Assessment of aflatoxin intake in São Paulo, Brazil


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A R T I C L E   I N F O

Article history:
Received 30 October 2012
Received in revised form 6 February 2013
Accepted 12 February 2013

Keywords:
Aflatoxin intake
Milk
Cheese
Peanuts
Corn
Beans

A B S T R A C T

In this study, the aflatoxin consumption was assessed by aflatoxin determination in peanut, corn, bean, milk and derivatives collected directly from home of residents of Pirassununga São Paulo, Brazil, and a Food Frequency Questionnaire (FFQ) answered by volunteers. Samples were collected four times every three months, totaling 240 samples from 34 volunteers who answered the FFQs. Aflatoxins B1, B2, G1, G2 and M1 were determined by high performance liquid chromatography using a previously validated method with immunospecific column clean-up. Thirty-five percent of peanut samples tested positive for aflatoxin (AFB1 + AFB2 + AFG1 + AFG2) at levels of 0.05–36.7 µg kg⁻¹. Corn derivatives showed 42% positive samples for aflatoxins (0.05–8.3 µg kg⁻¹), with a higher incidence in corn meal. Bean samples had the highest incidence of aflatoxins (75% positive samples), but at lower levels (0.025–0.042 µg kg⁻¹). Forty percent of fluid milk samples tested positive for AFM1 at concentrations from 0.009 to 0.069 µg L⁻¹. AFM1 was quantitated in 30% of positive cheese at levels from 0.091 to 0.30 µg kg⁻¹. Yoghurt samples had no detectable levels of AFM1. Aflatoxin intake was calculated considering the aflatoxin concentration values in the food products and the individual FFQ results. Peanut products had the highest contribution to aflatoxin intake, with mean probable daily intake (PDI) of 1.56 ng kg⁻¹ body weight (b.w.) day⁻¹. Milk was the main source of AFM1 in the diet of individuals, which PDI values ranged from up to 0.10 ng kg⁻¹ b.w. day⁻¹. Although low levels of aflatoxin were found in all types of samples, the consumption data reported indicate that the food products evaluated may contribute significantly for the overall human exposure in the population studied.

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1. Introduction

Aflatoxins are highly toxic, teratogenic, mutagenic and carcinogenic metabolites produced by fungi of the genus Aspergillus, mainly by species Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius (Moss, 1998). These fungi are distributed worldwide and may grow in various agricultural commodities like cereals, tree nuts and spices in the field and in the storage conditions. Pre-harvest contamination is favored by high temperatures, prolonged drought conditions and high insect activity while post-harvest production of aflatoxins is mostly promoted by warm temperatures and high humidity (Soler et al., 2010).

There are four major aflatoxins, namely B1, B2, G1 and G2, which can directly contaminate feed and foodstuffs. However, aflatoxin B1 (AFB1) is the most commonly occurring and has the greatest toxicogenic potential (Coulombe, 1991). Aflatoxin M1 (AFM1) is found in milk of lactating cows that have consumed feeds contaminated

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maximum aflatoxin levels allowed in foodstuffs. Foodstuffs' characteristic of each country, frequency of consumption of these items and climate characteristics apparently influence the maximum limits adopted in each region, although there is a consensus that these limits should comply with the ALARA (as lowest as reasonable accepted) criterion recommended by the Food and Agriculture Organization (2004).

In Brazil, until 2011 only peanuts and corn had a maximum tolerable limit of 20 g kg\(^{-1}\) established by legislation for the sum of AFB\(_1\), AFB\(_2\), AFG\(_1\) and AFG\(_2\). In fluid milk and powder milk, 0.5 g L\(^{-1}\) and 5.0 g kg\(^{-1}\) were allowed for AFM\(_1\), respectively. A recent revision in legislation introduced other types of foods like cheeses, beans, dehydrated fruits, spices and cocoa products. A special attention was given to cereal-based baby food and infant formulas with a maximum tolerable limit of 1 g kg\(^{-1}\) for the sum of aflatoxins (Agência Nacional de Vigilância Sanitária, 2011, pp. 72–73). Assessment of aflatoxin intake by Brazilian population has been usually estimated by analyzing a single type of food product like peanut, corn or milk collected separated in retail markets (Oliveira, Gonçalves, Rosim, & Fernandes, 2009; Iha, Barbosa, Favaro, & Truckssess, 2011; Iha, Barbosa, Okada, & Truckssess, 2011; Magrine et al., 2011). In this study, the aflatoxin intake was measured by aflatoxin determination in peanut, corn, beans, milk and derivatives collected simultaneously and directly from home of residents in Pirassununga, São Paulo, Brazil, and from data of a Food Frequency Questionnaire (FFQ) answered by volunteers.

