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ORIGINAL PAPER

Physicochemical Characteristics and Aflatoxin Levels in Two Types of Sudanese Sesame Oil

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Abstract The physicochemical characteristics and aflatoxin levels of two types of sesame oil [Walad (W) and Normal (N)] were determined. A total of 104 sesame oil samples were collected during two seasons (I and II) from traditional mills in five states of Sudan. Levels of aflatoxin B_1, B_2, G_1, and G_2 were determined using HPLC. The physicochemical characteristics of W and N samples were significantly ($P \leq 0.05$) different: samples of W and N from the five states had fluctuations in physicochemical characteristics in the two seasons. The highest percentage of contamination (recorded in Khartoum followed by Kordofan state) by aflatoxin B_1 during season II occurred in normal sesame oil which was 80.77 %, followed by Walad sesame oil which was 76.92 %. These percentages of contamination in season I were lower than 59.26 % for normal sesame oil and lower than 52.0 % for Walad sesame oil in season II. Aflatoxin B_2 contamination recorded the highest incidence in season II (3 out of 26 samples, 11.54 %) of normal sesame oil, followed by Walad sesame oil (2 out of 26 samples, 7.69 %). These percentages were lower than the 7.40 and 4.0 % of normal and Walad sesame oils in season I, respectively. Aflatoxin B_1 and B_2 levels in sesame oil ranged from 0.5 to 9.8 and 0.5 to 1.3 μg/kg, respectively.

Keywords Sesame oil · Normal · Walad · Traditional mills · Afaltoxins

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Introduction

Sesame (*Sesamum indicum* l.) is an oil crop belonging to family Pedaliaceae. The sesame seed contains 54 % oil, 20 % protein, 13.4 % carbohydrate, 3.2 % crude fiber, and 3.7 % ash [1]. Sesame seeds are an important source of edible oil and high quality protein; most of these seeds are used for oil extraction and food preparations [2]. Sesame seed oil shows a remarkable stability to oxidation [3, 4], due to its high content of natural antioxidants, e.g., tocopherols, sesamol, sesamolin, and sesamin [5, 6]. The oil composition depends on climatic conditions, soil type, maturity of plant, crop variety, and method of processing, while physicochemical properties of sesame seed oil affected by their lipids and glyceride composition [7].

Mycotoxins are secondary metabolites produced by fungi with small molecular weight. About 300–400 compounds have been recognized as mycotoxins, most of them are potentially hazardous to human and animals [8]. The hot and humid climate of Sudan as a tropical country provides suitable condition for the growth of toxigenic moulds. Aflatoxins have been shown to cause various types of cancer in different species. B_1, B_2, G_1, and G_2 are the main four aflatoxins, while M_1 and M_2 are metabolites of $B_1$and B_2 that are found in the milk of animals fed aflatoxin-contaminated diets. Humans are exposed to aflatoxins by consuming contaminated food due to the growth of aflatoxin-producing fungi, because fungal growth in food is

difficult to control. Many consumers do not take care with these contaminated foods due to their poor education and socio-economic levels [9]. Contamination of sesame seeds and sesame products with aflatoxins has been reported in several studies carried in Turkey, China, Iran, and Senegal [10–13]. Broad bean meal is a popular Sudanese breakfast meal which contains 9.98 % of sesame oil [14]. The annual consumption of sesame oil in Sudan is 20,000 tons, and no sesame oil produced in Sudan in factories or traditional mills is refined and therefore could be contaminated with aflatoxins [15]. Idris et al. [15] analyzed a small number of sesame oil samples and found 43.75 % of them contaminated with aflatoxins. Studies on aflatoxin exposure and incidence of liver cancer in places like China and West Africa showed that the situation was alarming [16]. A study by Nilüfer and Boyaclu [17] emphasized the need for control of aflatoxin contamination of foods involving sesame seeds as an ingredient. Liver cancer in Sudan could be reduced by decreasing the contamination of food with aflatoxins to its international accepted levels [15]. The aim of this study is to investigate the physico-chemical characteristics of two types of sesame oil, and the levels of aflatoxins in oil samples collected from five states of Sudan.

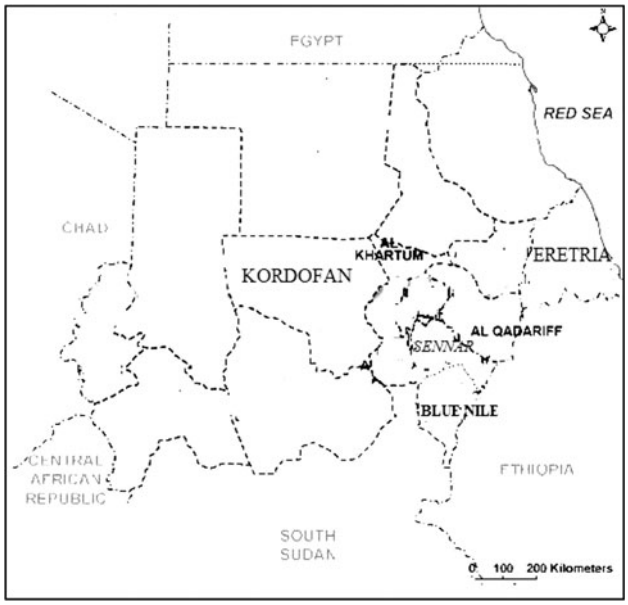

Fig. 1 Locations of Khartoum, Kordofan, Algadarif, Sennar, and Blue Nile states in Sudan

Materials and Methods

Materials

Source of Samples

A total of 104 sesame oil samples (approximately 500 ml each) were collected from five Sudanese states which were considered as major sesame oil producing and consuming states, in two seasons: season I (50 samples) seeds that were harvested in November 2008, were stored for 7 months and the oil was extracted and analyzed in June 2009, while season II (54 samples) seeds that were harvested in November 2009 and the oil was extracted in January 2010 and then analyzed. The states are Khartoum (24 samples, 12 normal sesame oil, 12 Walad sesame oil), Al gadarif (24 samples, 9 normal sesame oil, 15 Walad sesame oil), Kordofan (22 samples, 15 normal sesame, 7 Walad sesame), Blue Nile (16 samples, 8 normal sesame, 8 Walad sesame), and Sennar (18 samples,10 normal sesame, 8 Walad sesame) Fig. 1. The samples were collected from traditional mills and packed in plastic bottles and stored at refrigerator temperature (approximately 4 °C) prior to analysis.

