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Reduction of fumonisin B₁ in extruded corn breakfast cereals with salt, malt and sugar in their formulation

Abstract

The objective of this study was to determine the effect of added sodium chloride, barley malt and sucrose on the stability of fumonisin B₁ (FB₁) present in corn flour. Two levels of both sodium chloride (0.4 and 2%) and barley malt (0.8 and 5%) were added to the unextruded corn flour and six levels of sucrose (from 3 to 10%) were used. Addition of sucrose at the lowest salt content (0.4%) as well as addition of salt, either at 0.4 or 2%, led to a significant decrease of FB₁ levels in extruded samples whereas malt, either at 0.8 or 5%, did not significantly affect FB₁ stability. Decontamination rates depended on the concentrations of added ingredients and ranged from 2 to 92%. The greatest reductions in FB₁ content were achieved with extrusion cooking with high salt content whilst the lowest reductions were the result of processing corn flour with low contents of both salt and sucrose. Salt at 2% was the most effective ingredient in reducing FB₁ content of the final extruded food.

Keywords: barley malt, extrusion cooking, fumonisins B₁, salt, sucrose.

Introduction

Fumonisin are a group of related polar metabolites produced mainly by *F. verticillioides* and *F. proliferatum*, the most ubiquitous seed-borne fungi associated with corn (Weidenbörner 2001). They have been shown to be the causative agents of several toxicoses, such as equine leucoencephalomalacia (ELEM), porcine pulmonary edema (PPE), toxicity to poultry and hepatosis and nephrotoxicity in rodents (Norred & Voss 1994). Epidemiological evidence has also linked fumonisin contaminated corn consumption with the high incidence of human esophageal cancer in Transkei region of South Africa, Lixian country of China and north eastern Italy (Sydenham et al. 1990, 1991, Chu & Li 1994, Doko & Visconti 1994). Current investigations are also aiming to

1 clarify the effect of fumonisins on the heart. It has been suggested that cardiac effects
2 may occur at relatively low levels of exposure (Pitt & Tomaska 2001). The International
3 Agency for Research on Cancer (IARC) evaluated fumonisins and classified them in
4 Group 2B (possibly carcinogenic to humans) (IARC, 1993).

5 Fumonisin predominantly contaminate corn and corn based feed and food products. It
6 has been demonstrated that the highest levels of fumonisin B₁ (FB₁), which is the most
7 abundant and toxic contaminant in corn-based foods and feeds among the several
8 structurally related homologues (Lu et al. 2002), are found in those products that
9 undergo only a physical milling or grinding process, such as cornflour, corn polenta,
10 muffin mix, and corn grits. By contrast, low levels have been detected in more highly
11 processed products such as cornflakes, corn tortillas, and popcorn, where thermal
12 (baking, roasting, extrusion cooking) and alkali (nixtamalization) processes are involved
13 (Patel et al. 1997, Solovey et al. 1999, Machinski & Soares, 2000, Velluti et al. 2001).

14 Extrusion cooking was developed in the 1940s to make puffed snacks and ready-to-eat
15 cereals (RTE) from cereal flours or grits. In the 1960s, the process found numerous new
16 applications and during the 1970s, extension of its applications occurred (increasing
17 numbers of RTE cereals, salty and sweet snacks, croutons for soups and salads, dry pet
18 foods, biscuits, etc.). This technology is known as high temperature short time
19 technology (HTST) which produces a wide variety of final and intermediate foods and
20 feeds. It offers several advantages over traditional processes such as versatility, high
21 quality of resulting products, processing of relatively dry viscous materials, minimum
22 nutrient degradation and significant improvement in digestibility and biological value of
23 proteins (Riaz 2001).

24 The high shear, pressure and temperature reached inside the screw channel result in
25 mixing and forming the material and lead to several chemical reactions, such as
26 gelatinization of starch, denaturation of proteins, inactivation of many food enzymes
27 and reduction of microbial counts (Gopalakrishna & Jaluria 1992). Due to the evident
28 advantages over the traditional cooking techniques, extrusion cooking is being widely
29 applied in the industrial cereal processing plants. Although its main aim is not that of
30 decontamination of raw material, it has been demonstrated that extrusion cooking can
31 lead to reduction in mycotoxin levels in different cereals (Cheftel 1989, Accerbi et al.
32 1999, Piñeiro et al. 1999, Castells et al. 2006). As the potential of extrusion cooking in

1 decontaminating cereal based foods was already demonstrated in earlier studies, the
2 present study tried to assess the effect of industrially used ingredients, salt, barley malt
3 and sucrose, on the stability of FB₁ during extrusion cooking of naturally contaminated
4 corn flour.

