Introduction

Fumonisins are a group of toxic metabolites produced primarily by the filamentous fungi *Fusarium verticillioides* and *Fusarium proliferatum*, which most often attack maize crops worldwide. Taking into consideration the toxicity of fumonisins, the most prominent ones are fumonisin B₁ (FB₁), fumonisin B₂ (FB₂), and fumonisin B₃ (FB₃) [1]. Among 15 forms of fumonisins, which have so far been isolated from the filamentous fungi or food based on corn, fumonisin B₁ is considered the most widespread and most toxic [2]. Fumonisin B₁ was classified by the International Agency of Research on Cancer (IARC) in group 2b as a potential carcinogen in humans [3]. Moreover, fumonisins belong to neurotoxins due to the fact that they can damage the biosynthesis pathway of sphingosine, which is a component of brain and nervous tissue [4]. They cause damage in DNA and induce cell apoptosis [5-6]. Fumonisins are also a cause of kidney...
and intestine damage [7-8] and demonstrate carcinogenic activity [7].

Fumonisins occur worldwide mainly in a grain of maize and products of its processing intended for food and feeding purposes [9]. The contamination of crops with fumonisins depends on the geographical location, in which the plants are cultivated and season or conditions in which the growth and harvest of maize take place, as well as storage [2]. The type of maize grain and such characteristics as types of endosperm, chemical composition, and stage of development may also influence fungal infections and then the production of fumonisins. However, the varieties of maize characterized by vertical corncobs, tight corn shells, thin pericarp of grains, and its increased tendency for dividing may be more susceptible to infections [10]. Furthermore, the cereals grown in tropical and subtropical regions are more susceptible to mycotoxin contamination due to a long and warm growing season [2].

Consumption of forage contaminated with fumonisins by horses causes softening of the brain (leukoenephalomalacia, or LEM), which eventually may lead to death [11-12]. Moreover, liver damage occurs in horses fed with maize contaminated with mould metabolites [12]. Pigs fed with forage that contains the fungus F. verticillioides were diagnosed with porcine pulmonary edema [13].

For people in South Africa, Asia, and Central America, where maize is the basis of the diet, exposure to fumonisin is linked to a more frequent prevalence of primary liver cancer and oesophagal cancer [3, 14]. In some countries of Central America exposure to fumonisin is in turn linked to a bigger number of cases of neural tube defect in children [3], since fumonisin B1 inhibits the absorption of folic acid in various cell lines [14].

Contamination of food and forage with fumonisins poses a serious problem for the health of humans and animals as well as being a significant obstacle in national and international trade. In order to decrease exposure to potential contamination of food and forage with fumonisins, the limits of fumonisin content in food products have been set, which are included in Regulation (EU) No. 1881/2006 of the European Parliament and of the Council of 19 December 2006 setting the maximum allowed levels of some contaminants in food and its subsequent amendments [15]. The Food and Agriculture Organization of the United Nations (FAO) together with the World Health Organization (WHO) have set the maximum tolerable daily consumption limit of fumonisins at 2 μg/kg of body weight daily for FB1, FB2, and FB3 occurring separately, or for the total amount of fumonisins [16].

Aim

The aim of the study was to assess the fumonisin contamination level of maize grain and corn-processed products marketed on the area of the Silesian Region.

Materials and Method

Preparing Samples for FB1 and FB2 Assay

The material for the study was constituted by food product samples that were collected in trade points in Silesian Voiwdship in 2010-14. The analysis comprised 46 samples of maize flour, 13 samples of corn grits, 15 samples of cornflakes, 10 samples of products for babies and small children, 13 samples of popcorn, and nine samples of maize grain. The samples were analyzed in an accredited laboratory of the Voiwdship Sanitary and Epidemiological Station in Katowice in the Liquid Chromatography Department.

All samples used in the study were homogenized, which ensured homogeneity. The samples that required crumbling were ground with a laboratory mill, while the homogenous samples underwent dry homogenization by careful mixing up to the moment of obtaining a homogenous aggregate sample.