The Chemicals and Reagents

All chemicals and solvents used were of reagent grade. Chemicals (starch, potassium iodide, sodium thiosulphate, potassium hydroxide, phenolphthalein, cupric carbonate, sodium chloride) and solvents (acetonitrile, 95 % ethanol, dichloromethane, benzene, hexane, Tri-Floro-Acetic acid, methanol, petroleum ether, and chloroform) and acids (glacial acetic acid, hydrochloric acid), were supplied by Merck (Darmstadt, Germany).

HPLC-grade water was obtained from Prime for Scientific Services, Khartoum, Sudan. The aflatoxin B_1, B_2, G_1, and G_2 were from Sigma (St. Louis, MO, USA). Following Idris et al. [15], stock standard solutions of aflatoxins with concentrations of 100 μg/ml were prepared in benzene–acetonitrile (98:2, v/v), wrapped in aluminum foil to prevent gradual break-down of aflatoxins under UV light, and kept at −20 °C.

Methods

The processing of normal and Walad sesame oils was carried out in traditional mills, the detection of aflatoxin in sesame oils and detection of sesame oil was carried out and the physicochemical characteristics of sesame oil were determined, these include Refractive Index (RI), Viscosity, Color, Specific Gravity, Peroxide Value (PV), Acid Value (AV), and statistical analysis for data generated.

Normal and Walad Oil Processing

All sesame oil samples were obtained from camel traditional oil processing mills (Assara) in Al gadarif, Sinnar, Blue Nile, Kordofan, and Khartoum State. A camel traditional milling process was used to produce Normal and Walad sesame oils. The camel traditional mill is composed

of a wooden bowl (Asara) approximately 75 cm diameter and 100 cm length, a wooden stick (Walad), a wooden connector (Hawam) and a camel. Milling is started by putting enough cleaned sesame seeds (approximately 50 kg) in the bowl then they are pressed by the stick (Walad) which is driven by a blindfolded camel plodding round and round, which squeezes the oil out of the seeds, which forms into two layers: a clean, light color upper one, which is known as Normal sesame oil, and the lower layer which produces a cloudy, dark-colored and strong-flavored oil known as Walad sesame oil [18].

Detection of Sesame Oil

The method of AOAC [19] was used for detection of sesame oil. In brief: 0.1 g of cane sugar was dissolved in 10 ml hydrochloric acid (sp.gr.1.19), 20 ml of oil sample was added and the solution was shaken well for 1 min, and left to stand. The presence of sesame oil is indicated by a crimson color in the lower (acid) layer. To check whether samples were adulterated with other edible oils, all the samples were submitted to a modified method of the Baoudouin test to identify sesame oil.

Physical Characteristics of Sesame Oil

Refractive Index

The refractive index (RI) was determined by an Abbe 60 refractometer according to the AOAC method [20] at 30 °C.

Viscosity

The oil viscosity was determined using an Ostwald-U-tube viscometer according to AOAC method [20].

Color

The color intensity of oils was recorded using a Lovibond Tintometer as units of red, yellow, and blue according to the AOCS method [19].

Specific Gravity

The specific gravity of the oils was determined at 60 °C following the AOCS official method [19].

Chemical Characteristics of Sesame Oil

Peroxide Value (PV) and Acid Value (AV)

The AOCS method [19] was followed to determine the PV of the oil samples. Five grams of the oil were accurately weighed in a conical flask. 30 ml of glacial acetic acid and chloroform (3:2) were added, and the solution was swirled gently to dissolve the oil. Then, 0.5 ml of 0.1 N KI was added to the flask, and the contents of the flask were left to stand for 1 min before adding 30 ml of distilled water. After a while, the contents were titrated with 0.1 N sodium thiosulphate until the yellow color almost disappeared. Next, 0.5 ml of 1 % starch solution was added, and the titration continued with vigorous shaking until the blue color completely disappeared. The numbers of ml 0.01 N sodium thiosulphate (a) were recorded. The same process was reported for blanks. The numbers of ml 0.01 N sodium thiosulphate (b) were recorded.

Calculation: Peroxide value = $\frac{(b-a)\times N\times 100}{S}$

where, b−a reading of blank (ml) − reading of oil sample (ml), N is the normality of sodium thiosulphate solution, and s is the original weight of sesame oil (g).

The AOCS method [19] was followed to determine the AV of the oil samples. Ten grams of oil were dissolved in 50 ml of ethanol and and ether:solvent mixture (1:1), then titrated while swirling with 0.1 N KOH solution using phenolphthalein as indicator until a pink color persisted for 15 s, the number of ml of 0.1 N KOH required (AV) was calculated as follows:

Acid value = $\frac{56.1\times V\times N}{W}$ where V is the volume in ml of KOH, N is the normality of the KOH, and W is the weight of the sample in grams.

