Occurrence of Aflatoxins in Syrian Foods and Foodstuffs: A Preliminary Study

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ABSTRACT

Sixty-three samples of nineteen food commodities of Syrian origin were analyzed for the first time for aflatoxin contamination. Fifteen commodities, including staple foodstuffs such as wheat and its two main products, two kinds each of legumes, nuts, dried fruits and vegetables, five types of cheese, and a few local popular foods showed no contamination. Seven out of the sixteen samples comprising peanuts, dried figs, lentils and the secondary dairy product Koshk contained aflatoxins in detectable levels. The lowest level of contamination was that of aflatoxin M1 in Koshk (0·19 µg/kg), and the highest was of B1 in dried figs (11·8 µg/kg). Aflatoxin B2 was found only in roasted shelled peanuts whilst G1 and G2 were not detected in any commodity.

INTRODUCTION

Aflatoxins are secondary mould metabolites shown to be highly toxic and potently carcinogenic. They have been detected in various food commodities from many areas of the world (Smith & Moss, 1985), and are presently considered as one of the most dangerous contaminants of food and feed. A number of surveys and monitoring programmes have been carried out in several countries attempting to obtain a general pattern of the extent of food contamination (Yndestad & Underdal, 1975; Boutrif *et al.*, 1977; Girgis *et al.*, 1977; Sanchis *et al.*, 1986; MAFF, 1987; Piva *et al.*, 1987; Lotter &

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261

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Krohm, 1988; Tabata & Kamimura, 1988). Furthermore, regulations for the control of aflatoxins in food exist in at least fifty different countries throughout the world, and maximum amounts are specified from zero tolerance (in practice, the detection limit of the method) up to $50 \,\mu g/kg$ (Van Egmond, 1989).

The humid summer and rainy winter in the Mediterranean coastal regions of Syria, as well as the poor storage conditions prevalent in most food stores and markets, may promote the growth of moulds and the production of aflatoxins. Nevertheless, apart from the analyses of peanuts performed for some importers, no analysis of aflatoxins has been carried out on Syrian foodstuffs. The work described in this paper is a preliminary investigation of the occurrence of aflatoxins in a variety of Syrian foods and foodstuffs.

MATERIALS AND METHODS

Sixty-three samples of nineteen food commodities of Syrian origin were obtained in January 1989 from the city of Lattakia, Syria. They were all collected as single random purchases from retail outlets of the food market except one home-made sample of a secondary dairy product called Koshk. The obtained commodities included wheat and its two main products (bread and Borghol), legumes, nuts, dried fruits and vegetables, various cheeses and a few other local popular foodstuffs. The following is a brief description of the unfamiliar names: Borghol is parboiled wheat, sundried then ground and the outer layer or pericarp removed. Akkawi, Halloum and Shelal are three standard white soft or semisoft types of cheese normally made from sheep's milk. Sorkeh and Shankleesh are cheeses usually made from the defatted yogurt of goat's milk; the first is consumed raw and the second after ripening by natural microorganisms for a few months. Falafel is a ground mixture of chickpeas and broad beans with or without spices. Teheena is sesame butter. Halawa is a confectionery made principally of Teheena and sugar and usually relished with nuts. Za'tar is a mixture of roasted sesame seeds and powdered oregano and nuts. Qamaruddin is a sheet-like product of sundried apricot pulp. Koshk is a sundried mixture of Borghol and yogurt.

The sample weight varied depending on the nature of the commodity and its packaging condition, but ranged between 250 g for homogeneous commodities such as cheese and 1 kg for the others like grains and nuts. The samples reached the laboratory within a few days of their collection and were either tested upon arrival or stored at $4^{\circ}C(-20^{\circ}C$ for cheese) to arrest any aflatoxin formation afterwards. Prior to analysis, the samples were ground or homogenized, thoroughly mixed, and duplicate 50 g subsamples (20 g for cheese) were taken for analysis. Aflatoxins B1, B2, G1 and G2 were analyzed by the liquid chromatographic method of Paulsch *et al.* (1988) which includes extraction with chloroform, followed by a two step-cleanup on Sep-Pack Florisil and Sep-Pack C18 cartridges, respectively. The CB extraction and cleanup procedures of the AOAC (1984) method were used with a few commodities for comparison with the previous method. In all cases, the final extract was submitted to reverse phase high performance liquid chromatography with water-methanol-acetonitrile (130 + 70 + 40, v/v/v) as mobile phase and postcolumn derivatization with iodine. Aflatoxin M1 was determined in cheese and Koshk according to the method of Bijl *et al.* (1987) which includes two cleanup steps on Sep-Pack Silica and Sep-Pack C18 cartridges, respectively. The original ratio of acetonitrile-water (30 + 70) used by Bijl *et al.* was modified to (20 + 80) in order to obtain better resolution of peaks.

