A SURVEY OF THE OCCURRENCE OF AFLATOXINS IN GROUNDNUTS (PEANUTS) AND GROUNDNUT PRODUCTS IN SÃO PAULO STATE /BRAZIL IN 1994*

Myrna SABINO**
Emiko Ikejiri INOMATA**
Leda Conceição Antonia LAMARDO**
Thaïs Valéria MILANEZ**
Sandra Aparecida NAVAS**
Maria Ângela Pompeu ZORZETTO**

RIALA 6/851

SABINO, M; INOMATA, E.I.; LAMARDO,L.C.A.; MILANEZ, T.V.; NAVAS, S.A. & ZORZETTO, M.A.P.- A survey of the occurrence of aflatoxins in groundnuts (peanuts) and groundnut products in São Paulo State/ Brazil in 1994. *Rev.Inst.Adolfo Lutz*, 58 (1): 53-57, 1999.

ABSTRACT: Survey results of 321 samples of groundnuts (peanuts) and groundnut products obtained from the Inspection Service of the Sanitary Guidance of the Health Secretary of São Paulo (totaling 321 samples) collected in an one-year period (1994) showed levels of aflatoxins B_1 and G_1 above 30.0 $\mu g.kg^{-1}$ for 36% of the samples. All samples were analyzed by thin-layer chromatography and results quantified by visual analysis. The aflatoxins concentration of the samples varied from 5.0 to 2440.0 $\mu g.kg^{-1}$ and the 90th percentil was 489.0 $\mu g.kg^{-1}$. A total of 116 samples (36%) showed concentrations greater than permitted by Brazilian Legislation which, for aflatoxins $B_1 + G_1$, is 30.0 $\mu g.kg^{-1}$ for food (domestic consumption). The limit of determination of the method was 5.0 $\mu g.kg^{-1}$.

This survey reconfirmed the extent and level of occurrence of aflatoxins in groundnuts and groundnut products in São Paulo and also showed that the mycotoxin problem still exists, mainly due to conditions of temperature and humidity prevalent in the State of São Paulo that are favorable to the growth of toxigenic moulds.

DESCRIPTORS: aflatoxins, peanuts, peanuts products, thin-layer chromatography.

INTRODUCTION

Aflatoxins are a group of secondary metabolites produced by three species, *Aspergillus nomius*, *Aspergillus flavus* and *Aspergillus parasiticus* [7]. They are toxic to different animal species, and aflatoxin B_1 is the most toxic of this group. It is hepatotoxic and was classified as class 1 human carcinogen[8] . Aflatoxin production is favoured by temperature of 25 to 30° C, high humidity and high water activity (0.86-0.96). If the moisture content of the commodity goes above a certain level aflatoxin formation may occur[3,6].

Aflatoxin contamination is a large problem in groundnuts and groundnut products in Brazil, a tropical country where mould growth is favored by the conditions of high humidity and temperature, cultivation , harvest, transportation and storage [9].

Most Brazilian groundnut are grown in São Paulo State. There are yearly two crops of grounduts, the first one, the rain crop, is harvested from January to March during the summer. The second one is smaller and it is cal-

* Realizado na Seção de Química Biológica
** Do Instituto Adolfo Lutz

led the dry crop, it goes from May to June. Temperatures and humidities are high in the west of São Paulo State which is the main groundnut producing area. Table 1 shows its area and production of groundnut first crop (rain crop - the main one). Measures needed to improve the situation are often difficult to introduce, because an efficient extension service is still being developed.

TABLE 1

Area and production of groundnut first harvest, São Paulo State, outstanding Regional Agriculture Divisions (RADs). Harvest 1993/94 and 1994/95.

	AREA(ha)			PRODUCTION (MI BAG 25 Kg)		
RADS	1994	1995	VARIATION%	1994	1995	VARIATION%
Marília	9,000	10,325	14.7	660	365	-44.7
Ribeirão Preto	21,630	26,250	21.4	2,200	2,630	19.5
São Carlos	6,970	10,300	47.8	560	815	45.5
Vale do Paranapanema	3,345	3,652	9.2	185	270	45.9
Presidente Prudente	3,695	3,520	- 4.7	300	260	-13.3
Others	8,460	7,583	-10.4	565	590	4.4
State	53,100	61,630	16.1	4,470	4,930	10.3

Source: Instituto de Economia Agrícola (IEA) and Coordenadoria de Assistência Técnica Integral (CATI)

Groundnut production in Brazil was 956,000 tons in 1972, an increase of 100% over that in 1960. However investments in soybean cultivation in the early 1970s caused groundnut production to decrease to levels equal to or less than in 1958. Groundnut production has decreased so much that it is no longer competitive with soybean as an oil crop [4,5] (Table 1) Groundnut cultivation is mainly done by small farmers using rudimentary systems which are vulnerable to mould infection, and aflatoxin production.

