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Food Additives and Contaminants



Transfer of aflatoxin B₁ and fumonisin B₁ from naturally contaminated raw materials to beer during an industrial brewing process

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4 Transfer of aflatoxin B₁ and fumonisin B₁ from naturally contaminated raw 5 materials to beer during an industrial brewing process.

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12 Abstract

The aim of this research was to determine the fate of aflatoxins (AFs) and fumonisins (FBs) naturally occurring in raw materials (maize grit and malted barley) during four industrial brewing processes. The aflatoxin B₁ (AFB₁) level in raw materials varied from 0.31 to 14.85 μ g kg⁻¹, while the fumonisin B_1 (FB₁) level (only in maize grit) varied from 1146 to 3194 µg kg⁻¹. The concentration in finished beer ranged from 0.0015 to 0.022 μ g l⁻¹ for AFB₁ and from 37 to 89 μ g l⁻¹ for FB₁; the other aflatoxins and fumonisin B_2 were not found in beer samples. The average percentage of toxins recovered in finished beer, referring to the amounts contained in raw materials, were 1.5±0.8% for AFB₁ and 50.7±4.7% for FB₁. These results were mainly due to the different solubility of the two mycotoxins during the mashing process. If raw materials comply with the limits fixed by the EC regulation, the contribution of a moderate daily consumption of beer to AFB₁ and FB₁ intake does not contribute significantly to the exposure of the consumer.

25 Keywords: aflatoxin B₁, fumonisin B₁, brewing, beer, maize grit, malted barley.

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In Italy it is common practice to brew beer from a mixture of malted barley and maize grit; according to the Italian Regulation (Gazzetta Ufficiale della Repubblica Italiana 1962) and its subsequent modifications, adjuncts (usually maize-derived products), can replace malted barley up to a maximum of 40%. The natural occurrence in maize of mycotoxins such as aflatoxins, fumonisins, trichothecenes and zearalenone, has been reported in various studies. Nearly 91% of the Italian production of maize is grown in northern Italy (Istat 2006). In this area, which ranges from Piedmont to Friuli Venezia Giulia, the mycotoxins that deserve special attention are aflatoxins, for their extreme acute and chronic toxicity, and fumonisins for their high and widespread contamination, that cause several disease states in animals and could affect human health (Pietri et al. 2004; Battilani et al. 2005).

Aflatoxins B_1 , B_2 , G_1 and G_2 are mainly produced by Aspergillus flavus and A. parasiticus, fungal species that grow in warm conditions in tropical and sub-tropical regions of the world. Aflatoxin B_1 (AFB₁), the prevalent and most toxic aflatoxin, is a potent genotoxic carcinogen in laboratory animals and there is strong evidence that it is a liver carcinogen in humans. The International Agency for Research on Cancer (IARC) has classified AFB₁ as a Class 1 (human carcinogen) (IARC 1993). Food and health bodies have not set a tolerable daily intake (TDI) as AFB₁ is a genotoxic carcinogen; therefore, contamination in food should be reduced to the lowest possible level.

Fumonisins B₁, B₂ and B₃ 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. Fumonisins have been shown to be hepatocarcinogenic in male rats and female mice and
nephrocarcinogenic in male rats (Voss et al. 2001). Fumonisin B₁ (FB₁), the prevalent and most toxic
fumonisin, has 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 FB₁

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might increase the risk of neural tube defects in populations consuming large amounts of fumonisincontaminated maize (Missmer et al. 2006). FB₁ has been categorised by the IARC as class 2B (possible human carcinogen) (IARC 2002).

