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Stability of mycotoxins during food processing

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Abstract

The mycotoxins that commonly occur in cereal grains and other products are not completely destroyed during food processing operations and can contaminate finished processed foods. The mycotoxins most commonly associated with cereal grains are aflatoxins, ochratoxin A, fumonisins, deoxynivalenol and zearalenone. The various food processes that may have effects on mycotoxins include sorting, trimming, cleaning, milling, brewing, cooking, baking, frying, roasting, canning, flaking, alkaline cooking, nixtamalization, and extrusion. Most of the food processes have variable effects on mycotoxins, with those that utilize the highest temperatures having greatest effects. In general the processes reduce mycotoxin concentrations significantly, but do not eliminate them completely. However, roasting and extrusion processing show promise for lowering mycotoxin concentrations, though very high temperatures are needed to bring about much of a reduction in mycotoxin concentrations. Extrusion processing at temperatures greater than 150 °C are needed to give good reduction of zearalenone, moderate reduction of alfatoxins, variable to low reduction of deoxynivalenol and good reduction of fumonisins. The greatest reductions of fumonisins occur at extrusion temperatures of 160 °C or higher and in the presence of glucose. Extrusion of fumonisin contaminated corn grits with 10% added glucose resulted in 75–85% reduction in Fumonisin B_1 levels. Some fumonisin degredation products are formed during extrusion, including small amounts of hydrolyzed Fumonisin B_1 and *N*-(Carboxymethyl) — Fumonisin B_1 and somewhat higher amounts of *N*-(1-deoxy-D-fructos-1-yl) Fumonisin B_1 in extruded grits containing added glucose. Feeding trial toxicity tests in rats with extruded fumonisin contaminated corn grits show some reduction in toxicity of grits extruded with glucose.

Keywords: Mycotoxins; Extrusion; Thermal Processing; Aflatoxins; Fumonisins; Deoxynivalenol

Cereal grains may become contaminated by molds while in the field and during storage. Some of these molds can produce mycotoxins. In general, mycotoxins are stable compounds. The mycotoxins that occur commonly in cereal grains are not destroyed during most food processing operations, which may lead to contamination of finished cereal based foods. The mycotoxins that are most common in cereal grains are aflatoxins, ochratoxins, fumonisins, deoxynivalenol and zearalenone. These mycotoxins and the foods or commodities that they commonly contaminate are given in Table 1.

Food processes that may have effects on mycotoxins include sorting, trimming, cleaning, milling, brewing, cooking, baking, frying, roasting, canning, flaking, alkaline cooking, nixtamalization (tortilla process) and extrusion.

1. Sorting, trimming and cleaning

Sorting and trimming may lower mycotoxin concentrations by removal of contaminated material. However, these operations do not destroy mycotoxins. Cleaning grains removes kernels with extensive mold growth, broken kernels and fine materials, which helps to reduce mycotoxin concentrations. Fumonisin concentrations in corn were reduced by 26–69% by cleaning according to Sydenham et al. (1994). Cleaning can also be used to remove scab infested wheat and barley kernels which

Table 1
Mycotoxins of concern and the commodities in which they may occur

Mycotoxin	Food/commodity
Aflatoxins	Maize (corn), cottonseed, peanuts, tree nuts, milk
Ochratoxin	Wheat, coffee beans, grapes, raisins
Zearalenone	Maize
Deoxynivalenol (DON, Vomitoxin)	Wheat, barley, maize
Fumonisins	Maize

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can reduce deoxynivalenol concentrations by 5.5 to 19% in wheat in preparation for milling (Abbas et al., 1985). On the other hand, only 2–3% reduction of ochratoxin A in barley was achieved by cleaning (Scudamore et al., 2003). Physical cleaning, where mold-damaged kernels, seeds or nuts are removed from the intact commodity, may result in 40–80% reduction of aflatoxins (Park, 2002). While sorting, trimming and cleaning may reduce mycotoxin concentrations in commodities, these operations may not completely remove all of the contamination. The initial condition of the grain, or commodity, and extent of the contamination will have an effect on cleaning efficiency.