6 **Material and methods**

7 *Corn flour*

8 Naturally contaminated corn flour with a concentration of FB₁ of $1.74 \pm 0.25 \mu\text{g g}^{-1}$ (dry
9 weight basis) and a moisture content of ca. 14% was used. The moisture content of the
10 flour was raised from initial 14 to 24% by adding distilled water to the flour. A
11 preliminary curve was constructed to show the relationship between the volume of
12 added water and the resulting moisture content (%). Based on the resulting linear
13 regression, 0.098 mL of water per gram of corn flour was added for a moisture content
14 of 24%. Rehydrated flour was placed into sealed containers and allowed to equilibrate at
15 5 °C with periodic hand-shaking during 48 h.

16 Barley malt was supplied by Moravia S.A. (malting company), Lleida, Spain. Salt
17 (Prolabo, VWR International, Fontenay-sous-Bois, France) and sucrose (Prolabo, VWR
18 International, Fontenay-sous-Bois, France) were thereafter added to rehydrated corn
19 flour. Finally, the flour was thoroughly mixed until a homogenous dough was obtained.

20 *Extrusion cooking*

21 A laboratory-scale single screw extruder (Compact E 19/25 D; Brabender, Duisburg,
22 Germany) with a 19/25D screw, a compression ratio of 3:1 and a 3-mm-diameter
23 cylindrical die was used.

24 Corn flour wetted to 24% was extruded at 140 °C at a screw speed of 100 rpm. A
25 temperature of 140 °C was chosen because in a preliminary study where temperatures of
26 140, 160 and 180 °C were set up, it was observed that lowest temperature resulted in a
27 product whose texture resembled industrially processed RTE breakfast cereals at most.
28 A screw speed of 100 rpm resulted in 45 s of the dough inside the barrel, an
29 intermediate level of residence time, as measured by interpolating the screw speed value

1 in a curve previously obtained which correlated screw speed values with residence times
2 (data not shown).

3 A three-factorial design with salt, barley malt and sucrose concentrations as factors
4 affecting the stability of FB₁ was carried out. Two levels of both salt (0.4 and 2%) and
5 malt (0.8 and 5%) were set up whereas six levels were used for sucrose (3, 4.4, 5.8, 7.2,
6 8.6 and 10%). These levels were chosen based on a patent on breakfast cereals
7 processing (Vollink 1962). This design yielded 24 treatments which were carried out in
8 triplicate.

9 For each replicate, 650 g of corn flour was extruded. The extruder was neither stopped
10 nor cleaned between samples hence a portion of the next sample was used to purge the
11 extruder. For each extruded sample, 150 g was collected, ground and stored at -18 °C
12 until analyzed.

13 *Moisture analysis*

14 The air oven method was used to measure the moisture content of the extruded and
15 unextruded samples; each sample (10 g) was dried in an oven at 105 °C for 17 h. FB₁
16 concentrations were then calculated in a dry weight basis.

17 *Extraction and determination of FB₁*

18 FB₁ in corn flour before and after extrusion was determined by high performance liquid
19 chromatography (HPLC). The CEN (European Committee for Standardization) method
20 (2002) was adopted with slight modifications. Briefly, 20 g ground sample was
21 extracted with 40 mL 75+25 methanol:water for 15 min using a magnetic stirrer. A 15-
22 25 mL aliquot of the extract was filtered through a fluted filter paper.

23 Bond-Elut SAX (strong anion exchange) columns (Varian, Harbor City, CA., USA)
24 were attached to a vacuum manifold and conditioned with 5 mL of methanol followed
25 by 5 mL of 75+25 methanol:water solution. Aliquots of 10 mL of the filtered sample
26 extracts were then loaded into the columns and allowed to flow at a rate of no more than
27 2 mL min⁻¹. The cartridges were then washed with 8 mL of methanol:water (75+25)
28 followed by 3 mL of methanol. The rinses were discarded and fumonisins were eluted
29 with 10 mL methanolic acetic acid 1% at a flow rate of 1 mL min⁻¹.

1 The eluate was evaporated to dryness in a rotavapor, redissolved in methanol,
2 transferred to a vial, evaporated to dryness again under a gentle stream of nitrogen at ca.
3 40 °C and dissolved in 1 mL methanol for HPLC.

4 50 µL of the extract were derivatized with 450 µL o-phthalaldehyde reagent (OPA)
5 prepared according to the method. The samples were injected into HPLC within 1 min
6 after derivatization.

