

## OCCURRENCE OF AFLATOXINS IN PEANUTS AND PEANUT PRODUCTS CONSUMED IN THE STATE OF SÃO PAULO/BRAZIL FROM 1995 TO 1997

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### ABSTRACT

One hundred and thirty seven samples of peanuts and peanut containing foods were collected in markets in the State of São Paulo, Brazil, between January 1995 and December 1997. Most of the samples were collected by the Inspection Service of São Paulo Secretary of Health. The foods included raw peanuts, peanut candies ("paçoca" and "pé de moleque"), peanut butter, fried/roasted salted peanuts, "torrone", chocolate coated peanuts and salt-coated peanuts. The samples were analyzed for aflatoxins using a thin-layer chromatographic method. About 45% of the samples were positive for aflatoxins and 27% exceeded the limits of the Brazilian legislation ( $30.0 \mu\text{g.kg}^{-1}$  for aflatoxins  $B_1+G_1$ ). The aflatoxins were confirmed by derivatization with trifluoroacetic acid. The 90<sup>th</sup> percentile was 110.0 in 1995, 60.0 in 1996 and  $118.0 \mu\text{g.kg}^{-1}$  in 1997. The aflatoxins concentration in the raw peanut samples ranged from 5.0 to  $382.0 \mu\text{g.kg}^{-1}$  and 27.1% were above the legal limits. Contamination in peanut candies was above the limit in 32.8% of the samples and the aflatoxins levels ranged from 6.0 to  $494.0 \mu\text{g.kg}^{-1}$ . Contamination of salty peanuts was less frequent, around 10% of the samples and the toxin levels were usually below  $10 \mu\text{g.kg}^{-1}$ . The maximum level of contamination,  $536.0 \mu\text{g.kg}^{-1}$ , was found in a sample of peanut with a salty coat ("amendoim japonês"). Results of previous studies in peanuts and peanut products in the city of São Paulo from 1980-1987 had 68.75% of the samples with levels greater than the limit  $30.0 \mu\text{g.kg}^{-1}$  and the 90<sup>th</sup> percentile ranged from 42.0 to  $333.0 \mu\text{g.kg}^{-1}$ . In 1994, 36.0% of the samples showed results above the limit and the 90<sup>th</sup> percentile was  $489 \mu\text{g.kg}^{-1}$ . The results show that aflatoxins contamination in peanuts is decreasing but it is still a serious problem in Brazil, a country where the climate, the agricultural practices and storage conditions favour fungal growth.

**Key words:** aflatoxins, peanuts, peanut products, thin-layer chromatography

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### INTRODUCTION

Aflatoxins were discovered almost forty years ago and aflatoxin  $B_1$  is the most toxic substance of the group. It can cause liver damage, impaired productivity

and reproductive efficiency in different animal species (12).

Aflatoxin contamination is the main problem in peanuts and peanut products in Brazil (2, 5, 6, 8, 9, 10). The World Health Organization recommends a

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systematic control of aflatoxin level in the population diet, mainly in countries located in tropical and sub-tropical areas, where the climatic conditions favour the growth of aflatoxin-producing fungi (13). Given the necessary conditions for mould growth and toxin production, agricultural commodities may be contaminated in the field or after harvest during storage, processing and transport. Once the commodities are contaminated they become not only a public health hazard but also a financial loss (4).

Since 1976 the Brazilian regulatory limit in food is 30.0  $\mu\text{g. kg}^{-1}$  for the sum of the concentrations of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) and aflatoxin G<sub>1</sub> (AFG<sub>1</sub>) (1). More recently MERCOSUL, a group for common market among Argentina, Brazil, Paraguay and Uruguay, through the Resolution 56/94, established the maximum limit of 20.0  $\mu\text{g. kg}^{-1}$  for the sum of the aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. This resolution was already internalized by the Brazilian Ministry of Agriculture (Portaria n<sup>o</sup> 183 of March 21, 1996) and the same is expected to happen by the Brazilian Ministry of Health.

The Instituto Adolfo Lutz monitors the levels of aflatoxins in peanuts and peanut products consumed in São Paulo area through a joint program with The Sanitary Guidance of the Secretary of Health of São Paulo State. The present work reports the results from the 1995-1997 inspection. The study aims to collect data in order to furnish the State government adequate information, so it can act accordingly.

## MATERIALS AND METHODS

One hundred and thirty seven samples of raw peanuts and peanut containing foods, collected from January 1995 to December 1997 in São Paulo markets, were included in this study.

About 3.0 kg of sample of the lot under inspection

were collected and divided into three 1 kg sub-samples designated 1, 2 and 3. One of them was finely ground in a blender (Waring - Model 31BL.91) and homogenized. A sub-sample of 50 g was taken for the aflatoxins determination.

