AFLATOXINS, OCHRATOXIN A AND ZEARALENONE IN MAIZE-BASED FOOD PRODUCTS

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ABSTRACT

The occurrence of aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2), ochratoxin A (OA) and zearalenone (ZEA) was evaluated in 121 maize-based food samples collected in the commerce of Maringá City, Paraná State, Brazil. The study was carried out between January 2002 and February 2003. Thin-layer chromatographic method was used to determine the mycotoxins. The recovery averages were 106.6%, 109.4%, 106.6%, 109.4%, 101.8% and 101.7% to AFB1, AFB2, AFG1, AFG2, OA and ZEA, respectively. Three samples (2.5%) were positive to AFB1 (8 to 59 μg/kg), two (1.7%) to AFB2 (2.4 μg/kg), one (0.8%) to OA (64 μg/kg) and one (0.8%) to ZEA (448 μg/kg). The greater frequency of positive samples and also the highest concentration of AFB1 were found in popcorn samples (8.3%, 59 μg/kg). The data showed a low frequency of mycotoxins in products based on maize traded in Maringá, but the Probable Average Daily Intake (PDI) of AFB1 in them was high. Therefore, it is necessary to accomplish an active vigilance of these mycotoxins in such food products in order to provide safety to Brazilian people health.

Key words: mycotoxins; risk; Brazilian products derived from maize

INTRODUCTION

Zummo and Scott (51) demonstrated that maize (Zea mays L.) is constantly exposed to the risk of fungi development for having ideal nutrients composition. Moreover, tropical and subtropical climate countries have favorable environmental conditions to the development of the main types of genotoxicant fungi, Aspergillus, Fusarium and Penicillium. Among the mycotoxins which are found in maize, aflatoxins, zearalenone and ochratoxin A are detached both for the concerning showed by the researchers due to their possible toxicant effect in human beings and animals and for economical reasons (6).

Aflatoxins are a group of mycotoxins produced by clumps of Aspergillus flavus (Link) and Aspergillus parasiticus (Speare). Four aflatoxins stand out: B1 (AFB1), G1 (AFG1), B2 (AFB2) and G2 (AFG2). Aflatoxin B1 is considered one of the most powerful human carcinogenic and also hepatic (11).

Ochratoxin A (OA) is mainly produced by fungi Aspergillus and Penicillium genus (50), where A. ochraceus (Wilhelm) and P. verucosum (Dierckx) are the main productive species of this mycotoxin. Ochratoxin A (OA) is mutagenic, teratogenic and nephrotoxic and has been involved in etiology of Balkan Endemic Nephropathy (7).

Zearalenone (ZEA) presents an estrogenic and anabolic action in several animals. Swine specie is the most affected (18), being caused endometrial hyperplasias, ovarium atrophy, nymphomania (8), pseudogestation, vulvovaginitis (42) and embryonic loss (27). In young male swines the toxin causes prepuce oedema, testicle atrophy and increasing of mammary gland (12). In relation to the toxic effects in human beings, there are cases in which ZEA was reported because it caused premature puberty in children aged between 7 and 8 years (35) and cases in Puerto Rico (41). This mycotoxin is mainly produced by Fusarium graminearum (Schwabe), Fusarium proliferatum (T Matsushima) Niremberg and Fusarium culmorum (Wm G Sm) Sacc.

Some reports have shown the occurrence of mycotoxins in maize in Brazil. Sabino et al. (40) found different levels of ZEA: from not detected (nd) to 9830 μg/kg in 358 maize samples; in respect to aflatoxins 44 samples were positive to AFB1, from 5 to 900 μg/kg. In another survey, Henningen and Dick (19) did not
detect ZEA in the 23 maize samples stocked in silos. However, they found aflatoxins in 34.8% of the samples and the sum of AFB1 + AFG1 varied from 12 to 906 μg/kg. Machinski Jr. et al. (29) demonstrated a high aflatoxin contamination in the new crop maize. The authors found 54.5% of positive samples in a total concentration (AFB1 + AFB2 + AFG1 + AFG2) varying from 0.6 to 1792 μg/kg.

