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OCCURRENCE OF AFLATOXINS IN RAW SESAME SEEDS AND ITS PROCESSED PRODUCTS IN EGYPT

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ABSTRACT

A total of 105 samples consisting of 70 raw sesame seed samples were collected from different places of Egyptian Governorates (Cairo, Giza, Qalubia, Gharbia, Portsaid, Ismailia, and Dakahlia). Additional, 35 randomly Halaw Tehinia (HT.) and Tehina (T.) samples processed by some authorized registered(R) and non authorized unregistered(UR) Egyptian producing factories were purchased from Cairo retail markets. The samples were collected during autumn and winter seasons of 2004/2005.

All samples were examined for the contamination of aflatoxins. The chemical composition, fatty acids pattern of the oils extracted from raw sesame seed, HT. and T. were studied. Also amino acids concentrations in the main products were determined.

Results showed that raw sesame seeds, R.HT., and R.T. samples were almost free from aflatoxins in most samples. On the other hand, most of H.T. samples produced from illegal factories (UR.HT.) were contaminated with aflatoxin. The reason of such contamination were studied. The discussion is presented showing that, it is highly probably that peanut and palm oil were used in the manufacturing of UR.HT. to replace part of sesame seed.

Keywords: Raw Sesame seeds, Halawa Tehinia, Tehina, Aflatoxin.

INTRODUCTION

Sesame (*Sesamum indicum L.)* is an important annual oilseed crop grown specially in Middle Eastern countries as a rich source of oil, protein, calcium and phosphorus (*Salunkhe et al., 1991 and Uzun et al., 2002*). There are many foods in which sesame is an ingredient. Sesame seed is the main ingredient in manufacturing of Halawa Tehinia (HT.) and Tehina (T.). Some special varieties of bread and cakes use sesame seed. Sesame seed oil is used in salad and cooking. Tehina is produced by squeezing roasted sesame seeds. Tehina is used mainly in preparing special salad. Halawa Tehinia is manufactured mainly by mixing Tehina with hot solution made up from *Saponria officinalis*, sugar and citric acid or tartaric acid. Halawa Tehinia (Halva in Turkish) is a sweet paste popular in Egypt and most of Middle Eastern countries. The word Halawa is derived from the Arabic word "Helw" meaning sweet. It is composed of 25-30% oil, 10-15% protein, and 45-50% sugar *(Kabak and GÖk,2005)*.Aflatoxin (AF), a class of mycotoxins produced by fungal species of the genus *Aspergillus* (*A. flavus*, *A. parasiticus* and *A. nomius*) (JECFA, 2001).

Aflatoxins have been found in many commodities such as cereal grains, figs, nuts, tobacco, oilseeds and their products (*Diener et al., 1987, Abdelhamid, 1990 and Abdelhamid et al., 1996*). In fact almost all foods are susceptible to mold growth during some stage of production, processing, storage or transport (*Farag et al., 1986 and Abdelhamid,2000*). The contamination of food and feed materials with aflatoxins which have toxic,

carcinogenic and mutagenic activity, causes important health problems and economic losses (*Sweeney and Dobson,1998 and Abdelhamid et al.,1999*).

Several surveys studies showed that AFB_1 can be found in sesame seeds and tehina which is the main constituent of HT. (*Nilüfer and Boyacioğlu, 2002*). *Kabak and GÖk (2005)* reported that information available on aflatoxins in T. and HT. is little. Consequently, the aim of the present study is to carry out an investigation on the presence of aflatoxins in sesame, H.T., and T. produced and consumed in Egypt.

MATERIALS AND METHODS

The main target of the present work is to carry out a simple survey on sesame seeds (S.S), halawa tehinia (HT.) and tehina (T.) in different localities and from different producers.

Sesame seed samples:

Seventy sesame seed (S.S) samples were collected from 7 Governorates, i.e., Cairo, Giza, Qalubia, Gharbia, Portsaid, Ismailia, and Dakahlia, 10 samples from each governorate.

Halawa tehinia (HT.):

Twelve samples of H.T., were collected from markets in Cairo and Giza, produced in a packed form by 5 known registered authorized factories (abbreviated to R. HT.). Other eleven samples of HT. produced by unknown, unnamed, unregistered fabrics were collected from different places (abbreviated to UR. HT.).

Tehina (T.):

Ten samples of T. produced by some known registered authorized factories were collected from Cairo and Giza markets, (abbreviated to R.T.).

Two samples of T. produced by unknown, unnamed, unregistered fabrics, were collected from Giza markets, (abbreviated to UR.T.).