As a consequence of the use of maize adjuncts, normally maize grit, in the brewing process, aflatoxins and fumonisins may potentially occur in beer; moreover, aflatoxins can be occasionally found also in malted barley (Fukal et al. 1990). Surveys on different types of beer reported that AFB₁ was detected in 5 of 8 samples imported from Mexico and in one from Brazil (0.0028-0.049 μ g l⁻¹) (Scott and Lawrence 1997), in 2 of 22 (0.0005 and 0.0008 µg l⁻¹) and in 11 of 94 (0.0005-0.0831 µg l⁻¹) Japanese and imported beer samples (Nakajima et al. 1999). Successively, in a survey on 304 samples of Canadian and imported beer, the toxin was found in 4 of 5 samples from India (0.096- $0.230 \ \mu g \ l^{-1}$, 2 of 6 from Spain, 1 of 5 from Portugal and in 5 of 49 from Mexico (0.0044-0.018 $\ \mu g \ l^{-1}$) (Mably et al. 2005). Regarding FB₁, several surveys reported concentrations generally lower than 100 µg l⁻¹ (Scott and Lawrence 1995; Kawashima et al. 2007; Torres et al. 1998; Hlywka and Bullerman 1999; Dasko et al. 2005). Generally, with the exception of a survey on 75 bottled Kenyan lager beers (Mbugua and Gathumbi 2004), much higher levels of AFB₁ and FB₁ were found in some types of traditional African beer (aflatoxins: 200-400 μ g l⁻¹; FB₁: 38-1066 μ g l⁻¹) (Scott 1996; Odhav and Naicker 2002; Shephard et al. 2005).

Since aflatoxins (AFs) and fumonisins (FBs) can be present in beer, the aim of this study was to investigate, during full scale industrial brewing process, the carry-over of AFs and FBs from naturally contaminated raw materials to the different intermediate and final products.

Materials and methods

Brewing process

The study was carried out in an industrial brewery, located in northern Italy, that produces bottom fermented pilsner type beer from a mixture of malted barley and de-germed maize grit (Figure 1) with

a particle size > 500 µm. First, finely ground malted barley and maize grit were mashed in order to convert starch into fermentable sugars and dextrins and to provide mashing liquors with nitrogenous fractions which can be assimilated by yeast. The mashing process was performed according to a single decoction process, which requires two different kettles to be completed. In the adjunct kettles, a fraction of the finely ground malted barley and maize grit were boiled with water (cooked mash); in the second tun, finely ground malted barley and water were mixed and heated at two different saccharification rest temperatures according to a specific protocol (malt mash). In this process the content of the cooked mash was added back to the main malt mash to increase the main malt mash temperature up to the first saccharification rest (decoction process). The following are the essentials of the mashing operations. Malted barley (≅800 kg), maize grit (≅5000 kg) and water (≅15000 l) were mixed in the adjunct kettle and held at 65 °C for 17 min. Then, the temperature was increased to 99.5 °C in 40 min and the mash was boiled for 20 min. In the main mash tun, finely ground malted barley (≅7000 kg) and water (≅20000 l) were mixed at 37 °C for 15 min and the mixture was allowed to stand at 37 °C for 15 min. Then, the cooked mash was added and the temperature was raised to 63 °C. A first stand for 45 min was programmed. Successively, the temperature was increased to 69 °C and a second shorter stand (20 min) programmed. Finally, heating up to 78 °C (mashing off) in 20 min preceded the separation of the sweet wort. During the process, the pH of the malt mash was adjusted to 5.6±0.1 by means of phosphoric acid. No addition of exogenous enzymes occurred during mashing nor acidification by means of lactic acid bacteria.

The mashing liquors were separated from spent grains by means of a Meura filter, which is a thin bed membrane-assisted mash filter (*lautering*). Lautering occurred in two stages: 1) the running off of the first wort, that is the aqueous solution of the extract recovered from mashing; 2) the sparging of the spent grains at 78 °C to wash out the extract retained by the spent grains. Sparging was performed until the last run off wort concentration was about 1.6 °P. At the end of the filtration process, spent grains were discharged. Sweet wort and spargings were gathered in a buffer tank which served the

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wort boiling kettle. Successively, sweet wort was boiled for 1 h and hopped (boiling and hopping) using a combination of CO₂ hop extract (53.4% α -acids per 500 g of product) and type 90 pellets (15% α-acids per 1 kg of product) in a fixed proportion, in order to obtain a cast wort of 35 European Brewery Convention (EBC) bitter units. During boiling, a small amount of water evaporated (maximum 4%). Then, the cast wort was treated in a whirlpool at 90 °C, for 20 min in order to separate protein-polyphenol aggregates formed during wort boiling (hot trub). After removal of the hot trub (about 4000 l), the wort was cooled at 15 °C, aerated to avoid adverse yeast behaviors during cell division and pitched (pitching rate 0,007 kg l^{-1}) with a lager strain of Saccharomyces cerevisiae, originating from a former Hürlimann brewery collection (fermentation).