The present paper studied the occurrence of aflatoxins, ochratoxin A and zearalenone in food products based on maize, commercialized in the city of Maringá, Paraná, Brazil, in the period from January 2002 to February 2003. The exposure of Brazilian people to the mycotoxins was also evaluated according to the consumption of maize and its derivatives.

MATERIALS AND METHODS

Samples

Between January 2002 and February 2003, a hundred and twenty one samples of maize-based food products were collected by the Municipal Sanitary Vigilance in markets in Maringá City, Paraná, Brazil. The collected samples were: 37 packs of degerminated corn, 17 packs of corn flour, 10 packs of corn flakes, 26 packs of corn meal, 24 packs of popcorn and 7 packs of corn grits. The minimum size of the pack of each sample was 500 g. The samples were ground, homogenized and stocked in a temperature of -20ºC until the analysis.

Analytical standards

The standards of mycotoxins were obtained from Sigma Chemical Co. (USA) and the preparation of the standard solutions was accomplished according to the Manual of Official Methods of Analysis of Association of Official Analytical Chemists (3). From the individual stock solutions to each toxin and after determining the exact concentration by ultraviolet spectrometry, a work solution with a mixture of toxins was prepared: 5 μg/mL to AFB1 and G1, 1.5 μg/mL to AFB2 and G2, 10 μg/mL to OA and 40 μg/mL to ZEA in benzene-acetonitrile (98:2, v/v).

Extraction, cleanup, quantification and confirmation by thin-layer chromatography

The determination of aflatoxins, ochratoxin A and zearalenone was accomplished according to the method described by Soares and Rodriguez-Amaya (45) by thin-layer chromatography. Fifty grams of sample were homogenized in a blender with 270 mL of methanol and 30 mL of 4% potassium chloride during 5 minutes. The mixture was filtered in common paper filter. A hundred and fifty mL of the filtered mixture were transferred to a glass where 150 mL of 30% ammonium sulfate and 50 mL of Celite during 5 minutes. The mixture was filtered in common paper filter. A hundred and fifty mL of the filtered mixture were transferred to a separation funnel and were added 150 mL of water and partitioned twice with 10 mL of chloroform. Five mL from the first and from the second partition of the chloroform were combined. It evaporated because of the dryness in a bath with water at a temperature of 80ºC. Five ¼L from the extract were applied in the chromatoplate (silicagel 60G, MN). The standards were applied separately. The plate was placed in an unsaturated tank containing toluene-ethyl acetate-chloroform-formic acid (70:50:50:20, v/v/v/v). The aflatoxins and ochratoxin A were visualized by incidence of the long UV light. Then the chromatoplate was revealed with a aluminum chloride solution to evaluate the presence of zearalenone. Known volumes of sample and standards were applied in the chromatoplates to the quantification. To the quantification of aflatoxins the plates were developed in the previous described solvent system; to ochratoxin A, toluene-ethyl acetate-chloroform-formic acid was used (5:4:1, v/v/v) and to zearalenone, toluene-ethyl acetate-chloroform-formic acid was used (60:40:0.5, v/v/v) and posterior revealing with aluminum chloride. All calculations were done according to the Manual of Official Methods of Analysis of AOAC (3).

The toxins identity was confirmed by reactions of derivation and evaluated with the standards by thin-layer chromatography. Aflatoxins were derived with trifluoroacetic acid according to Przybylski (38). Zearalenone was acetylated with acetic anhydride in the presence of pyridine as described by Golinski and Grabarkiewicz-Szczena (16). Ochratoxin A was methylated with boron trifluoride in methanol according to the procedure of Hunt et al. (21).

Analytical quality control

All samples were analyzed in duplicate with each duplicate on a different day. Each group of nine determinations was accompanied by a spiked, that is, to the samples extracted in the same day an amount of mycotoxins was added to a known sample in the day before the extraction. These samples were used to evaluate the recovery and the quantification, so that all results could be corrected by the found recovery. The following analytical quality criteria were defined: recovery should be between 65 and 135% and the coefficient of variation between the duplicates should be less than 30% according of Horwitz et al. (20).

