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Research Note

Effect of Industrial Processing on the Distribution of Fumonisin B_1 in Dry Milling Corn Fractions

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ABSTRACT

The aim of this study was to investigate the distribution of fumonisin B_1 in various corn milling fractions processed by an industrial plant. Corn kernels and six derived milling fractions (germ, bran, large and small grits, animal feed flour, and flour) were sampled. In addition, in order to evaluate the effect of cooking, samples of polenta were prepared starting from naturally contaminated flour obtained from the industrial processing cycle. The industrial plant worked continuously at a rate of 60 tons per day. Two sublots of 5 tons each were investigated with samples of derived products taken at regular time intervals. Due to a similar heterogeneous distribution of fumonisin B_1 with other mycotoxins, such as aflatoxins, the sampling scheme was derived from the European Directive 98/53 for aflatoxins. Both lots of kernels showed fumonisin contamination at 4.54 and 5.09 mg/kg, respectively. Germ, bran, and animal feed flour showed contamination levels, namely 8.92 mg/kg (lot 1) and 9.56 mg/kg (lot 2), 7.08 mg/kg (lot 1) and 8.08 mg/kg (lot 2), and 9.36 mg/kg (lot 1) and 6.86 mg/kg (lot 2) higher than large and small grits and flour (0.39 mg/kg [lot 1] and 0.42 mg/kg [lot 2], 0.60 mg/kg [lot 1] and 1.01 mg/kg [lot 2], and 0.40 mg/kg [lot 1] and 0.45 mg/kg [lot 2], respectively). These results seem to account both for the industrial yields of the derived products and the distribution of fumonisin contamination in a kernel. The cooking of polenta in a domestic pressure cooker did not affect fumonisin contamination because the mycotoxin concentrations were similar to those of the starting flour (0.40 and 0.45 mg/kg).

Fumonisins are mycotoxins produced mainly by *Fusarium verticillioides* (Sacc.) Nirenberg (former *F. moniliforme* Sheldon) and *Fusarium proliferatum* (T. Matsushima) Nirenberg, which often occur in maize and maize-based food products at levels that could affect human and animal health. In particular, fumonisins have been shown to be hepatotoxic and nephrotoxic to most animal species tested and to cause leukoencephalomalacia in horses *(2, 23)*, pulmonary oedema in swine *(7)*, and hepatocarcinoma in rats *(6)*. Recently, the U.S. National Toxicology Program showed clear evidence of carcinogenic activity of fumonisin B_1 (FB_1) in male rats and female mice based on the increased incidences of renal tubule and hepatocellular neoplasms, respectively *(21)*. On the basis of the toxicological evidence of fungal cultures containing high concentrations of fumonisins, the International Agency for Research on Cancer has classified *F. verticilloides* toxins as potentially carcinogenic to humans (class 2B carcinogens) *(8)*. In order to set realistic tolerance levels of fumonisins, in addition to toxicological evaluations of known or potential adverse health effects resulting from human and animal exposure to contaminated foodstuffs and feed, reliable information on the exposure to the mycotoxins should be available.

Numerous studies on natural occurrence of fumonisins in corn and corn-based foods have been so far conducted as reported by Shephard et al. *(17)*. Furthermore, in order to assess the extent of the public health problem for the European Union population from exposure to fumonisin B_1 (FB_1), Scientific Co-operation Programme (SCOOP) launched an ad hoc Task (3.2.10) whose final report came out in 2003 (Collection of occurrence data of *Fusarium* toxins in food and assessment of dietary intake by the population of EU member states, available at: http://europa.eu.int/comm/food/fs/scoop/task3210.pdf). As for toxicological aspects, the Scientific Committee for Food of the European Union has recently put forward an evaluation on the toxicity of fumonisins *(5)*. At present, an official tolerance value of 1 mg/kg for FB_1 plus FB_2 has been issued by the Swiss Federal Office of Public Health *(18)* for dry maize products and the French Council of Public Hygiene has recommended a maximum level of 3 mg/kg for cereals *(4)*.