The fermentation was performed in 140000 l horizontal stainless steel vessels at 15 °C for an average period of 10 days; normally a small amount of water was added (about 2500 l). Fermentation time may vary according to the reduction of the diacetyl level in green beer below 50 μ g kg⁻¹. Then, the green beer was cooled at 7 °C for 2 days. At the end of fermentation, beer was centrifuged. Then, beer was clarified and stabilized by using diatomaceous earth and polyvinyl polypyrrolidone (PVPP) as the main filter aids, respectively. A sheet type filter was used for clarification, supplied with a fully automatic kieselguhr dosing unit. Stabilization with PVPP was performed in a recycling process. The stabilizing solution was made dissolving 350 kg of PVPP in 5000 l of water at 80 °C; PVPP dosing into beer was set at 0.35 g l^{-1} (*stabilization*). Successively, dilution to the final beer alcohol content was performed by means of de-aerated water. A trap filter, installed downstream, helped retain kieselguhr particles that bleed through the sheet filter and PVPP fines. Finally, the finished beer was recovered in 100000 l horizontal bright beer tanks; after bottling, the beer was tunnel pasteurized; the bottled beer was kept at 64 °C for 20 min and then cooled to room temperature.

24 Samples

In accordance with the brewing program, four brews (A, B, C and D) were brewed on a 65000 l

scale. Wort A and B were fermented in two different horizontal fermentors with a capacity of 140000 1. Wort C and D were jointly fermented using a horizontal fermentor of equal full capacity. The utilization of fermentors of such size, usually used for the production of extremely limited volumes of specialty beer in substitution of classical 450000 l cylindroconical units, helped us optimize the sampling plan and avoided there being an overwhelming number of samples to be monitored for each brew at the fermentation stage. Sampling of malted barley and maize grit was performed according to the EC Regulation 401/2006 (Commission of the European Communities 2006a). Primary samples of about 200 g of malted barley and maize grit were separately collected in continuum at a fixed and constant flow rate by means of a sampling flux deviator applied to the raw material conveyors, which connected the weighing machines to the silos. Using this flux deviator, 40 incremental samples were collected in a time period as long as the time required for the off-take of a single brew; two aggregate samples of about 8 kg of malted barley and maize grit were obtained. Samples of intermediate products and finished beer were taken at the end of the corresponding process (numbered in figure 1). For these products, two samples of 1.5 kg or liter were collected and put together. Solid samples (malted barley and maize grit) were ground with a hammer mill (1 mm sieve) and homogenized for 10 min using a horizontal mixer. From the industrial plant were collected: malted barley (1), maize grit (2), malt mash (3), sweet wort (4), boiled wort (5), wort after whirlpool process (6), hot trub (7), green beer (8), beer after kieselguhr filtration (9), beer after PVPP stabilization (10), beer after water dilution and trap filtration (11), bottled beer (12). All the samples were stored at -20 °C until the time of analysis. For each brewing, the amounts of malted barley and maize grit and the volumes of water, wort and beer are shown in table 1. The volumes were measured in 1 with an approximation of ± 200 l. These data derived from measurements carried out by plant-technicians at the time of brewing.

24 Final product

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The bottled beer was a standard product, whose main parameters are shown in table 2. The parameters were measured by means of an Alcolyzer Plus (Anton Paar GmbH, Graz, Austria) coupled with a DMA 4500 density meter and with a SP-1m automatic sample changer. Bitterness and diacetyl were measured in compliance with Analytica EBC methods 9.8 and 9.24.1, respectively (European Brewery Convention, 1998).

7 Analytical Standards

Aflatoxin and fumonisin standards were obtained from Sigma-Aldrich (St. Louis, MO, USA). For each aflatoxin, a stock solution of 5-8 μ g 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, 2005a). The working standard solution was prepared after evaporation under nitrogen of an aliquot (50 µl) of the stock solution and re-dissolution in chloroform (10 ml) by ultrasonication. An aliquot (50 or 100 µl) of this solution was evaporated under nitrogen and re-dissolved in the HPLC mobile phase (1-25 ml), to obtain calibrant solutions at individual concentrations of AFB₁, AFB₂, AFG₁, AFG₂ between 0.1 and 6 μ g l⁻¹. FB₁ and FB₂ (10 mg) were separately dissolved in 50 ml acetonitrile:water (1:1, v/v). These solutions were diluted to obtain HPLC calibrant solutions in acetonitrile:water (30:70, v/v) at individual concentrations of FB₁ and FB₂ between 2.5 and 50 μ g l⁻¹.