Chemicals

Sovents and salts are from Merck S.A. (Rio de Janeiro, Brazil).

RESULTS AND DISCUSSION

The average recovery of the method was 106.6% to aflatoxin B1, 109.4% to B2, 106.6% to G1, 109.4% to G2, 101.8% to ochratoxin A and 101.7% to zearalenone, according to Table 1. The detection limits were 2, 0.96, 2, 0.48, 6.4 and 76.8 μg/kg respectively to AFB1, AFB2, AFG1, AFG2, OA and ZEA.

The aflatoxins were detected in 2.5% (3/121) of all analyzed products, in a total concentration (AFB1 + AFB2) which varied twice with 10 mL of chloroform. Five mL from the first and from the second partition of the chloroform were combined. It evaporated because of the dryness in a bath with water at a temperature of 80ºC. Five ¼L from the extract were applied in the chromatoplate (silicagel 60G, MN). The standards were applied separately. The plate was placed in an unsaturated tank containing toluene-ethyl acetate-chloroform-formic acid (70:50:50:20, v/v/v/v). The aflatoxins and ochratoxin A were visualized by incidence of the long UV light. Then the chromatoplate was revealed with a aluminum chloride solution to evaluate the presence of zearalenone. Known volumes of sample and standards were applied in the chromatoplates to the quantification. To the quantification of aflatoxins the plates were developed in the previous described solvent system; to ochratoxin A, toluene-ethyl acetate-chloroform-formic acid was used (5:4:1, v/v/v) and to zearalenone, toluene-ethyl acetate-chloroform-formic acid was used (60:40:0.5, v/v/v) and posterior revealing with aluminum chloride. All calculations were done according to the Manual of Official Methods of Analysis of AOAC (3).

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from 10.4 to 59 μg/kg. Among the positive samples three of them presented AFB1 in concentration varying from 8 to 59 μg/kg and two of them had AFB1 in concentration of 2.4 μg/kg. The result of the positive samples is summarized in Table 2. Among the analyzed samples only two popcorn samples and one of corn grits presented positive results to aflatoxins.

The results found to aflatoxins were similar to other results found by various authors. Furlong et al. (14) reported that 7.7% of 39 analyzed samples presented contamination with aflatoxins. Pich et al. (37) found levels from 3 to 24 μg/kg in 29 samples of corn flour analyzed. Soares and Rodriguez-Amaya (45) verified that 5 samples among 130 analyzed ones contained 20 to 47 μg/kg of AFB1 in several products derived from maize.

The products derived from maize showed low contamination by aflatoxins; just 2.5% of the samples were positive and these results were partially similar to investigations in other parts of the world, like the results reported by Solovey et al. (46) that among 37 samples of food products derived from maize, originating from Argentina, there was not any detection of aflatoxins and also like the results from Abdulkadar et al. (1) that observed a contamination of 1 to 2 μg/kg in three of the 54 samples of maize and derivatives, originating from Qatar.

The contamination level of products derived from maize with aflatoxins is considerably lower than the found level to the non-processed maize; several studies prove this assertion like Ali et al. (2) who reported a total of 69% of samples from Indonesia which were contaminated in average levels of 119 μg/kg; Bhat et al. (4) considered 25.8% of contaminated samples with amounts over than 30 μg/kg in India; Glória et al. (15) verified 33% of contamination in samples originating from five Brazilian states; Vargas et al. (48) reported 38.3% of contamination in samples from Brazilian different regions with average levels of 9.4 μg/kg; Henningen and Dick (19) mentioned 30.5% of samples originating from South of Brazil, with variable levels from 10 to 906 μg/kg; Li et al. (26) reported 35% of contaminated samples with average levels of 82 μg/kg in China; Machinski Jr. et al. (29) found 54.5% of cultivated samples in experimental fields in Brazil, with levels varying from 6 to 1600 μg/kg; Medina-Martinez and Martinez (31) verified contamination in 16.6% of analyzed samples originating from Venezuela; Ono et al. (34) reported 11.3% of contaminated samples from Brazil with average levels of 190 μg/kg; Nepote et al. (33) mentioned 10% of contamination in samples originating from Argentina with concentrations over 20 μg/kg also in Argentina, González et al. (17) did not report any contamination by aflatoxins in 30 analyzed samples.