Studies on the distribution of the mycotoxin in the various part of the kernel and on the effects of food processing are also fundamental in order to set appropriate tolerance levels of fumonisins in foodstuffs directly intended for human consumption. From the literature, so far, a limited number of articles dealing with the distribution of FB_1 in corn milled fractions is available. More specifically, some studies have been conducted in the United States and in Argentina *(1, 9)*, but actually no study has been performed in Europe. The study by Katta et al. *(9)* was conducted on laboratory-scale processing apparatus and the research of

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Broggi et al. *(1)* dealt with different corn milled fractions in comparison with the ones produced in Europe. Furthermore, a review dealing with the effect of processing on FB_1 in corn and corn milled products, including studies aimed at a better individuation of potential critical control points throughout the food chain production, has recently been published *(14)*.

Therefore, the aim of this work was to investigate, in a dry-milling process, the distribution of FB_1 in corn milling fractions as derived from the processing in an Italian industrial plant.

The methodology for FB_1 detection used by most laboratories is based on extraction with aqueous methanol, purification of the extracts through a strong anion exchange column, and final determination by reversed-phase liquid chromatography with fluorometric detection after precolumn derivatization with *o*-phthaldialdehyde/2-mercaptoethanol *(19, 22)*. This method has been adopted by AOAC International as an official first action method for corn analysis *(19)*; however, the method could not be successfully applied for the analysis of corn-based food products such as corn bran flour, corn bran breakfast cereals, mixed baby cereals, and cornflakes due to low recoveries and inadequate cleanup *(10, 15, 16)*. Recently, the use of immunoaffinity columns for the cleanup step has been reported to produce higher fumonisin recovery *(3)*.

In this study, an automatic method was carried out to process a high number of samples, according to principles of Quality Assurance *(11)* and used for the determination of FB_1 in all analyzed samples. Among fumonisins, only FB_1 was considered because it has a major impact on the food chain.

MATERIALS AND METHODS

Milling process. The industrial process is based on a technology of dry milling coupled with a wet degermination, incorporated with various stages of preparation of the raw material and selection and calibration of the finished product.

The raw material is cleaned (stage 1) through a dry stoner, an intensive horizontal scourer, and a vibrating aspirator (stage 2). In this step, corn is separated from waste (stones, earth, and other foreign matter) and from other types of foliage (cob, broken kernels, etc.). The foliage is then sent to the hammermill for production of animal feed flour.

After the cleaning process, the corn's moisture level is raised to 20% (stage 3) by adding water in order to obtain a softer and swollen kernel to facilitate the degermination and peeling processes at the conical degerminator (stage 4).

Two channels are available at the exit of the degerminator, the first conveying animal feed flour mixed with small-size germ, bran, and grits, the second conveying large-size germ and grits. The first channel leads to a conical turbo aspirator (stage 5) separating the animal feed flour from the other products that are in turn joined to the products, conveyed by the second channel, dried, and transported through a pneumatic system to the plansifter (stage 6). The products are then separated according to their size in grits and germ of small and large dimensions. They are then sent to two different gravity tables (stages 7a and 7b), where the germ is separated from the grits.

Four different products are obtained: large grits, small grits, large germ, and small germ, which are then stored in three different cells—two for large and small grits and one for the gathered germ.

The small grits can be processed again using a cyclical process along a horizontal rollermill (stage 8a) and plansifter (stage 8b) in order to obtain flour with the desired granulometry. Undersized flour is sent, together with the rest of the waste from stages 1 and 5, to the hammer mill for the production of animal feed flour.

The reprocessed small grits are transformed into edible flour by additional horizontal rollermills and a plansifter. The corn grits may also be transformed into precooked flour by a steam cooker, a flake rolling press, and a flour dryer. At the time of this study, the production of precooked flour was not operating. A flow diagram of the corn milling process is given in Figure 1.

According to the process described above the following products were obtained: corn kernels, germ (fat content 20 to 22%), bran, large grits (sized from 3.350 to 6.000 μm), small grits (sized from 2.500 to 4.000 μm), flour (sized from 300 to 800 μm), and animal feed flour (sized from 300 to 850 μm).