2. Milling

In the milling process mycotoxin contamination may be redistributed and concentrated in certain mill fractions, but there is no step or operation that destroys mycotoxins. Mycotoxins tend to be concentrated in germ and bran fractions in the dry milling process (Abbas et al., 1985; Katta et al., 1997; Park, 2002; Scudamore et al., 2003; Brera et al., 2004). Katta et al. (1997) showed that during the dry milling of corn, fumonisin B_1 was found in highest amounts in the bran fraction that is used as animal feed, followed by the germ fraction, which may be used as animal feed or for oil extraction. Fractions used for food production, including flaking grits and flour, had the least amount of fumonisin contamination (Fig. 1). Brera et al. (2004) also observed this while studying the effect of industrial processing on the distribution of fumonisin B_1 in dry milled corn fractions. Also with the dry milling of wheat, barley, and other cereals, DON, zearalenone, aflatoxins and ochratoxin A were found in highest amounts in fractions of the commodity that are less likely to be used for food production (germ and bran fractions) (Chelkowski et al., 1981; Scott et al., 1984; Abbas et al., 1985; Alldrick, 1996; Park, 2002; Scudamore et al., 2003).

In the wet milling of corn, mycotoxins may be dissolved into the steep water or distributed among the byproducts of the process, but not destroyed. By the end of the wet milling process mycotoxins, including aflatoxin, zearalenone and fumonisins, can be found in the steep water, gluten fiber and germ, while the starch tends to be relatively free of these mycotoxins (Bennett et al., 1996a; Lauren and Ringrose, 1997; Park, 2002; Ryu et al., 2002). Bennett et al. (1996b), found that during wet milling, fumonisin in contaminated corn was also dissolved into the steepwater or distributed to the gluten, fiber and germ fractions, leaving no detectable amounts in the starch.

3. Brewing

Aflatoxin B_1, ochratoxin A, zearalenone, DON, and fumonisins B_1 and B_2 may be transferred from contaminated grains into beer, in the brewing process. The source of these mycotoxins could be the malted grain or adjuncts (Scott, 1996). In brewing, corn in the form of grits or syrup, rice grits, unmalted barley, wheat starch, or sorghum grits may be used as adjuncts to provide fermentable carbohydrates for the yeast (Hoseney, 1994). While studying the stability of aflatoxin B_1 and ochratoxin A in the brewing process, Chu et al. (1975) found that the patterns for the loss of these mycotoxins were similar. Both mycotoxins were relatively stable at boiling temperatures of the mash cooking step, but were more sensitive to mash malting (protein hydrolysis), wort boiling, and final fermentation, with removal of 12 to 27%, 20 to 30%, and 20 to 30%, respectively in these steps. Scott et al. (1995) observed that about a 2–13% reduction of ochratoxin A, 3–28% of FB_1 and 9–17% of FB_2 occurred during the fermentation of wort to which ochratoxin A and fumonisins B_1 and B_2 had been added.

4. Thermal processing

The application of heat to cook and preserve products is the basis of all thermal processes. These processes include ordinary cooking, frying, baking, roasting and canning. Extrusion, which is also a thermal process, will be considered separately. The stability of several mycotoxins during various methods of thermal processing have been reported (Boudra et al., 1995; Jackson et al., 1996a,b; Ryu et al., 2003; Pineda-Valdes and Bullerman, 2000).

