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Distribution of aflatoxins and fumonisins in dry-milled maize fractions

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Abstract

The aim of this study was to evaluate the distribution of aflatoxins and fumonisins in fractions derived from dry-milling of contaminated maize. Two maize lots with different contamination levels were processed and sampled: the first (maize 1) had aflatoxin B_1 (AFB_1) and fumonisin B_1 (FB_1) levels of 3.6 and 5379 μg kg^{-1}, respectively; the second (maize 2) had corresponding levels of 91.1 and 8841 μg kg^{-1}, respectively. The cleaning step reduced AFB_1 and FB_1 levels by 8 and 11% in maize 1 and by 57 and 34% in maize 2. The subsequent removal of bran and germ led to a further decrease in contamination levels in the products destined for human consumption. In the latter, AFB_1 was uniformly distributed, while FB_1 was concentrated in the finer size fractions. Contamination of raw maize 1 (3.6 μg kg^{-1}) was below the EU AFB_1 limit of 5 μg kg^{-1} for unprocessed maize, but among the final products only coarse flour (1.7 μg kg^{-1}) was within the EU limit of 2 μg kg^{-1}, while grits and fine flour showed higher levels (2.7 and 2.5 μg kg^{-1}, respectively). As regards

cleaned maize, a different distribution of the two toxins was observed in the kernels: AFB_1 contamination was more superficial and concentrated in germ, while FB_1 contamination affected the inner layers of the kernels.

Keywords: aflatoxins, fumonisins, maize, dry-milling process, milling fractions

Introduction

Maize is one of the most important agricultural crops in the world, both for its potential yield per hectare and its nutritional value. In Italy, the area dedicated to maize cultivation is about 1.1 million hectares, located mainly in Northern Italy, while maize production is about 9.7 million tonnes (Istat, 2006). Although grown principally for animal feed, about 4% of Italian maize production is destined for the milling industry and is processed into products for human consumption.

The natural occurrence in maize of mycotoxins such as aflatoxins, fumonisins, trichothecenes and zearalenone, has been reported in various studies (Vargas et al. 2001; Adejumo et al. 2007; Abbas et al. 2002; Ali et al. 1998; Domijan et al. 2005; Gonzalez et al. 1999). As regards maize produced in Italy, mycotoxins that deserve special attention are: aflatoxins for their extreme acute and chronic toxicity and because a toxic metabolite (aflatoxin M_1) is excreted into the milk of cows fed contaminated feeds; fumonisins for their toxicity and high and widespread contamination that cause several disease states in animals and could affect human health (Pietri et al. 2004; Logrieco et al. 2002; Battilani et al. 2005). Aflatoxins B_1, B_2 , G_1 and G_2 are mainly produced by two fungal species: *Aspergillus flavus* and *A. parasiticus*, mould species that grow in

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warm conditions in tropical and sub-tropical regions of the world. Aflatoxin B_1, (AFB_1) the most toxic aflatoxin, is a potent genotoxic carcinogen in laboratory animals and there is strong evidence that it is a liver carcinogen in humans. Fumonisins B_1, B_2 and B_3 are a group of closely related compounds mainly produced by *Fusarium verticillioides* and *F. proliferatum*, fungi that commonly contaminate maize in many growing regions of the world; studies demonstrate the importance of insect damage, drought and temperature stress on fumonisin synthesis. Fumonisins have been shown to be hepatocarcinogenic in male rats and female mice and nephrocarcinogenic in male rats (Voss et al. 2001). Fumonisins have also been associated with the high incidence of human oesophageal cancer in some regions of South Africa and China (Sydenham et al. 1990; Sun et al. 2007). Recent findings suggest that they might increase the risk of neural tube defects in populations consuming large amounts of fumonisin-contaminated maize (Missmer et al. 2006).

Aflatoxin B_1 and *Fusarium verticillioides* toxins have been categorised by the International Agency for Research on Cancer (IARC) as class 1 (human carcinogen) and 2B (possible human carcinogen), respectively (IARC 1993). In a later evaluation in 2002, the IARC declared fumonisin B_1 (FB_1) to be a group 2B carcinogen (IARC 2002). The EU legislation fixed maximum admissible levels for AFB_1, total aflatoxins (sum of AFB_1, AFB_2, AFG_1 and AFG_2) and fumonisins (sum of FB_1 and FB_2) in cereals and derived products for direct human consumption (Commission of the European Communities 2006a, 2007). Current limits relevant to maize are given in Table 1.