The system of HPLC used in this study was a Perkin-Elmer Series 10 Liquid Chromatograph equipped with LS-4 Fluorescence Spectrometer and a Varian 4290 electronic integrator. A Chrompack column ($150 \times 4.6 \text{ mm}$) packed with 5 μ m Chromsphere C18, and an ESC-3511 Degasser from Erma Inc. were also used throughout this study. The qualitative and quantitative analysis of aflatoxins was carried out by comparing retention times and peak areas with those of aflatoxin standards purchased from Sigma Chemicals. Further confirmation of positive results was done by comparison of the chromatograms with those of the standards after derivatization with trifluoroacetic acid according to Bijl *et al.* (1987) for aflatoxin M1 and to Beebe (1978) for aflatoxin B1.

The detection limit attainable for each of the aflatoxins B1, B2, G1 and G2 was lower than $0.1 \,\mu$ g/kg in the CB procedure of the AOAC and than $1 \,\mu$ g/kg in the original method of Paulsch *et al.* (1988). However, an additional step of concentrating the final 50 ml eluate at 40°C under vacuum (introduced into the latter method) was found to decrease its detection limit by more than one order of magnitude. Nevertheless, no quantitation was attempted on samples with levels of contamination lower than $0.1 \,\mu$ g/kg. The detection limit of aflatoxin M1 was in the range of 20 ng/kg and its recovery from cheese spiked at 0.5 ng/g was 97%. Recoveries of aflatoxins B1, B2, G1 and G2 determined in various commodities at two spiking levels of 0.4 and $4 \,\mu$ g/kg varied between 76 and 94%. All results, however, were not corrected for recovery.

RESULTS AND DISCUSSION

The liquid chromatographic method of Paulsch et al. (1988) originally developed for the determination of aflatoxins in feedstuffs containing citrus

Commodity	Number of samples
Wheat	3
Bread	3
Borghol	3
Chickpeas	3
Faba beans	3
Almonds (shelled)	3
Walnuts (shelled)	3
Raisins	3
Okra (sun dried)	3
Qamaruddin	2
Cheese: Akkawi	2
Halloum	2
Shelal	2
Sorkeh	2
Shankleesh	2
Falafel	2
Teheena	2
Halawa	2
Za'tar	2
Total	47

TABLE 1Syrian Food Commodities Found Free of AflatoxinContamination (detection limit 0.1 µg/kg for each of B1,B2, G1, G2 and 0.02 µg/kg for M1)

pulp was found satisfactory for the analysis of the various foodstuffs tested in this study. None of the commodities showed any interferences in the aflatoxin elution zone in the chromatogram. Analysis of a few samples of some contaminated and noncontaminated commodities by this method in comparison with the CB extraction and cleanup procedures of the AOAC method gave similar results. The former method, however, was simpler, faster, and gave cleaner chromatograms than the latter.

The results of the analysis are summarized in Tables 1 and 2. Aflatoxins were not detected in fifteen out of the nineteen commodities analyzed. The noncontaminated commodities comprised 47 samples and included staple and most commonly used Syrian foodstuffs; wheat and its two main products, two major legumes, five types of cheese, two of nuts, three dried fruit and vegetable products and four other local popular foodstuffs. Although one should always guard against making generalizations based on limited numbers of samples analyzed, the present results indicate that aflatoxin contamination of the major staple Syrian foodstuffs seems to be low and does not contribute much to the aflatoxin intake in the diet.

Commodity (number of samples in parentheses)	Number of positive samples	Type and level of aflatoxin (µg/kg)
Peanuts: (7)	3	B1 & B2
Raw in-shell (2)	0	_
Raw shelled (2)	1	B1(2·7)
Roasted shelled (3)	2	B1(1·9) & B2(0·6) B1(0·4) & B2(0·3)
Dried figs: (4)	2	B1(11.8 & 2.5)
Lentils: (4)	1	B 1
Intact (2)	0	
Ground (2)	1	B1(1·8)
Koshk: (1)	1	M1(0·19)

 TABLE 2

 Aflatoxins and Their Levels in the Contaminated Food Commodition

" Results are the means of duplicate subsamples differing by less than 10%.

Four commodities, namely, peanuts, figs, lentils and Koshk were found to contain aflatoxins in detectable levels (Table 2). The toxin types present were B1, B2 and M1. Aflatoxins G1 and G2 could not be detected in any sample. The highest level of contamination was found in one sample of figs (11.8 μ g B1/kg) and the lowest was in Koshk (0.19 μ g M1/kg). Yogurt usually provides about half of the total solids of Koshk. If moisture contents of the final product and its ingredients are accounted for, then the yogurt used in the preparation of this sample must have had a content of 0.06 μ g aflatoxin M1/kg. This concentration, however, is a rather moderate level of contamination.