Detection of Aflatoxins

A mixture of benzene:acetonitrile (98:2) solution was used to dissolve the crystalline aflatoxins and concentrations of 0.5 μg/ml B_1 and G_1 and 0.25 μg/ml for B_2 and G_2 were obtained. Appropriate aliquots were taken to give specific concentrations of the individual aflatoxins.

Extraction and Analysis of Aflatoxins

The AOAC [20] and Idris et al. [15] methods were followed for the purification of aflatoxins from collected samples, in brief: 250 ml of methanol:water (55:45) were added to each oil sample (50 g), then 50 ml 0.1 N HCl were added, the mixture was blended and then centrifuged, then filtered through 24-cm Whatman No. 1 paper. Fifty milliliters of filtrate were transferred into a 250-ml separator funnel, then 50 ml 10 % NaCl solution were added and swirled, and finally 50 ml of hexane were added and gently shaken for 30 s. The separated lower aqueous layer phase was drained into another 250-ml separator; then extracted by 3 × 25 ml dichloromethane and added to the aqueous phase and shaken vigorously for 30 s. Then, the

phases separated and the lower dichloro-methane layer was drained, collected, and evaporated on a boiling water bath to dryness.

Derivatization

Hexane (200 ml) and 50 ml Tri-Floro-Acetic acid (TFA) were added to the extract and mixed on a Vortex for 30 s, allowed to stand for 5 min, then 1.950 ml water:acetonitrile (9:1) was added and mixed vigorously on the Vortex for 30 s, and the layers allowed to separate for 10 min, and the lower layer of the acetonitrile:water phase was taken in a vial for HPLC determination [15].

HPLC Determination of Aflatoxins

The HPLC equipment was a Shimadzu reverse phase HPLC system (Shimadzu, Japan) with a Macherey–Nagel, C-18, 25 cm, 4.6 mm column, with the system set at 360 nm excitation and 460 nm emission. The mobile phase was water:methanol:acetonitrile (4:1:1) and the flow rate was 1.0 ml/min. The aflatoxin concentrations in the sample extract were determined and quantified by the retention time and peak areas, respectively [15].

Calculation:

$$\text{Aflatoxin}\,(\mu g) = P_x/P_s \times C_s \times \tfrac{2}{10} \times 1000 \times D$$

where P_x is the peak area of the sample, P_s the peak area of the standard, C_s the concentration of the standard, and D the dilution factor

Statistical analysis

Data generated were subjected to the SAS software package. Three-factors RCD was performed, where factor (A) = season (two seasons), factor (B) = type of oil (two types), and factor (C) = location (five locations). Means were then tested and separated using DMRT as reported by Steel et al. [21].

Results and Discussion

Detection of Sesame Oil

All the samples were submitted to the Baoudouin test to identify sesame oils which gave positive results. The Baoudouin test has been reported for the detection of sesame oil up to extent 0.2 %, and this color test performs readily when a small quantity of sesame oil containing some other oil and fat is allowed to react with 2-thiophen carboxaldehyde in the presence of hydrochloric acid, when a very fine pink or deep red color reaction is specific to sesame oil. It has been observed by experimentation [22] that the other oils and fats do not react to this test at all. This test ensured the absence of any adulteration in the studied samples and that these samples were 100 % sesame oil and free of any other edible oils.

Changes in Physical Properties of Sesame Oil as Affected by Location, Type of Oil and Season

Refractive Index (RI)

The RI of sesame oil samples from five states are shown in Table 1. The lowest values (1.471) were recorded in samples from Kordofan and Algadarif states in seasons I and II, the values for N samples ranged between 1.472 and 1.474 in seasons I and I), while for W samples values ranged between 1.471 and 1.474 in season I and 1.471–1.473 in season II which was lower than samples N. No significance difference between two types of samples from the state within the same season, but for the same type of oil from different states, a significant difference was observed, which suggests it is due to variation in the sesame type used. Codex standards [23] gave values of 1.465–1.469 for the RI of sesame oil at 40 °C. The values having different superscript letter(s) in each column and row in Table 1 differ significantly ($P \leq 0.05$). Rahman [7] suggested that the refractive index is related to the molecular structure and degree of the unsaturation of the oil, and he also suggested that the same type of oil, varying due to unsaturation, is greater than those varying due to other sources, which is in agreement with our result obtained from two types of crude sesame oil from different locations.

Changes in Viscosity

Table 2 shows viscosity of crude sesame oil obtained from different locations and seasons at ambient temperature (34 ± 5 °C). The increase in viscosity of the oil within two seasons was significant ($P \leq 0.05$). The highest levels recorded 24.71 and 24.68 cp in Khartoum state for samples N and W, respectively in season I, samples N ranged between 17.98 and 24.71 cp in season I, and ranged between 19.24 and 23.47 cp in season I), while for W samples ranged between 17.75 and 24.68 cp in season I, and 19.63 and 23.10 cp in season II. The observed decrease in viscosity in season II is in agreement with Murwan [24] who reported that the viscosity of sesame seed oil ranges between 18.90 and 26.43 cp at 32 °C. No significant difference observed in viscosity of the same type of oil from same state, but significantly different between two types of oil from the same state was observed, and it was also observed that the same type of sesame oil differ significantly ($P \leq 0.05$) within the states. These results indicate that the viscosity of sesame oil was affected by variety, soil

Table 1 Refractive index of sesame oil as affected by location, type of oil and season