Aflatoxin contamination is a public health concern, so the Instituto Adolfo Lutz monitorizes the incidence of these substances in groundnuts and groundnut products periodically. This paper is a complement toof others surveys done before [2,15,16] in order to know the real condition of these products concerning aflatoxins.

MATERIAL AND METHODS

Sample collection

Representative 1 kg were taken from 321 samples of groundnuts and groundnut products from different markets and milling factories located in São Paulo State by the Inspection Service of the Sanitary Guidance of Health Secretary of São Paulo State, from January to December, 1994.

Extraction

Aflatoxins were determined using the TLC method of Soares & Rodriguez-Amaya[18] as follows: the finely ground sub sample (50g) was blended with a mixture of 270 mL methanol and 30 mL of 4% KC1. After blending for 5 min. the mixture was filtered throught fluted paper. Then, 150 mL of the extract was cleaned up by adding 10% $CuS0_4$ plus Hyflo Super Cel, stirred with a glass rod and filtered through fluted paper. A 150 mL volume of this filtrate was extracted with $CHCl_3$ (10mL x 2). This combined chloroform extract was evaporated to near dryness in a steam bath , transferred quantitatively with $CHCl_3$ to a sample vial (ca 1-2 g) and evaporated to dryness in a steam bath under a stream of nitrogen.

TLC determination

The amount of 5.0 µl of the chloroform extract was spotted on the same plate with the standards at different concentrations. The mobile phase used was toluene: ethyl acetate:formic acid 90% (50 + 40 + 10) v/v. . The developed plate was dried and then observed under long wave ultraviolet light (366 nm). The intensities of the fluorescent spots were compared with the aflatoxin standards by visual observation. The detection limit of this method was 2.5 µg kg^{-1} . The identity of aflatoxins B_1 and G_1 were confirmed by the formation of the characteristic derivatives after reaction with trifluoroacetic acid.[13] The determination limit of the method was 5.0 µg.kg^{-1}.

RESULTS AND DISCUSSION

A summary of the results from the survey of aflatoxin contamination is show in Table 2. The natural incidence of aflatoxins in these samples was high. About 36% (116) of the samples studied showed contamination exceeding that tolerated by Brazilian legislation, a maximum level of 30.0 µg. kg^{-1} for aflatoxin B_1 plus aflatoxin G_1. 44% (142) of the samples showed levels of aflatoxins (B_1 + G_1) that varied from 5.0 to 2440.0 µg.kg^{-1}. The amount of aflatoxins in most of the positive samples (42/142) ranged from 400 to 2440 µg.kg^{-1}. However, 12 samples contained more than 1000 µg aflatoxins kg^{-1} with a 90th percentile value of 489 µg.kg^{-1}.

TABLE 2

Incidence of AFB_1 and AFG_1 in groundnuts and groundnuts products from several regions in São Paulo State

TOTAL SAMPLES	Nº SAMPLES >5μg.kg^{-1}	Nº SAMPLES 5-30μg.kg^{-1}	Nº SAMPLES >30μg.kg^{-1}	AVERAGE OF POSITIVE SAMPLES (μg.kg^{-1})	90th% (μg.kg^{-1})	RANGE MIN-MAX (μg.kg^{-1})
321	142 (44.2%)	26 (8.1%)	116 (36.1%)	305	489	5-2440

