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

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ABSTRACT

The occurrence of aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2), 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 AFB_1, AFB_2, AFG_1, AFG_2, OA and ZEA, respectively. Three samples (2.5%) were positive to AFB_1 (8 to 59 µg/kg), two (1.7%) to AFB_2 (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 AFB_1 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 AFB_1 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: B_1 (AFB_1), G_1 (AFG_1), B_2 (AFB_2) and G_2 (AFG_2). Aflatoxin B_1 is considered one of the most powerful human carcinogenic and also hepatoxic (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 AFB_1, from 5 to 900 µg/kg. In another survey, Henningen and Dick (19) did not

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detect ZEA in the 23 maize samples stocked in silos. However, they found aflatoxins in 34.8% of the samples and the sum of $AFB_1 + AFG_1$ 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 ($AFB_1 + AFB_2 + AFG_1 + AFG_2$) 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 AFB_1 and G_1, 1.5 µg/mL to AFB_2 and G_2, 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 a 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 reveled 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 B_1, 109.4% to B_2, 106.6% to G_1, 109.4% to G_2, 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 76.8¼g/kg respectively to AFB_1, AFB_2, AFG_1, AFG_2, OA and ZEA.

The aflatoxins were detected in 2.5% (3/121) of all analyzed products, in a total concentration ($AFB_1 + AFB_2$) which varied

Table 1. Recovery of mycotoxins from some maize matrices.

Products	Recovery (%)					
	AFB_1	AFB_2	AFG_1	AFG_2	OA	ZEA
1. Yellow degerminated corn	100 133	133 133	100 133	133 133	100 133	100 133
2. White degerminated corn	100 88 100	100 89 100	100 88 100	100 89 100	100 90 100	100 89 100
3. Corn flour	100	100	100	100	100	100
4. Corn flakes	100	100	100	100	100	100
5. Corn meal	100 100	100 100	100 100	100 100	100 100	100 100
6. Popcorn	100 100	100 100	100 100	100 100	100 100	100 100
7. Corn grits	131 133	133 133	131 133	133 133	100 100	100 100
Average (%)	106.6	109.4	106.6	109.4	101.8	101.7

from 10.4 to 59 µg/kg. Among the positive samples three of them presented AFB_1 in concentration varying from 8 to 59 µg/kg and two of them had AFB_2 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 AFB_1 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 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.

Products	Incidence (nº of positive/total samples)				AFB_1		AFB_2		OA		ZEA	
	AFB_1	AFB_2	OA	ZEA	Average of positive samples	Range (µg/kg)	Average of positive samples	Range (µg/kg)	Average of positive samples	Range (µg/kg)	Average of positive samples	Range (µg/kg)
Corn flour	nd	nd	1/17	nd	-	-	-	-	64	64	-	-
Popcorn	2/24	1/24	nd	1/24	33.5	8-59	2.4	2.4	-	-	448	448
Corn grits	1/7	1/7	nd	nd	21.3	21.3	2.4	2.4	-	-	-	-

nd = not detected (detection limit: AFB_1 2 ppb, AFB_2 0.96 ppb AFG_1 2 ppb, AFG_2 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 B_1 of 0.15 ng/kg bw/day. Considering that the average weight of adult population is 70 kg and the average concentration of aflatoxin B_1, 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 B_1 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 B_1 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 B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) e G_2 (AFG_2), 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 AFB_1, AFB_2, AFG_1, AFG_2, OA e ZEA, respectivamente. Três amostras (2,5%) foram positivas para AFB_1 (8 a 59 µg/kg), duas (1,7%) para AFB_2 (2,4 µg/kg), uma (0,8%) para OA (64 µg/kg) e uma (0,8%) para ZEA (448 µg/kg). A maior freqüência de amostras positivas e também a mais alta concentração de AFB_1 foi encontrada nas amostras de pipoca (8,3%, 59 µg/kg). Os dados demonstraram uma baixa freqüência de micotoxinas em produtos à base de milho comercializados em Maringá, mas a Ingestão Diária Provável Média (IDP_M) de AFB_1 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