Current legislation being very detailed, for maize millers it is vital to know the extent of decontamination that can be obtained by the first step (cleaning) of the process and how

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these mycotoxins are distributed in final products. Physical procedures generate little heat other than that caused by the operation of the machinery for cleaning, de-husking and abrasion so no significant thermal breakdown of mycotoxins would be expected at this stage. However, moulds and mycotoxins are often concentrated in dust and broken grains or in the outer seed coat of grains. The removal of this material can thus result in a considerable reduction of the mean mycotoxin concentration.

Fumonisin distribution was studied on a laboratory-scale processing apparatus (Katta et al. 1997) or sampling, over 9 months, maize products in a commercial dry-mill (Broggi et al. 2002). Brera et al. (2004, 2006) carried out a study, in a commercial dry-milling plant, of FB_1, aflatoxins and zearalenone distribution in milled corn fractions from two maize lots showing medium-low contamination levels. These studies indicated that contamination increased, with respect to unprocessed maize, in products like bran, germ and flour of low particle size (animal feed flour), while it decreased in grits and flour of higher particle size, destined for human consumption. No data are available on the effect of the cleaning step.

The aim of our work was to investigate in an industrial dry-mill: (a) aflatoxin and fumonisin distribution in milling products deriving from the processing of two maize lots, that showed (with respect to the Italian situation) medium levels of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2) and fumonisins (FB_1 and FB_2) in the first and high levels in the second (about 5 and 8000 µg kg^{-1} for the first lot, 120 and 13000 µg kg^{-1} for the second, respectively); (b) the efficacy of maize cleaning steps, carried out at the beginning of the process, on aflatoxin and fumonisin contamination levels. Further, a

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mass balance of the mycotoxins was carried out, in order to verify the accuracy of the results.

[insert Table 1 about here]

Materials and methods

Dry-Milling Process

The study was carried out in an industrial dry-milling plant able to process 10 tonnes per hour of maize grain and performed de-germination under partially wet conditions by a conical de-germinator. First, the unprocessed maize was cleaned through three different steps (Figure 1), which included a separator with aspirator (winnower), a dry de-stoner and an intensive scourer coupled with an aspirator. The cleaned maize was soaked in warm water (70°C) for 10 min, to increase moisture to 22%. In these conditions, the germ becomes soft and elastic; also, partial detachment of bran occurs. After soaking, the maize was decorticated and de-germinated in the conical de-germinator, then sifted through a 5-mm sieve. The fraction (large size grits and germ) retained on the sieve was directly conveyed to the plan sifter, while the sieved fraction (flours, bran, small size grits and fractions of germ) was dried to 14% moisture content and conveyed to a turbo-sifter, where the flour of a particle size <2 mm (fine flour), was separated and destined to animal feed. The fraction with a particle size >2 mm was conveyed to a winnower, which aspirated the bran (lighter) sending it to animal feed, while fractions of germ and small size grits were sent to the plan sifter, together with large size grits and germ of particle size >5 mm. The plan sifter separated the products according to their size: those of size >5.5 mm, were conveyed back to the de-

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germinator, while the smaller ones were separated through a 4 mm sieve. The fractions which were obtained were sent to different gravity tables, to separate germ from small and large size grits. Fine grits were processed again using a horizontal roller mill and transformed into flour, that was conveyed to a plan sifter and divided between coarse flour (300-850 µm), fine flour (<300 µm) and residual germ (about 1%). Undersized flour, bran and cleaning waste, were processed with a hammer mill for the production of animal feed flour.