Samples. The industrial plant could process 5 tons of maize per hour continuously and the samples were drawn from the opening slits of the plant. Samples of each product listed above were taken for the analysis of FB_1. Because a specific sampling plan for fumonisins is not yet available, the adopted sampling procedure was derived from the European Directive CEE 98/53/CE *(12)* dealing with sampling procedures for another group of mycotoxins, namely aflatoxins, that show a similar heterogeneous distribution within a bulk lot. In order to obtain representative information of the distribution of the toxin throughout the production cycle, two different lots (5 tons each) of the same bulk corn were tested. According to the Directive, for a lot of 5 tons and for each of the seven groups of products, 40 incremental samples, 100 g each, were collected in 1 h, at regular intervals. All incremental samples (40 incremental samples × 7 different matrices × 2 lots) were mixed, ground with a mill, and stored at 4°C until analyzed. The entire sampling procedure lasted 1 h per lot and was repeated on the same day for the two lots.

Corn flour was used to prepare polenta samples. For their preparation, a commonly used cooking technique (pressure cooker) was applied for approximately 30 min. The water/polenta ratio was 3/1 m/m.

Apparatus. The high-performance liquid chromatography (HPLC) system consisted of a Gilson 321 pump (Gilson Inc., Middleton, Wis.), a Gilson ASPEC XL as sample processor, fitted with a Rheodyne 7010 injector, a Gilson 402 Dilutor and a Jasco FP1520 fluorescence detector set at an excitation wavelength of 335 nm and at an emission wavelength of 440 nm (Jasco Corporation, Tokyo, Japan). The detector, the Gilson pump, and the ASPEC XL system were linked to a Gilson 506 C system interface module. The control of the HPLC pump and the chromatographic data acquisition was performed by Gilson Unipoint System Software. The control of the ASPEC XL system was performed by Gilson 735 System Software. The analytical column (C18 reverse phase 250 by 4.6 mm packed with 3.5-μm particle size) was maintained at 40°C.

Analysis. The employed analytical method was a combination of manual and automated procedures. Sample preparation and extraction phases were performed manually, while the steps from cleanup by immunoaffinity column to the HPLC analysis were in turn analyzed by an automatic sample processor.

FB_1 was analyzed according to the R-Biopharm Rhone Ltd. method *(13)*, briefly described as follows. Twenty-five grams of