Location	Sample				$Lsd_{0.05}$	SE
	N		W			
	Season					
	I	II	I	II		
Khartoum ($n_1 = 12$; $n_2 = 12$)	$^{A}1.474 \pm 0.03^{a}$	$^{C}1.472 \pm 0.01^{b}$	$^{A}1.474 \pm 0.03^{a}$	$^{B}1.472 \pm 0.01^{b}$	0.0005955*	0.0001822
Sennar ($n_1 = 10$; $n_2 = 8$)	$^{C}1.472 \pm 0.01^{b}$	$^{B}1.473 \pm 0.02^{a}$	$^{B}1.473 \pm 0.02^{a}$	$^{A}1.473 \pm 0.02^{a}$	0.0005952*	0.0001821
Blue Nile ($n_1 = 8$; $n_2 = 8$)	$^{A}1.474 \pm 0.03^{a}$	$^{A}1.474 \pm 0.03^{a}$	$^{A}1.474 \pm 0.03^{a}$	$^{A}1.473 \pm 0.02^{b}$	0.0005956*	0.0001824
Kordofan ($n_1 = 10$; $n_2 = 12$)	$^{B}1.473 \pm 0.02^{a}$	$^{C}1.472 \pm 0.01^{b}$	$^{C}1.472 \pm 0.01^{b}$	$^{C}1.471 \pm 0.00^{c}$	0.0005957*	0.0001827
Algadarif ($n_1 = 12$; $n_2 = 12$)	$^{C}1.472 \pm 0.01^{a}$	$^{C}1.472 \pm 0.01^{a}$	$^{D}1.471 \pm 0.00^{b}$	$^{C}1.471 \pm 0.00^{b}$	0.0005954*	0.0001823
$Lsd_{0.05}$	0.0005953*	0.0005951*	0.0005952*	0.0005951*		
SE	0.0001825	0.0001827	0.0001824	0.0001822		

N Normal sesame oil, *W* Walad sesame oil, *I* First season, *II* Second season, n_1, n_2 number of samples in two seasons

Differences marked * are significantly different

Mean ± SD value(s) having different superscript letter(s) within rows (small letters) and columns (capital letters) differ significantly ($P \leq 0.05$)

Table 2 Changes in viscosity of sesame oil as affected by location, type of oil and season

Location	Sample				$Lsd_{0.05}$	SE
	N		W			
	Season					
	I	II	I	II		
Khartoum ($n_1 = 12$; $n_2 = 12$)	$^{A}24.71 \pm 0.41^{a}$	$^{B}20.80 \pm 0.08^{b}$	$^{A}24.68 \pm 0.17^{a}$	$^{B}20.79 \pm 0.21^{b}$	0.0952*	0.0638
Sennar ($n_1 = 10$; $n_2 = 8$)	$^{C}17.98 \pm 0.08^{b}$	$^{A}23.47 \pm 0.28^{a}$	$^{C}17.95 \pm 0.25^{b}$	$^{A}23.10 \pm 0.19^{a}$	0.0387*	0.0824
Blue Nile ($n_1 = 8$; $n_2 = 8$)	$^{AB}23.51 \pm 0.41^{a}$	$^{BC}20.93 \pm 0.11^{c}$	$^{AB}23.12 \pm 0.13^{a}$	$^{AB}22.17 \pm 0.23^{b}$	0.0126*	0.0121
Kordofan ($n_1 = 10$; $n_2 = 12$)	$^{BC}18.03 \pm 0.05^{b}$	$^{C}19.24 \pm 0.10^{a}$	$^{C}17.75 \pm 0.33^{b}$	$^{BC}19.63 \pm 0.23^{a}$	0.0247*	0.0069
Algadarif ($n_1 = 12$; $n_2 = 12$)	$^{B}21.52 \pm 0.09^{a}$	$^{B}20.85 \pm 0.09^{b}$	$^{B}20.89 \pm 0.28^{b}$	$^{B}20.62 \pm 0.13^{b}$	0.0395*	0.0145
$Lsd_{0.05}$	0.9325*	0.8745*	0.9152*	0.7633*		
SE	0.0067	0.0095	0.0013	0.00325		

N Normal sesame oil, *W* Walad sesame oil, *I* First season, *II* Second season, n_1,n_2 number of samples in two seasons

Differences marked * are significantly different

Mean ± SD value(s) having different superscript letter(s) within rows (small letters) and columns (capital letters) differ significantly ($P \leq 0.05$)

type and location. The crude sesame oil has a higher viscosity than the refined oils. Hui [25] reported that saturation and long chain fatty acids or polymerized break-down products in oils tend to increase the viscosity of oils. There is a relationship between viscosity and temperatures, Mariod et al. [26] studied the viscosity of four Sudanese conventional oils in comparison with three unconventional ones, they found that the viscosity was decreased with increase of temperature in all vegetable oils studied.

Changes in Color

Table 3 shows the changes in the Lovibond tintomiter values for the oils obtained from the five states. All samples recorded values (0 tintometer) for blue color, oil from Blue Nile state had the highest level for yellow color (10.30 and 10.60 tintometer) for samples N and W, respectively, in season I, while the lowest level (3.83 tintometer) in yellow color was observed in samples N of Khartoum state in season I. In other states, the values ranged between 4.00 and 8.70 tintometer for samples N and W in two seasons: the lowest level of red color was recorded in Khartoum state for samples N in season I, while the highest level was also reported in the same state for the same samples in season II. All samples recorded values ranging between 0.99 and 1.27 tintometer for the red color in two seasons. The values obtained from different states and two types of oil were significantly different ($P \leq 0.05$) in two seasons as affected by the period of storage. In these results, the variation in yellow and red color unit values were due to differences in period of storage and sources of sesame oil. Higher numbers of red

units indicate darker oil color and this could be due to the presence of pigments in the oil. Chlorophyll pigments (mainly pheophytins) are extracted into crude oils and impart a greenish color to the crude oil and become indicators of quality. Thermal decomposition of chlorophyll gives a pheophytin pigment which results in dark-colored oil. This pigment contributes to an off-flavor and may also promote the oxidation of the oil, thus reducing its storage stability Diosady [27].