[insert Figure 1 about here]

Samples

Two maize lots (maize 1 and 2), with different aflatoxin and fumonisin contamination, were processed and sampled. Both were FAO 600 class hybrids, with flinty endosperm. Samples of unprocessed maize and derived fractions were drawn from twelve opening slits of the plant (numbered in Figure 1). In order to obtain a dynamic representative sampling (corresponding to the flow of different products), a primary sample of about 300 g was collected from each slit every 90 seconds for one hour (40 samples), according to the Regulation 2006/401/EC (Commission of the European Communities 2006b). From the cleaning plant were collected: unprocessed maize (1), waste of the winnower (2), of the dust filter (3), of the intensive scourer (4) and cleaned maize (5). From the de-germination process were collected: bran (6), germ (7), coarse grits (8), fine grits (9) and animal feed flour (12). From the refining step were collected: coarse (10) and fine flour (11). For each collecting-slit, the 40 primary samples were mixed, ground with a hammer mill (1 mm sieve), and homogenized for 10 min using a

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horizontal mixer. For each product, two sub-samples of 1.5 kg were withdrawn and stored at –20°C until the time of analysis. The percentage yields of the different fractions were calculated taking into account the technical specifications of the plant and from measurements carried out by plant-technicians during processing of the two maize lots, before sampling.

Analytical Standards

Aflatoxin and fumonisin standards were obtained from Sigma-Aldrich (St. Louis, MO, USA). For each aflatoxin, a stock solution of 5-8 µg ml^{-1} was prepared in benzene:acetonitrile (98:2, v/v, 2 ml) and stored at –20°C. The solution was calibrated spectrophotometrically at 350 nm (AOAC International, 2005). The working standard solution was prepared after evaporation under nitrogen of an aliquot (100 µl) of the stock solution and re-dissolution in chloroform (10 ml) by ultrasonication. An aliquot (100 µl) of this solution was evaporated under nitrogen and re-dissolved in the HPLC mobile phase (0.5-5 ml), to obtain calibrant solutions at individual concentrations of AFB_1, AFB_2, AFG_1, AFG_2 between 0.3 and 6 µg l^{-1}. FB_1 and FB_2 (1 mg) were separately dissolved in 10 ml acetonitrile:water (1:1, v/v); the concentration was calculated using the weight indicated by the manufacturer. These solutions were diluted to obtain HPLC calibrant solutions in acetonitrile:water (30:70, v/v, acidified with 0.4% acetic acid) at individual concentrations of FB_1 and FB_2 between 2.5 and 50 µg l^{-1}.

Analysis for aflatoxins and fumonisins

Aflatoxins were analysed according to the method of Stroka et al. (1999). Aflatoxins were extracted from 25 g of sample with 250 ml methanol:water (80:20, v/v), using a

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rotary-shaking stirrer for 45 min. After filtration through a folded filter-paper, an aliquot of the filtrate (5 ml) was diluted with distilled water (45 ml) and the solution was purified through an immunoaffinity column (R-Biopharm Rhône LTD, Glasgow, Scotland, UK). After washing of the column with 5 ml distilled water, aflatoxins were eluted into a graduated glass vial with methanol (2.5 ml). The eluate, concentrated to 1.0 ml under a gentle stream of nitrogen, was brought to 2 ml with acetonitrile:water (25:75, v/v), and vortex-mixed for a few seconds; then, the extract was filtered (Millipore Corporation, Bedford, Massachussetts, USA, HV 0.45 µm) and injected (30 µl). Analysis was performed using an HPLC instrument, consisting of two PU-1580 chromatographic pumps, an AS 1555 sampling system, a FP 1520 fluorescence detector and a post-column derivatization system (Jasco Corporation, Tokyo, Japan); the instrument was controlled by Borwin 1.5 software (Jasco). A Superspher RP-18 column (4 µm particle size, 125x4 mm i.d., Merck) was used at ambient temperature, with a mobile phase of water:methanol:acetonitrile (64:23:13, v/v/v), at 1.0 ml min^{-1}. A solution of pyridinium bromide perbromide (25 mg in 500 ml of HPLC-grade water) was used as a derivatizing agent. The flow of the post-column derivatizing solution was set at 0.1 ml min^{-1} and the reaction tubing was 500 µl. The detector was set at λ_{ex}=365 nm and λ_{em}=440 nm.