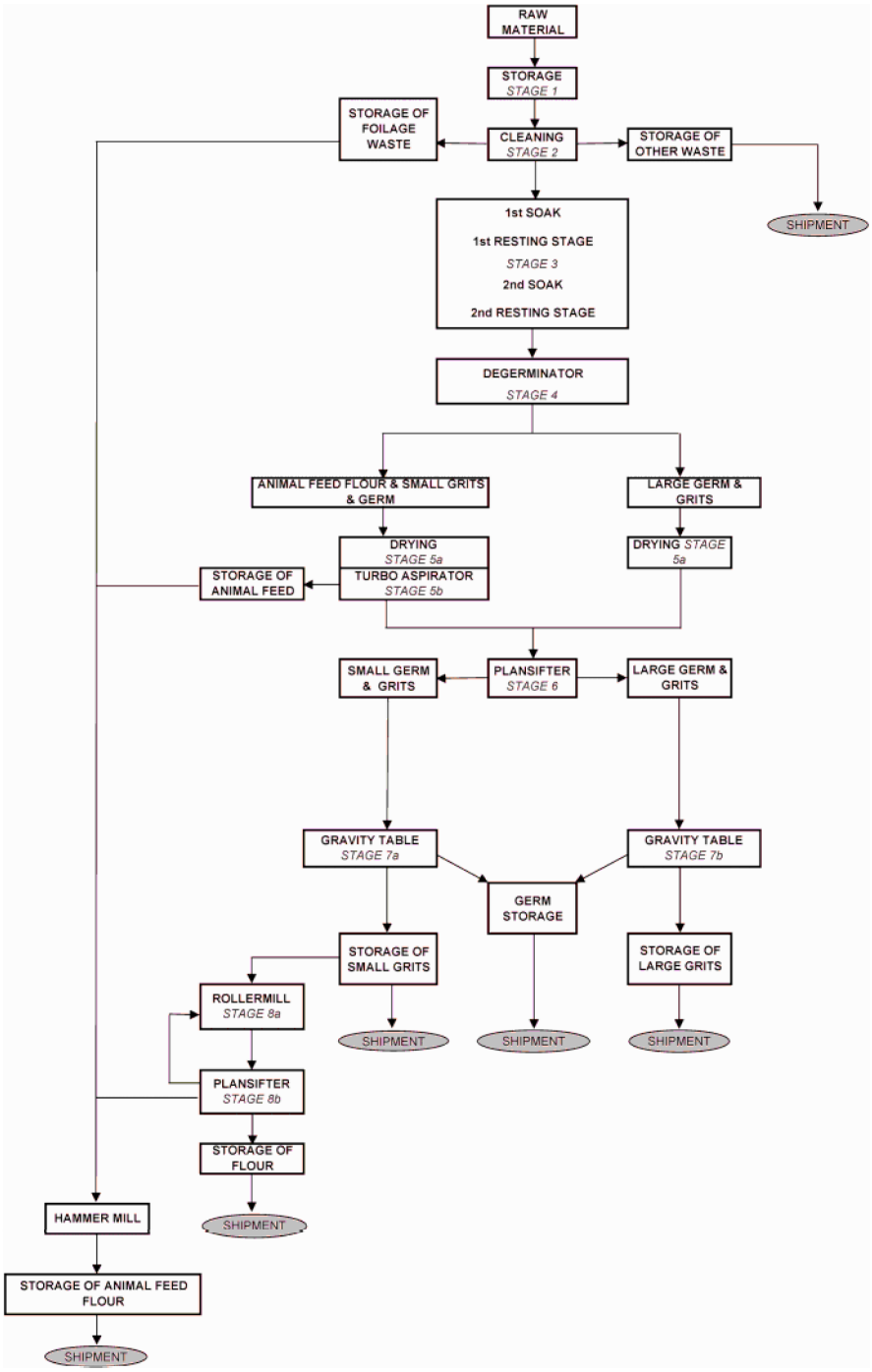
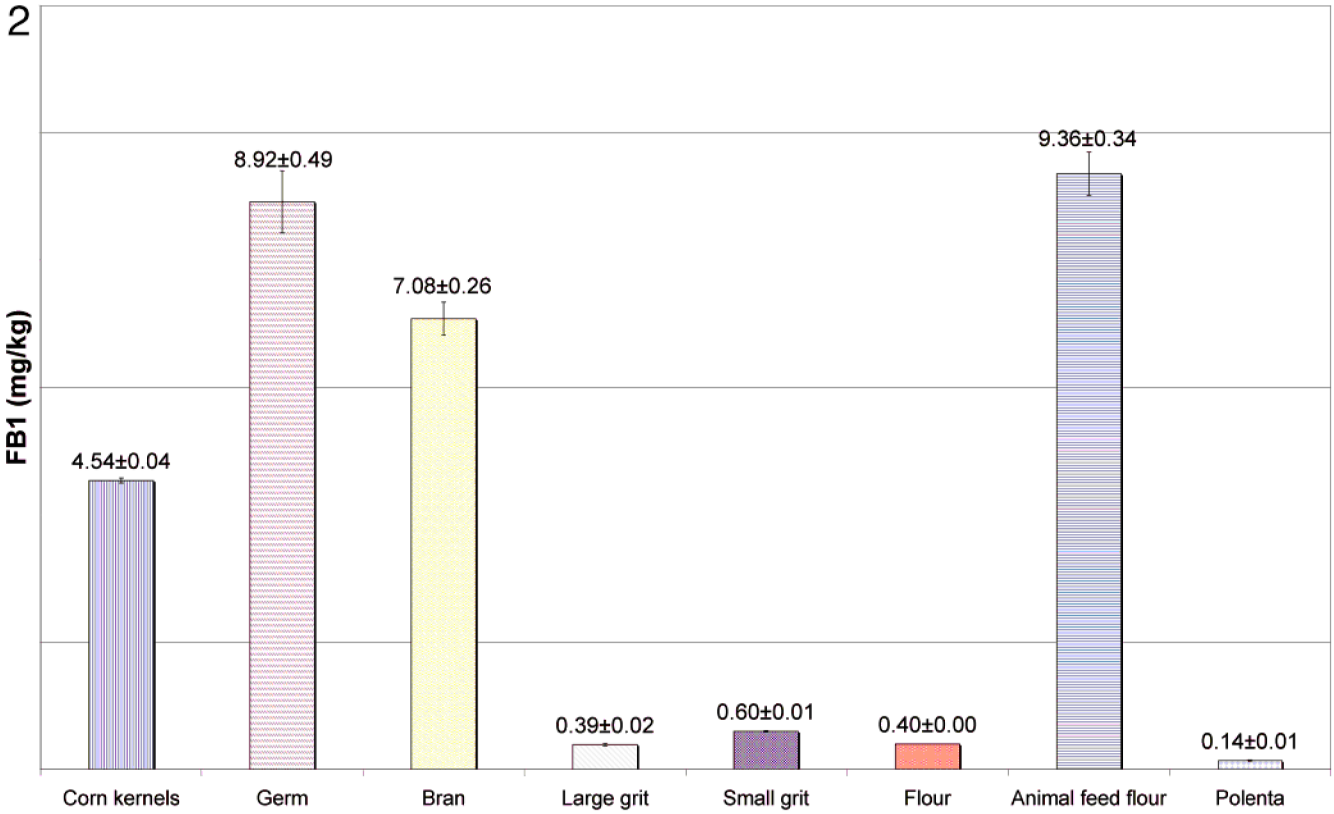