Changes in Specific Gravity

The specific gravity (SG) of sesame oil samples from five states is shown in Table 4. Oil from Sennar state had the highest values (0.9533, 0.9535 %) in season I for samples N and W, respectively, while the values for samples N ranged between 0.9176 and 0.9533 % in season I, and 0.9154 and 0.9192 % in season II, samples W ranged between 0.9172 and 0.9535 % in season I and 0.9153 and 0.9193 % in season II,. All the values decreased in season II: the values of two types of sesame oil differ significantly ($P \leq 0.05$) within the states, but no significant difference was found for the same type of oil within the same state. The values are in good agreement with SSMO [28] sesame oil standards. Also, Murwan [24] reported that the increase in specific gravity determined the purity of edible oils during processing and storage. The specific gravity at given temperature increases with the decrease of the molecular weight, and increase of the degree of unsaturation [29]. It was concluded that the specific gravity of sesame oil varies

Table 3 Changes in color (tintometer) of sesame oil as affected by location, type of oil and season

Location	Sample				$Lsd_{0.05}$	SE
	N		W			
	Season					
	I	II	I	II		
Yellow						
Khartoum ($n_1 = 12$; $n_2 = 12$)	$^{D}3.83^{c} \pm 0.00$	$^{C}5.17^{a} \pm 0.43$	$^{D}4.70^{b} \pm 0.37$	$^{CD}4.00^{bc} \pm 0.23$	0.4384*	0.0183
Sinnar ($n_1 = 10$; $n_2 = 8$)	$^{BC}8.00^{a} \pm 0.71$	$^{CD}4.25^{b} \pm 0.46$	$^{B}8.00^{a} \pm 0.71$	$^{C}4.25^{b} \pm 0.31$	1.2840*	0.0329
Blue Nile ($n_1 = 8$; $n_2 = 8$)	$^{A}10.30^{ab} \pm 0.94$	$^{A}8.00^{b} \pm 0.71$	$^{A}10.60^{a} \pm 0.92$	$^{A}7.00^{bc} \pm 0.65$	0.3906*	0.0647
Kordofan ($n_1 = 10$; $n_2 = 12$)	$^{C}5.22^{bc} \pm 0.46$	$^{B}6.88^{ab} \pm 0.57$	$^{C}7.00^{a} \pm 0.65$	$^{B}5.28^{b} \pm 0.47$	0.1165*	0.0099
Algadarif ($n_1 = 12$; $n_2 = 12$)	$^{B}8.70^{a} \pm 0.76$	$^{CD}4.17^{bc} \pm 0.29$	$^{B}C7.77^{ab} \pm 0.69$	$^{BC}4.50^{b} \pm 0.34$	0.3681*	0.0078
$Lsd_{0.05}$	1.8265**	0.8547*	1.6983*	0.6255*		
SE	0.2743	0.1936	0.1774	0.0834		
Red						
Khartoum ($n_1 = 12$; $n_2 = 12$)	$^{BC}0.93^{bc} \pm 0.01$	$^{A}1.30^{a} \pm 0.06$	$^{A}1.07^{b} \pm 0.03$	$^{A}1.20^{ab} \pm 0.05$	0.0845*	0.0032
Sinnar ($n_1 = 10$; $n_2 = 8$)	$^{A}1.14^{a} \pm 0.04$	$^{B}1.15^{a} \pm 0.04$	$^{AB}1.06^{b} \pm 0.03$	$^{AB}1.13^{a} \pm 0.04$	0.0469*	0.0017
Blue Nile ($n_1 = 8$; $n_2 = 8$)	$^{AB}1.13^{a} \pm 0.04$	$^{BC}1.10^{ab} \pm 0.03$	$^{A}1.08^{b} \pm 0.03$	$^{AB}1.13^{a} \pm 0.04$	0.0387*	0.0024
Kordofan ($n_1 = 10$; $n_2 = 12$)	$^{B}0.99^{bc} \pm 0.02$	$^{B}1.13^{a} \pm 0.04$	$^{B}1.00^{b} \pm 0.02$	$^{B}1.10^{b} \pm 0.03$	0.0364*	0.0016
Algadarif ($n_1 = 12$; $n_2 = 12$)	$^{A}1.15^{ab} \pm 0.04$	$^{AB}1.27^{a} \pm 0.06$	$^{A}1.07^{b} \pm 0.03$	$^{BC}1.07^{b} \pm 0.03$	0.0975*	0.0038
$Lsd_{0.05}$	0.0463*	0.0678*	0.0591*	0.0720*		
SE	0.0087	0.0054	0.0021	0.0068		
Blue						
Khartoum ($n_1 = 12$; $n_2 = 12$)	$^{A}0.00^{a} \pm 0.00$	$^{A}0.00^{a} \pm 0.00$	$^{A}0.00^{a} \pm 0.00$	$^{A}0.00^{a} \pm 0.00$	$0.001^{n.s}$	0.00
Sinnar ($n_1 = 10$; $n_2 = 8$)	$^{A}0.00^{a} \pm 0.00$	$^{A}0.00^{a} \pm 0.00$	$^{A}0.00^{a} \pm 0.00$	$^{A}0.00^{a} \pm 0.00$	$0.001^{n.s}$	0.00
Blue Nile ($n_1 = 8$; $n_2 = 8$)	$^{A}0.00^{a} \pm 0.00$	$^{A}0.00^{a} \pm 0.00$	$^{A}0.00^{a} \pm 0.00$	$^{A}0.00^{a} \pm 0.00$	$0.001^{n.s}$	0.00
Kordofan ($n_1 = 10$; $n_2 = 12$)	$^{A}0.00^{a} \pm 0.00$	$^{A}0.00^{a} \pm 0.00$	$^{A}0.00^{a} \pm 0.00$	$^{A}0.00^{a} \pm 0.00$	$0.001^{n.s}$	0.00
Algadarif ($n_1 = 12$; $n_2 = 12$)	$^{A}0.00^{a} \pm 0.00$	$^{A}0.00^{a} \pm 0.00$	$^{A}0.00^{a} \pm 0.00$	$^{A}0.00^{a} \pm 0.00$	$0.001^{n.s}$	0.00
$Lsd_{0.05}$	$0.001^{n.s}$	$0.001^{n.s}$	$0.001^{n.s}$	$0.001^{n.s}$		
SE	0.00	0.00	0.00	0.00		