FB_1 and FB_2 content was analysed according to the method proposed by Visconti et al. (2001). Fumonisins were extracted from 10 g of sample in a plastic centrifuge bottle with 50 ml of acetonitrile:methanol:water (25:25:50, v/v/v). After extraction for 45 min using a rotary-shaking stirrer and centrifugation at 4500 *g* for 6 min, the supernatant was poured into a flask; another 50 ml of the same solution was added to the residue in

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the centrifuge bottle, and a second extraction performed for 30 min. The combined extracts were filtered through a folded filter-paper. An aliquot of 2 ml was diluted with 20 ml of 0.1 M phosphate buffered saline (PBS, pH=7.4) and purified through an immunoaffinity column (R-Biopharm Rhône Ltd, Glasgow, Scotland, UK); after washing the column with PBS (2 ml), the fumonisins were slowly eluted (0.5 ml min^{-1}) with methanol (6 ml) into a graduated glass vial; subsequently, the eluate was concentrated to 2 ml under a gentle stream of nitrogen. Analysis was carried out using a LC-MS/MS system, consisting of a LC 1.4 Surveyor pump (Thermo-Fisher Scientific, San Jose, CA, USA), a PAL 1.3.1 sampling system (CTC Analitycs AG, Zwingen, Switzerland) and a Quantum Discovery Max triple-quadrupole mass spectrometer; the system was controlled by Excalibur 1.4 software (Thermo-Fisher). After dilution of the extract (0.1 ml brought to 1 ml) with acetonitrile:water (30:70 v/v, acidified with 0.4% acetic acid), the fumonisins were separated on a Betasil RP-18 column (5 µm particle size, 150 x 2.1 mm, Thermo-Fisher) with mobile-phase gradient acetonitrile-water (both acidified with 0.4% acetic acid) from 25:75 to 55:45 in 9 min, then isocratic for 3 min; the flow rate was 0.2 ml min^{-1}. Ionisation was carried out with an ESI interface (Thermo-Fisher) in positive mode as follows: spray capillary voltage 4.0 kV, sheath and auxiliary gas 35 and 14 psi, respectively, temperature of the heated capillary 270 °C. The mass spectrometric analysis was operated in selected reaction monitoring (SRM). For fragmentation of $[M+H]^+$ ions (722 *m/z* for FB_1, 706 *m/z* for FB_2), the argon collision pressure was set to 1.5 mTorr and the collision energy to 36 V. The selected fragment ions were: 704, 352 and 334 *m/z* for FB_1, 688, 336 and 318 *m/z* for FB_2. Quantitative determination was performed using LC-Quan 2.0 software (Thermo-Fisher).

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Both for aflatoxins and fumonisins, four replicates were analysed for each sample.

Mass balance

Both for aflatoxins and fumonisins, mass balances of the cleaning step [$\Sigma amount_{(cleaning\ waste+cleaned\ maize)}/amount_{(unprocessed\ maize)}$]*100, of the de-germination process [$\Sigma amount_{(animal\ feed\ flour\text{-}cleaning\ waste+grits+germ)}/amount_{(cleaned\ maize)}$]*100 and of the total milling process [$\Sigma amount_{(animal\ feed\ flour+coarse\ grits+flours+germ)}/amount_{(unprocessed\ maize)}$]*100, were calculated.

Results and discussion

Yields of the milling-corn fractions

Yields of the different fractions, calculated with an approximation of 1%, are reported in Table 2. The two maize lots had different characteristics, maize 2 being visually dustier and with a higher amount of broken kernels with respect to maize 1; as a consequence, the cleaning waste was 6 vs. 3% of the processed product for maize 2 and 1, respectively. Further, maize 2 yielded a lower amount of undersized flour deriving from the turbo sifter, with respect to maize 1 (17 vs. 20%). As a consequence, the yield of animal feed flour was the same for maize 1 and 2 (28%). For both maize lots, the ratio between coarse and fine flour, after refining of fine grits, was 85:15.

[insert Table 2 about here]

Performance of the methods

Recovery percentages were evaluated by spiking known blank samples at levels of 2

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and 1000 µg kg^{-1} for aflatoxins and fumonisins, respectively. Average recovery values were: 97.8∀1.6% for AFB_1 and over 93% for the other aflatoxins, 95.5∀1.9% for FB_1 and 93.6∀2.1% for FB_2. A certified reference material (ground corn, R-Biopharm Rhône LTD), with an AFB_1 contamination of 4.1∀1.0 µg kg^{-1}, was also analysed; the result was 4.2∀0.2 µg kg^{-1}. The results of the analyses were not corrected for recovery. The limit of detection (LOD, signal-to-noise ratio of 3:1) and of quantification (LOQ, signal-to-noise ratio of 10:1) were respectively 0.02 and 0.05 µg kg^{-1} for aflatoxins, 10 and 30 µg kg^{-1} for fumonisins.