Aflatoxins B<sub>1</sub> and G<sub>1</sub> were determined by thin-layer chromatography (TLC), according to the method described by Soares and Rodriguez-Amaya (11). It involved extraction with a mixture of methanol and 4% KCl (9+1,v/v), followed by a clarification step with 10% CuSO<sub>4</sub> and partition to chloroform. The limit of quantification obtained was 5.0  $\mu\text{g. kg}^{-1}$ . The chemical confirmation of AFB<sub>1</sub> and AFG<sub>1</sub> was performed with trifluoroacetic acid (7).

## RESULTS AND DISCUSSION

From 137 samples, 45.3% (62 samples) were positive for aflatoxins and 27.0% (37 samples) presented levels of AFB<sub>1</sub> + AFG<sub>1</sub> above the maximum limit allowed by the Brazilian Legislation (30.0  $\mu\text{g. kg}^{-1}$ ) (Tables 1 and 2). The 90<sup>th</sup> percentile was 110.0  $\mu\text{g. kg}^{-1}$  in 1995, 60.0  $\mu\text{g. kg}^{-1}$  in 1996 and 118.0  $\mu\text{g. kg}^{-1}$  in 1997 (Table 1). Comparing these values with the results obtained in the 1980-1987 study (9), which ranged from 42.0 to 333.0  $\mu\text{g. kg}^{-1}$ , the present results were lower but still of concern. In the present study the aflatoxins concentration in the raw peanuts samples ranged from 5.0 to 382.0  $\mu\text{g. kg}^{-1}$  and 27.1% were above the regulatory limits while 32.8% of the peanuts candies samples were above the limit and the concentration of aflatoxins B<sub>1</sub>+G<sub>1</sub> ranged from 6.0 to 494.0  $\mu\text{g. kg}^{-1}$ . Contamination in salted peanuts samples were around 10.0%. The toxins concentrations were mostly below 10.0  $\mu\text{g. kg}^{-1}$  except for one sample of salt coated peanut ("amendoim japonês") that presented a level of 536.0  $\mu\text{g. kg}^{-1}$ .

**Table 1.** Levels of AFB<sub>1</sub> and AFG<sub>1</sub> in samples of peanuts and peanut containing foods ( $\mu\text{g. kg}^{-1}$ )

Year	n <sup>o</sup> . of samples	> 30 $\mu\text{g. kg}^{-1}$	< 30 $\mu\text{g. kg}^{-1}$ > 5 $\mu\text{g. kg}^{-1}$	median	90 <sup>th</sup> percentile	maximum level ( $\mu\text{g. kg}^{-1}$ )
1995	58	18 (31.0%)	10 (17.3%)	<5	110	494 <sup>a</sup>
1996	50	09 (18.0%)	08 (16.0%)	<5	60	469 <sup>a</sup>
1997	29	10 (34.5%)	07 (24.1%)	<5	118	536 <sup>b</sup>
TOTAL	137	37 (27.0%)	25 (18.3%)	<5	123	536

a - sample of candy made with peanut ("paçoca")

b - sample of peanut with salty cover ("amendoim japonês")

**Table 2.** Distribution of aflatoxins B<sub>1</sub> and G<sub>1</sub> in peanuts and peanut containing food samples according to the present Brazilian legislation (%).

year	raw peanut		peanut containing food			
	unshelled		candies		salted	
	App.	Cond.	App.	Cond.	App.	Cond.
1995	68.7	31.3	60.6	39.4	100.0	0.0
1996	89.5	10.5	72.7	27.3	88.9	11.1
1997	53.8	46.2	75.0	25.0	75.0	25.0
TOTAL	72.9	27.1	67.2	32.8	90.9	9.1