In a study of corn muffins made from cornmeal naturally contaminated with aflatoxins, 87±4% of the initial amount of aflatoxin B_1 in the cornmeal was found in the baked muffins (Stoloff and Trucksess, 1981). However, ordinary cooking of rice contaminated with aflatoxin B_1 showed an average

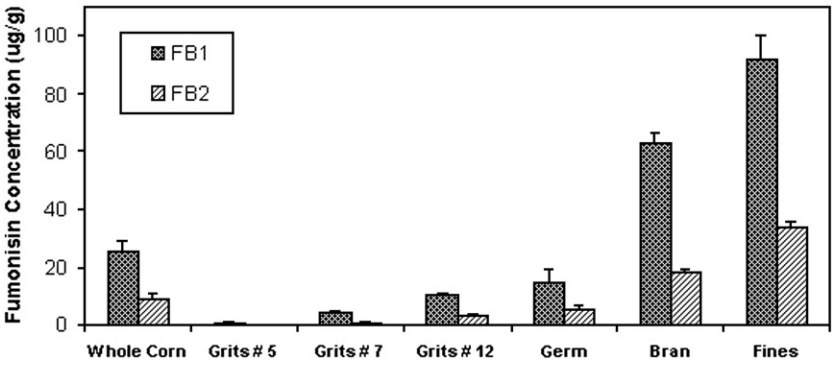

Fig. 1. Levels of fumonisin B_1 and B_2 (FB_1 and FB_2, respectively) in different fractions of dry-milled yellow corn. Data from Katta et al. (1997).

reduction of 34%. Even further reduction was obtained with pressure cooking (78–88%) (Park et al., 2005, Park and Kim, 2006). In another study boiling corn grits gave an average reduction of aflatoxins of 28%, while frying the boiled grits gave 34–53% total reduction (Stoloff and Trucksess, 1981). Roasting pistachio nuts at 90, 120, and 150 °C for 30, 60, and 120 min reduced the aflatoxin content of the nuts by 17–63%, with the reduction being dependent on time and temperature (Yazdanpanah et al., 2005). The reduction of aflatoxins during coffee bean roasting was also dependent on the type and temperature of roasting with reductions of about 42 to 56% achieved (Soliman, 2002). Production of tortillas by alkaline cooking and steeping of the corn, followed by further processing into tortilla chips, and corn chips, led to reduction of initial aflatoxin contamination by approximately 52% in the tortillas, 84% in the tortilla chips, and 79% in the corn chips (Torres et al., 2001).

Ochratoxin is stable during bread baking, with no loss or reduction of the concentration of ochratoxin (Scudamore et al., 2003, Subirade, 1996). However, baking of biscuits resulted in about two-thirds of the toxin being destroyed or immobilized (Subirade, 1996). Pressure cooking beans, in water, resulted in up to 84% reduction of ochratoxin A (Milanez and Leitão, 1996). Autoclaving oatmeal with 50% water gave a 74% reduction of ochratoxin, while autoclaving dry oatmeal or rice cereal gave greater losses of 86–87.5% (Trenk et al., 1971). Roasting coffee gave 13–93% reduction of ochratoxin (Pérez de Obanos et al., 2005), and when the processing conditions were those applied to obtain a typical espresso coffee brew the elimination of ochratoxin A was greater than 90%, in samples with both high and low levels of contamination (Romani et al., 2003).

Baking regular bread, cookies and biscuits gave variable reductions in deoxynivalenol of 24–71% in bread and a 35% reduction in cookies and biscuits, but baking Egyptian flat bread gave no reduction of deoxynivalenol (El-Banna et al., 1983; Scott et al., 1983; Scott, 1984). Canning of cream style corn gave a slight (12%) reduction of deoxynivalenol while canning baby food and dog food gave no reduction (Wolf-Hall et al., 1999).

Fumonisin B_1 (FB_1) is a fairly heat stable compound that is stable at boiling temperatures. No loss of FB_1 was observed when *Fusarium verticillioides* culture material was boiled in water for 30 min and dried at 60 °C for 24 hours (Alberts et al., 1990). However, when higher temperatures are used some reduction may be observed. Castelo et al. (1998a) showed a significant decrease in fumonisins when canning was applied to whole kernel corn, as well as with canned cream-style corn and baked corn bread, but corn-muffin mix artificially contaminated with 5 μg/g of FB_1 and naturally contaminated corn-muffin mix showed no significant losses of fumonisins upon baking. However, roasting cornmeal samples both artificially contaminated (5 μg/g of FB_1) and naturally contaminated cornmeal samples at 218 °C for 15 min resulted in almost complete loss of fumonisins (Castelo et al., 1998a). In another study, baking corn muffins at 175 °C and 200 °C resulted in 16 and 28% reductions of fumonisin, respectively. At both temperatures, losses of FB_1 were greater at the surface than at the core of the muffins (Jackson et al., 1997). Frying corn masa at 140–170 °C (0–6 min) gave no reduction of fumonisins; while frying tortilla chips at 190 °C (15 min) resulted in a 67% reduction of fumonisin (Jackson et al., 1997). Dry heat appears to be more destructive than moist heat.