In order to assay the content of B1 and B2 fumonisins in the above-mentioned food products, the methods described in the norm PN-EN 14352:2005 were applied for assaying B1 and B2 fumonisins in corn food products using the HPLC method with immune affinity purification and our own modifications.

One-hundred ml of extraction solvent was added to 20 g of homogenized sample (acetonitrile/methanol/water, 25:25:50, v/v/v) shaken for 20 minutes in a shaker, then centrifuged for 10 minutes and filtered through a fluted filter paper. 10 ml of the extract was measured and then 40 ml of PBS was added and then mixed and filtered through a glass fiber filter. 10 ml of the filtered extract was poured into the immunoaffinity column (Fumonitest WB, VICAM), which was then washed with 10 ml of PBS. Fumonisins B1 and B2 were eluted with 1.5 ml of methanol. The obtained eluate was evaporated to dryness in a stream of nitrogen at 50°C. The dry residue was dissolved in 200 μl of acetonitrile-water (50:50, v/v). All samples were analyzed in duplicate.

High-performance Liquid Chromatography (HPLC)

The obtained extracts were analyzed using high-performance liquid chromatography (HPLC) with fluorescent detection after prior derivatization. In order to create derivatives of fumonisins with the automatic control program system, 10 μl of each calibration solution of the fumonisin mixture was mixed with 10 μl of the reagent in order to obtain the OPA/MCE (phthalaldehyde/dimethylformamide) derivatives, and was poured into the chromatographic column in the volume of 20 μl in the repeatable time of one minute directly before analysis. The FB1 and FB3 derivatives were analyzed on the Alliance 2695 liquid chromatraph produced by Waters with a Waters 2474 fluorescent detector with excitation at wavelength 335 and emission at wavelength 44 nm. The conditions of chromatographic analysis were as follows:
Waters Xterra MS C18 4, 6x150 nm chromatographic column with analogical pre-column, mobile phase; mixture of methanol and 1 mol/l of sodium dihydrogenphosphate solution at a ratio of 77:23 by volume brought to pH 3.35 with o-phosphoric acid (V); and flow rate of mobile phase was 1 ml/min, temperature of column thermostat 25ºC, temperature of autosampler thermostat 4ºC, and dosing volume 20 μl, time of analysis was 15 minutes.

The applied analytical method was checked and validated. The limit of detection, quantification, operating range, linearity, sensitivity, accuracy, repeatability, recovery, and uncertainty were set. The obtained values fulfill the analytical performance criteria determined for analytical methods required in official controls of the level of mycotoxins in food products [17].

Specification of the Analytical Method

In order to determine the linearity of the method a triple calibration curve consisting of four points was prepared: 0.025, 0.063, 0.250, and 0.500 ng/ml. Linearity was determined by the analysis of linear regression and was expressed as linear correlation coefficient r, which for FB1 amounted to 0.99980 and for FB2, 0.99989. On the other hand, the coefficient of calibration variation for FB1 was 2.51, while for FB2 it amounted to 1.87. The detection limit of the method was 12.5 μg/kg, while the quantification limit was 25 μg/kg. The content of fumonisin B1 and B2 in samples was calculated from the calibration curve prepared prior to each analysis.

The recovery for corn grits, cornflakes, corn flour, and products for babies and small children (for the level of 100 μg/kg) was performed for 50% of the maximum allowed level (MAL) for a given matrix (Table 1). The recovery for maize grain was checked in the upper range of the method, while products for babies and small children (level 25 μg/kg) were in the lower range of the method.

The sum of fumonisins B1 and B2 is the sum of the concentration of those that exceeded the limit of quantification. When the concentration of B1 or B2 is between the detection and quantification levels, and another one is above the limit of quantification, the sum of fumonisins B1 and B2 is synonymous with that concentration of fumonisin that exceeds the limit of quantification (Table 2).

Estimating Tolerable Weekly Intake (TWI)

The calculations were based on the average levels of the sum of fumonisins FB1 and FB2 found in samples and the maximum concentration (95th percentile), which were multiplied by the daily consumption of corn-based food per capita in Eastern Europe (FAO, 2013) and calculated for average body weight of an adult.