7 The HPLC system was equipped with a 474 fluorescence detector (Waters, Mildford,
8 MA, USA) (λ_{exc} 335 nm; λ_{em} 440 nm) and a C₁₈ column (Waters Spherisorb 5 µm,
9 ODS2, 4.6x250 mm), all under control of Waters Millennium³² software. The analysis
10 was performed under isocratic conditions at a flow rate of 1 mL min⁻¹ of the mobile
11 phase (methanol:0.1M sodium phosphate (75+25) adjusted to pH 3.35 with ortho-
12 phosphoric acid). A standard curve was constructed with FB₁ levels ranging from 0.1 to
13 2 µg mL⁻¹. FB₁ was quantified by interpolating peak areas of the sample extracts in the
14 standard curve. The detection limit of the method was 0.04 µg g⁻¹.

15 The recovery rates obtained by spiking corn flour samples with 0.1, 1 and 10 µg FB₁ g⁻¹
16 in triplicate were 119, 108 and 100%, respectively. Regarding repeatability, the RSDr
17 was 21.1, 16.7 and 7.4 for 0.1, 1 and µg FB₁ g⁻¹, respectively.

18 *Statistical analysis*

19 In order to determine the effect of added salt, malt and sucrose on FB₁ content present
20 in corn flour, final FB₁ concentrations resulting from each treatment were transformed
21 to a percentage according to the initial content in unextruded corn flour, which were
22 analyzed by analysis of variance (ANOVA) using SAS program ($\alpha = 0.05$) version 8.2
23 (SAS Institute Inc., Cary, NC, USA). Significant mean differences were assessed by
24 Duncan's test ($\alpha = 0.05$).

26 **Results and discussion**

27 Salt, malt and sucrose concentrations were foreseen as factors affecting the stability of
28 FB₁ due to their common use in industrial extrusion processing plants. These ingredients
29 are basic in ready-to-eat breakfast cereal production at different concentrations.

1 Extruded samples were significantly affected by the addition of salt and sucrose while
2 malt did not affect them (Table I). The more obvious effect observed was that the higher
3 the salt content, the greater the decontamination achieved in the extruded product
4 (Figure 1).

5 (Insert Table I about here).

6 (Insert Figure 1 about here).

7 The mean reduction in FB₁ levels when processing with 2% salt was as high as 91%
8 whereas when processing corn flour with 0.4% of salt, the mean reduction was 24% of
9 the initial FB₁ content. The biggest differences in decontamination rates between
10 processing with high and low salt levels were observed with corn flour containing 3%
11 sucrose; in this case, the reduction in FB₁ which was achieved when processing with 2%
12 salt was almost 10-fold the reduction achieved at 0.4%. The fact that salt addition
13 significantly affected FB₁ initial content is consistent with studies carried out by
14 previous authors who also observed a significant effect of different types of salts in
15 decontaminating cereals. Abramson et al. (1995) demonstrated that levels of
16 deoxynivalenol (DON) were significantly reduced when sodium carbonate was added to
17 contaminated barley, which was heated at 80 °C for different time periods. As observed
18 in the present study, reductions with the highest salt content were greater than those
19 reached with the lowest ones. Trenholm et al. (1992) also demonstrated the
20 effectiveness of sodium carbonate to reduce DON and zearalenone (ZEN) present in
21 both barley and corn. Washing contaminated grain with sodium carbonate increased
22 DON and ZEN removal above that reached with water alone. In the case of extrusion
23 cooking, the effect of the addition of sodium metabisulphite, ammonium hydroxide and
24 ammonium bicarbonate on aflatoxin stability was studied. Greater aflatoxin
25 decontamination rates were found when extrusion cooking of corn and peanut meal
26 containing the above mentioned additives than when processing raw material alone
27 (Cheftel 1989, Hameed 1993, Cazzaniga et al. 2001). By contrast, other additives used
28 during extrusion processing, such as calcium hydroxide, were not found to affect the
29 stability of total aflatoxins present in extruded corn meal (Hameed 1993).

30 Statistical analysis also demonstrated that sucrose addition had a significant effect on
31 FB₁ reduction; its effect, however, depended on the added salt concentration, as

1 demonstrated by the two-way interaction between salt and sucrose. Even though the
2 main effect of sugar addition proved to be significant, reduction of FB₁ was not
3 significantly affected by sucrose addition when corn flour contained 2% salt. By
4 contrast, when processing with 0.4% salt, sucrose significantly affected FB₁ stability, as
5 seen in Figure 1. Nevertheless, and even though the observed trend was that greater
6 decontamination rates were reached as sucrose concentration increased,
7 decontamination rates reached in samples containing medium sucrose levels (5.8 to
8 8.6%) and low salt (0.4%) levels were not significantly different.