The effects of processing time and temperature on the heat stability of Fumonisins B_1 and B_2 in aqueous buffered systems at pH 4, 7 and 10 showed that the rate and extent of FB_1 and FB_2 decomposition increased with processing temperature and that both compounds were least stable at pH 4, followed by pH 10 and 7, respectively (Jackson et al., 1996a,b). At >175 °C, more than 90% of FB_1 and FB_2 were both lost after processing for 60 min, regardless of pH.

The effects of corn starch, zein and glucose as individual matrix components on the fate of FB_1 in aqueous solutions heated to 100–150 °C showed that the greatest losses of FB_1 occurred in solutions containing glucose compared with solutions containing corn starch or zein (Hlywka, 1997).

A stable reaction product of heating FB_1 in the presence of reducing sugars, *N*-(carboxymethyl)-fumonisin B_1 (NCM-FB_1), was reported by Howard et al. (1998). The reaction likely proceeds through a common Maillard (nonenzymatic browing) reaction between FB_1 (an aliphatic primary amine) and a reducing sugar (Murphy et al., 1996), in a manner similar to the reaction of amino acids with reducing sugars. The first reaction product of FB_1 and D-glucose has been isolated and identified as *N*-(1-deoxy-D-fructos-1-yl) fumonisin B_1 (Polling et al., 2002; Seefelder et al., 2003) which, following the general Maillard reaction scheme, is further converted to NCM-FB_1 (Howard et al., 1998; Lu et al., 2002). Lu et al. (2002) reported that reacting FB_1 with fructose eliminated the FB_1 toxicity, possibly by blocking the amine group of FB_1 by the sugar in a Maillard reaction.

5. Corn flake process

The effect of the corn flake process on aflatoxin and fumonisins has been studied (Castelo, 1999; De Girolamo et al., 2001; Meister, 2001). With aflatoxin, cooking the grits with and without sugars resulted in 64–67% reduction of aflatoxin. After toasting the flakes with and without sugar the reductions in aflatoxin ranged from 78 to 85% (Lu et al., 1997). Ochratoxin was also reduced by processing of breakfast cereals such as in the corn flake process (Aish et al., 2004). The stability of fumonisin B_1 and B_2 in the corn flake process was studied by De Girolamo et al. (2001), and they found about 60–70% reduction in fumonisin content during the entire process, and only 30% of those losses were attributed to the extrusion step, where the material was subjected to 70–170 °C for 2–5 min. In another study, Meister (2001) evaluated the effects of extrusion cooking, gelatinization, and cornflaking on the stability of fumonisins B_1 and B_2, and reported that cooking extrusion and gelatinization were able to reduce fumonisin levels to ~30–55%, while cooking the grits for flaking reduced contamination to ~20–65%, and roasting the flakes reduced fumonisin content to ~6–35%. Castelo (1999) found that corn flake processing without sugars resulted in 53.5 and 48.7% losses of FB_1 after cooking and toasting, respectively, whereas processing in the