2. Material and methods

2.1. Sampling design

The study was conducted in households from employees of the University of São Paulo at Pirassununga, Brazil from June 2011 to March 2012. Residents (N = 34) were invited to participate as volunteers, providing food samples four times every three months. Twenty-four families agreed to participate and were instructed to collect peanuts and corn products, bean, liquid or powder milk, cheese and yoghurt if available on their residences on the day of samples collection. Food samples were packed in polyethylene bags or vessels for fluid milk and yoghurt, and sent to the laboratory. Solid samples were homogenized and finely ground. All samples (N = 240) were kept frozen at −20 °C until analysis.

2.2. Sample preparation

Analyses of aflatoxins were performed using immunoaffinity columns, described as follows. Identification and determination of aflatoxins B\(_1\), B\(_2\), G\(_1\) and G\(_2\) in peanut and corn samples were carried out by high performance liquid chromatography (HPLC). An aliquot of the sample (25 g or 50 g for peanut or corn, respectively) was weighted in an Erlenmeyer flask containing 5 g of sodium chloride and 125 mL of methanol/water (70:30, v/v) for peanut or 100 mL of methanol/water (80:20, v/v) for corn were added. The flask was placed in an orbital shaker (Tecnal, Piracicaba, Brazil) for 30 min, filtered, and to an aliquot (20 or 10 mL for peanut or corn, respectively), 20 mL or 40 mL of ultra-pure water were added and the mixture homogenized. An aliquot (10 mL) was then passed through the immunoaffinity column (Vicam, Watertown, MA, USA) at flow rate of 1–2 drops per second. After washing with 20 mL of ultra-pure water, aflatoxins were eluted from the column with 1 mL of methanol and the eluate was collected in an amber vial. The eluate was evaporated to dryness under nitrogen flow. Derivatization of AFB\(_1\) and AFG\(_1\) was obtained by adding 200 µL of n-hexane and 200 µL of trifluoroacetic acid (TFA). The mixture was kept at 40 °C for 10 min, evaporated to near-dryness and diluted in 1 mL of methanol:water (50:50, v/v). Final extracts were filtered through a 0.45 μm PTFEmembrane prior to injection into HPLC column.

Bean samples were extracted exactly by the same method described for corn samples, except that 20 mL were eluted through the immunoaffinity column.

AFM\(_1\) was determined in milk as proposed by the manufacturer of the immunoaffinity columns (Aflatest, Vicam, Watertown, MA, USA) with minor modifications. Forty milliliter of sample was heated at 37 °C for 10 min, added of 1 g of sodium chloride and centrifuged at 3500 rpm for 5 min. The upper fat layer was removed and the sample was centrifuged again to remove remaining fat. The sample was filtered under vacuum through Whatman no. 4 filter paper and 30 mL applied to immunoaffinity column, which was connected to a glass syringe and a vacuum system (flow of 2–3 mL min\(^{-1}\)). The column was washed with 20 mL of ultra-pure water and AFM\(_1\) was eluted with 1 mL of methanol. The eluate was evaporated to near-dryness under nitrogen flow and the dried extract was resuspended in 1 mL of methanol/water (50:50, v/v).

The determination of AFM\(_1\) in yoghurt and cheese was performed by the method proposed by Iha, Barbosa, Favaro, et al. (2011) and Iha, Barbosa, Okada, et al. (2011), with minor modifications. Eight g of grinded cheese or yoghurt were added of 2 g of sodium chloride, 22 mL of methanol and 13 or 12 mL of water for cheese or yoghurt, respectively. The mixture was homogenized in a mixer for 1 min, centrifuged at 3500 rpm for 10 min. The supernatants of cheese samples were filtered through Whatman no. 4 filter paper. Twenty mL of previous extract were diluted with 40 mL of ultra-pure water and passed through immunoaffinity column. The following extraction steps were the same as described for milk samples.

2.3. Reagents and solutions

All reagents were of analytical grade and water was purified by deionization (Milli-Q system, Millipore, Bedford, MA, USA). HPLC-grade acetonitrile and methanol (JT Baker, Xalostoc, Mexico) were used for chromatographic analyses.