Aflatoxin M1 is not destroyed in milk processing, and its concentration in cheese is usually four times higher than in milk from which it is prepared (Van Egmond, 1983). The presence of aflatoxin M1 in Koshk and its absence in the five types of cheese analyzed appear to be peculiar. It can be explained, however, by the use of yogurt made from cow's milk in Koshk processing, and the presence of aflatoxin in the subsidized imported mixed feed normally rationed for dairy cows. The five types of cheese analyzed are processed from the milk of sheep or goats, both of which feed on local forage or by grazing.

Peanut is the commodity in which aflatoxin contamination was first discovered (Sargeant *et al.*, 1961). Numerous surveys carried out afterwards have shown it to be generally contaminated throughout the world with regular indications of disturbing levels of aflatoxin (Campbell, 1978; Smith & Moss, 1985). Our results showed that three out of seven samples of Syrian

peanuts are contaminated with aflatoxins; two with B1 and B2 and one with B1 only. The levels of contamination, however, are rather low and within the acceptable limits. Roasting is known to moderately decrease the levels of aflatoxins in peanuts (Scott, 1984), but this may or may not be the reason for the lower level of contamination found in the roasted samples. One of the two raw shelled samples contained $2.7 \mu g$ aflatoxin B1/kg, and it is of interest to note that both the nuts and their shells in the two raw in-shell samples have been found free of aflatoxins. This may indicate that shelled peanuts are more prone to aflatoxin contamination during storage than the unshelled ones.

The occurrence of aflatoxin and its production in figs during ripening and drying have been reported several years ago (Anon., 1974; Buchanan *et al.*, 1975). However, the recent study of Steiner *et al.* (1988) on aflatoxin contamination in dried figs and its correlation with fluorescence under UV light has renewed the interest in this commodity. Furthermore, the high level of contamination occasionally found in figs imported from Turkey into a number of European countries has prompted continuous surveillance, specific control measures and guidelines along with the consideration or the implementation of tolerance limits ranging between 5 and 10 μ g/kg (Akerstrand & Moller, 1989; Rasmussen & Pedersen, 1989; Gilbert, 1989; Mathot, 1989). The present study showed two out of four samples of Syrian dried figs to be contaminated with aflatoxin B1 at the levels of 2·5 and 11·8 μ g/kg, respectively. In view of these results, the degree of contamination of Syrian dried figs should be investigated further.

Girgis et al. (1977) reported the presence of aflatoxin B1 (3.1 μ g/kg) in one of three samples of Egyptian lentils stored for 12 months, but no toxin was found in three freshly produced samples. Qutet et al. (1983) analyzed four samples of lentils stored under unfavourable conditions and found $72 \,\mu g$ aflatoxin B1/kg in one of them. One out of twenty-six samples tested by Amodio et al. (1984) was found contaminated with aflatoxin at a level of $8-10 \mu g/kg$. El-Maraghy (1988) studied aflatoxin and fungal flora in lentils and reported the presence of 20 μ g total aflatoxins/kg in one sample out of twenty. He concluded that this commodity may be a poor substrate for toxin contamination. In this study, two samples of intact lentils were found free of aflatoxin while one of two ground samples contained a rather low level of aflatoxin B1 (1.8 μ g/kg). It is reasonable to find ground lentils more contaminated than intact lentils because grinding destroys the protective outer testa and thus facilitates the colonization of the rich nutrients inside by the toxigenic fungi. Ground lentils are customarily manufactured from low quality seeds and commonly utilized in Syria for making soup.

The results of this study show that aflatoxins B1, B2 and M1 were detected for the first time in certain Syrian food commodities. The level of contamination, however, was rather low, but in view of the limited number of samples analyzed, and the highly skewed distribution known for aflatoxin contamination in agricultural commodities, further studies and a statistically based nation-wide survey should be conducted to evaluate the actual extent of aflatoxin contamination in Syrian foods and feed. Special consideration should be given to the commodities already found contaminated in this study.