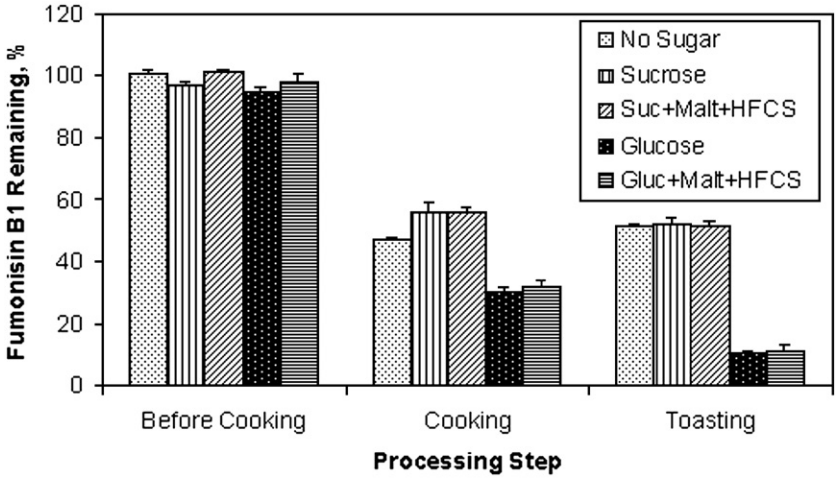

Fig. 2. Percentage (%) FB_1 remaining in spiked grits before cooking, after cooking and after toasting during the production of corn flakes with and without sugars. S+M+HF=Sucrose in combination with maltose and high fructose corn syrup. G+M+HF=Glucose in combination with maltose and high fructose corn syrup. Data from Castelo (1999).

presence of glucose gave 86–89% reduction of FB_1. Losses of FB_1 in the presence of sucrose, maltose and high fructose corn syrup were similar to reductions in corn flakes made without sugars (Fig. 2). While reductions of fumonisins during corn flake manufacture occurred, the presence of so called "hidden" or "masked" fumonisins (protein bound) has been reported in commercial corn flake samples obtained from retail outlets (Kim et al., 2003).

6. Extrusion processing

Extrusion processing is used extensively in the production of breakfast cereals, snack foods and textured foods. During extrusion cooking very high temperatures can be reached (Linko et al., 1984). During processing through the extruder, a dough-like mixture is forced through a stationary metal tube or barrel by a rotating screw shaft. As this occurs, heat can be added in the form of steam and is also generated by the mechanical energy of the turning screw and the friction of the barrel. As a result, very high temperatures (>150 °C) can be reached inside the barrel. In addition, very high pressures and severe shear forces are generated that contribute to chemical reactions and molecular modification of compounds and accomplish the cooking process in a short period of time (Harper, 1992; Francis, 2000). Extruders may have a single screw or may have a double screw.

The stability of mycotoxins during extrusion processing and the ability of extrusion processes to lower or reduce the concentration of a mycotoxin in extruded products have been studied. The amount of reduction of the mycotoxin concentration in the finished product depends on several factors including extruder temperature, screw speed, moisture content of the extrusion mixture, and residence time in the extruder. Of these factors, extrusion temperature and residence time seem to have the greatest effect. The greatest reductions in mycotoxin concentrations in extruded products occur at temperatures of 160 °C or greater and longer residence times.

The effect of extrusion on aflatoxin content appears to be influenced by the presence or absence of additives, moisture content and temperature (Cheftel, 1989; Hameed, 1993). Hameed (1993) showed that extrusion alone was able to reduce aflatoxin content by 50–80%, and with addition of ammonia, either as hydroxide (0.7 and 1.0%) or as bicarbonate (0.4%) the aflatoxin reduction achieved was superior to 95%. Cheftel (1989) reported similar results when peanut meal was subjected to extrusion cooking in the absence (23–66% reduction) or presence of 2–2.5% ammonium hydroxide (87% reduction).

The effect of temperature, moisture content, screw speed, and residence time on the stability of ochratoxin A during extrusion of contaminated wholemeal wheat flour was studied by Scudamore et al. (2004). Screw speed had little effect on the breakdown of ochratoxin A, while residence time, temperature and moisture affected the loss of ochratoxin. With higher temperature and moisture content, more ochratoxin breakdown was observed. When moisture content was 30%, at 116–120 °C 12% reduction was observed and at 133–136 °C 23.5% loss in ochratoxin A was noted. When the moisture content was 17.5% at 157–164 °C the average loss in ochratoxin A was 13.4%, while at 191–196 °C the average loss recorded was 31%. Degradation was also increased by longer residence time, when lower mass flow rates were applied, because the time the product spent in the extruder was increased. However, the maximum loss observed was no greater than 40% of the initial amount of ochratoxin A.