In the determination of different concentrations inspected nutrients or contaminants, certified reference materials had been used and all steps of ISO/IEC 17025 to prove and confirm the resulted figures.

a. Proximate Chemical Composition:

Moisture, ash, crude protein, fat and fiber contents were determined according to (A.O.A.C. 2000).

b. Analysis of aflatoxins:

I- Extraction of aflatoxins:

Extraction of aflatoxin was conducted according to *Roos et al., (1997)*. The samples were mixed with celite (25g), chloroform (250ml) and distilled water (25ml) using a shaker for 30min. The resultant mixture was filtered through Whatman No.4 filter paper. An aliquot of the chloroform filtrate (10ml) was evaporated to dryness using a rotary evaporator. The residue was reconstituted in 5ml methanol and the volume was made up to 50ml by distilled water. This solution was passed through an immuno affinity column (Waters company) at a flow rate of 2ml/min. using a slight vacuum. The column was washed once with distilled water (10ml) using a slight vacuum. The aflatoxin was then eluted from the column twice with methanol (1ml each) at a flow rate of 1 drop/second. The eluate was collected in 10ml

volumetric flask and made up to the mark with distilled water. An aliquot (250µl) from this solution was injected into HPLC apparatus (Agillent 1100).

II- Quantitative determination:

Aflatoxins were determination using HPLC technique (Agillent 1100 Series U.S.A with column C18,Lichrospher 100 RP-18,5 µmx25cm) according to the following technique. The mobile phase consisted of water : methanol : acetonitrile (54:29:17,v/v/v) at flow rate of I ml/min. C18 column was used The excitation and emission wavelengths for all aflatoxins were 362 and 460 nm (Florences detector), respectively *(Roos et al,. 1997)*

The aflatoxins were calculated using the formula:

Aflatoxin content (µg/Kg) = (S.Y.V.Z)/(X.W)

Where:

S= sample peak area.
Y= Concentration of aflatoxin standard.
V= ml. of final dilution of the sample.
Z= ml. of the extraction solution of the sample.
X= Peak area of the standard aflatoxin.
W= Weight of the sample (g).

c. Lipid extraction:

Lipids were extracted using petroleum ether (B.P. 60-80) in a Soxhlet apparatus for 4 hrs. The solvent was evaporated under reduced pressure *(A.O.A.C.2000)*.

d. Determination of fatty acids:

Saturated and unsaturated fatty acids were determined in 6 samples of sesame seed, HT., T., and oil samples using methyl esters boron trifluoride method *(A.O.A.C. 2000)*. The oil is saponified with sodium hydroxide in methanol. The fatty acids were methylised with boron fluoride in methanol, extracted with heptane and determined on a gas chromatography,Shimadzu,modelGC-2010,Kyoto,Japan.Capillary column silicon-based polymers (Polysiloxanes, model DB-Wax,USA and FID detector (PE Auto System XL) with auto sampler and Ezchrom integration system. Carrier gas (He):ca.25 psi-air 450ml/min- hydrogen 45ml/min-split 100ml/min. Oven temperature 200°C injector and detector 250°C.

e. Amino acids determination:

Individual and total amino acids were determined in 5 samples of sesame seed ,HT., and T. oil, according to the method described by *Widner and Eggum (1966)*. Oxidation was carried out using performing acid, to protect methionine and cysteine from destruction during acid hydrolysis, which was then hydrolyzed using 6N HCl in the oven at 110°C for 24 hours. High performance amino acid analyzer, Beckman 7300 was used for amino acid determination.

RESULTS AND DISCUSSION

Proximate analyses of sesame seeds, HT. and T. for the collected samples are shown in Table 1. The chemical composition shown in the present work is within the range of previous published work *(Farag et al. 1986)*. It is clear that S.S and it's products are good sources of energy and protein.

Table 1: Average proximate composition of samples for sesame seeds, halawa tehinia and tehina.

Analysis%	S.S	R. HT.	UR.HT.	R.T.
Moisture	4.60	5.60	5.00	7.00
Protein (NX6.25)	34.80	14.50	15.20	36.80
Ether Extract	43.00	27.36	23.94	40.00
Crude fiber	6.45	5.04	8.26	3.20
Ash	2.80	2.50	2.60	3.00
Nitrogen free extract	8.35	45.00	45.00	10.00

Concentration of total aflatoxins in raw sesame seeds are shown in Table 2. Total aflatoxins were not detected in 48 raw sesame seed samples collected during autumn and winter season of 2004/2005, from different places of Egyptian governorates while only 22 samples contained little amount of aflatoxins ranging between 1.0 to 3.0 ppb less than the perinisible limits according to Egyptian and international standards. This could be explained by the low moisture content which is unsuitable for fungal growths . These results are in agreement with those obtained by *El-Gohary (1995)*.