App. Approved  
Cond. Condemned

Sabino (8) analyzed 572 samples of food products including peanuts in the period 1971 to 1975 and reported higher levels such as 7,800 µg.kg<sup>-1</sup> and a mean of 1131.0 µg.kg<sup>-1</sup>. In the 1980-1982 period, Scussel and Rodriguez-Amaya (10) analyzed 241 samples of peanuts and peanut products. They found 128 to be positive, of which 92 exceeded the maximum limit of 30.0 µg.kg<sup>-1</sup>. Sabino *et al.* (9) analyzed 1,374 samples during the period 1980-1987 and found 68.7% of the samples of peanuts and its products with concentrations of aflatoxins B<sub>1</sub> + G<sub>1</sub> greater than the limit allowed. Prado *et al.* (6) analyzed 400 samples of rawpeanuts consumed in Belo Horizonte (MG-Brazil), 61.5% were positive ranging from 4 to 1032 µg.kg<sup>-1</sup> of AFB<sub>1</sub>. Oliveira *et al.* (5) found aflatoxins B<sub>1</sub> and G<sub>1</sub> above the maximum limit in 34.6% of 104 samples of peanuts collected in retail stores of Goiania/GO, Brazil. Brigido *et al.* (2) found 47% of 96 samples of peanuts and products from Campinas to exceed the maximum limit. All these studies detected a very high percentage of contaminated peanut samples. Specially of concern are candies made with peanuts (“paçoca” and “pé-de-moleque”) because they are consumed basically by youngsters. Frequently these products are sold inside or nearby schools. According to the present regulations (B<sub>1</sub> + G<sub>1</sub> = 30.0 µg.kg<sup>-1</sup>) about 30% of the samples of candies with peanuts were found to be over the limit, this percentage rises to about 50% if the MERCOSUL limits are considered. The Codex Alimentarius Commission (3) has recommended a level on 5.0 µg.kg<sup>-1</sup> for aflatoxin in food. In the present survey, 45% of the samples were found to be contaminated with AFB<sub>1</sub> and AFG<sub>1</sub> exceeding this recommended level, whereas only 27% of the samples exceeded the present Brazilian tolerance limit. Probably when the MERCOSUL limits (20.0 µg.kg<sup>-1</sup> for the sum of

aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>) is internalized by the Health Ministry the percentage of condemned samples will be higher.

Suggestions to change the present situation may include: a) motivation of agriculture extension services to educate farmers on the problems of mycotoxins and to encourage farmers to use good agricultural practices in order to minimize field, harvest and post-harvest contamination; b) increasing government awareness of its responsibilities concerning the marketing and consumption of peanut and peanut products.

## RESUMO

### Ocorrência de aflatoxinas em amendoim e produtos contendo amendoim consumidos no Estado de São Paulo/ Brasil no período 1995-1997

Cento e trinta e sete amostras de amendoim e produtos contendo amendoim, obtidas no período de janeiro de 1995 a dezembro de 1997, a grande parte delas coletadas pela Vigilância Sanitária da Secretaria de Saúde do Estado de São Paulo, foram submetidas à determinação de aflatoxinas. Foram incluídas amostras de amendoins cru, doces de amendoim (“paçoca” e “pé-de-moleque”), pasta de amendoim, amendoins salgados (frito e torrado), “torrone” e amendoins com cobertura de chocolate ou cobertura salgada (“amendoim japonês”). As amostras foram analisadas por cromatografia em camada delgada.

Sessenta e duas amostras (45,3%) foram positivas para aflatoxinas e 37 amostras (27,0%) apresentaram valores de aflatoxinas B<sub>1</sub> + G<sub>1</sub> acima do limite máximo da legislação brasileira (30,0 µg. kg<sup>-1</sup> para aflatoxinas B<sub>1</sub>+G<sub>1</sub>). A identidade destas aflatoxinas foi confirmada usando-se ácido trifluoro acético. O 90<sup>th</sup> percentil foi 110,0 em 1995, 60,0 em 1996 e 118,0 µg. kg<sup>-1</sup> em 1997. A concentração de aflatoxinas nas amostras de amendoim cru variou de 5,0 a 356,0 µg.kg<sup>-1</sup> e 27,1% delas acima do limite. Quanto à contaminação por aflatoxinas nas amostras de doces de amendoim 32,8% delas estavam acima do limite e as concentrações variaram de 6,0 a 536,0 µg. kg<sup>-1</sup>. A contaminação nos amendoins salgados foi menos frequente, cerca de 10% das amostras e os níveis de toxina geralmente abaixo de 10,0 µg. kg<sup>-1</sup>, porém uma das amostras com cobertura (“amendoim japonês”) apresentou 536,0 µg. kg<sup>-1</sup>. Comparando com os dados

de incidência de 1980-1987 em amendoim e produtos de amendoim na cidade de São Paulo, quando 68,75% das amostras mostraram valores maiores que o limite permitido e o 90<sup>th</sup> percentil variou de 42,0 a 333,0  $\mu\text{g.kg}^{-1}$ , e de 1994 quando 36,0% das amostras mostraram resultados acima do limite e o 90<sup>th</sup> percentil foi 489,0  $\mu\text{g.kg}^{-1}$ , os resultados deste trabalho mostram que a contaminação por aflatoxinas está diminuindo. Entretanto, mostram também que a contaminação por aflatoxinas em amendoim continua um problema sério no Brasil mas que deve-se levar em conta não somente as condições climáticas (umidade e altas temperaturas) mas também as práticas de agricultura e as condições de estocagem.

**Palavras-chave:** aflatoxinas, amendoim, produtos contendo amendoim, cromatografia em camada delgada

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