There have been several previous studies of aflatoxin contamination of peanuts in Brazil. Sabino[15] analysed 300 samples of many products including groundnuts and groundnut products and observed that, in the period 1971-1975, the levels ranged from not detected to 7,800 μg.kg^{-1}, with a mean of 1,131 μg.kg^{-1}. 37% of the samples exceeded the limit established by Brazilian legislation. Scussel & Rodriguez-Amaya[17] analysed samples of groundnuts and groundnut products purchased at random in different supermarkets and stores in Campinas (São Paulo State) in 1980-1982. Of the 241 samples analysed, 128 gave positive results, of which 92 exceeded the 30.0 μg.kg^{-1} limit. The highest levels encountered for aflatoxins B_1 and G_1 were 1,282 and 476.0 μg.kg^{-1} for "paçoca" (candy made with peanuts),1,904 and 69.0 μg.kg^{-1} for raw shelled peanuts and 1,026 and 366 μg.kg^{-1} for soypeanut. Sabino et al.[16] observed aflatoxin contamination in 1,374 samples of groundnut and groundnut products from São Paulo during the period 1980-1987. The incidence rate and the aflatoxin levels varied from year to year with no definied pattern. The highest mean level was obtained in 1983 when 49% of 198 samples were positive with a mean level of 333 μg.kg^{-1} ; the range was 8-864 μg.kg^{-1}. The most contaminated sample, belonging to the 1985 lot, had 6,561 μg.kg^{-1}. During this year, aflatoxins were detected in 28% of 275 samples, the mean being 91 μg.kg^{-1}. The highest incidence occurred in 1982 when 71% of 132 samples were positive; the range was 8 - 2,500 μg.kg^{-1}. Ricciardi and Ferreira[14] analysed 49 samples of groundnuts and 38 of groundnut candies for aflatoxins, collected from the Ribeirão Preto region - São Paulo. Aflatoxin B_1 was detected in 67.2% of the samples, 24.5% being above and 32.7% below the regulatory limit. All these studies show the continuing problem of aflatoxin contamination in groundnuts, even when the contamination occurred so high as years ago[2,10,11,12,15,16,17,18]. But the problem still exists. In years when rain is copious during harvesting, 40-80% of the crop may be contaminated with aflatoxins [12]. The products offered for export have been rejected by importing countries because of this contamination. It appears that environmental conditions in Brazil favor aflatoxin contamination of stored grains. For this reason there must be strict control in the handling of unprocessed peanut in order to avoid its contamination and the subsequent danger to the consumers.

CONCLUSIONS

This survey shows that aflatoxins B_1 and G_1 are present in about 44% of the samples analysed and 36% of the total exceeded the maximum tolerable limits established by the present Brazilian regulation. It reconfirms the extent and the level of occurrence of aflatoxins in groundnuts and groundnut products in Brazil. It also showed that the mycotoxin problem still exists, some years more pronouncially, others years not so much. It is necessary to motivate agriculture technicians to educate farmers on the prevention of this kind of contaminant and also encourage good agriculture practices to minimize field, harvest and post-harvest groundnut contamination. The authorities should be aware about their responsibilities and act concerning the marketing and utilization of this commodity.

RIALA 6/851

SABINO, M; INOMATA, E.I.; LAMARDO,L.C.A.; MILANEZ, T.V.; NAVAS, S.A. & ZORZETTO, M.A.P.- Dados de ocorrência de aflatoxinas em amendoim e produtos de amendoim no Estado de São Paulo/Brasil em 1994. *Rev. Inst. Adolfo Lutz*, 58 (1): 49-53, 1999.

RESUMO:Foram analisadas 321 amostras de amendoim e produtos de amendoim, comercializados no Estado de São Paulo, em 1994 e colhidos pela Vigilância Sanitária da Secretaria de Saúde do Estado de São Paulo quanto ao teor de aflatoxinas. Um total de 116 amostras (36%) mostraram níveis de aflatoxinas $B_1 + G_1$ acima do limite permitido pela legislação brasileira (30 $\mu g.kg^{-1}$). As amostras foram analisadas por cromatografia em camada delgada e o resultado, quantificado visualmente. As concentrações variaram de 5 a 2.440 $\mu g.kg^{-1}$ e o 90^{th} percentil foi de 489 $\mu g.kg^{-1}$. O limite de quantificação do método é de 5 $\mu g.kg^{-1}$. Os dados confirmam a extensão e o nível de ocorrência de aflatoxinas em amendoim e produtos de amendoim em São Paulo e também mostra que este problema ainda existe, principalmente em condições de temperatura e umidade alta, clima predominante no Estado de São Paulo favorável ao crescimento de fungos toxigênicos.