19 Analysis for aflatoxins

Regarding solid samples, aflatoxins were extracted from 25 g of sample with 250 ml acetone:water (85:15 v/v) according to the AOAC method 2003.02 (AOAC International 2005b). For liquid samples with solid materials (cooked and malt mash), 120 ml of acetone was added to a homogenized aliquot of 50 g. The extraction was performed using a rotary-shaking stirrer for 45 min. After filtration of the extracts 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).

For liquid samples (wort and beer), 20 ml was diluted with 20 ml of phosphate buffered saline (PBS, pH 7.4) and the solution was shaken for about 1 min. The pH was controlled and adjusted, if necessary, between 6.5 and 7.5 using NaOH 0.1 N; then the solution was purified through the immunoaffinity column.

After washing of the column with 5 ml of distilled water, aflatoxins were eluted into a graduated glass vial with methanol (2.5 ml). The eluate, evaporated under a gentle stream of nitrogen, was redissolved in 1 ml of 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 μ m) and injected (30 or 100 μ l).

Analysis was performed using an HPLC instrument, consisting of two PU-1580 chromatographic pumps, an AS 1555 sampling system, an 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.

21 Analysis for fumonisins

Regarding solid samples (malted barley, maize grit, spent grains), fumonisins were analysed according to the method proposed by Visconti et al. (2001). Fumonisins were extracted from 20 g of sample in a plastic centrifuge bottle with 100 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

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supernatant was poured into a flask. Another 100 ml of the same solution was added to the residue in
the centrifuge bottle, and a second extraction performed for 30 min; then, the extracts were combined.
For liquid samples with solid materials (cooked and malt mash), 40 ml of acetonitrile:methanol (50:50
v/v) were added to a homogenized aliquot of 50 g; the extraction was performed using a rotaryshaking stirrer for 45 min. After filtration of the extracts through a folded filter-paper, an aliquot of the
filtrate (2 ml) was diluted with 20 ml of PBS and purified through an immunoaffinity column (RBiopharm Rhône LTD, Glasgow, Scotland, UK).

8 For liquid samples, 20 ml was diluted with 20 ml of PBS and the solution was shaken for about 1 9 min. The pH was controlled and adjusted, if necessary, between 6.5 and 7.5 using NaOH 0.1 N; then 10 the solution was purified through the immunoaffinity column.

After washing the column with PBS (2 ml), 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), fumonisins were separated on a Betasil RP-18 column (5 µm particle size, 150x2.1 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 and 706 m/z for FB₁ and FB₂, respectively), the argon collision pressure was set to 1.5 mTorr and the collision

1 energy to 36 V. The selected fragment ions were 704, 352 and 334 m/z for FB₁ and 688, 336 and 318

m/z for FB₂. Quantitative determination was performed using LC-Quan 2.0 software (Thermo-Fisher).

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

5 Other mycotoxins

6 Malted barley and maize grit were also analyzed for deoxynivalenol (DON), zearalenone (ZEA) and 7 ochratoxin A (OTA) by methods routinely used in our laboratory (Rossi et al. 2006; Pietri et al. 2004); 8 since very low levels were found in all samples (DON < 200 μ g kg⁻¹, ZEA < 20 μ g kg⁻¹ and OTA < 9 0.2 μ g kg⁻¹), the carry-over of these mycotoxins was not investigated.

Results and discussion

12 Performance of the methods

Percentage recoveries were determined by spiking known blank maize grit samples at levels of 2 and 14 1000 μ g kg⁻¹ and blank beer samples at levels of 0.1 and 50 μ g kg⁻¹ for aflatoxins and fumonisins, 15 respectively. Average recovery values were: 97.8 ±1.6% and 98.3±1.5% for AFB₁, 94.9±2.3% and 16 97.6±1.8% for AFB₂, 95.5±1.9% and 97.6±1.7% for FB₁, 93.7±3.0% and 95.5±1.9% for FB₂, in maize 17 grit and beer, respectively. Recovery values higher than 90% were also obtained for malt mash and 18 boiled wort; for the other liquid intermediate products, being very similar to boiled wort and beer, 19 recovery experiments were not carried out.