FIGURE 1. *Flow diagram of the industrial corn milling process.*

ground sample was added to 2.5 g of sodium chloride and extracted with 125 ml of acetonitrile-methand-water (25/25/50 vol/vol/vol) in a blender at high speed for 5 min. The supernatant was filtered through Whatman filter paper No 4. Ten milliliters of extract was diluted with 40 ml of phosphate-buffered saline, mixed well, and filtered through a microfiber filter paper. Ten milliliters of diluted extract was passed through the immunoaffinity column (Fumoniprep, R-Biopharm Rhone Ltd., Glasgow, UK) at a flow rate of 1 to 2 drops per s. The column was washed with 10 ml of phosphate-buffered saline at a flow rate of 1 to 2 drops per s and air was forced through the column to push out all of the washing solution. Methanol (1.5 ml) was passed through the column to elute the bound fumonisin and the eluate was collected in a sample vial. Water (1.5 ml) was passed through the column and collected in the same sample vial to give a total volume of 3 ml. Fifty microliters of the eluate was derivatized with 450 μl of *o*-phthaldialdehyde solution (120 mg of *o*-phthaldialdehyde solved in 3 ml of methanol, 15 ml of a 0.1-M solution of disodium tetraborate, and 150 μl of 2-mercaptoethanol), and allowed to react by mixing for 30 s; 100 μl of the derivatized eluate was injected into the HPLC system. The mobile phase consisted of a solution of methanol–0.1 M sodium dihydrogen phosphate 77/23 (vol/vol), adjusted to pH 3.35 with *o*-phosphoric acid. The flow rate was set at 1 ml/min.