Results and Discussion

The conducted study indicated the presence of toxic metabolites produced by filamentous fungi in products marketed on the Polish consumer market.

Among all 106 analyzed corn food products, 37 samples (34.9%) were contaminated with the total amount of fumonisins B1 and B2. Fumonisin B1 was assayed in 48 samples (45.3) while B2 was present in 18 samples (17.0%). In one of the samples the maximum allowed level (MAL) was exceeded (317% MAL). In the other samples the average content of fumonisins B1 and B2, and the sum of fumonisins FB1 and FB2, was, respectively: 21-479 μg/kg, 20-243 μg/kg, and 39-740 μg/kg.

In 11 samples the concentration of fumonisin B1 and in three samples the concentration of fumonisins B2 were assayed at levels between the detection and quantification limits (i.e., 12.5-25.0 μg/kg). The highest average concentration of total fumonisins B1 and B2, amounting to 740 μg/kg, was assayed in corn flour. The average level of contamination of maize grain for total amount of fumonisins B1 and B2 amounted to 614 μg/kg. Moreover, for the samples of popcorn the average content of total amount of fumonisins amounted to 222 μg/kg. However, the samples of cornflakes and corn grits samples showed

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Level (μg/kg)</th>
<th>Recovery (%)</th>
<th>RSD* (%)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn flour</td>
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<td>-</td>
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<td>13</td>
</tr>
<tr>
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<td>1,400</td>
<td>97.3</td>
<td>6.38</td>
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<td>-</td>
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<td>4.4</td>
<td>81.6</td>
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<td>12.6</td>
<td>93.6</td>
<td>17.5</td>
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<td>Cornflakes</td>
<td>400</td>
<td>95.6</td>
<td>13.1</td>
<td>86.0</td>
<td>13.6</td>
</tr>
<tr>
<td>Products for infants and young children</td>
<td>25</td>
<td>92.7</td>
<td>5.0</td>
<td>96.0</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>98.5</td>
<td>5.2</td>
<td>88.3</td>
<td>12.0</td>
</tr>
</tbody>
</table>

*RSD: relative standard deviation

Table 1. Recovery and repeatability for selected matrices.
the average content of total amount of FB₁ and FB₂, respectively, at the levels of 75 μg/kg and 39 μg/kg.

Current EU regulations allow for the concentration of total B₁ and B₂ fumonisins in unprocessed maize intended for the first degree of processing at the level of 4,000 μg/kg [15]. In five out of nine samples of maize grain (55.6%) the average content of total fumonisins ranged between 29-1,792 μg/kg, and in none of these trials was the maximum allowed level exceeded (Table 2). B₁ was found in six out of nine analyzed products (66.7%) while B₂ was assayed in three samples (33.3%). The maximum concentrations of B₁ and B₂ assayed in maize grains amounted to 1,400 μg/kg and 392 μg/kg, respectively, while the average content of assayed mycotoxins was 334 μg/kg for FB₁ and 172 μg/kg for FB₂ Table 2. Shepard et al. assayed FB₁, FB₂, and FB₃ in 11 samples of maize intended for animal feed and in eight samples of corn cobs intended for human consumption. All samples analyzed by Shephard were contaminated with mycotoxins. In corn intended for forage the concentration of FB₁ was assayed at the level of 1,270-3,980 μg/kg, the concentration of FB₂ at the level of 190-1,180 μg/kg, and the concentration of FB₃ at the level of 150-960 μg/kg. The total amount of fumonisins ranged from 1,625 to 6,115 μg/kg with a recommendation in horse forages of maximum allowed level of fumonisins lower than 5,000 μg/kg [18]. The study conducted by Klaric et al. indicated on a high level the sum of fumonisins B₁, B₂, and B₃ in maize samples. The highest levels of fumonisins were found in the range 200 μg/kg to 20,700 μg/kg [19]. Corn cobs intended for human consumption were infected with fumonisin B₁ at the level of 10-590 μg/kg, while fumonisin B₂ and B₃ were at the level of 5 to 80 μg/kg [18].