The aflatoxins standards AFB\(_1\), AFB\(_2\), AFG\(_1\), AFG\(_2\) and AFM\(_1\) were purchased from Sigma (Sigma, St Louis, MO, USA). Individual stock solutions were prepared in toluen/acetonitrile (9:1, v/v), evaluated according to Scott (1990) (AOAC method 971.22), and stored in an amber glass paper and 30 mL applied to immunoaffinity column, which was connected to a glass syringe (A V. Jager et al. / Food Control 33 (2013) 87–92 89) until analysis. Final extracts were filtered through a 0.45 μm PTFEmembrane prior to injection into HPLC column.

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2.4. Instruments

All chromatographic analyses were conducted on the Shimadzu 10VP liquid chromatograph (Kyoto, Japan) with a 10 AXL fluorescence detector (excitation at 360 nm and emission above 440 nm). A Kineticex C18 column (Phenomenex, Torrance, CA, USA) 4.6 × 150 mm, 2.6 µm particle size and an in-line filter of 0.5 µm were used. The isotropic mobile phase consisted of methanol/water/acetonitrile (61.4:28.1:10.5, v/v/v) with a flow rate of 0.50 mL min\(^{-1}\).

2.5. Validation procedures

The limits of detection (LOD) and quantification (LOQ) were calculated for each method of analysis based on signal:noise ratio of
respectively. Linearity was evaluated by verifying the coefficient of determination ($r^2$) and visual inspection of residual plots of analytical curves built separately for each aflatoxin. TriPLICATE injection of each concentration level was used.

Recovery and precision for milk analyses were assessed by adding AFM1 at concentrations of 0.1, 0.25 and 0.5 µg L$^{-1}$. Cheese and yoghurt samples were fortified with AFM1 at 0.5 and 2.5 µg kg$^{-1}$ and 0.2 and 0.5 µg kg$^{-1}$, respectively. Bean samples were added of AFB1 at 0.5 and 5.0 µg kg$^{-1}$. AFB1 was added to corn and corn flour at concentration levels of 2 µg kg$^{-1}$ and 20 µg kg$^{-1}$. All these fortified samples were prepared and extracted in triplicate and final concentration calculated by triplicate injection of each extract.

2.6. Food frequency questionnaire

The FFQ was elaborated on the basis of the population consumption habits of foods susceptible to aflatoxin contamination and regulated in Brazil (Agência Nacional de Vigilância Sanitária, 2011, p. 72). Some food items like corn flour are used to prepare different processed foods and thus these foods were also included in the questionnaire aiming to cover as many foods consumed as possible. Consumption frequency was measured by a range starting from less than once a month until twice every day. Portion sizes were chosen for each food item according to usual measures, cups for milk, slices for cheese and tablespoon for cereals. A blank space was also available if the volunteers needed to express a different consumption pattern. The FFQ was applied to thirty-five individuals living in residences from where food samples were collected. The body weight of each individual was also recorded to calculate the PDI value by the following expression: PDI = (mean concentration of aflatoxins × mean consumption of food type)/(each individual body weight).

3. Results and discussion

3.1. Method performance

The LOD and LOQ values for AFM1 in milk were 0.075 and 0.25 µg L$^{-1}$ at sample extract, respectively, which represent 0.0025 and 0.0080 µg L$^{-1}$ in the original sample. Coefficient of determination was 0.9996 and residuals plot did not show tendency or deviation from linearity. Mean recoveries and coefficients of variation (CV) obtained for the three replicates were 100% (CV = 2%), 90% (CV = 7%) and 118% (CV = 4%) for 0.1, 0.25 and 0.50 µg AFM1 L$^{-1}$, respectively. The same LOD and LOQ for milk sample extracts are valid for cheese and yoghurt. However, due to differences in sample extraction procedures the LOD and LOQ of AFM1 in the original samples were 0.017 and 0.055 µg kg$^{-1}$, respectively. Mean recoveries found for cheese fortified with 0.5 µg AFM1 kg$^{-1}$ and 2.5 µg AFM1 kg$^{-1}$ were 73% (CV = 6%) and 77% (CV = 6%), respectively. Yoghurt samples added of AFM1 at 0.2 µg kg$^{-1}$ and 0.5 µg kg$^{-1}$ showed recoveries of 72% (CV = 8%) and 93% (CV = 2%), respectively. These values were similar to those found by Iha, Barbosa, Favaró, et al. (2011) and Iha, Barbosa, Okada, et al. (2011) for yoghurt and cheese and the method was considered suitable and applied to all samples.