1. Abdulkadar, A.H.W.; Abdulla, A.A.; Al-Jedah, J.H. Occurrence of aflatoxin in commodities imported into Qatar, 1997-2000. *Food Addit. Contam.*, 19(7), 666-670, 2002.
2. Ali, N.; Sardjono, Yamashita, A.; Yoshizawa, T. Natural co-occurrence of aflatoxins and Fusarium Mycotoxins (fumonisins, deoxynivalenol, nivalenol and zearalenone) in corn from Indonesia. *Food Addit. Contam.*, 15(4), 377-384, 1998.
3. AOAC. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 16th ed. Method 971.22; 985.17. The Association: Arlington, Washington, 1995.
4. Bhat, R.V.; Vasanthi, S.; Rao, S.B.; Rao, N.R.; Rao, S.V.; Nagaraja, K.V.; Bai, N.R.; Prasad, K.; Vanchinathan, S.; Roy, R.; Saha, S.; Mukherjee, A.; Ghosh, P.K.; Toteja, G.S.; Saxena, B.N. Aflatoxin B_1 contamination in maize samples collected from different geographical regions of India - a multicentre study. *Food Addit. Contam.*, 14(2), 151-156, 1997.
5. Caldas, E.D.; Silva, S.C.; Oliveira, J.N. Aflatoxinas e ocratoxina A em alimentos e riscos para a saúde humana. *Rev. Saúde Pública*, 36(3), 319-323, 2002.
6. CAST. *Mycotoxins: Economic and Health Risks*. Council for Agricultural Science and Technology Task Force Report, nº 116, 1A, 1989.
7. Castegnaro, M.; Barstch, H.; Chernozemsky, I. Endemic nephropathy and urinary tract tumors in the Balkans. *Cancer Res.*, 47, 3608-3609, 1987.
8. Chang, K.; Kurtz, H.J.; Mirocha, C.J. Effects of the mycotoxin zearalenone on swine reproduction. *Am. J. Vet. Res.*, 40, 1260-1267, 1979.
9. Creppy, E.E. Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicol. Lett.*, 127, 19-28, 2002.
10. Doko, M.B.; Canet, C.; Brown, N.; Sydenham, E.W.; Mpuchane, S.; Siame, B.A. Natural co-occurrence of fumonisins and zearalenone in cereals and cereal-based foods from Eastern and Southern Africa. *J. Agric. Food Chem.*, 44(10), 3240-3243, 1996.
11. Eaton, D.L.; Groopman, J.D. (Eds.. *The Toxicology of Aflatoxins: Human Health, Veterinary and Agricultural Significance*, Academic Press, San Diego, 1994.
12. Edwards, S.; Cantley, T.C.; Day, B.N. The effects of zearalenone on reproduction in swine II. *Theriogenol.*, 28, 51-58, 1987.
13. Eppley, R.M.; Stoloff, L.; Trucksess, M.W.; Chung, C.W. Survey of corn for *Fusarium* toxins. *J. Assoc. Off. Anal. Chem.*, 57, 632-635, 1974.
14. Furlong, E.B.; Soares, L.A.S.; Vieira, A.P.; Dadalt, G. Aflatoxinas, Ocratoxina A e Zearalenona em alimentos da região Sul do Rio Grande do Sul. *Rev. Inst. Adolfo Lutz*, 58, 105-111, 1999.
15. Glória, E.M.; Fonseca, H.; Souza, I.M. Occurrence of mycotoxins in maize delivered to the food industry in Brasil. *Trop. Sci.*, 37, 107-110, 1997.
16. Golinski, P.; Grabarkiewicz-Szczesna, J. Chemical confirmatory tests for ochratoxin A, citrinin, penicillic acid, sterigmatocystin, and zearalenone performed directly on thin layer chromatographic plates**.** *J. Assoc. Off. Anal. Chem.*, 67(6), 1108-1110, 1984.
17. González, H.H.L.; Martínez, E.J.; Pacin, A.M.; Resnik, S.L.; Sydenham, E.W. Natural co-occurrence of fumonisins, deoxyvalenol, zearalenone and aflatoxins in field trial corn in Argentina. *Food Addit. Contam.*, 16(12), 565-569, 1999.
18. Haggler, W.M. JR.; Towers, N.R.; Mirocha, C.J.; Eppley, R.M.; Bryden, W.L. Zearalenone: Mycotoxin or mycoestrogen? *Fusarium: Paul E. Nelson Memorial Symposium*, edited by B.A. Summerell, J.F. Leslie, D. Bachouse, W.L. Bryden; L.W. Burges (St. Paul: APS Press), p. 321-331, 2001.
19. Henningen, M.R.; Dick, T. Incidence and abundance of mycotoxins in maize in Rio Grande do Sul, Brazil. *Food Addit. Contam.*, 12(5), 677-681, 1995.
20. Horwitz, W.; Albert, R.; Nesheim, S. Reliability of mycotoxin assays – an update. *J. AOAC Int.*, 76, 461-491, 1993.