N Normal sesame oil, *W* Walad sesame oil, *I* First season, *II* Second season, n_1,n_2 number of samples in two seasons

Differences marked * are significantly different

Differences marked ** are not significantly different

Mean ± SD value(s) having different superscript letter(s) within rows (small letters) and columns (capital letters) differ significantly ($P \leq 0.05$)

Table 4 Changes in specific gravity of sesame oil as affected by location, type of oil and season

Location	Sample				$Lsd_{0.05}$	SE
	N		W			
	Season					
	I	II	I	II		
Khartoum ($n_1 = 12$; $n_2 = 12$)	$^{C}0.9183 \pm 0.02^{a}$	$^{BC}0.9157 \pm 0.01^{b}$	$^{AB}0.9183 \pm 0.02^{a}$	$^{B}0.9159 \pm 0.01^{ab}$	0.0005952*	0.0001823
Sennar ($n_1 = 10$; $n_2 = 8$)	$^{A}0.9533 \pm 0.04^{a}$	$^{B}0.9180 \pm 0.02^{b}$	$^{A}0.9535 \pm 0.04^{ab}$	$^{AB}0.9181 \pm 0.01^{b}$	0.0005951*	0.0001825
Blue Nile ($n_1 = 8$; $n_2 = 8$)	$^{B}0.9192 \pm 0.03^{a}$	$^{A}0.9192 \pm 0.03^{a}$	$^{AB}0.9184 \pm 0.00^{b}$	$^{A}0.9193 \pm 0.03^{a}$	0.0005953*	0.0001824
Kordofan ($n_1 = 10$; $n_2 = 13$)	$^{CD}0.9176 \pm 0.01^{b}$	$^{AB}0.9184 \pm 0.02^{a}$	$^{BC}0.9172 \pm 0.01^{bc}$	$^{AB}0.9182 \pm 0.02^{ab}$	0.0005957*	0.0001822
Algadarif ($n_1 = 12$; $n_2 = 12$)	$^{BC}0.9185 \pm 0.02^{a}$	$^{C}0.9154 \pm 0.01^{b}$	$^{B}0.9182 \pm 0.02^{ab}$	$^{BC}0.9153 \pm 0.01^{b}$	0.0005950*	0.0001823
$Lsd_{0.05}$	0.0005958*	0.0005952*	0.0005951*	0.0005953*		
SE	0.0001827	0.0001824	0.0001823	0.0001822		

N Normal sesame oil, *W* Walad sesame oil, *I* First season, *II* Second season, n_1,n_2 number of samples in two seasons

Differences marked * are significantly different

Mean ± SD value(s) having different superscript letter(s) within rows (small letters) and columns (capital letters) differ significantly ($P \leq 0.05$)

with the source and season of the sesame seeds which reflect on the quality of sesame oil. These may be lipochromes originated from tissues or artifacts causes by degradation by thermal processing and treatment.

Changes in Chemical Properties of Sesame Oil as Affected by Location, Type of Oil and Season

Changes in peroxide value

As shown in Table 5, the changes in peroxide value (PV) of crude sesame oil of five states recorded the highest level (22.55 meq/Kg oil) observed in Blue Nile state in season I for W samples, while the lowest level (0.220 meq/Kg oil) was observed in Sennar state in season II for W samples. The values in season I for N samples ranged between 0.8643 and 16.92 meq/Kg oil, while W samples recorded 0.6650–22.55 meq/Kg oil, which was higher values than samples N. The values in season II for N samples recorded 0.2167–1.764 meq/Kg oil, while for W samples they ranged between 0.220 and 1.066 meq/Kg oil, which was lower values than samples N. In these results, the variation in PV due to the difference in seasons of sesame oil and the location of the sesame seeds and which may also refer to their storage periods, showed large fluctuations during the same season for the same type of oil from different states and less fluctuation during two seasons for two types from the same state. The values are in agreement with the 1.43–29.55 reported by Fadl Elseed [30] for oil extracted from sesame seed cultivars stored for 12 months at ambient temperature, while SSMO [28] standards showed PV of unrefined sesame oil as 15 meq/Kg oil and refined sesame oil as 10 meq/Kg oil. Nzikou et al. [31] mentioned thatthe peroxide value of sesame oil increases with temperature and storage state. These results indicated that there are significant ($P \leq 0.05$) differences in the peroxide value of different samples.