One sample of corn four (0.8%) demonstrated an amount of 64 μg/kg of ochratoxin A (OA) that exceeded the maximum permitted limit of legislation from most of countries. Therefore, products derived from maize presented a low contamination by ochratoxin A. This result differ from the results obtained by Paolo and Tosi (36) who mentioned contents of 1250 to 2500 μg/kg in...

Table 1. Recovery of mycotoxins from some maize matrices.

<table>
<thead>
<tr>
<th>Products</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AFB1</td>
</tr>
<tr>
<td>1. Yellow degerminated corn</td>
<td>100</td>
</tr>
<tr>
<td>2. White degerminated corn</td>
<td>100</td>
</tr>
<tr>
<td>3. Corn flour</td>
<td>100</td>
</tr>
<tr>
<td>4. Corn flakes</td>
<td>100</td>
</tr>
<tr>
<td>5. Corn meal</td>
<td>100</td>
</tr>
<tr>
<td>6. Popcorn</td>
<td>100</td>
</tr>
<tr>
<td>7. Corn grits</td>
<td>131</td>
</tr>
<tr>
<td>Average (%)</td>
<td>106.6</td>
</tr>
</tbody>
</table>

Table 2. Content of aflatoxins, ochratoxin A and zearalenone found in food products based on maize purchased in markets of Maringá City, January/2002 to February/2003.

<table>
<thead>
<tr>
<th>Products</th>
<th>Incidence (nº of positive/total samples)</th>
<th>AFB1</th>
<th>AFB2</th>
<th>OA</th>
<th>ZEA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average of positive samples (μg/kg)</td>
<td>Range of positive samples (μg/kg)</td>
<td>Average of positive samples (μg/kg)</td>
<td>Range of positive samples (μg/kg)</td>
<td>Average of positive samples (μg/kg)</td>
</tr>
<tr>
<td>Corn flour</td>
<td>nd</td>
<td>nd</td>
<td>1/17</td>
<td>nd</td>
<td>-</td>
</tr>
<tr>
<td>Popcorn</td>
<td>2/24</td>
<td>1/24</td>
<td>nd</td>
<td>1/24</td>
<td>33.5</td>
</tr>
<tr>
<td>Corn grits</td>
<td>1/7</td>
<td>1/7</td>
<td>nd</td>
<td>nd</td>
<td>21.3</td>
</tr>
</tbody>
</table>

nd = not detected (detection limit: AFB1 2 ppb, AFB2 0.96 ppb, AFG1 2 ppb, AFG2 0.48 ppb, ochratoxin 6.4 ppb and zearalenone 76.8 ppb)
all samples of food products derived from maize originating from Argentina. This work corroborates the low contamination by ochratoxin A in food products derived from maize in Brazil. Such results may be compared to other studies accomplished by Caldas et al. (5), Furlong et al. (14), Hennigen and Dick (19), Soares (43), Soares and Furlani (44) and Soares and Rodriguez-Amaya (45).

Non-processed maize presents a level of contamination by ochratoxin A higher than the level found in products derived from maize. Veldman et al. (49) demonstrated a total of 16.7% of maize samples originating from Holland contaminated by OA with average level of 73 μg/kg. Ministry of Agriculture, Fishing and Food of United Kingdom (30) reported that 10.1% of crude maize samples originating from countries like France, Argentina, Spain, Hungary and Germany were contaminated with amounts less than 1.5 μg/kg of OA. In Brazil, Machinski Jr. et al. (29) mentioned two samples (1.8%) of maize contaminated with ochratoxin A (128 and 206 μg/kg).

From the obtained results zearalenone was registered in only one sample of popcorn, in concentration of 448 μg/kg. Knowing that the maximum limit of zearalenone in samples from most of countries is 200 μg/kg (30), we observe that, in spite of the low frequency of this mycotoxin in analyzed foods, the detected level in the positive sample must be considered as an important factor for it may cause toxic effect to human beings.