Aflatoxin distribution

AFB_1 and AFG_1 were detected in both maize lots, but AFB_2 and AFG_2 only in maize 2 (Table 3). The AFB_1 level of maize 1 (3.6 µg kg^{-1}) was lower than the value fixed by EC Regulation 1881/2006 for unprocessed maize (5 µg kg^{-1}); however, considering the final products, only coarse flour was within the limit for human consumption (2 µg kg^{-1}), while coarse and fine grits slightly exceeded this limit. After the cleaning steps, maize 1 showed an AFB_1 concentration slightly lower than the unprocessed one (3.3 against 3.6 µg kg^{-1}, -8%). Bran and germ deriving from de-cortication and de-germination steps, resulted as more contaminated than cleaned maize (+127 and +197%, respectively). Animal feed flour, that included cleaning waste, bran, and undersized flour ground together, showed an AFB_1 level close to raw maize. The results obtained for maize 1 basically accord with those reported by Brera et al. (2006): processing two maize lots with an AFB_1 concentration below 3 µg kg^{-1}, they observed an increase of contamination in bran, germ and animal feed flour and a consequent decrease in grits and flour destined for human consumption, that showed values below 2 µg kg^{-1}.

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Maize 2 was much more contaminated (91.1 µg kg^{-1}); after cleaning it showed an AFB_1 level decidedly lower (39.5 µg kg^{-1}) with respect to the unprocessed one (-57%), as cleaning steps removed the most contaminated fractions (broken kernels, dust, etc.). Bran and germ of maize 2 showed much higher AFB_1 concentrations (+379 and +629%, respectively) compared to cleaned maize. As a consequence, unlike maize 1, the contamination level decreased remarkably in grits (-80%) and increased (+141% with respect to raw maize) in animal feed flour; however, the latter could not be used, as the value (0.219 mg kg^{-1}) exceeded the limit of 0.02 mg kg^{-1} in force in the EU (Commission of the European Communities 2002). None of the milling-corn fractions destined for human consumption showed a concentration below the legal limit (2 µg kg^{-1}), but they could be used as feeds. Considering the AFB_1 percent distribution (Table 3), it is evident that cleaning steps led to very different results for the two maize lots, removing 11 vs. 59% of the toxin from maize 1 and 2, respectively. Analogously, very different results were observed in de-cortication and de-germination steps; toxin removal was 30 vs. 72% for cleaned maize 1 and 2, respectively. Consequently, the AFB_1 percentage remaining in coarse and fine grits, was 55 vs. 13%, for cleaned maize 1 and 2, respectively. Fine grits were subsequently processed to obtain coarse and fine flour, in the ratio 85:15; for maize 1, 61% of the AFB_1 amount in fine grits (7.2% of the total) was present in coarse flour and 16.1% in fine flour; for maize 2, the corresponding percentages were 64.3 and 9.0%. The lower figure was due to a small amount of remaining germ fraction (about 1% of unprocessed maize), more contaminated, that was removed in this step of the process. The different AFB_1 percentage (26.4% against 67.5% for maize 1 and 2, respectively) collected in animal feed flour, was mainly due to the different contribution made by the waste of the

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cleaning steps.

A similar trend was observed for the other aflatoxins.

