safety and supervision are of great importance. In the present study, aflatoxin content was within the Iranian national standard range; however, high contamination in the samples may pose severe threats to human health.

Acknowledgements

This research was supported by the Research Council of Isfahan University of Medical Sciences, Isfahan, Iran. The authors would like to thank the Food Security Research Center staff, Department of Food Science and Technology, School of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, Iran, for their laboratory supports.

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