Certified reference materials (ground corn, R-Biopharm Rhône LTD), with AFB₁ and fumonisin contamination of 4.1±1.0 μ g kg⁻¹ and 2500±600 μ g kg⁻¹ (FB₁=1600, FB₂=700, FB₃=200 μ g kg⁻¹), respectively, were also analysed; our results were $4.2\pm0.2 \ \mu g \ kg^{-1}$ for AFB₁, 1553±39 and 721±21 μg kg^{-1} for FB₁ and FB₂, respectively. The results of the analyses were not corrected for recovery; the relative standard deviations were always <13% for AFB₁ and <11% for FB₁. The limits of detection (LOD, signal-to-noise ratio of 3:1) and of quantification (LOQ, signal-to-noise ratio of 10:1) were

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respectively: 0.005 and 0.01 μ g kg⁻¹ for AFB₁, 0.01 and 0.02 μ g kg⁻¹ for AFB₂, 30 and 50 μ g kg⁻¹ for FB₁ and FB₂, in maize grit; 0.001 and 0.002 μ g l⁻¹ for AFB₁, 0.002 and 0.004 μ g l⁻¹ for AFB₂, 1 and 2.5 μ g l⁻¹ for FB₁ and FB₂, in beer.

AFB₁ ranged in maize grit from 0.31 to 14.85 µg kg¹; it was also found in malted barley, at levels ranging from 0.20 to 4.07 μ g kg⁻¹ (table 3). Malted barley and maize grit used for brewing A and maize grit for brewing B showed concentrations higher than $2 \mu g kg^{-1}$, the maximum level fixed by EC Regulation for all cereals and all products derived from cereals, including processed cereal products (Commission of the European Communities 2006b). As regards the other aflatoxins, only AFB₂ was detected in maize grits used for brew A and B production (1.2 and 0.7 μ g kg⁻¹, respectively); however, it resulted <LOD in the bottled beer and therefore the very low levels found in some intermediate products are not reported. For each stage of the brewing, the AFB₁ percentage in the product, referring to the total amount in the raw materials (TARM), was calculated ((conc_{product}*kg(or l)_{product})/(conc_{malted} barley*kgmalted barley+concmaize grit*kgmaize grit)*100). A relatively small amount of the toxin was transferred to the liquid phase during mashing; in fact, averaging the 4 brewing results, only 14.3±2.8% of the AFB₁ TARM was found in the liquor after mashing: this is probably due to the low solubility of the toxin in water. In the sweet wort the AFB₁ level was reduced both because of the dilution of the product, and mainly because of the filtration step: in effect, lautering caused a consistent reduction (- $45.4\pm14.3\%$) of the AFB₁ present in the liquor and only an average of $7.9\pm3.0\%$ of the AFB₁ TARM was found in the sweet wort. In the following steps, the reductions were more moderate (boiling -28.5±10.5%, whirlpooling -2.6±14.9%, fermentation -14.3±9.5%), while a further strong decrease (-59.6±17.3%) was observed passing from green to bottled beer. The percentages of AFB₁ TARM found in the bottled beer varied from 0.6 to 2.2% (mean $1.5\pm0.8\%$) and the concentrations, because of the high dilution, were in the low part-per-trillion level. These results are in agreement with a previous

study (Steiner and Länzlinger 1978), where about 5% of AFB₁ and AFB₂ were recovered in the final beer brewed from fortified malt. Differently, Chu et al. (1975), in a micro-brewing process, after addition of AFB₁ (dissolved in chloroform) at 1 and 10 μ g g⁻¹, at the beginning of mashing, found 18 and 27% of the toxin, respectively, in bottled beer; Okoye (1986) found a carry-over of 41% from red guinea corn into a Nigerian native beer. These higher recoveries were likely due to the modality of the trials and to the very high level of spiking.