[insert Table 3 about here]

Fumonisin distribution

Both maize lots turned out to be contaminated with FB_1 and FB_2 (Table 4) at levels exceeding the limit (4000 μg kg^{-1}) in force in the EU. Maize grains obtained after cleaning steps showed fumonisin concentrations lower (especially maize 2) than the unprocessed ones (-11 and -34% for FB_1). Bran showed higher contamination levels with respect to cleaned maize (+50 and +167% for FB_1); germ turned out to be less contaminated with respect to bran in both maize lots. Coarse and fine grits, fractions intended for human consumption, showed much lower FB_1 concentrations when compared to cleaned maize (-90 and –88% for maize 1 and –90 and –73% for maize 2). Besides, fine grits and fine flours, showed a level higher than the coarse ones. Therefore, as already found by Brera et al. (2004), contamination increases in lower particle size final products. Coarse grits for both maize lots, fine grits and coarse flour for maize 1, showed a contamination level (FB_1 + FB_2) below 1400 μg kg^{-1}, which is the limit fixed by the EU. Animal feed flour, which collected bran and cleaning waste, was three times more contaminated than unprocessed maize. Contamination levels found in different corn-milling fractions were similar to those found in previous studies (Katta et al., Broggi et al., Brera et al.), in which there was always reported an increase of concentration in bran, germ and animal feed flour and a decrease in products intended for human consumption, like grits and flours. As regards the FB_1 percentage

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distribution, the cleaning plant removed 14% and 38% of the toxin for maize 1 and 2, respectively; regarding cleaned maize, de-cortication and de-germination processes removed altogether 12% and 27% of the toxin, while 6.8% and 8.8% remained in coarse and fine grits. It is evident that the highest fraction of FB_1 remained in the undersized flour separated from the turbo sifter and conveyed into animal feed flour; this product gathers 83.3 and 90.9% of the total FB_1 present in uncleaned maize 1 and 2, respectively. A similar trend has been observed for FB_2.

[insert Table 4 about here]

Mass balance

Mass balances of aflatoxins and fumonisins for the cleaning step, for the degermination process and for the complete process are reported in Table 5. The mass balance of the complete process for AFG_1 in maize 1 was relatively low (79.3%), probably because of a high analytical error associated with the low level of the mycotoxin; except for this result, mass balances of mycotoxins in both processes were in the range 90-110%. Thus, it can be concluded that the dynamic sampling was effective and the results were altogether accurate.

[insert Table 5 about here]

Conclusions

The extent of decontamination obtained from the cleaning step was decidedly higher for a “dirty” maize (maize 2) with respect to a “normal” maize (maize 1). In this step, the

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percent toxin reduction in maize 2, if calculated in terms of both concentration and mass, was 57-59% and 34-38% for AFB_1 and FB_1, respectively. For maize 1, the corresponding values were 8-11% and 11-14% for AFB_1 and FB_1, respectively.

The extent of decontamination obtained from the cleaning step of maize 2, was higher for aflatoxins than for fumonisins. Probably, during harvesting, handling and transportation, a fraction of the aflatoxin contaminated kernels broke and produced powder; therefore their removal contributed significantly to decontamination. For maize 2, discarding of the cleaning waste would decrease the yield of animal feed flour from 28% to 22%, but the total amount of AFB_1 and FB_1 residual in the product would decrease from 67.5% to 8.2% and from 90.9% to 53.2%, respectively. Generally, it is evident that an alternative use of cleaning waste would substantially improve the quality of animal feed flour.

As regards products after the cleaning step, a different distribution of the two toxins was observed. There was a large amount of AFB_1 in the germ fraction, of 18.5% and 46.7% of the total for maize 1 and 2, respectively; for bran the corresponding values were 11.8% and 25.6%; most of the remaining AFB_1 was in coarse grits. As regards FB_1, 4.3% and 12.7% occurred in germ, 7.7% and 14.1% in bran, for maize 1 and 2, respectively; the amount remaining in coarse grits was low (5.5-5.6%), while most was collected in undersized flour (81-64%), that was intended for animal feed. It is evident that aflatoxin contamination of maize kernels was more superficial and concerned mainly the germ, while fumonisin contamination affected the inner layers of kernels.

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Table 1. EU maximum admissible limits for AFB_1, total aflatoxins (sum of AFB_1, AFB_2, AFG_1, AFG_2) and fumonisins (sum of FB_1 and FB_2) in maize and derived products.

	AFB_1 ($\mu g\ kg^{-1}$)	Sum of AFB_1, AFB_2, AFG_1 and AFG_2 ($\mu g\ kg^{-1}$)	Sum of FB_1 and FB_2 ($\mu g\ kg^{-1}$)
Unprocessed maize	5.0	10.0	4000
Maize for direct human consumption	2.0	4.0	1000
Maize-based breakfast	-	-	800
Milling fractions of maize with particle size > 500 micron	-	-	1400
Milling fractions of maize with particle size # 500 micron	-	-	2000

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Table 2. Yield of maize milling fractions.