Table 2: Incidence of aflatoxins in raw sesame seed samples

Concentration of total aflatoxins (ppb)						
Cairo	Giza	Qalubia	Gharbia	PortSaid	Ismailia	Dakahlia
0.0	1.0	0.0	1.0	0.0	0.0	3.0
0.0	0.0	1.0	1.0	0.0	0.0	1.0
0.0	1.0	0.0	0.0	2.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	1.0
0.0	1.0	0.0	0.0	1.0	0.0	1.0
1.0	0.0	1.0	1.0	0.0	0.0	0.0
0.0	0.0	1.0	1.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	1.0
1.0	1.0	0.0	0.0	1.0	0.0	0.0
0.0	1.0	0.0	0.0	0.0	0.0	0.0

Table 3 shows the results of total aflatoxins in 23 processed H.T. consisting of 12 samples purchased in their original packages of the five H.T. illegal factories (R.HT.), and 11 non-packed illegally produced HT. (UR.HT.) samples collected from different places of retail market. The highest incidence of contamination with aflatoxins occurred in non-packed H.T. samples (UR.HT.). While only 2 packed H.T (R.HT.) samples obtained from company (E), out of 12 tested samples, were found to have 10 ppb aflatoxins. The highest detectable level of aflatoxins, i.e. 150 ppb, was found in the illegal non-packed H.T. samples.

Packed tehina samples (R.T.) and those produced from unknown illegal fabrics (UR.T.) have very low concentration of aflatoxins (Table 4). The highest concentration of aflatoxins was shown in samples drawn from company A being 6 ppb.

Table 3 : Aflatoxins concentration (ppb) of processed Halawa Tehinia.

HT.product source	Packed HT. (R.HT.)					Non-packed HT. Illegal product (ILL.HT)
	Company (A)	Company (B)	Company (C)	Company (D)	Company (E)	
No. of samples	2	3	3	2	2	11
Concentration of total aflatoxin (ppb)	1 1	0 1 0	1 0 1	1 1	10 10	125 1 120 8 10 10 140 150 1 1 130

Table 4: Aflatoxins distribution of processed sesame Tehina.

Tehina product source	Packed Tehina					Non-packed Tehina
	Company (A)	Company (B)	Company (C)	Company (D)	Company (E)	
No. of samples	2	2	2	2	2	2
Concentration of total aflatoxin (ppb)	6 6	2 1	1 1	1 1	1 1	1 1

DISCUSSION

The results of the present work show that most of the samples of unpacked H.T., produced by unregistered factories (UR.HT.) have high content of aflatoxins. If these products were produced using the main ingredient, i.e. sesame seeds, which are almost free from aflatoxins (Table 2), it would be expected to be free or have low content of aflatoxins. Accordingly it is rational to suggest that the manufacturing of these products included other ingredients than those normally used by the legally produced products.

Table 5: Main fatty acids content of materials under study

Fatty acids	SS	R.HT.	UR.HT.	R.T	PN	(1)Palmoil
Palmitic 16:0	9.03	9.67	25.99	8.52	12.16	27.30
Stearic 18:0	5.50	6.13	4.53	4.96	2.41	6.10
Oleic 18:1	42.13	42.73	51.04	43.77	45.30	58.50
Linoleic 18:2	40.64	30.05	10.88	40.85	33.25	11.40
Arachidic 20:0	0.61	0.52	1.19	0.69	1.22	-
Gondoic 20:1	0.19	0.22	0.77	0.26	1.02	-
Behenic 22:0	0.23	0.14	1.94	0.27	2.30	-

(1) *Clegg (1973)*

Consulting the trends of fatty acids concentration (Table 5) in the products under study, it can be noticed that:

a. UR.HT. fat has high content of palmitic acid (16:0), being 25.99%, compared to R.HT. ,RT. and sesame seed fats being 9.67, 8.52, and 9.03%, respectively.

b. The extracted fat from UR.HT. contained 10.88% linoleic acid (18:2), while those of sesame seed, R. HT. and R.T. fats have concentrations of 40.64, 30.05 and 40.85%,respectively.

c. Arachidic acid (20:0) content of sesame seed oil is shown to be 0.61% (Table 5). Its values in both R. HT and R.T. were 0.52 and 0.69% respectively.The illegal production of H.T. showed a concentration of 1.19% arachidic acid in its oil, almost double the content of sesame seed oil.

d. The differences between sesame seed, registered production H.T. and T. oils on one hand and that of H.T. (unregistered production), on the Other hand, are clearly shown manly with two fatty acids i.e. gondoic20:1 and behenic 22:0. Gondoic and behenic acids compose 0.19% and 0.23% respectively in sesame seed oil (Table 5). H.T. and T. produced by legal companies have concentration of 0.22% and 0.26% gondcic and 0.14% and 0.27% behenic respectively.