DESCRITORES: aflatoxinas, amendoim, produtos de amendoim, cromatografia em camada delgada.

REFERENCES

1. BRASIL, Leis , decretos,etc.- Resolução nº 34/76 da Comissão Nacional de Normas e Padrões para Alimentos. Diário Oficial, Brasília,19 de janeiro 1977. Sec. I pt. I, p.710.
2. BRIGIDO, B.M.; BADOLATO, M.I.C. & FREITAS, V.P.S.- Contaminação de amendoim e seus produtos comercializados na região de Campinas-SP, por aflatoxinas durante o ano de 1994. *Rev. Inst.Adolfo Lutz,* **55**(2):85-90, 1995.
3. BULLERMAN,L.B.; SCHROEDER,L.L. and PARK, K-Y - Formation and control of mycotoxins in food. *Journal of Food Protection*, **47**(8): 637-646,1984.
4. CACEX, 1984: Informação semanal nº 909 (Agosto).
5. CACEX, 1985: Informação semanal nº 960 (Agosto).
6. DIENER, U.L. and DAVIS, N.D.- Limiting temperature and relative humidity for growth and production of aflatoxin and free fatty acids by *Aspergillus flavus* in sterile peanuts. *Journal of American Oil Chemists' Society*, **44**: 259-263,1967.
7. FRISVAD,J.C. and THRANE,U.- Mycotoxin production by food-borne fungi. In: *Introduction to Food-Borne Fungi*, edited by R.A. Samson, E.S. Hoekstra, J.C. Frisvad and O. Filtenborg (C.B.S.) 1995,p. 251-260.
8. IARC.- Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins, *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans,* Volume 56 (Lyon: International Agency for Research on Cancer),1993, p. 245-395.
9. MILLER, J.D.- Fungi and mycotoxin in grain: implications for stored product research. *Journal of stored Product Research*, **31**(1): 1-16,1995.
10. OLIVEIRA, V.; MESQUITA, A.J.; SERAFINI, A.B.; RIBEIRO, J.L.; SILVA, M.R.R. - Ocorrência de aflatoxinas B_1 e G_1 em amendoim comercializado em Goiânia - GO, Brasil. *Revista de Microbiologia*. **22:** 319-322, 1991.
11. PRADO, G. - Incidência de aflatoxina B_1 em alimentos. *Revista . Farmacia Bioquimica*. **5:** 147-157, 1983.
12. PRADO, G.; MATTOS, S.V.M.; PEREIRA, E.C. - Níveis de aflatoxinas em alguns alimentos consumidos em Belo Horizonte no período de 1983 a 1988. *Ciência e Tecnologia de Alimentos* **9:** 138-147, 1989.
13. PRZYBYLSKI, W.- Formation of aflatoxin derivatives on thin-layer chromatographic plates. *Journal of the Association of Official Analytical Chemists*, **58**: 163-164, 1975.
14. RICCIARDI, J.A. and FERREIRA, J.F.- Dosagem de aflatoxina B_1 em amendoim e em doces de amendoim . *Revista Brasileira de Farmácia*,**67**: 111-117, 1986.
15. SABINO, M.- Variações de níveis de aflatoxina B_1 em alimentos e rações animais no período de 1971 a 1979. *Revista do Instituto Adolfo Lutz*, **4**(2): 153-158, 1980.

16. SABINO, M., ZORZETTO, M.A.P., PEDROSO, M.O., MILANEZ, T.V.- Incidência de aflatoxinas em amendoim e produtos derivados consumidos na cidade de São Paulo, no período de 1980 a 1987. *Revista do Instituto Adolfo Lutz*, **49**: 41-44, 1989.

17. SCUSSEL, V.M. and RODRIGUEZ-AMAYA, D.B.- Teores de aflatoxinas em amendoim e seus produtos comercializados em Campinas em 1980-82, Boletim *SBCTA*, **19**: 109-119, 1985.

18. SOARES, L.V. and RODRIGUEZ-AMAYA, D.B.- Survey of aflatoxins , ochratoxin A, zearalenone and sterigmatocystin in some Brazilian foods by using a multitoxin thin-layer chromatographic method. *Journal of the Association of Official Analytical Chemists*, **72**(1):22-26, 1989.

Recebido para publicação em 04/06/1998.