The LOD values for AFB1, AFB2, AFG1 and AFG2 were 0.015, 0.009, 0.06 and 0.015 µg kg$^{-1}$, respectively, while the LOQ values were 0.05, 0.03, 0.2 and 0.05 µg kg$^{-1}$ in the original samples of peanuts and corn derivatives. Beans samples had a LOD and LOQ twice than obtained for peanut and corn derivatives, since 20 mL of extract were passed through the immunoaffinity column. Coefficients of determination were higher than 0.9995 for all analytical curves and residual plots inspection did not show deviations from linearity. Mean recoveries of bean samples fortified with AFB1 at concentrations levels of 0.5 and 5.0 µg kg$^{-1}$ were 82% (CV = 10%) and 81% (CV = 4%), respectively. Corn fortified with 2 µg kg$^{-1}$ and 20 µg kg$^{-1}$ of AFB1 showed recoveries of 89% (CV = 2%) and 108% (CV = 12%), respectively. In corn flour samples, mean recoveries were 80% (CV = 8%) for 2 µg AFB1 kg$^{-1}$ and 86% (CV = 13%) for 20 µg AFB1 kg$^{-1}$. Recoveries and precision studies for aflatoxins in peanut and products had been already performed and reported before (Oliveira et al., 2009). Recoveries ranged from 71 to 126% for all aflatoxins and coefficients of variation between 2 and 39% were obtained.

3.2. Analyses of samples

Two hundred and forty samples were analyzed and results of AFM1 determined in fluid and powder milk, yoghurt and cheese, and the sum of AFB1, B2, G1 and G2 quantified in peanut, corn and beans are summarized in Table 1. Forty percent of milk samples showed AFM1 concentration values above LOQ. Cheese samples had 30% of positive samples, while 100% of yoghurt samples tested negative for AFB1. All milk and dairy products samples had AFM1 concentration below the tolerance limit established in Brazil. AFM1 incidence and concentration values determined in this work are lower than previous studies in Brazil. Shundo, Navas, Lamardo, Ruvieri, and Sabino (2009) investigated 125 liquid and powder milk samples and 95.2% were found positive for AFM1 with concentrations ranging from 0.010 to 0.200 µg kg$^{-1}$. Raw milk samples from dairy farms were analyzed by Oliveira, Sebastião, Fagundes, Rosim, and Fernandes (2008) and AFM1 concentration varied between 0.010 and 0.645 µg L$^{-1}$, with one sample above the tolerance limit of 0.5 µg kg$^{-1}$ adopted in Brazil. Iha, Barbosa, Favaró, et al. (2011) and Iha, Barbosa, Okada, et al. (2011) analyzed 53 yoghurt

<table>
<thead>
<tr>
<th>Type of food</th>
<th>N</th>
<th>Positive samples</th>
<th>MPL</th>
<th>n &gt; MPL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut products</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn products</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn flour (flakes)</td>
<td>28</td>
<td>21</td>
<td>75</td>
<td>0.051–2.8</td>
</tr>
<tr>
<td>Corn (for popcorn)</td>
<td>18</td>
<td>0</td>
<td>–</td>
<td>&lt; LOD</td>
</tr>
<tr>
<td>White hominy</td>
<td>3</td>
<td>3</td>
<td>100</td>
<td>0.064–2.5</td>
</tr>
<tr>
<td>Bean (in nature)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk products</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquid milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Powder milk</td>
<td>4</td>
<td>2</td>
<td>50</td>
<td>0.50–0.81</td>
</tr>
<tr>
<td>Cheese</td>
<td>10</td>
<td>3</td>
<td>30</td>
<td>0.091–0.30</td>
</tr>
<tr>
<td>Yoghurt</td>
<td>7</td>
<td>0</td>
<td>–</td>
<td>&lt; LOD</td>
</tr>
</tbody>
</table>

N: total number of samples analyzed.

n: Samples showing concentrations above the limit of determination (LOD).