The oil of the unregistered produced H.T. has gondic and behenic acids with the values 0.77% (4 times of oil sesame seed values) and 1.94 (8.4 times of sesame seed oil value) respectively.

From the foregoing discussion, it is apparent that the H.T. produced by the illegal enterprises (UR.HT.) used materials other than the legally accepted ingredient, i.e. sesame seeds.

To get high content of palmitic and oleic acids and low content of linoliec acid in UR.HT. than those in R.T. and R.HT. it is suggested that other sources of oils than that of sesame seed were used in its production. The oil that can produce such variation is palm oil, where palmitic acid is three times and oleic acid 1.3 times higher than those in SS, R.HT. and R.T. Linoleic acid in palm oil is almost one third of its concentration in R.HT. (Table 5).

The high content of gondoic and behenic acids in UR.HT. than those in SS, R.HT. and R.T. (4 and 8 times, respectively) point out that an ingredient rich in such fatty acids was used in the manufacturing of UR.HT. The oil that has high content of both gondoic and Behenic acids is the peanut (NP) oil (Table 5).

The presence of peanut oil suggests that peanut was used in the production of UR.HT. to replace part of SS. With this finding, the amino acids of the materials studied beside peanut (PN) should be considered, to be able to give a final statement of materials used in the manufacturing of UR.HT. Table 6 presents the percentage of 7 amino acids in the materials studied. It is apparent that PN proteins are lower than SS proteins in the amino acids ALA, ILE, LEU, MET, THR and VAL and are higher in LYS. If PN was used to replace part of SS or R.T. to produce UR.HT. , the result will be similar to the amino acid concentrations shown in Table 6 for UR.HT.

Table 6: Amino Acids concentration (g/100g protein =16gN) in SS,L.T., R.HT., PN. and UR.HT.

A. A	SS	R.T.	R.HT.	PN	UR.HT
ALA	5.83	5.54	5.31	3.46	4.28
ILE	4.57	4.29	3.52	3.06	2.96
LEU	8.59	8.23	7.03	6.09	6.12
LYS	2.64	2.39	1.86	3.00	2.30
MET	2.67	2.53	1.66	0.66	0.79
THR	4.77	4.59	3.17	1.55	2.37
VAL	5.98	5.63	4.48	1.35	3.62

Combining the discussion in respect to fatty acids and amino acids, it could be expected that the PN was used in the manufacturing of UR.HT. Because PN contains about 35% oil while SS contains about 50% oil, the manufacture of UR.HT. , using palm oil to raise the oil content of PN. It is also rational to suggest that the high content of aflatoxins in UR.HT. samples is due to the inclusion of PN in the process of UR.HT. production. As it is known that PN and not SS, is liable to be attacked by *Aspergillus flavus* producing aflatoxins. The present work could show that fatty acid and amino acid determination is a good method for detecting adulteration of oil seed products. It is also be concluded that processed products from unregistered factories are unsafe for human consumption.

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تواجد سموم الأفلاتوكسينات في بذور السمسم و منتجاته المصنعة في مصر

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جمعت ٧٠ عينة من بذور السمسم في فترة خريف و شتاء عامي ٢٠٠٤، ٢٠٠٥ و ذلك من أماكن مختلفة من محافظات القاهرة ،الجيزة، القليوبية، بورسعيد، الإسماعيلية و الدقهلية ، كما تم تجميع ٣٥ عينة من منتجات الحلاوة الطحينية و الطحينة من الأسواق المحلية و التي يدخل السمسم في تصنيعها ، و قد تم اختبار جميع العينات السابقة لتواجد سموم الأفلاتوكسين طبيعيا بها، كذلك تم تقدير كلا من المكونات الكيميائية و الأحماض الدهنية و الأحماض الأمينية.

و قد أوضحت النتائج أن معظم عينات السمسم الخام كانت خالية من سموم الأفلاتوكسين او تحتوى على نسبه ضئيله جدا اقل من الحد المسموح به في حين كانت الحلاوة الطحينية مجهولة المصدر و المنتجة من مصانع غير مرخصة ملوثة بالأفلاتوكسينات الكليه حيث وصلت نسبة التلوث بالأفلاتوكسين في بعضها إلى ١٥٠ جزء في البليون.

كذلك توصلت نتائج هذه الدارسة بوجود احتمالات كبيرة على أن الحلاوة الطحينية المنتجة من مصانع غير مرخصة و التي ثبت تلوثها بنسب عالية من سموم الأفلاتوكسين قد استخدم في تصنيعها كل من بذور الفول السوداني و كذلك زيت النخيل بديلا عن جزء من بذور السمسم الداخلة في التصنيع الأصلي لهذه المنتجات.