Brief communication

Determination of aflatoxin levels in Sudanese edible oils

Yousif M.A. Idris a,*, Abdalbasit A. Mariod a, Ibrahim Alfaig Elnour a, Adam Ali Mohamed b

a Department of Food Science and Technology, College of Agricultural Studies, Sudan University of Science and Technology, P.O. Box 71, Khartoum North, Sudan

b National Health Laboratory, Federal Ministry of Health, Khartoum, Sudan

A R T I C L E   I N F O

Article history:
Received 20 March 2010
Accepted 10 May 2010

Keywords:
Aflatoxins
Groundnut oil
Sesame oil
Cottonseed oil
HPLC

A B S T R A C T

Fifty-six samples of groundnut, sesame and cottonseed oils form factories, and traditional mills were collected from several localities in Kordofan, Gezira and Khartoum states, Sudan and assessed for aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2), using high performance liquid chromatography (HPLC). Aflatoxin B1 (AFB1) was detected in eight samples representing 14.3%, the highest incidence of aflatoxin contamination occurred in sesame (7 out of 16 samples, 43.75%) followed by groundnut (1 out of 28 samples, 3.57%) while no aflatoxin contamination was detected in cottonseed oil. Aflatoxin B1 levels in sesame oil samples ranged from 0.2–0.8 μg/kg and were 0.6 μg/kg in groundnut oil samples. All aflatoxin contaminated samples are unrefined. This paper reports the findings of the first exploratory investigation on presence of aflatoxins in Sudanese edible oils collected from three states.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Mycotoxins are poisonous organic compounds produced by several species of fungi. They pose a health risk for humans and can be considered as a causative agent for liver cancer in man. Aflatoxins are a major group of mycotoxins, the four major types of which are B1, B2, G1, and G2 while M1 and M2 are metabolites of B1 and B2 which are found in milk of mammals fed aflatoxin contaminated diets. Aflatoxin B1 is a potent hepatocarcinogenic and mutagen (Oye- lamí et al., 1998; Sylla et al., 1999). Omer et al. (2001) reported that aflatoxin B1 causes liver cancer in Sudan. High aflatoxin content (25–600 ppb) was reported in groundnut in Sudan (Omer et al., 1998). Younis and Malik (2003) studied aflatoxin contamination in Sudanese groundnut and groundnut products and found that percentage of aflatoxin contamination was 2%, 64%, 14% and 11% for kernels, butter, cake and roasted groundnuts, respectively. They confirmed that aflatoxin B1 was predominant in all samples followed by G1, B2, and G2. The occurrence of liver cancer in Sudan could be substantially reduced by lessening contamination of food with aflatoxins to internationally accepted levels (Omer et al., 2004).

Sudanese groundnut exports are governed by strict European and other countries very low acceptable levels of aflatoxins. This resulted in through sorting of the products to eliminate contaminated kernels to ensure acceptability. The contaminated kernels may find their way in the local market particularly for oil processing factories. The majority of oil produced in these factories is not refined and as such could be contaminated with aflatoxins. Several vegetable oils including groundnut, cottonseed, sesame and sunflower oil are produced in Sudan and consumed by almost all segments of the population. According to Eltahir et al. (2005) the average Sudanese consumption of sesame oil with the popular meal, broad beans was found to be 9.58%. Elzupir et al. (2010) reported aflatoxin levels in vegetable oils in Khartoum state, Sudan. The contamination was found in 98.8% of their samples, with total AF levels (AFB1 + AFB2 + AFG1 + AFG2) of 0.43–339.9 μg/kg and mean level of 57.5 μg/kg. They found that all sesame oils had total AF levels that were much higher than the acceptable limit of 20 μg/kg. The percentage of samples with total AF values <20 μg/kg in other oils varied and was 57.1% in peanut oil, 36.8% in sunflower oil, 66.7% (mixed oil from factory A), and 91.7% (mixed oil from factory B). However, no information is available about presence or levels of aflatoxins in oil consumed in Sudan. Consequently the magnitude of health risk resulting from consumption of aflatoxins contaminated oils is unknown.