MPL: maximum permitted level adopted by Brazilian regulations.

a Samples analyzed for $\Sigma$ aflatoxins $B_1 + B_2 + G_1 + G_2$.

b Samples analyzed for aflatoxin $M_1$.

c Results expressed in µg L$^{-1}$. 

samples and 72% were positive for AFM1 with concentration ranging from 0.010 to 0.529 µg kg\(^{-1}\). Brazil has not established tolerance limits for AFM1 in yoghurt or dairy drinks. Cheese samples were also examined for AFM1 in recent years and incidence (27.1–67%) and concentration values (0.010–0.66 µg kg\(^{-1}\)) were mostly higher than the ones found in the present study. Nevertheless, concentrations above the tolerance limit of 2.5 µg kg\(^{-1}\) applied since 2011 have not been found in Brazil (Oliveira et al. (2009), where 44% of peanut and derivatives samples showed above the tolerance limit. Similar results were obtained by Oliveira (2009), where 44% of peanut and derivatives samples showed concentrations of aflatoxins (>0.05 µg kg\(^{-1}\)) and only one sample showed concentration of 36.7 µg kg\(^{-1}\), above the tolerance limit adopted in Brazil. Thirty-five percent of peanut and derivatives samples had quantifiable concentrations of aflatoxins (>0.05 µg kg\(^{-1}\)) and only one sample showed concentration of 36.7 µg kg\(^{-1}\), above the tolerance limit adopted in Brazil (20 µg kg\(^{-1}\)). As noticed for milk and cheese samples, these values are also below the observed in preceding studies. Recently, Magrine et al. (2011) found 50% of peanut samples (N = 100) positive for aflatoxins and concentration values as high as 113 µg kg\(^{-1}\). Thirteen percent of samples were above the tolerance limit. Similar results were obtained by Oliveira et al. (2009), where 44% of peanut and derivatives samples showed concentration values ranging from 0.5 to 103.8 µg kg\(^{-1}\), with nine positive samples (3.7%) above the tolerance limit.

Consuming corn and derivatives samples, 42% tested positive for aflatoxins with higher incidence in corn flour samples (75% of positive samples) but at low levels (0.051–2.8 µg kg\(^{-1}\)). The highest concentration for corn samples was found in a popcorn sample, with 8.3 µg kg\(^{-1}\) for the sum of aflatoxins. None of analyzed samples showed aflatoxins concentration above the Brazilian tolerance limit. In a previous evaluation, 60 samples of corn meal and flour samples were analyzed by Bittencourt, Oliveira, Dilkin, and Corrêa (2005) but none were positive for aflatoxins. Low occurrence of aflatoxins in processed corn products has also been observed by Amaral, Nascimento, Sekiyama, Janeiro, and Machinski (2006), who analyzed 123 samples of corn-based food products and only seven samples (5.7%) were positive for aflatoxins. These results are comparable with our data, although both studies of Bittencourt et al. (2005) and Amaral et al. (2006) used thin-layer chromatography (TLC) for quantification of aflatoxins, and therefore had higher LOQs varying from 0.5 to 3.2 µg kg\(^{-1}\).

Similar to corn flour, bean samples demonstrated high incidence of aflatoxins (75%) at low levels (0.025–0.042 µg kg\(^{-1}\)). The Brazilian tolerance level of aflatoxins in bean is 5.0 µg kg\(^{-1}\), therefore all samples were far below this value. Aflatoxins in bean samples have been scarcely evaluated in Brazil. Silva et al. (2002) analyzed thirty bean samples by TLC and only one sample tested positive for aflatoxins at level of 2.5 µg kg\(^{-1}\).

Aflatoxins levels in food samples presented above seem to indicate a tendency to decreasing values, as was already observed before by Oliveira et al. (2009) regarding the evaluations carried out in the 90’s. In February 2011, Brazilian regulation limits were enforced not only for aflatoxins, but also for other mycotoxins like fumonisins, ochratoxin A, deoxynivalenol (DON), zearalenone and patulin. These regulations are expected to promote further reductions and stimulate investigations of other mycotoxins levels in coming years.

### 3.3. Estimation of food consumption

The Food Frequency Questionnaire was applied to thirty-five volunteers, of which 17 were women (48.5%) and 18 were men (51.5%) with ages ranging from 17 to 55 years old. All these individuals are residents from homes that supplied food samples. Food ingestion habits declared were tabulated and minimum, average and maximum consumption values of each type of food are presented in Table 2. Food intake was calculated on a monthly basis because not all food types under evaluation are usually consumed daily by Brazilian population. Table 2 shows that from all food products evaluated, the most frequently consumed by this group of individuals are beans, cheese, liquid milk and yoghurt. Corn products showed low importance in the diet of the sampling population. Peanuts and derivatives also occur in large amount in the diet of volunteers. Although 91% of volunteers declared to consume peanuts at least once a month, the variation in consumption is of great importance when aflatoxins intake is calculated, since peanut represents the samples contaminated at higher levels as stated in Table 1. It is also noteworthy that beans are part of the diet of 100% of volunteers in a large amount, as can be verified by the mean and standard deviation of consumption.