The moisture content of grits did not affect the reduction of zearalenone by extrusion cooking, when either mixing or non-mixing screws were used. When the mixing factor was evaluated, detoxification of zearalenone was significantly higher using a mixing screw (66–83%) than was achieved using a non-mixing screw (65–77%). The reduction of zearalenone achieved at 120 and 140 °C was 73–83%, while at 160 °C it was only 66–77% (Ryu et al., 1999).

Cazzaniga et al. (2001) studied the effect of extrusion cooking on the stability of deoxynivalenol in the presence and absence of additives (sodium metabisulphite). In all conditions

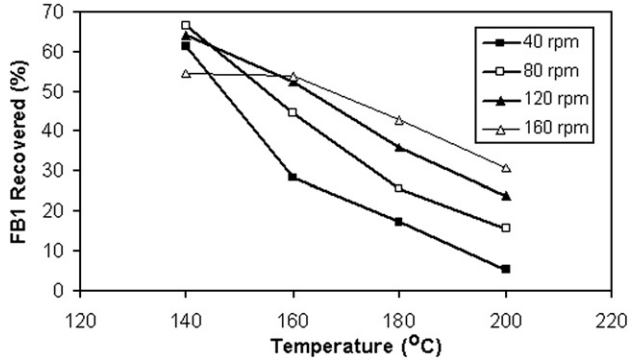

Fig. 3. Effects of temperature and screw speed on fumonisin B_1 (FB_1) recovery during extrusion cooking of corn grits. Data from Katta et al. (1999).

applied, which included moisture contents of 15 and 30%, temperatures of 150 and 180 °C, and sodium metabisulphite concentrations of 0 and 1% the detoxification achieved was higher than 95%.

The stability of fumonisins during extrusion cooking have been studied in a series of different experiments. Extrusion cooking resulted in more apparent loss of FB_1 with mixing screws than non-mixing screws (Castelo et al., 1998b). Katta et al. (1999) evaluated corn grits spiked with FB_1 at a level of 5 μg/g when extruded in a co-rotating twin-screw extruder at different temperatures (140, 160, 180 and 200 °C) and screw speeds (40, 80, 120 and 160 rpm). The FB_1 losses increased with an increase in temperature and a decrease in screw speed. The amounts of FB_1 lost from cooking grits at the different extrusion parameters used in this study ranged from 34 to 95% (Fig. 3).

The effect of sugars on the stability of Fumonisins during extrusion processing has also been studied by Castelo et al. (2001). Corn grits spiked with FB_1 (5 μg/g), and sugars (glucose, fructose, sucrose) added individually at 2.5 and 5.0% were extruded. Extrusion cooking of the grits resulted in significant reductions of FB_1 in all treatments relative to unextruded controls, but use of glucose resulted in greater reductions (44.8–66.6%) than with fructose (32.4–52.2%) or sucrose (26–42.7%). In a follow up experiment, spiked corn grits were extruded at 160 °C using different glucose concentrations (2.5, 5.0, 7.0, and 10.0%) and screw speeds (40, 60, and 80 rpm). Both the screw speed and glucose concentration significantly affected the extent of FB_1 reduction in extruded grits, with greater reductions of FB_1 (up to 92.7%) observed at lower screw speeds and higher glucose concentrations (Fig. 4). The work showing reduction of FB_1 by extrusion processing and added glucose was based on analyses of FB_1 concentrations by high performance liquid chromatography (HPLC) and enzyme-linked immunosorbant assay (ELISA). These methods rely on the chemical structures of the toxin. If the structure is modified or changed by extrusion or if the FB_1 combines with the corn matrix as a result of extrusion it may not be detectable or quantifiable by either HPLC or ELISA methods alone. Therefore, these methods are not able to prove conclusively that the toxicity or biological activity of FB_1 is destroyed by extrusion processing and not converted to another or bound form that may remain toxic. Bullerman et al. (2007) studied this using liquid chromatography-mass spectrometry (LC-MS) to identify degredation products and the rat kidney as a biosensor to detect residual toxicity. In this study corn grits were contaminated with FB_1 by direct addition (spiking) and by mold growth. The spiked grits contained about 30 μg/g of FB_1 and grits fermented with *F. verticillioides* contained 40–50 μg/g FB_1. The grits were extruded at 160 °C and 60 rpm screw speed. A mass balance approach was used to account for all of the FB_1. With 10% glucose in the extrusion mixture the main FB_1 derivative formed was *N*-(1-Deoxy-D-Fructos-1-y1) FB_1. The bioassay showed that rats fed non extruded spiked and fermented grits and spiked and fermented grits extruded without glucose showed significantly lower kidney weights and lesions characteristic of fumonisin exposure than rats fed spiked and fermented grits extruded with glucose added to the mix. Histopathological examinations of kidney tissue showed that lesions were least severe in rats given fumonisin contaminated