Miller et al. (1985) used a simple method for determination of aflatoxins in both crude and de-gummed vegetable oils. The oil sample, dissolved in hexane, was applied to a silica column and washed with ether, toluene, and chloroform; aflatoxins were eluted from the column with chloroform–methanol (97:3). They quantified the eluted aflatoxins by thin layer chromatography and liquid chromatography, the oils analyzed contained aflatoxin B1 at levels of 5–200 μg. Ndiaye et al. (1999) determined aflatoxins contamination levels of peanut oil prepared by small scale production in areas of Kaolack and Diourbel in Senegal. High performance liquid chromatography (HPLC) analysis of different samples showed that 80% of were contaminated in the areas of Kaolack.

* Corresponding author. Tel.: +249 (0) 185 311886; fax: +249 (0) 185 311889.
E-mail address: yousifidris@yahoo.com (Y.M.A. Idris).

0278-6915/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2010.05.021
and Diourbel. Aflatoxin B1, B2, G1 and G2 has been detected with a profile of contamination almost identical in both areas. Aflatoxin B1 was prevalent and has been found in over 85% of samples. Mean contents of this mycotoxin is about 40 ppb, a value superior to acceptable levels.

The main aim of this study is to analyze groundnut, sesame, and cottonseed oils for evaluation of aflatoxins contamination levels. The specific objectives are to detect presence and determine levels of aflatoxins B1, B2, G1, and G2 in crude and refined vegetable oils.

2. Materials and methods

2.1. Chemicals

Acetonitrile, methanol, benzene, hexane, Tri-Floro-Acetic acid, cupric carbonate, NaCl, HCl, dichloromethane, and chloroform were supplied by Merck (Darmstadt, Germany). HPLC-grade water was obtained from Prime for scientific services, Khartoum, Sudan. The aflatoxin B1, B2, G1, and G2 were purchased from Sigma (St. Louis, MO, USA). 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 under protected conditions at –20 °C. All other inorganic chemicals and organic solvents were of reagent grade or higher.

2.2. Materials

Fifty-six samples (approximately 500 ml each) were collected from the three major vegetable oil producing states. The states are Khartoum (22 samples, 11 groundnut, five sesame and six cottonseed oil), Gezira (15 samples, nine groundnut, six cotton), and North Kordofan (19 samples, nine groundnut and 10 sesame). The samples were collected from 33 factories and 23 mills. Samples were coded as follows: FG = factory groundnut, MG = mill groundnut, FC = factory cottonseed, MS = mill sesame, while states were coded by their first letter as KH, G, and K for Khartoum, Gezira and Kordofan, respectively.

2.3. Methods

The crystalline aflatoxins were dissolved in benzene acetonitrile (98:2) to obtain a concentration of 0.5 μg/ml aflatoxin B1 and G1, and 0.25 μg/ml for B2 and G2. Appropriate aliquots were taken to give specific concentration of the individual aflatoxins.

2.4. Extraction and analysis of aflatoxins

The AOAC method (970-40 1990) was 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 and swirled; 50 ml of hexane were added and gently shake for 30 s. The separated lower aqueous layer phase was drained into another 250 ml separator; then extracted by 3 × 25 ml dichloromethane and were added to aqueous phase and shacked vigorously for 30 s. Then the phases separated and the lower dichloromethane layer was drained, collected and evaporated on a boiling water bath to dryness.

2.5. Derivatization

Hexane (200 μl) were added to extract and 50 μl Tri-Floro-Acetic acid (TFA) and mixed on a Vortex for 30 s; allowed to stand for 5 min then 1.950 μl water–acetoniitrile (9 + 1) was added. Mixed vigorously on Vortex for 30 s, and allowed layers to separate for 10 min; and lower layer of acetoniitrile water phase was taken in vial for HPLC determination. The HPLC equipment was a Shimadzu reverse phase HPLC system (Shimadzu, Japan) with a Machery-Nagel, C-18, 25 cm, 4.6 mm column, the system was 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.