Maize milling fractions	Yield maize 1 (%)	Yield maize 2 (%)
Unprocessed maize	100	100
Waste of aspirator	2	3
Waste of dust filter	0,2	1,5
Waste of intensive scourer	0,8	1,5
Cleaned maize	97	94
Bran	5	5
Germ	6	6
Coarse grits	55	55
Fine grits	11	11
from which:		
coarse flour	85	85
fine flour	15	15
Undersized flour	20	17
Animal feed flour (waste+bran+undersized flour)	28	28

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Table 3: Aflatoxin levels (mean of 4 replicates, μg kg^{-1}), relative standard deviation (RSD, %) and AFB_1 relative distribution (%) in maize-milling fractions referred to unprocessed maize and (between parentheses) to cleaned maize.

Maize-milling fractions	Maize 1					Maize 2								
	AFB_1			AFG_1		AFB_1			AFB_2		AFG_1		AFG_2	
	μg kg^{-1}	RSD %	Distribution %	μg kg^{-1}	RSD %	μg kg^{-1}	RSD %	Distribution %	μg kg^{-1}	RSD %	μg kg^{-1}	RSD %	μg kg^{-1}	RSD %
Unprocessed maize	3.6	11.1	100	1.7	5.9	91.1	3.8	100	3.4	8.8	26.3	1.5	1.1	9.1
Waste of aspirator	3.4	11.8	1.9	1.8	11.1	138.3	3.9	4.6	38.1	3.4	26.3	9.5	1.6	12.5
Waste of dust filter	17	9.4	1.0	8.3	8.4	1296	2.0	21.3	57.8	2.4	290.3	2.3	9.1	5.5
Waste of intensive scourer	11.5	12.2	2.6	6.1	8.2	1554	0.8	25.6	54.5	2.9	285.5	2.5	9.4	5.3
Cleaned maize	3.3	15.1	88.7 (100)	1.1	9.1	39.5	6.1	40.7 (100)	1.8	11.1	13.9	4.3	0.7	14.3
Bran	7.5	9.3	10.5 (11.8)	3.9	7.7	189.4	4.5	10.4 (25.6)	7.3	10.9	42.7	3.5	1.1	9.1
Germ	9.8	9.2	16.4 (18.5)	5.2	9.6	287.9	1.0	19.0 (46.7)	16.7	3.0	81.9	1.5	5.0	6.0
Coarse grits	2.7	11.1	41.7 (47.0)	1.1	9.1	7.6	3.9	4.6 (11.3)	0.4	25.0	1.9	5.3	0.3	16.7
Fine grits	2.3	8.7	7.2 (8.1)	1.0	10	7.8	5.1	0.9 (2.2)	0.7	14.3	2.4	8.3	0.6	16.7
from which:														
coarse flour	1.7	11.8	61.0	0.8	12.5	5.9	5.1	64.3	0.6	16.7	2.0	10.0	0.5	10.0
fine flour	2.5	12.0	16.1	1.2	8.3	4.7	6.4	9.0	0.5	20.0	1.4	14.3	0.4	12.5
Animal feed flour	3.4	11.8	26.4	1.1	9.1	219.6	2.5	67.5	8.8	3.4	48.5	7.4	1.7	11.8

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Table 4: Fumonisin levels (mean of 4 replicates, μg kg^{-1}), relative standard deviation (RSD, %) and FB_1 relative distribution (%) in corn-milling fractions referred to unprocessed maize and (between parentheses) to cleaned maize.