The results presented by Queiroz et al. indicate contamination of all analyzed samples (40/40) with the total amount of fumonisins in the concentration range between 230-6,450 μg/kg, which in turn implies that the exceedance of the maximum allowed level was noted. Although in most samples concentration of fumonisins were below 4,000 μg/kg, the acceptable European Communities limit for unprocessed, 33 of the 40 maize samples analysed (82.5%) were contaminated at levels above 1,000 μg/kg [20].

In all the maize grain samples (12/12) analyzed by Krnjaja et al., B₁ ranged from 880 to 2,950 μg/kg [21]. Another study reported that 39.7% of all maize samples were contaminated with fumonisins, both FB₁ and FB₂. The contamination levels of fumonisins in all maize samples ranged as follows: the maximum levels were 15,221 μg/kg, 7,141 μg/kg, and 22,362 μg/kg, and the mean levels were 340 μg/kg, 157 μg/kg, and 497 μg/kg for FB₁, FB₂, and total fumonisins, respectively [22]. The results obtained by Chilaka et al. showed that fumonisins were the most dominant mycotoxins occurring at high levels and incidence rates in all food types, especially in maize samples. From the total of 136 samples, B₁ was detected in 88 samples (65%) and B₂ was detected in 73 samples (54%). The highest assayed concentration

| Table 2. Contents of fumonisin B₁ and B₂ and their sums in the tested foods. |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| **Matrix**     | **Number of contaminated samples** | **Number of test samples** | **FB₁** | **FB₂** | **Sum of FB₁ and FB₂** | **FB₁** | **FB₂** | **Sum of FB₁ and FB₂** | **FB₁** | **FB₂** | **Sum of FB₁ and FB₂** | **MAL** | **LOD** | **LOQ** | **X̄** | **>MAL** | **>LOQ** | **>LOD** |
| **Maize**       | 9               | 6               | 3           | 6           | 10               | 5           | 3           | 8               | 5           | 3           | 8               | 21           | 7           | 2           | 3           | 0           | 0           | 0           | 0           | 0           | 0           |
| **Popcorn**     | 15              | 5               | 2           | 4           | 10               | 5           | 8           | 6               | 3           | 6           | 10               | 13           | 12           | 4           | 10           | 12           | 4           | 10           | 12           | 4           | 10           | 12           |
| **Cornflakes**  | 13              | 8               | 2           | 8           | 5               | 11          | 5           | 0               | 0           | 0           | 8               | 2           | 8           | 5           | 11          | 5           | 0           | 0           | 0           | 0           | 0           |
| **Products for infants and young children** | 5               | 0               | 0           | 0           | 0               | 0           | 0           | 0               | 0           | 0           | 0               | 0           | 0           | 0           | 0           | 0           | 0           | 0           | 0           | 0           |
| **Corn grits**  | 46              | 24              | 11           | 19           | 22               | 35           | 27           | 5               | 11           | 19           | 22               | 35           | 27           | 5           | 11           | 19           | 22           | 35           | 27           | 5           | 11           |
| **Corn flour**  | 46              | 24              | 11           | 19           | 22               | 35           | 27           | 5               | 11           | 19           | 22               | 35           | 27           | 5           | 11           | 19           | 22           | 35           | 27           | 5           | 11           |
| **MAL by Commission Regulation (EU) No. 1881/2006 of 19 December 2006 as amended setting maximum levels for certain contaminants in foodstuffs.** | **LOD** | **LOQ** | **X̄** | **>MAL** | **>LOQ** | **>LOD** |
| **Min. content (μg/kg)** | **Max. content (μg/kg)** | **Sum of FB₁ and FB₂ (μg/kg)** | **FB₁ (μg/kg)** | **FB₂ (μg/kg)** | **FB₁ (μg/kg)** | **FB₂ (μg/kg)** | **FB₁ (μg/kg)** | **FB₂ (μg/kg)** | **FB₁ (μg/kg)** | **FB₂ (μg/kg)** | **FB₁ (μg/kg)** | **FB₂ (μg/kg)** | **FB₁ (μg/kg)** | **FB₂ (μg/kg)** | **FB₁ (μg/kg)** | **FB₂ (μg/kg)** | **FB₁ (μg/kg)** | **FB₂ (μg/kg)** | **FB₁ (μg/kg)** | **FB₂ (μg/kg)** |
| **Fumonisin B₁** | 334               | 172          | 614          | 614          | 23               | 61           | 29           | 1,400          | 392           | 0              | 334           | 172          | 614          | 614          | 23               | 61           | 29           | 1,400          | 392           | 0              |
| **Fumonisin B₂** | 185              | 148          | 222          | 222          | 25               | 121           | 25           | 508            | 175           | 765            | 0              | 185           | 148          | 222          | 222          | 25               | 121           | 25           | 508            | 175           | 765            |
| **Sum of fumonisin B₁ and B₂** | 519               | 320          | 836          | 836          | 48               | 380           | 30           | 1,900          | 764           | 0              | 519           | 320          | 836          | 836          | 48               | 380           | 30           | 1,900          | 764           | 0              |