### 3.4. Assessment of aflatoxin intake

The mean aflatoxin level found in each food types expressed in Table 1 together with the mean consumption of each type of food expressed in Table 2 and each individual body weight were used to calculate the probable daily intake of aflatoxins, summarized in Table 3. Peanuts and derivatives are clearly the main source of aflatoxin intake by population. The highest PDI of aflatoxins through peanuts ingestion (13.7 ng kg\(^{-1}\) b.w. day\(^{-1}\)) is higher than the value found for Magrine et al. (2011) of 10.4 ng kg\(^{-1}\) b.w. day\(^{-1}\) for high peanuts consumers. A difference that may be considered is that Magrine et al. (2011) considered just AFB1 consumption and in our approach was selected because AFB2, AFG1 and AFG2 are also toxic for the sum of aflatoxins, but also for other mycotoxins like patulin. These regulations are expected to promote further reductions and stimulate investigations of others mycotoxins levels in coming years.

### Table 2

<table>
<thead>
<tr>
<th>Type of food</th>
<th>n</th>
<th>Consumption (g month(^{-1}))</th>
<th>Variation(^{b})</th>
<th>Mean ± SD(^{c})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut products</td>
<td>32</td>
<td>22–3382</td>
<td>382 ± 608</td>
<td></td>
</tr>
<tr>
<td>Corn products</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn flour</td>
<td>26</td>
<td>10–60</td>
<td>32 ± 18</td>
<td></td>
</tr>
<tr>
<td>Corn flakes</td>
<td>23</td>
<td>5–360</td>
<td>41 ± 72</td>
<td></td>
</tr>
<tr>
<td>Corn (for popcorn)</td>
<td>79</td>
<td>25–300</td>
<td>77 ± 75</td>
<td></td>
</tr>
<tr>
<td>White hominy</td>
<td>19</td>
<td>12–100</td>
<td>26 ± 20</td>
<td></td>
</tr>
<tr>
<td>Bean (in natura)</td>
<td>35</td>
<td>25–3000</td>
<td>1038 ± 1000</td>
<td></td>
</tr>
<tr>
<td>Milk products</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquid milk</td>
<td>30</td>
<td>200–24,000</td>
<td>7313 ± 6316</td>
<td></td>
</tr>
<tr>
<td>Powder milk</td>
<td>12</td>
<td>20–480</td>
<td>118 ± 172</td>
<td></td>
</tr>
<tr>
<td>Cheese</td>
<td>32</td>
<td>25–3000</td>
<td>519 ± 622</td>
<td></td>
</tr>
<tr>
<td>Yoghurt</td>
<td>28</td>
<td>200–6000</td>
<td>1764 ± 2089</td>
<td></td>
</tr>
</tbody>
</table>

n: Number of individuals who declared to consume the type of food at least once a month.

\(^{a}\) Number of volunteers who answered the questionnaire: 35.

\(^{b}\) Variation expressed for individuals who declared to consume the type of food at least once a month.

\(^{c}\) Mean calculated from values different from zero.
with higher aflatoxin levels found in peanuts products analyzed, the final result was a lower PDI.