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REFERENCES

- Akerstrand, K. & Moller, T. (1989). Examination of figs imported to Sweden in 1987 and 1988 and measures to be taken in the processing plants. Paper presented at the International Symposium on Dried Figs and Aflatoxins, Izmir, Turkey, 4-8 April 1989.
- Amodio, R., Del Prete, U., D'Errico, M. M. & Fiore, P. (1984). Sulla presenza di micotossine in alcuni alimenti vegetali. Nuovi Annali di Igiene e Microbiologia, 35, 51-5.
- Anon. (1974). Imported figs recalled because of aflatoxin. Food Chem. News, 16, 17--8.
- AOAC (1984). Official Methods of Analysis, 14th edn. Association of Official Analytical Chemists, Washington, DC, Sections 26.029 and 26.030.
- Beebe, R. M. (1978). Reverse phase high pressure liquid chromatographic determination of aflatoxins in foods. J. Assoc. Off. Anal. Chem., 61, 1347-52.
- Bijl, J. P., van Peteghem, C. H. & Dekeyser, D. A. (1987). Fluorimetric determination of aflatoxin M1 in cheese. J. Assoc. Off. Anal. Chem., 70, 472-5.
- Boutrif, E., Jemmali, M., Campbell, A. D. & Pohland, A. E. (1977). Aflatoxin in Tunisian foods and foodstuffs. Ann. Nutr. Alim., 31, 341-4.
- Buchanan, J. R., Sommer, N. F. & Forlage, R. J. (1975). Aspergillus flavus infection and aflatoxin production in fig fruits. Appl. Microbiol., 30, 238-41.
- Campbell, A. D. (1978). Food mycotoxins surveys and monitoring programs. Ann. Nutr. Alim., 31, 403-10.
- El-Maraghy, S. S. M. (1988). Aflatoxins and fungal flora in lentil (*Lens esculenta* L.). *Mycopathologia*, **102**, 31–5.
- Gilbert, J. (1989). Surveillance and control of aflatoxin contamination of dried figs and fig paste imported into the United Kingdom. Paper presented at the International Symposium on Dried Figs and Aflatoxins, Izmir, Turkey, 4–8 April 1989.

- Girgis, A. N., El-Sherif, S., Rofael, N. & Nesheim, S. (1977). Aflatoxin in Egyptian foodstuffs. J. Assoc. Off. Anal. Chem., 60, 746-7.
- Lotter, L. H. & Krohm, H. J. (1988). Occurrence of aflatoxins in human foodstuffs in South Africa. Bull. Environ. Contam. Toxicol., 40, 240-3.
- Mathot, Ir. P. J. (1989). Approach in The Netherlands concerning aflatoxin in dried figs. Paper presented at the International Symposium on Dried Figs and Aflatoxins, Izmir, Turkey, 4–8 April 1989.
- Ministry of Agriculture, Fisheries and Food (MAFF) (1987). Mycotoxins, The Eighteenth Report of the Steering Group on Surveillance, The Working Party on Naturally Occurring Toxicants in Food: Sub-Group on Mycotoxins. HMSO, London.
- Paulsch, W. E., Sizoo, E. A. & van Egmond, H. P. (1988). Liquid chromatographic determination of aflatoxins in feedstuffs containing citrus pulp. J. Assoc. Off. Anal. Chem., 71, 957-64.
- Piva, G., Pietri, A., Gallazi, L. & Curto, O. (1987). Aflatoxin M1 in dairy products marketed in Italy. Food Additives and Contaminants, 5, 133-9.
- Qutet, S. M., El-Tahey Shehata, M. & Mesallam, A. S. (1983). Occurrence of aflatoxins in some Egyptian food crops collected from two coastal regions. *Food Chem.*, 10, 149-53.
- Rasmussen, G. & Pedersen, E. (1989). Experience with aflatoxins in figs in Denmark. Paper presented at the International Symposium on Dried Figs and Aflatoxins, Izmir, Turkey, 4–8 April 1989.
- Sanchis, V., Sala, N., Palomes, A., Santamarina, P. & Burdaspal, P. A. (1986). Occurrence of aflatoxin and aflatoxigenic molds in foods and feed in Spain. J. Food Prot., 49, 445-8.
- Sargeant, K., Sheridan, A., O'Kelly, J. & Carnaghan, R. B. A. (1961). Toxicity associated with certain samples of groundnuts. *Nature (London)*, 192, 1096-7.
- Scott, P. M. (1984). Effects of food processing on mycotoxins. J. Food Prot., 47, 489-99.
- Smith, J. E. & Moss, M. O. (1985). *Mycotoxins Formation, Analysis and Significance*. John Wiley & Sons, Chichester, UK, pp. 85-6.
- Steiner, W. E., Rieker, R. H. & Battaglia, R. (1988). Aflatoxin contamination in dried figs: Distribution and association with fluorescence. J. Agric. Food Chem., 36, 88-91.
- Tabata, S. & Kamimura, H. (1988). Survey of aflatoxin contamination in Tokyo's commercial foods and foodstuffs. Proceedings of the Japanese Association of Mycotoxicology. Supplement No. 1. pp. 63-4.
- Van Egmond, H. P. (1983). Mycotoxins in dairy products. Food Chem., 11, 289-307.
- Van Egmond, H. P. (1989). Current situation on regulations for mycotoxins. Overview of tolerances and status of standard methods of sampling and analysis. Food Additives and Contaminants, 6, 139-88.
- Yndestad, M. & Underdal, B. (1975). Aflatoxin in foods on the Norwegian market. Nordisk Veterinaermedicin, 27, 42-8.