*MAL: Minimum admissible level; LOD: Limit of detection; LOQ: Limit of quantitation; X̄: Average concentration value taking into account the results above LOD.
of B1 amounted to 8,222 µg/kg, while fumonisins B2 was 2,885 µg/kg [23].

B1 and B2 were detected in 61.5% (8/13) and 15.4% (2/13) of the popcorn samples analysed. A similar level of contamination is observed in other studies, where 88.2% of popcorn samples were contaminated by fumonisins, ranging from 89 to 1,170 µg/kg (B1) and 57 to 211 µg/kg (B2), respectively [24].

The maximum allowed level of total fumonisins for cornflakes, which equals 800 µg/kg, was not exceeded in any of the 15 analyzed samples, while the content of fumonisin was assayed in four samples (26.7%) in the range between 28 and 132 µg/kg. In 5 samples FB1 was assayed at an average level of 64 µg/kg, while FB2 was found in two samples at an average level of 20 µg/kg (Table 2). The analysis conducted by the Cano-Sanchez et al. showed contamination sum of fumonisins in 28 of 72 samples (39%). The average level of fumonisin contamination in samples of cornflakes was 78.9 µg/kg [25]. In other studies fumonisins were present in 50% of cornflakes samples (2/4), and the mean value was 30.5 µg/kg [26]. In Martin’s et al. studies, cornflakes showed significantly higher contamination by fumonisins, where the average values of B1 and B2 were 341 µg/kg and 87 µg/kg, respectively [24]. Bryta et al. analyzed 19 samples of cornflakes, from which nine were contaminated by B1 and B2 in the range of 13 to 248 µg/kg [27].

In none of the samples from the products intended for consumption for babies and small children were fumonisins detected (Table 2). However, other studies have shown contamination by fumonisins 7 with 30 samples of baby food (23.3%), where the average concentration of fumonisin was 36.4 µg/kg [25]. Girolamo et al. showed the presence of fumonisins in six out of 19 maize-based baby foods at levels up to 53 µg/kg [28]. In the case of maize milling fractions and the products of maize milling of 500 microns in size (not intended for direct consumption), the maximum concentration was set at the level of 1,400 µg/kg. Only in one out of 13 samples of corn grits (7.7%) was the total amount of fumonisins assayed at the level of 39 µg/kg. B1 was assayed in five products at an average level amounting to 21 µg/kg. In four out of five samples in which B1 was assayed, the concentration level ranged between the detection and quantification limits. The analyzed samples of corn grits were free from contamination with B2 (Table 2). Martins et al. observed fumonisins in 19 samples of corn grits (67.9%), where high levels of B1 and B2 were found in concentrations ranging from 91 to 3,462 µg/kg and from 45 to 886 µg/kg, respectively [24].