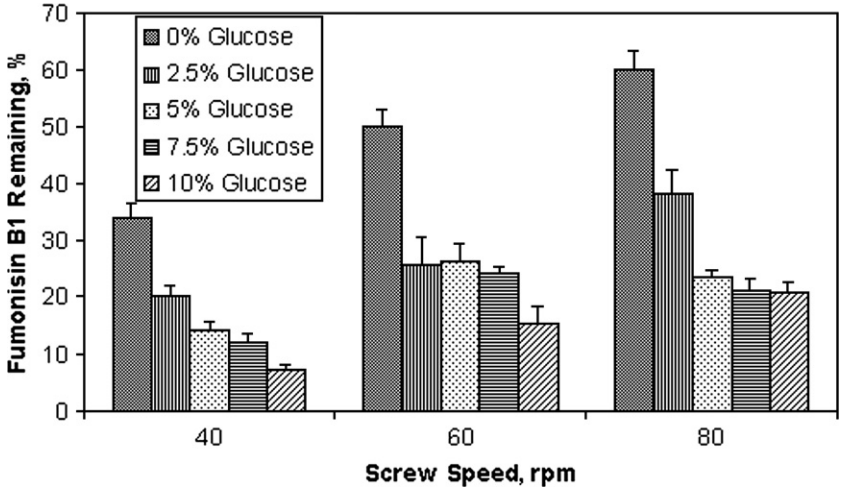

Fig. 4. Percent (%) FB_1 remaining in corn grits extruded at 160 °C. Glucose concentration (0, 2.5, 5, 7.5, and 10%) and screw speed (40, 60, and 80 rpm) were variables. Data from Castelo et al. (2001).

grits extruded with glucose, suggesting that *N*-(1-Deoxy-D-Fructos-1-y1) FB_1 is less toxic than unmodified FB_1.

7. Conclusions

Food processing has an impact on mycotoxins. Cleaning removes broken and moldy grain kernels. The milling processes dilute and distribute mycotoxins into certain fractions that most commonly become animal feed. However, some toxins in animal feed fractions may have the potential to become residues in animal products (i.e. aflatoxins, ochratoxin A) and still enter the human food chain. High temperature processes cause varying degrees of reduction of mycotoxin concentrations, but most mycotoxins are moderately stable in most food processing systems. Aqueous cooking and steeping reduces mycotoxin concentrations. Roasting and extrusion cooking at high temperatures (above 150 °C) appear to reduce mycotoxin concentrations. However, in the case of fumonisins, the fate of the toxin is unclear since it may be modified or matrix bound and be non-recoverable, but retain toxicity. Studies of the breakdown products of FB_1 extruded in the presence of 10% glucose show the formation *N*-(1-Deoxy-D-Fructos-1-y1) FB_1 and toxicity studies with rat kidneys indicate that this compound is less toxic than FB_1.

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