3. Results and discussion

The levels of aflatoxins found in modern factories, traditional mills of the three states are presented in Tables 1 and 2. Table 1 shows the results of aflatoxin analyses of 56 oil samples collected from Khartoum, Gezira and North Kordofan states of Sudan, Table 2 shows aflatoxin contamination according to oil type and processing method. The only detected aflatoxin was B1 which was found in eight samples, corresponding to 14.3% of the total samples analyzed and found in the range of 0.2–0.8 μg/kg. B2, G1 and G2 were not detected in any of the 56 oil samples. Five of the contaminated samples were from Kordofan and three from Khartoum, no aflatoxins contamination was found in oil samples collected from Gezira state. Elzupir et al. (2010) reported 98.8% of aflatoxin contamination in vegetable oils collected in Khartoum state. One out of the eight contaminated samples was unrefined groundnut oil (from modern factories) and seven unrefined sesame oil (from traditional oil mills) and no any contamination was detected in cottonseed oil samples. These results indicate that unrefined oils were more susceptible to aflatoxins contamination; hence protective measures must be followed in these mills. As cottonseed oil was the only oil that was fully refined so the results confirmed the importance of oil refining in reducing aflatoxin contamination. Parker and Melnick (1966) were the first to confirm that refining eliminates aflatoxin from oils obtained from moldy peanut not suitable for human consumption. The results were confirmed using corn oil obtained from corn germ deliberately contaminated in the laboratory with Aspergillus flavus. Omer et al. (1998) reported high aflatoxin content (25–600 ppb) in groundnut in Sudan, and Younis and Malik (2003) studied aflatoxin contamination in Sudanese groundnut and groundnut products (oil was not included) and found that percentage of aflatoxin contamination was 2%, 64%, 14% and 11% for kernels, butter, cake and roasted groundnuts, respectively. They confirmed that aflatoxin B1 was predominant in all samples followed by G1, B2, and G2. Ablaka (1984) detected aflatoxins B1, B2, G1, and G2 in crude groundnut.

Table 1

<table>
<thead>
<tr>
<th>State</th>
<th>Total no. of samples</th>
<th>AFB1 contaminated samples</th>
<th>% of contaminated samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khartoum</td>
<td>22</td>
<td>3</td>
<td>13.64</td>
</tr>
<tr>
<td>Gezira</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kordofan</td>
<td>19</td>
<td>5</td>
<td>26.32</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>8</td>
<td>14.3</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Oil type</th>
<th>No. of samples</th>
<th>AFB1 contaminated samples</th>
<th>AFB2 contaminated samples</th>
<th>AFG1 contaminated samples</th>
<th>AFG2 contaminated samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sesame (unrefined)</td>
<td>16</td>
<td>70</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Groundnut (unrefined)</td>
<td>8</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Groundnut (partially refined)</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Groundnut (refined)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cottonseed (refined)</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Notes:

a 0.2–0.8 μg/kg AFB1.

b 0.2 μg/kg AFB1, limit of detection 0.1 μg/kg.
oil and crude cottonseed oil and G1 in crude palm oil in three factories in Nigeria. However, B1, and G2 were detected in two samples of refined groundnut oil and no aflatoxin was detected in refined cottonseed and palm oils. Cavaliere et al. (2007) detected aflatoxin B1 in 3 out of 15 commercial virgin olive oils samples in Italy. Hong Kong Food and Environmental Hygiene Department (2001) analyzed 245 vegetable oil and fat samples and found that nine samples were contaminated with aflatoxins with levels of 0.1–5.8 μg/kg.

The maximum permissible level of B1 as well as other mycotoxins in food materials has been regulated in many countries. The legal limits may vary from one country to another, depending on the degree of development and economic consideration. The Scientific Commission of the European Community has regulated the maximum allowable level of 2 μg/kg for B1 (Commission of the European Communities, 2001). In the US, the FDA has set a maximum admissible level of 20 μg/kg for total aflatoxins in all foods for human consumption (Creppy, 2002). The detected B1 level in this study was found to be lower than the legal limit that regulated in Sudan. This indicated that the majority of Sudanese edible oils samples in this study are safe. This is the first data on the presence of aflatoxin in edible oils in Sudan.

4. Conclusions

It could be concluded that some unrefined sesame and groundnut oils are contaminated with AFB1 at levels of 0.2–0.8 μg/kg, suggesting that refining is an essential process for elimination of aflatoxins from edible oils. The higher incidence of AFB1 contamination of sesame oil warrants further investigation as this oil is widely consumed in Sudan without refining. A wide investigation of aflatoxin levels in edible oils consumed in Sudan is necessary.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The authors thank the Sudanese Standards and Metrology Organization (SSMO) for the financial support for this research.

References