Maize-milling fractions	Maize 1					Maize 2				
	FB_1			FB_2		FB_1			FB_2	
	μg kg^{-1}	RSD %	Distribution %	μg kg^{-1}	RSD %	μg kg^{-1}	RSD %	Distribution %	μg kg^{-1}	RSD %
Unprocessed maize	5379	10.5	100	2576	7.7	8841	7.9	100	4234	8.4
Waste of aspirator	17353	4.6	6.4	6539	5.6	48425	5.5	16.5	11465	6.5
Waste of dust filter	42222	2.9	1.6	31389	2.1	50872	3.7	8.7	34103	4.3
Waste of intensive scourer	43402	2.8	6.4	24653	1.8	48565	3.0	8.3	24746	4.1
Cleaned maize	4770	8.5	86.0 (100)	2360	6.7	5862	3.9	62.3 (100)	2975	5.3
Bran	7154	5.3	6.6 (7.7)	2559	6.5	15647	1.0	8.8 (14.1)	5597	2.2
Germ	3332	7.2	3.7 (4.3)	1237	6.6	11674	8.1	7.9 (12.7)	4334	8.2
Coarse grits	458	11.1	4.7 (5.5)	128	11.7	563	8.0	3.5 (5.6)	157	13.4
Fine grits	556	8.1	1.1 (1.3)	163	11.0	1592	2.8	2.0 (3.2)	500	8.4
from which:										
coarse flour	292	10.6	44.6	96	9.4	1266	7.5	67.6	416	7.9
fine flour	1922	7.7	51.8	635	5.7	3058	5.8	28.8	2975	5.6
Animal feed flour	16011	2.1	83.3	8155	3.7	28712	5.9	90.9	14624	2.4

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Table 5: Aflatoxin and fumonisin mass balance (%) for maize 1 and 2.

Mass balance	AFB_1	AFB_2	AFG_1	AFG_2	FB_1	FB_2
Cleaning step *Maize 1* *Maize 2*	94.2 96.3	- 109.1	93.8 96.2	- 96.0	100.4 95.6	104.0 102.2
De-germination process *Maize 1* *Maize 2*	97.2 99.5	- 108.0	110.0 99.5	- 99.5	91.2 114.6	89.8 106.1
Complete milling process *Maize 1* *Maize 2*	90.0 91.7	- 110.0	79.3 92.1	- 92.1	92.9 104.3	95.0 106.2

Figure 1. Layout of the industrial plant showing sampling points (numbered) and final products (bold type).

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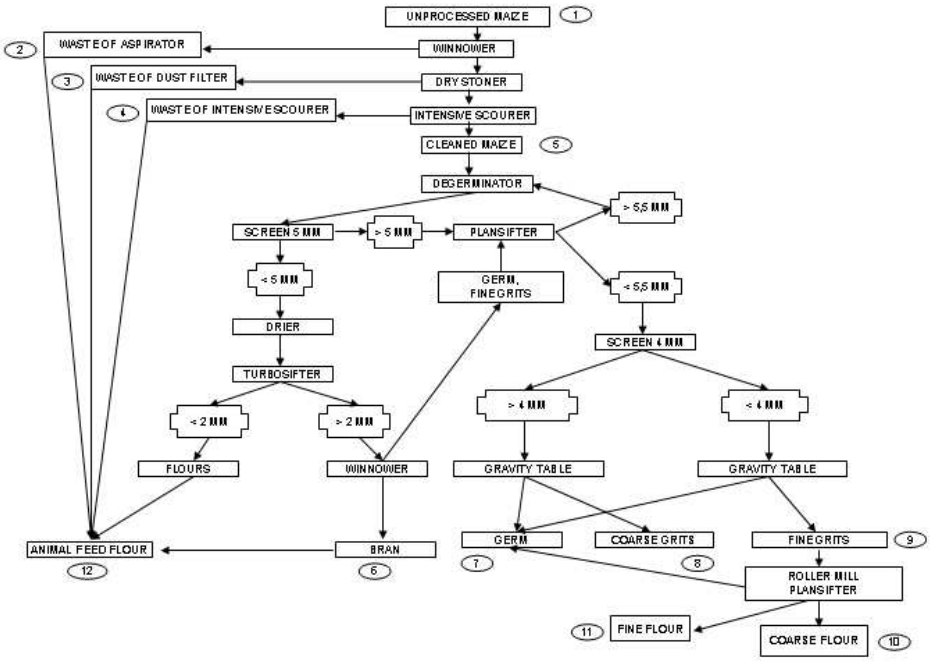

Figure 1.

Figure 1. Layout of the industrial plant showing sampling points (numbered) and final products (bold type).
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