The highest level of aflatoxin found of 36.7 ng g\(^{-1}\) and the highest peanut consumption declared by a volunteer led to a worst case of aflatoxin intake of 59.1 ng kg\(^{-1}\) b.w. day\(^{-1}\), which is the highest estimative of aflatoxin intake reported in Brazil. Higher and lower PDI values have been reported in surveys worldwide. Among much lower aflatoxin intake are the data reported in France by Leblanc, Tard, Volatier, and Verger (2005), where values of PDI were 0.117 ng kg\(^{-1}\) b.w. day\(^{-1}\) and 0.345 ng kg\(^{-1}\) b.w. day\(^{-1}\) for mean and high adult consumers, respectively. Lower PDI values of 0.15 ng kg\(^{-1}\) b.w. day\(^{-1}\) and 0.26 ng kg\(^{-1}\) b.w. day\(^{-1}\) were reported in Australia and United States, respectively (World Health Organization, 1998). A recent evaluation conducted in Spain found a PDI of 0.072 ± 0.167 ng kg\(^{-1}\) b.w. day\(^{-1}\) for adult males and 0.077 ± 0.208 ng kg\(^{-1}\) b.w. day\(^{-1}\) for adult females (Cano-Sancho, Sanchis, Marín, & Ramos, 2013). An intermediate PDI of 0.8 ng kg\(^{-1}\) b.w. day\(^{-1}\) for aflatoxins was estimated in Sweden by Thuander et al. (2001). Park, Kim, and Kim (2004) evaluated the PDI of aflatoxins by Koreans and the range was 1.19–5.79 ng kg\(^{-1}\) b.w. day\(^{-1}\), being the rice the main source of AFB1 ingestion. The highest PDI of aflatoxins was reported in China, with values ranging from up to 91 ng kg\(^{-1}\) b.w. day\(^{-1}\) (World Health Organization, 1998).

As expected for its high consumption, milk was the main source of AFM\(_1\) in the diet of population studied, with a mean PDI of 0.10 ng AFM\(_1\) kg\(^{-1}\) b.w. day\(^{-1}\). This value is close to the PDI of as 0.09 ng kg\(^{-1}\) b.w. day\(^{-1}\) estimated by Leblanc et al. (2005) for the French population. The authors considered the mean individual consumption of milk products as 229 g day\(^{-1}\), while in this study the mean consumption declared was 319 g day\(^{-1}\). There is no previous report in Brazil on the assessment of AFM\(_1\) intake using FFQ and data from analyses of different types of milk products as performed in the present survey. Shundo et al. (2005) estimated the PDI of AFM\(_1\) as 0.188 ng kg\(^{-1}\) b.w. day\(^{-1}\) only by liquid milk consumption. The low PDI of AFM\(_1\) observed in this study was due to low levels of AFM\(_1\) found in milk and milk products. If milk and derivatives analyzed had AFM\(_1\) concentration close to Brazilian legislation limits of 0.5 \(\mu\)g L\(^{-1}\) for milk, 2.5 \(\mu\)g kg\(^{-1}\) for cheese and 5.0 \(\mu\)g kg\(^{-1}\) for powder milk the mean PDI around 2.6 ng kg\(^{-1}\) b.w. day\(^{-1}\) would be obtained.

French maximum permitted level for AFM\(_1\) in milk and derivatives is established at 0.05 \(\mu\)g L\(^{-1}\), it means 10 times lower than Brazilian limits for liquid milk.

In summary, PDI for the population studied in Brazil was higher than previous data reported in Brazil for the sum of AFB\(_1\) + B\(_2\) + G\(_1\) + G\(_2\) and lower for the PDI of AFM\(_1\). In comparison to recent assessment in other countries, Brazilian PDI for aflatoxins were also higher than reported in Spain and French and lower than reported in Korea.

Although there is no consensus for tolerable daily intake of AFB\(_1\), Kuiper-Goodman (1998) attempted to indicate the provisional maximum tolerable daily intake for aflatoxin (PMTDI) of 1.0 ng kg\(^{-1}\) b.w. day\(^{-1}\) for adults and children without hepatitis B virus and 0.4 ng kg\(^{-1}\) b.w. day\(^{-1}\) for adults carrying hepatitis B virus. The PDI values obtained for the population evaluated in this study was higher than established for both carriers and non-carriers hepatitis B virus, hence indicating the need for further studies to evaluate the overall health risks associated to human exposure to aflatoxins in Brazil.

4. Conclusion

Results of this trial indicate that the most susceptible food commodities to aflatoxin contamination including peanut, corn, bean, fluid milk and dairy products had higher frequencies of positive samples but concentrations below the Brazilian tolerance limits. Peanuts products and fluid milk were the major source of total aflatoxins and AFM\(_1\) in the diet, respectively. The overall mean PDI of total aflatoxins and AFM\(_1\) were 1.58 ± 2.49 ng kg\(^{-1}\) b.w. day\(^{-1}\) and 0.14 ± 0.10 ng kg\(^{-1}\) b.w. day\(^{-1}\) respectively. The fact that AFB\(_1\) and AFM\(_1\) are potent hepatocarcinogens warrants concern about the human exposure levels through the foods products evaluated, especially those intended for infant populations.

Acknowledgments

The authors thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) of Brazil for financial support and fellowships.

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