RESULTS AND DISCUSSION

Analytical methodology. The automated analytical method employed was reliable and time-saving and the detection limit of 0.03 mg/kg was suitable for control pur-



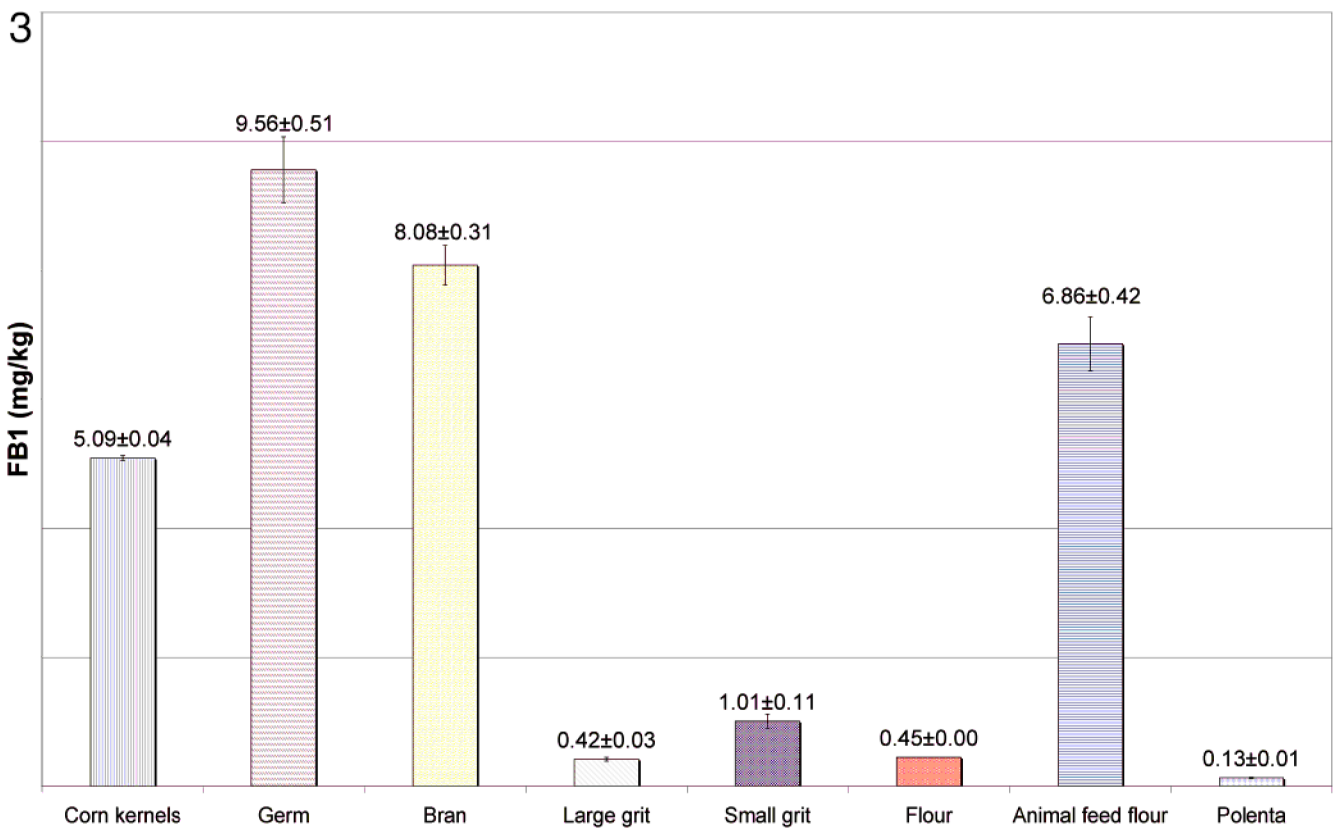


FIGURE 2. *Distribution of FB_1 contamination levels (mg/kg ± SD) in raw corn kernels, corn milling fractions, and polenta samples (lot 1 [number of replicates = 3]).*

FIGURE 3. *Distribution of FB_1 contamination levels (mg/kg ± SD) in raw corn kernels, corn milling fractions, and polenta samples (lot 2 [number of replicates = 3]).*

poses. Each sample was analyzed in triplicate and is reported as the average concentration and standard deviation.

Effect of processing. In order to estimate the effect of technological process on the fate of FB_1 throughout the milled fractions, two distinguished corn lots (lot 1 and lot 2) have been taken into consideration and processed in the described industrial plant. For both lots, the analysis of FB_1 on the raw material and on the derived corn milling fractions was carried out and the obtained results are described in Figures 2 and 3. Contamination levels in the starting materials were remarkably high (4.54 and 5.09 mg/kg for lots 1 and 2, respectively), especially in comparison with the maximum tolerable limits currently existing in some countries. FB_1 concentration in the derived products showed a rather differentiated pattern of distribution.