Changes in Acid Value

Table 6 shows the changes in acid value (AV) of crude sesame oil of five states. The acid value had the highest level (3.680) observed in oil from Algadarif state in season I for W samples, while the lowest level (0.7433) was observed in oil from Kordofan state in season II for W samples. The values in season I for N samples ranged between 1.367 and 2.783 % while W samples recorded 1.580–3.680 % which were higher values than samples N. The values in season II for N samples recorded 0.8800–2.440 % while W samples ranged between 0.7433 and 2.197 % which was lower values than samples N. These results indicated that the two types of sesame oil differed in acid value within seasons and location due to variation in storage time and source of sesame seeds. Free fatty acid and acid values are usually considered as rancidity parameters used in evaluating the quality of oil during storage and heating. Increases in free fatty acid and acid value decrease shelf life during storage. These results indicated that there is a significant difference in acid value of different samples at $P \leq 0.05$.

Aflatoxin Content of Two Types of Sesame Oil as Affected by Location and Season

The percentage of aflatoxins contamination were calculated in 104 samples of two types (W and N) of sesame oil collected from traditional mills from five states (Khartoum, Kordofan, Algadarif, Blue Nile and Sennar). The results

Table 5 Changes in peroxide value of sesame oil as affected by location, type of oil and season

Location	Sample				$Lsd_{0.05}$	SE
	N		W			
	Season					
	I	II	I	II		
Khartoum ($n_1 = 12$; $n_2 = 12$)	$^{D}1.020 \pm 0.02^{a}$	$^{B}0.311 \pm 0.01^{b}$	$^{CD}0.92 \pm 0.10^{ab}$	$^{B}0.301 \pm 0.02^{b}$	0.10314*	0.03192
Sennar ($n_1 = 10$; $n_2 = 8$)	$^{B}13.26 \pm 0.38^{a}$	$^{C}0.244 \pm 0.02^{c}$	$^{B}10.17 \pm 0.07^{b}$	$^{CD}0.220 \pm 0.00^{cd}$	0.36723**	0.00287
Blue Nile ($n_1 = 8$; $n_2 = 8$)	$^{A}16.92 \pm 0.17^{b}$	$^{CD}0.2167 \pm 0.03^{cd}$	$^{A}22.55 \pm 0.32^{a}$	$^{C}0.2699 \pm 0.02^{c}$	0.13685**	0.00334
Kordofan ($n_1 = 10$; $n_2 = 12$)	$^{C}3.394 \pm 0.00^{a}$	$^{A}1.764 \pm 0.02^{bc}$	$^{C}1.923 \pm 0.02^{b}$	$^{A}1.066 \pm 0.01^{c}$	0.15762*	0.00816
Algadarif ($n_1 = 12$; $n_2 = 12$)	$^{DE}0.8643 \pm 0.57^{a}$	$^{BC}0.2627 \pm 1.59^{cd}$	$^{D}0.665 \pm 0.94^{b}$	$^{BC}0.278 \pm 0.07^{c}$	0.00241*	0.00183
$Lsd_{0.05}$	0.0523**	0.0429*	0.0568**	0.0109*		
SE	0.0274	0.0186	0.0199	0.00013		

N Normal sesame oil, *W* Walad sesame oil, *I* First season, *II* Second season, n_1,n_2 number of samples in two seasons

Differences marked * are significantly different

Differences marked ** are not significantly different

Mean ± SD value(s) having different superscript letter(s) within rows (small letters) and columns (capital letters) differ significantly ($P \leq 0.05$)

Table 6 Changes in acid value of sesame oil as affected by location, type of oil and season

Location	Sample				$Lsd_{0.05}$	SE
	N		W			
	Season					
	I	II	I	II		
Khartoum ($n_1 = 12$; $n_2 = 12$)	$^{B}1.367 \pm 0.11^{b}$	$^{BC}1.370 \pm 0.09^{bc}$	$^{CD}1.580 \pm 0.09^{a}$	$^{CD}1.240 \pm 0.06^{c}$	0.1283*	0.0517
Sennar ($n_1 = 10$; $n_2 = 8$)	$^{BC}2.353 \pm 0.15^{ab}$	$^{A}2.440 \pm 0.01^{a}$	$^{C}2.347 \pm 0.06^{b}$	$^{A}2.197 \pm 0.29^{c}$	0.1660*	0.1031
Blue Nile ($n_1 = 8$; $n_2 = 8$)	$^{A}2.783 \pm 0.22^{a}$	$^{B}1.750 \pm 0.25^{ab}$	$^{B}C2.567 \pm 0.11^{b}$	$^{B}1.757 \pm 0.01^{ab}$	0.2752*	0.0284
Kordofan ($n_1 = 10$; $n_2 = 13$)	$^{C}2.330 \pm 0.06^{b}$	$^{CD}0.880 \pm 0.14^{bc}$	$^{B}3.057 \pm 0.00^{a}$	$^{D}0.743 \pm 0.19^{c}$	0.1981*	0.0655
Algadarif ($n_1 = 12$; $n_2 = 12$)	$^{AB}2.767 \pm 0.25^{b}$	$^{C}1.243 \pm 0.05^{cd}$	$^{A}3.680 \pm 0.01^{a}$	$^{C}1.347 \pm 0.01^{c}$	0.1027*	0.0231
$Lsd_{0.05}$	0.0251*	0.0179*	0.0346*	0.0298*		
SE	0.0063	0.0089	0.0016	0.0064		

N Normal sesame oil, *W* Walad sesame oil, *I* First season, *II* Second season, n_1,n_2 number of samples in two seasons