This result was similar to the studies carried out by Soares and Rodriguez-Amaya (45) who did not detect zearalenone in 296 samples of food collected in markets of Campinas, Brazil and Furlong et al. (14) reported 2.6% of samples originating from South of Brazil, with level of 163 μg/kg.

Zearalenone has been found in maize in many parts of the world (10,29,40,47). It is notorious that countries with hot climate have not been presented problems concerned to contamination by ZEA with maize. However, countries with cold and temperate climates have presented high levels and high percentages of contamination by this toxin in maize (13,19,25,28,32,39).

Even knowing that the occurrence of mycotoxins is low in foods derived from maize commercialized in Brazil, there is a necessity of a constant vigilance of these foods aiming to provide quality and safety to all consumers because there are many different factors which influence the development of fungi and production of toxins, such as temperature, humidity, levels of intergranule oxygen, mechanical damages to the grain, among others, and these results may vary in products originating from different crops.

Daily consumption of maize and its derivatives in Brazil is 42 grams per person (22). Kuiper-Goodman (24) established a Tolerable Daily Intake (TDI) to aflatoxin B1 of 0.15 ng/kg bw/day. Considering that the average weight of adult population is 70 kg and the average concentration of aflatoxin B1, in this study, is 0.7298 μg/kg, Probable Average Daily Intake (PDI) is 0.4379 ng/kg bw/day. Therefore, the estimated average consumption of aflatoxin B1 was high in the analyzed food products derived from maize. This may contribute to an increase of incidence of hepatocellular carcinoma, so there is a significant risk to the health of Brazilian people due to the chronic exposure to aflatoxin B1 in diets with foods derived from maize.

In respect to ochratoxin A, Temporary Tolerable Week Intake (TWI) is 0.1 μg/kg bw/week (23). Nevertheless, if we consider that the average concentration of ochratoxin A in the analyzed samples was 0.5289 μg/kg, the Average Probable Week Intake (PWI) will be 0.0022 μg/kg bw/week. Temporary Tolerable Maximum Daily Intake (TMDI) of ZEA is 0.5 μg/kg bw (9). Knowing that the average concentration of ZEA in the analyzed samples was 3.7025 μg/kg, we have the information that the Average Probable Daily Consumption will be 0.0022 μg/kg bw/day. We conclude that the food products derived from maize do not contribute much to the consumption of ochratoxin A and zearalenone by Brazilian people.

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RESUMO

Aflatoxinas, ocratoxina A e zearalenona em produtos alimentícios à base de milho

A ocorrência de aflatoxinas B1 (AFB1), B2 (AFB2), G1 (AFG1) e G2 (AFG2), ocratoxina A (OA) e zearalenona (ZEA) foi avaliada em 121 amostras de alimentos à base de milho, que foram coletadas no comércio da cidade de Maringá, PR, Brasil, entre os meses de Janeiro/2002 a Fevereiro/2003. A cromatografia em camada delgada foi empregada para a determinação das micotoxinas. As médias das recuperações foram 106,6%, 109,4%, 106,6%, 109,4%, 101,8% e 101,7% para AFB1, AFB2, AFG1, AFG2, OA e ZEA, respectivamente. Três amostras (2,5%) foram positivas para AFB1 (8 a 59 μg/kg), duas (1,7%) para AFB2 (2,4 μg/kg), uma (0,8%) para OA (64 μg/kg) e uma (0,8%) para ZEA (448 μg/kg). A maior frequência de amostras positivas e também a mais alta concentração de AFB1 foi encontrada nas amostras de pipoca (8,3%, 59 μg/kg). Os dados demonstraram uma baixa frequência de micotoxinas em produtos à base de milho comercializados em Maringá, mas a Ingestão Diária Provável Média (IDPM) de AFB foi alta nos produtos analisados. Portanto, torna-se necessário a realização de uma vigilância ativa destas micotoxinas nestes produtos alimentícios, a fim de proporcionar segurança à saúde da população brasileira.

Palavras-chave: micotoxinas, risco, produtos brasileiros derivados do milho
REFERENCES