Germ, bran, and animal feed flour showed a marked contamination, while lower concentrations were observed in large and small corn grits and in the flour samples intended for preparation of cooked polenta. Contamination levels found for grits and cooked polenta would comply with the existing limits mentioned above.

The observed differences in the FB_1 concentrations can be associated both to the industrial yield of the fractions deriving from the milling process and to the distribution of fumonisin in the various parts of the kernels. Average industrial yields obtained in the employed milling plant were 65, 10, 7, 5, and 13% for grits, flour, germ, bran, and animal feed flour, respectively. Therefore, the highest contamination level in bran and germ can be associated to their lower industrial yield. In fact, due to the low number of contaminated grains with respect to the total number of kernels in the lot, a concentration of the mycotoxin in bran and germ higher than in the remaining fraction, such as grits, is reasonable. The ratio of the contaminated fraction/total mass in bran and germ is higher than the equivalent ratio for grits.

According to the previous explanation, the low concentration of flour can be attributable to a comparative industrial yield of this product higher than the others in the milling process. In addition to the previously presented rationale for the distribution of FB_1 in the milled products, the concentrations found in germ and bran higher than in the grits and polenta can also be attributed to a relevant contamination in the external part of the kernels and to a poor transfer of the mycotoxin in the inner parts. The high mycotoxin concentration in germ is consistent with the composition of this fraction because the high content of fats is favorable to the attack of molds. However, the presence of mycotoxin in the derived corn oil and margarine is presumably negligible due to the well-recognized degradation of the mycotoxin following the alkaline treatment usually adopted in the processing of germ.

FB_1 concentration in animal feed flour can be considered as a result of the two factors mentioned above. Additionally, bran represents one of the main ingredients of the animal feed flour.

In addition, the hypothesis that the external parts of the kernels are contaminated at concentrations higher than those found in the inner parts is reasonable and consistent with the dynamics of mold attack, even though the different industrial yields do not allow quantification of the distribution of the mycotoxin in the kernel.

The demonstrated fate of fumonisins during corn processing implies relevant considerations with respect to the definition of maximum acceptable level of the mycotoxins in this cereal. According to the statement of European Commission Regulation 466/01, the limit for aflatoxins (AFs) in cereals has been stated at 2 (AFB_1) and 4 (total AFs) μg/kg, both in raw and in derived products, due to the lack of information on the fate of these mycotoxins during milling. With the aim of differentiating limits for different fractions, with particular attention to raw, corn kernels and edible food commodities (i.e., cooked polenta), a request for submission of relevant data was set in European Commission Regulation 257/2002. The differentiation of limits could relieve the raw material of too stringent legal requirements whenever the milling could lead to less-contaminated processed fractions.

Therefore, the results obtained in this study could be useful when the European Commission takes into consideration the setting of maximum tolerable limits for fumonisins in cereal products.

Effect of cooking. With the aim of testing the efficiency of cooking in the reduction of the mycotoxin from flour to cooked polenta, flour samples contaminated at 0.40 mg/kg (lot 1) and 0.45 mg/kg (lot 2) were used to prepare cooked polenta using a household pressure cooker. The final concentration of FB_1 in the cooked polenta sample was 0.14 mg/kg for lot 1 and 0.13 mg/kg for lot 2. Taking into consideration the flour/water ratio (1/3) used for the preparation of cooked polenta, it can be concluded that no reduction of the mycotoxin occurred after the cooking process.

Polenta represents a staple food in some northern parts of Italy, where an average consumption of 26 g per day per person is reported *(20)*. The intake of FB_1 derived from the consumption of polenta samples considered in this study accounts for 0.16 μg/kg of body weight per day, representing therefore a scarcely relevant contribution to the tolerable daily intake of 2 μg/kg of body weight per day suggested for fumonisin by the Scientific Committee for Food at European level *(5)*.

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