21. Hunt, D.C.; McConnie, B.R.; Crosby, N.T. Confirmation of ochratoxin A by chemical derivatisation and high-performance liquid chromatography. *Analyst*, 105, 89-91, 1980.
22. IBGE. *Estudo Nacional de Despesa Familiar*, Fundação Instituto Brasileiro de Geografia e Estatística, Rio de Janeiro, volumes 1-5, 1977.
23. JECFA. *Safety evaluation of certain food additives and contaminants – Ochratoxin A*. World Heath Organization, 1995.
24. Kuiper-Goodman, T. Mycotoxins: risk assessment and legislation. *Toxicol. Lett.*, 82/83, 853-859, 1995.
25. Lauren, D.R.; Agnew, M.P.; Smith, W.A.; Sayer, S.T. A survey of the natural occurrence of *Fusarium* mycotoxins in cereals grown in New Zealand in 1986-1989. *Food Addit. Contam.*, 8(5), 599-605, 1991.
26. Li, F.Q.; Yoshizawa, T.; Kawamura, O.; Luo, X.Y.; Li, Y.W. Aflatoxins and fumonisins in corn from the high-incidence area for human hepatocellular carcinoma in Guangxi, China. *J. Agric. Food Chem.*, 49, 4122-4126, 2001.
27. Long, G.G.; Dieckman, M.A. Characterization of effects of zearalenone in swine during early pregnancy. *Am. J. Vet. Res.*, 47, 184-187, 1986.
28. L'Vova, L.S.; Orlova, N.Y.; Bystryakova, Z.K.; Omel´Chenko, M.D., Remele, V.V. Occurrence of toxigenic fungi and mycotoxins in various crop grains. *Prikl. Biokhim. Mikrobiol.*, 29, 70-79, 1993.
29. Machinski Jr., M.; Soares, L.M.V.; Sawazaki, E.; Bolonhezi, D.; Castro, J.L.; Bortolleto, N. Aflatoxins, ochratoxin A and zearalenone in Brazilian corn cultivars. *J. Sci. Food Agric.*, 81, 1001-1007, 2001.
30. MAFF. *Survey for aflatoxins, ochratoxin A, fumonisins and zearalenone in raw maize*. Food Contaminants Division, 2002. Disponível; em: <http:://www.fssg.maff.gov.uk/.
31. Medina-Martínez, M.S.; Martínez, A.J. Mold occurrence and aflatoxin B_1 determination in corn samples in Venezuela. *J. Agric. Food Chem.*, 48, 2833-2836, 2000.
32. Milanez, T.V.; Atui, M.B.; Lázzari, F.A. Comparação entre imunoensaio e cromatografia em camada delgada na determinação de aflatoxinas, ocratoxina A e zearalenona em amostras de milho em grão e fubá. *Rev. Inst. Adolfo Lutz*, 57(1), 65-71, 1998.
33. Nepote, M.C.; Piontelli, L.E.; Saubois, A. Occurrence of *Aspergillus flavus* strains and aflatoxins in corn from Santa Fe, Argentina. *Archiv. Lat. Nutr.*, 47(3), 262-264, 1997.
34. Ono, E.Y.S.; Ono, M.A.; Funo, F.Y.; Medina, A.E.; Oliveira, T.C.R.M.; Kawamura, O.; Ueno, Y.; Hirooka, E.Y. Evaluation of fumonisin-aflatoxin co-occurrence in Brazilian corn-hybrids by Elisa. *Food Addit. Contam.*, 18(8), 719-729, 2001.
35. Painter, K. Puberty signs evident in 7-and 8-year old girls. *USA Today*, April 8, P.A-1, 1997.
36. Paolo, O.D.; Tosi, E.A. Contaminacion de harina de maiz con ochratoxina A, aflatoxina y zearalenona. *Alimentaria*, 67, 1998.
37. Pich, P.H.; Nordin, N.S.D.; Noll, I.B. *Detecção de aflatoxinas em produtos derivados de milho comercializados na região de Porto Alegre-RS*. IX Encontro Nacional de Micotoxinas, Florianópolis, 1998, p. 120.
38. Przybykski, W. Formation of aflatoxin derivatives on thin layer chromatographic plates. *J. Assoc. Off. Anal. Chem.*, 58(1), 163-164, 1975.
39. Rheeder, J.P.; Sydenham, W.E.; Marasas, W.F.O.; Thiel, P.G.; Shephard, G.S.; Schlechter, M.; Stockenström, S.; Cronje, D.W.; Viljoen, J.H. Fungal infestation and mycotoxin contamination of South African commercial maize harvested in 1989 and 1990. *South Afr. J. Sci.*, 91, 127-131, 1995.
40. Sabino, M.; Prado, G.; Inomata, E.I.; Pedroso, M.O.; Garcia, R.V. Natural occurrence of aflatoxins and zearalenone in maize in Brasil. Part II. *Food Addit. Contam.*, 6(3), 327-331, 1989.