9 This is not the first study reporting on the potential of sugars in decreasing mycotoxin
10 contamination; Murphy et al. (1996) concluded that the stability of FB₁ present in a
11 model system was affected by the presence of fructose and glucose due to the non-
12 enzymatic browning reaction of the reducing sugar with the amino group present in its
13 structure. Stability of FB₁ was also studied during the extrusion cooking of corn grits
14 containing three types of sugars at levels of 2.5 and 5% (Castelo et al. 2001). Significant
15 reductions were obtained when glucose was added into the corn grits, with FB₁
16 decontamination ranging from 40 to 92%. Contrary to the significant effect of sucrose
17 found in the present study, they demonstrated that neither added sucrose nor fructose
18 significantly affected FB₁ levels in extruded corn grits. However, they found that even
19 though the reduction resulting from processing with sucrose was not statistically
20 relevant, a decontamination rate of 19-39% was achieved. As sucrose is a non-reducing
21 sugar, covalent binding to FB₁ via the Maillard browning reaction is not possible, as
22 reported earlier (Murphy et al. 1996). The fact that in the present study levels of FB₁
23 were reduced during processing of corn flour mixed with sucrose may be the result of
24 the formation of conjugates between the sugar and FB₁ with a modified chemical
25 structure which could not be detected by the HPLC procedure used, as suggested by
26 Park et al. (2004). Seefelder et al. (2003) performed a model system in an attempt to
27 explain the fate of FB₁ during thermal food processing, where FB₁ was incubated with
28 sucrose at 150 °C for 40 min. They demonstrated that a covalent binding was formed
29 between sucrose and the mycotoxin. The formation of this conjugate was also studied
30 when spiked corn grits were extrusion cooked at 180 °C (Seefelder et al. 2001), where
31 reduction in FB₁ initial levels ranged from 68 to 77%. The potential of FB₁ to bind to
32 food matrix ingredients in heat-processed foods was also reported by Kim et al. (2003)

1 and Park et al. (2004). They demonstrated that there was 1.3 to 2.6 times more FB₁-
2 protein conjugate than native FB₁.

3 (Insert Figure 1 about here).

4 Contrary to what was observed for salt and sugar, malt addition was found not to reduce
5 FB₁ levels of extruded corn flour. Reductions in FB₁ levels (60%) with up to 5% malt,
6 irrespective of salt and sugar concentrations, were not statistically different (53%) from
7 those obtained at 0.8% malt content. Even though it was not statistically relevant, it was
8 observed that when corn flour contained the lowest salt content, higher reductions were
9 reached with 0.8% malt than with 5%. By contrast, at the highest salt content and at
10 4.4% of sucrose, greater reduction resulted from processing with 5% of malt, as can be
11 seen from Figure 1.

12 **Conclusions**

13 Our results demonstrate that the combination of extrusion cooking and the addition of
14 salt, malt and sucrose as food ingredients is an effective way that can decontaminate
15 corn flour by as much as 92% of FB₁. Nevertheless, it is important to emphasize that the
16 measured FB₁ concentrations in extruded samples might underestimate the toxicological
17 potential of the final extruded product due to the formation of conjugates resulted from
18 the binding of FB₁ to sucrose, with still an unclear toxicological activity. Therefore,
19 more research is needed to identify and chemically characterize fumonisin reaction
20 products resulted from the binding of FB₁ to food matrix ingredients such as sugars and
21 proteins.

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24 Ramón y Cajal Program).

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Table I. Effects of salt, malt and sucrose addition on FB₁ content in extruded corn flour product.

Source of variation	DF	MS	F-value
Salt	1	82527	1220.76*
Malt	1	155	2.30 ^{ns}
Sucrose	5	200	2.96*
Salt*malt	1	978	14.48*
Salt*sucrose	5	216	3.20*
Malt*sucrose	5	129	1.92 ^{ns}
Salt*malt*sucrose	5	115	1.71 ^{ns}

DF: degrees of freedom; MS: mean square. * significant $p < 0.05$; ^{ns} not significant

Figure 1. Percent (%) reduction of FB₁ in extruded samples containing different sucrose contents. Each bar (S: salt, M: malt) represents the average of 3 replicate runs. Bars with different letters (within each sucrose level) are significantly different ($p < 0.05$). Numbers above the bars indicate the mean FB₁ reduction level for each treatment.