8 Fumonisin B₁

 The FB₁ levels in maize grit varied from 1146 to 3194 μ g kg⁻¹ (table 4); FB₂ occurred as well, at levels from 347 to 908 μ g kg⁻¹. Since FB₂ was not detected in the bottled beer, the very low levels found in some intermediate products are not reported. Only in the case of maize grit used for brewing B (FB₁+FB₂=1493 μ g kg⁻¹), was the concentration of fumonisins close to the level fixed by the EC Regulation 1126/2007 (Commission of the European Communities 2007) for milling fractions of maize with particle size > 500 μ m not intended for direct human consumption (1400 μ g kg⁻¹); on the contrary, the fumonisin level exceeded the limit in the case of the grit used in the other brewing processes. Fumonisins were not detected in malted barley. For each stage of the brewing, the FB₁ percentage in the product, referring to the TARM, was calculated ((conc_{product}*kg(or l)_{product})/(conc_{maize} grit*kg_{maize grit})*100). Unlike AFB₁, most of the FB₁ entering the brewing was extracted into the mashing liquors (average $84.3 \pm 10.3\%$). During the following steps, the reduction was quite low: $-7.3\pm2.5\%$ after lautering, $-8.3\pm3.6\%$ at the end of the boiling process, $-10.3\pm2.5\%$ after whirlpooling. Likewise, the fermentation process did not appreciably decrease the amount of FB₁ (-4.2 \pm 2.6%); this was in agreement with the study of Scott et al. (1995), in which a loss of FB₁ in the range 3-28% was estimated. Finally, a further low reduction (on average -16.4±10.0%) was observed from green beer to bottled beer. The clarification and stabilization processes, which employed kieselguhr and PVPP, respectively, did not appreciably reduce the amount of FB1. These materials showed a nearly full

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retentive indifference towards FB₁; this held true also for celite used for the brewing process, as reported by Belajova et al. (2007). In bottled beer $50.7\pm4.7\%$ of the FB₁ TARM was recovered; the concentrations varied from 37 to 89 µg l⁻¹. These results indicate that FB₁ can occur in beer when maize-based solid adjuncts are used. FB₁ occurrence in finished beer is mainly due to its high solubility in water and to the relative stability to heat treatment.

7 Conclusions

The four industrial brewing processes gave quite similar results; as expected, AFB₁ and FB₁ showed different behaviors during brewing, mainly due to their different solubility in water; however, also the process determined a different transfer rate of the two solubilised toxins into the bottled beer (10.5 vs. 60.1% for AFB₁ and FB₁, respectively). Consequently, the concentration of AFB₁ retrieved in bottled beer was very low. In Italy the consumption of beer is about 30 l per person year⁻¹ (Assobirra 2007), corresponding to 0.082 l per person day⁻¹; if a consumer drinks beer with contamination like that obtained from brewing A, the AFB₁ intake would be 1.8 ng or 0.03 ng kg⁻¹ b.w., that is 3.6-0.5% of the estimated range of daily exposure to total aflatoxins in Italy (0.838-1.934 ng kg⁻¹ b.w.: EFSA 2007). When the EU limits for AFB_1 in raw materials are respected, like in brewing C+D, the corresponding figures for a consumer are 0.12 ng of AFB₁ or 0.002 ng kg⁻¹ b.w., that is 0.2-0.1% of the estimated range of daily exposure in Italy. In the latter case, the contribution of beer to exposure is negligible.

Analogous precautions should be taken for the fumonisin contamination in maize adjuncts. If the fumonisin contamination clearly exceeds the EU limit, as in maize adjuncts used in brewing C+D, a daily exposure of 0.12 μ g kg⁻¹ b.w. can be calculated for a consumer, that represents about 6% of the TDI of 2 μ g kg⁻¹ b.w., fixed by the Scientific Committee on Food (2000). If the EU limit is respected, as in maize adjuncts used in brewing B, the corresponding figure for a consumer is 0.05 μ g kg⁻¹ b.w. per day, that represents only about 2% of the TDI.

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However, it must be considered that in Italy about 2% of males and about 3% of young males (25-44 years) consume more than 0.5 l of beer; for this fraction of population, the calculated mean exposure must be multiplied by six or more.

It is evident that when the raw materials used in the brewing process exceed the EU limits for AFB₁ and FB₁, beer can become a more significant source of exposure to the two toxins.