41. Saenz De Rodriguez, C.A.; Bangiovanni, A.M.; Conde De Borrego, D. An epidemic of precocious development in Puerto Rican children. *J. Pediatr.*, 393-396, 1985.
42. Shier, W.T.; Shier, A.C.; Xie, W.; Mirocha, C.J. Structure-activity relationships for human estrogenic activity in zearalenone mycotoxins. *Toxicon*, 39, 1435-1438, 2000.
43. Soares, L.M.V. *Micotoxinas em milho de pipoca*. IX Encontro Nacional de Micotoxinas, São Paulo, 1990, p. 52.
44. Soares, L.M.V.; Furlani, R.P.Z. Micotoxinas em milho para pipoca. *Bol. Soc. Bras. Ciênc. Tecnol. Aliment.*, 26, 33-36, 1992.
45. Soares, L.M.V.; Rodriguez-Amaya, D.B. Survey of aflatoxins, ochratoxin A, zearalenone, and sterigmatocystin in some Brazilian foods by using multi-toxin thin-layer chromatographic method. *J. Assoc. Off. Anal. Chem.*, 72(1), 22-26, 1989.
46. Solovey, M.M.; Somoza, C.; Cano, G.; Pacin, A.; Resnik, S. A survey of fumonisins, deoxyvalenol, zearalenone and aflatoxins contamination in corn-based food products in Argentina. *Food Addit. Contam.*, 16(8), 325-329, 1999.
47. Tanaka, T.; Hasegawa, A.; Yamamoto, S.; Lee, U.S.; Sugiura, Y.; Ueno, Y. Worldwide contamination of cereals by the *Fusarium* mycotoxins nivalenol, deoxynivalenol, and zearalenone. 1. Survey of 19 countries. *J. Agric. Food Chem.*, 36(5), 979-983, 1988.
48. Vargas, E.A.; Preis, R.A.; Castro, L.; Silva, C.M.G. Co-occurrence of aflatoxins B_1, B_2, G_1 e G_2 zearalenone and fumonisin in Brazilian corn. *Food Addit. Contam.*, 18(11), 981-986, 2001.
49. Veldman, A.; Borggreve, G.J.; Mulders, E.J.; Van de Lagemaat, D. Occurrence of the mycotoxins ochratoxin A, zearalenona and deoxynivalenol in feed components. *Food Addit. Contam.*, 9(6), 647-655, 1992.
50. Vrabcheva, T.; Usleber, E.; Dietrich, R.; Märtlbaver, E. Co-occurrence of Ochratoxin A and Citrinin in Cereals from Bulgarian Villages with a History of Balkan Endemic Nephropathy. *J. Agric. Food Chem.*, 48, 2483-2488, 2000.
51. Zummo, N.; Scott, G.E. Interaction of *Fusarium moniliforme* and *Aspergillus flavus* on Kernel infection and aflatoxin contamination in maize ears. *Plant Dis.*, 76, 771-773, 1992.