For maize milling fraction and the products of maize milling of a size below 500 microns not intended for direct consumption, the maximum allowed level amounts to 2,000 µg/kg. Among 46 samples of corn flour, 19 were found with total B1 and B2 and 24 samples contained B1, while 11 contained B2. In one sample of corn flour the concentration of the analyzed mycotoxin was assayed at 6,342 µg/kg, which indicates a threefold exceedance of the maximum allowed level. In the other samples of corn flour, the sum of fumonisins was assayed in the range of 29-1,302 µg/kg. The average content of fumonisins amounted to 479 µg/kg for FB1 and 243 µg/kg for FB2, respectively (Table 2). The results obtained by Esposito et al. showed a high percentage of positive samples for FB1, FB2, and FB3 (85%). The highest concentration was recorded for the total of fumonisins for cornmeal and savory snacks, amounting to 961 µg/kg and 439 µg/kg, respectively [29]. Similarly, the study conducted by Brera et al. also showed the highest contamination in snacks (761 µg/kg) and flour (544 µg/kg) [30]. Martins et al. found 73.3% of corn flour samples where the levels of positive samples FB1 and FB2 ranged from 128 to 555 µg/kg and from 56 to 235 µg/kg, respectively [24]. However, in other studies samples of maize flour were contaminated by a sum of fumonisins in the range 13 to 1,688 µg/kg [27].

The studies also showed that 64% of samples of maize snacks (nine out of 14) were highly contaminated with fumonisins B1, B2, and B3 (13-3,297 µg/kg). However, in 12 out of 14 samples of noodles (86%) we detected a sum of fumonisins at an average level of 175 µg/kg [27]. In the study conducted by Mruczyk et al. we found the presence of fumonisin in four out of seven analyzed samples. In four samples of cereal preparations did not exceed the maximum allowed level and fell within the range of 25% of MAL. However, in the other three samples the content of mycotoxins did not exceed the limit of quantification of the method [31]. The low levels of fumonisins were detected in rice and sweet snacks, where concentrations of fumonisins were 31 µg/kg and 77 µg/kg, respectively [29]. Rubert et al. analyzed a total of 1,250 samples including 716 and 534 organic and non-organic products, respectively. B1 was detected in 11.4% of organic and 3.6% of conventional samples. The obtained concentration for FB1 ranged from 20.2 to 1,201.7 µg/kg for both organic and conventional cereals products. B2 was identified in 11.3% and 3.4% of the organic and conventional samples, respectively, at levels ranging from 21.7 to 1,010.5 µg/kg. The highest concentration of fumonisins was found in gofio organic samples of corn and wheat, amounting to 1,201.7 µg/kg and 1,001.4 µg/kg, respectively [32].

For the assessment of health risk from exposure to mycotoxins in corn food products available in the Polish consumer market the amount of mycotoxins taken in with these products was estimated and compared with the tolerable weekly intake (TWI) [16]. It was assumed that the average body weight of an adult consumer is 60 kg, and the average consumption of maize products in Eastern Europe is 18.05 g/capita/day (FAO, 2013) [33]. According to our study the average level of the sum of fumonisins FB1 and FB2, and the maximum concentration (95th percentile) in the analyzed samples were 176.2 and 890.0 µg/kg, respectively, representing 2.6% and 13.3% of TWI.

The most recent comprehensive risk assessment on health effects related to the presence of fumonisins in food was presented by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2014. The evaluation was...
considering the exceptional situation of high contamination of maize and maize-based products. The dataset used for the exposure assessment of fumonisins contained 3,654 analytical results obtained on food samples that were reported by 11 European countries and collected between 2000 and 2010. The calculated exposure to fumonisins showed that mean lower bound and upper bound exposure across age class and surveys was lower than the provisional maximum tolerable daily intake (PMTDI), whereas the 95th percentile exposure in toddlers and other children was above the PMTDI in several surveys [34].

In conclusion, the analyzed food products, except for the products for infants and small children, were characterized by contamination with fumonisins at different levels. The obtained results together with many bibliographical references indicate that there is a risk of exposure of general population to fumonisins by consumption of food products prepared on the basis of maize. Therefore, it is necessary to constantly monitor the presence of mycotoxins in food. Exposure to mycotoxins in the analyzed corn food products is not a significant health risk to consumers.

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References

2. MWALWAYO D.S., THOLE B. Prevalence of aflatoxin B₁ and fumonisins (B₁ + B₂) in maize consumed in rural Malawi. Toxicology Reports, 3, 173, 2016.
in family farm in Minas Gerais, Brazil. Food Control 28, 83, 2012.