7 Aknowledgement

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Table 1. Amounts (kg) of malted barley and maize grit and volumes (l) of wort and beer.

	brewing A	brewing B	brewing C	brewing D
Malted barley	7633	7885	7853	7857
Maize grit	4999	5036	5000	5000
Water added in mash	37500	35000	34600	35500
Sweet wort	69700	68000	70800	70700
Boiled wort	69500	66900	69000	69000
Wort	65700	62900	65000	65000
Hot trub	3800	4000	4000	4000
Green beer	67700	66000	133	000
Filtered beer	67500	65800	128	000
Stabilized beer	67500	65800	128	000
Diluted beer	81500	79400	156	500
Bottled beer	81500	79400	156	500

Table. 2. Main parameters of bottled beer.

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Table 3. Transfer of a flatoxin B_1 (mean of three replicates) from raw materials to the different intermediate and final products during the brewing
processes.

	brewing A		brewing B		brewing C		brewing D		
	µg kg⁻¹	Percentage	µg kg⁻¹	Percentage	µg kg⁻¹	Percentage	µg kg⁻¹	Percentage	
	or µg l ⁻¹	$(\%)^{(1)}$	or $\mu g l^{-1}$	$(\%)^{(1)}$	or $\mu g l^{-1}$	$(\%)^{(1)}$	or $\mu g l^{-1}$	$(\%)^{(1)}$	
Malted barley	4.07	100	1.36	100	0.38	100	0.20	100	
Maize grit	14.85	100	10.93	100	0.92	100	0.31	100	
Malt mash	0.437	15.6	0.202	10.7	0.038	17.3	0.012	13.6	
Sweet wort	0.178	11.8	0.046	4.8	0.009	8.4	0.003	6.8	
Boiled wort	0.114	7.5	0.033	3.4	0.008	7.3	0.002	4.2	
Wort	0.089	5.5	0.029	2.8	0.005	4.2	0.002	4.2	
Hot trub	0.141	0.5	0.024	0.2	0.008	0.4	0.003	0.4	
						brewii	ng C+D		
					μ	g l ⁻¹	Perce	ntage (%)	
Green beer	0.082	5.3	0.021	2.1	0	.003		3.6	
Filtered beer	0.060	3.8	0.019	1.9	0	.003		3.6	
Stabilized beer	0.058	3.7	0.019	1.9	0	.003		3.6	
Diluted beer	0.046	3.6	0.013	1.6	0	.002		2.9	
Bottled beer	0.022	1.7	0.005	0.6	0.	0015		2.2	

 $^{(1)} (conc_{product} * kg(or \ l)_{product}) / (conc_{malted \ barley} * kg_{malted \ barley} + conc_{maize \ grit} * kg_{maize \ grit}) * 100.$

Table 4. Transfer of fumonisin B_1 (mean of three replicates) from maize grit to the different intermediate and final products during the brewing processes.

	brewing A		brewing B		brev	wing C	brewing D		
	µg kg ⁻¹ Per		µg kg⁻¹	Percentage	µg kg⁻¹ or	Percentage	µg kg⁻¹	Percentage	
	or µg l ⁻¹	$(\%)^{(1)}$	or $\mu g l^{-1}$	$(\%)^{(1)}$	$\mu g l^{-1}$	$(\%)^{(1)}$	or $\mu g l^{-1}$	$(\%)^{(1)}$	
Malted barley	< 30	100	< 30	100	< 30	100	< 30	100	
Maize grit	1741	100	1146	1146		100	3194	100	
Malt mash	207	89.2	116	70.3	347	83.4	424	94.2	
Sweet wort	102	81.7	56	66.0	162	79.6	191	84.6	
Boiled wort	97	77.5	52	60.3	156	74.7	170	73.4	
Wort	95	71.7	50	54.5	145	65.4	159	64.7	
Hot trub	134	5.8	66	4.6	227	6.3	211	5.3	
						brewing	rewing C+D		
					μ	g l ⁻¹	Percer	ntage (%)	
Green beer	88	68.4	47	53.7	-	142		60.8	
Filtered beer	83	64.4	46	52.4		137		57.7	
Stabilized beer	81	62.8	45	51.3		132		55.6	
Diluted beer	62	58.1	38	52.3		98		50.5	
Bottled beer	59	55.2	37	50.9		89	4	45.9	

 $\label{eq:conc_product} ^{(1)}(conc_{product}*kg(or\ l)_{product})/(conc_{maize\ grit}*kg_{maize\ grit})*100.$

Page 23 of 23 Figure 1: layout of the industrial of difference of the industrial of



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