Differences marked * are significantly different

Mean ± SD value(s) having different superscript letter(s) within rows (small letters) and columns (capital letters) differ significantly ($P \leq 0.05$)

revealed that oil from Khartoum state showed the highest percentage of contamination, as 91.7 % of the 24 samples was contaminated with AFB_1 and 12.50 % with AFB_2, followed by oil from Kordofan where 68.2 % of the samples were contaminated with AFB_1 and 4.6 % with AFB_2. Oil from Sennar came in the third rank, as 66.7 % of the samples were contaminated with AFB_1 and 11.1 % with AFB_2, for Blue Nile, 62.0 % was contaminated with AFB_1 and no incidence for AFB_2, while 45.8 % of oil samples from Algadarif state were contaminated with AFB_1 and only 8.3 % of the samples were contaminated with AFB_2, which represented the lowest percentage of contaminated samples. No aflatoxin contamination with $AFG_1$and AFG_2 was found in any oil samples. These results are in good agreement with the 98.8 % that was reported by Elzupir et al. [32] for the aflatoxin level in vegetable oils collected from Khartoum state. In these results, it was observed that some aflatoxin-contaminated samples in each state had high levels of peroxide values for two types of oil in seasons I and II, which indicated a correlation between the storage period of the oil seeds and aflatoxin contamination.

The aflatoxin contamination according to oil type and seasons revealed that the aflatoxins detected were B_1 and B_2 while AFG_1 and AFG_2 were not detected in any of the oil samples. The B_1 was found in 70 samples, representing 67.31 % of the total samples analyzed, in the range of 0.5–9.8 μg/kg, while B_2 was found in 8 samples, corresponding to 7.69 % of the total samples analyzed and in the range of 0.5–1.3 μg/kg. Of the samples of Walad sesame oil (W), 13 of 25 (52 %) were contaminated with AFB_1 in

season I, 1 of them (4 %) was contaminated with AFB_2, and 20 out of 26 (76.92 %) were contaminated with AFB_1 in season II and 2 (7.69 %) were contaminated with AFB_2. Of the samples of Normal sesame oil (N), 16 of 27 (59.26 %) were contaminated with AFB_1 in season I and 2 (7.40 %) were contaminated with AFB_2, and 21 out of 26 (80.77 %) in season II were contaminated with AFB_1 and 3 (11.54 %) were contaminated with AFB_2.

The concentration of aflatoxin B_1 in the 104 sesame oil samples tested revealed that no aflatoxin was detected in 34 samples which represented more than 31.79 % of the samples. The contamination in the ranges 0.5–2.0, 2.1–4.0, 4.1–6.0, 6.1–8.0, and 8.1–10.0 μg/kg were detected in 15, 36, 11, 41, and 4 samples respectively, representing about 14.42, 34.62, 10.57, 3.85, and 3.85 % of sesame oil samples, respectively. The majority of the contaminated samples were within the aflatoxin B_1 concentration range of 2.1–4.0 μg/kg.

The results of this study indicated that sesame oils obtained from stored sesame seeds in the traditional mills were easily contaminated with aflatoxins, so these mills should use strong protective measurements. The level of aflatoxin in sesame seeds from production states and in sesame oil from consumption states (such as the contaminated Khartoum samples) in two seasons may occur due to infestation of the sesame seeds by *A. flavus* in the production or consumption areas, in the field preharvest or postharvest, or during the drying or storage periods due to high moisture [33]. Cotty and Lee [34] suggested that unacceptable aflatoxin levels may occur from unpreventable insect damage to the developing crop or from exposure of the mature crop to moisture, either prior to harvest or during storage, handling, and transporting. This suggests that the aflatoxin contamination in sesame oil may be due to improper storage of sesame seeds under favorable conditions for fungal growth and aflatoxin production. According to Reddy et al. [33], the level of AFB_1 contamination in sesame seeds ranges from 550.4 to 2,890.6 μg/kg. All sesame oil in this study was unrefined, and the aflatoxin levels observed in this study are in agreement with those reported by Idris et al. [15] who determined aflatoxin contamination levels of crude sesame oil samples collected from three state of Sudan (Kordofan, Gezira and Khartoum) in the range from 0.2 to 0.8 μg/kg, which was the highest incidence of aflatoxin contamination (representing 43.75 %) than the other samples in the study (groundnut and cottonseed oils). Cavaliere et al. [35] studied Italian commercial virgin olive oil samples and found 20 % of these samples were contaminated with aflatoxin B_1.

Many countries have regulated the maximum permissible levels of aflatoxin B_1 and other mycotoxins in food materials, and these levels vary from one country to another. The maximum allowable level for aflatoxin B_1 regulated by the Scientific Commission of the European Community is 2 μg/kg, while in the USA the maximum allowable level for total aflatoxins is 20 μg/kg in all foods for human consumption.

High aflatoxin contamination levels were observed in season II (long storage period) in normal sesame oil samples more than in Walad sesame oil samples, which indicates that the stored sesame oil more susceptible to aflatoxin contamination, and that the storage time is the essential factor for fungal growth and aflatoxin production in sesame oil.

Conclusions

Samples of sesame oil obtained from stored seeds are contaminated with AFB_1 at levels of 0.5–9.8 μg/kg and AFB_2 at levels of 0.5–1.3 μg/kg, suggesting that storage conditions are essential factors in aflatoxin contamination of sesame oils. The statistical analysis showed that there are significant differences between the various types of sesame oil, production seasons, and production locations in the physical and chemical properties of sesame oil. A correlation between the aflatoxin contamination and high levels of peroxide value was observed, which indicates aflatoxin production during the sesame seed storage period. The detected aflatoxin $B_1$and B_2 levels in this study were lower than previously reported values; however, it is necessary for the Sudanese authorities to set maximum levels of aflatoxins in vegetable oils and to improve postharvest processes to minimize the health hazards resulting from the consumption of alflatoxin-contaminated foods.

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