Effect of Nixtamalization on Mycotoxin-Contaminated Corn

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Corn (*Zea mays*) is a staple food in Mexico and both Central and South America with rapid growth in consumption in Europe and the United States (*2*, *10*). This agricultural commodity is prone to fungal damage due to inadequate pre- and post-harvest handling practices, which can lead to a reduction of yield and quality. Moreover, the presence of certain molds is often accompanied by mycotoxin contamination, compromising the safety of corn. Mycotoxins are defined as secondary metabolites of filamentous fungi (molds), predominantly belonging to the genera *Alternaria*, *Aspergillus*, *Claviceps*, *Fusarium*, and *Penicillium* (*9*).

Human and animal exposure to mycotoxins occurs primarily through food and can result in acute or chronic intoxications, known as *mycotoxicoses*, with symptoms varying depending on dosage, length of exposure, specific toxin, and the health of the individual (*31*).

Controlling mycotoxins poses a challenge worldwide, being most problematic in tropical and subtropical regions. The two primary fungal toxin groups affecting corn are aflatoxins and fumonisins (*3*, *25*). However, other toxins such as zearalenone, deoxynivalenol, citrinin, and ochratoxin have been reported in corn as well (*9*, *20*, *24*). More information regarding specific mycotoxicoses can be found in the NebGuide *Understanding Fungal (Mold) Toxins (Mycotoxins)*, G1513.

Originating in Mesoamerica, nixtamalization is the alkaline (lime, $Ca(OH)_2$) cooking of corn. The alkaline solution degrades and removes the pericarp, softens the
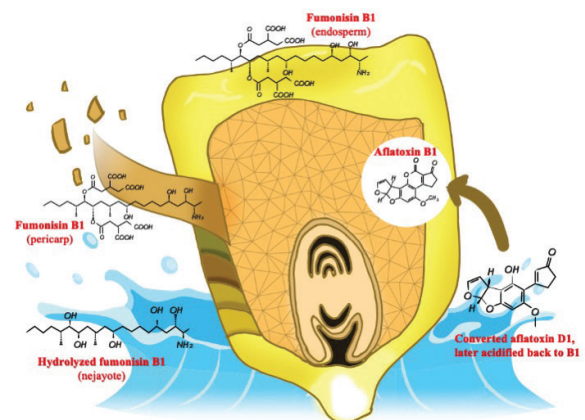

Fig. 1. Exemplification of hypothesized fate of some mycotoxins during corn nixtamalization. Some mycotoxins can be partially solubilized while in other cases they can be masked and still be present in food.

endosperm structure, and allows diffusion of water and calcium ions into the inner starch portion of the kernel which may have an influence on mycotoxin contamination (Figure 1). In the traditional nixtamalization process (Figure 2), after alkaline cooking, corn kernels are steeped for approximately 24 h, and are later washed to remove the remains of the pericarp and excess calcium (*8*, *10*, *19*).

The resulting product, called *nixtamal*, is ground to produce the masa dough, which is the base for several products such as tortillas, tamales, corn cakes (e.g. pupusas, arepas), liquid products (e.g. atole, pozole), and snacks.
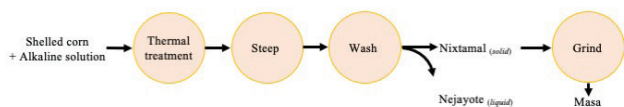
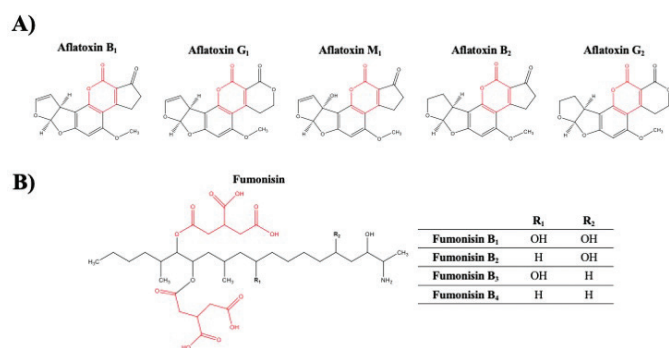Fig. 2. Traditional nixtamalization and masa production process.

Fig. 3. Examples of aflatoxin and fumonisin chemical structures. The aflatoxin lactone ring and tricarballylic acid moieties from fumonisin are marked in red.

Besides sensorial changes, nixtamalization enhances the nutritional value of the corn. As the calcium content is increased, niacin becomes available, and phytic acid levels are reduced (2, 27). In addition, nixtamalization can impact mycotoxin levels in the resulting food product. Effects on aflatoxins and fumonisins present in contaminated corn undergoing this alkaline thermal treatment are discussed in this publication.

Fumonisins

Fumonisins (Figure 3B) are mycotoxins produced mainly by *Fusarium verticillioides* and *F. proliferatum*. The forms generally present in contaminated food are members of the B series, fumonisin B_1 (FB_1), B_2 (FB_2), B_3 (FB_3), and B_4 (FB_4) (16). Of these, the International Agency for Research on Cancer (IARC) has classified FB_1 as a Group "2B carcinogen", indicating this toxin is possibly a carcinogenic to humans (4). Fumonisins are heat-stable (100–120°C), especially at neutral pH, and tolerate many of the commonly used thermal treatments in food processing and preparation (12, 13). Table 1 includes some findings of the effects of nixtamalization on fumonisins.

The high pH of the alkaline nixtamalization processing promotes ionization of starch hydroxyl groups, as well as the hydrolysis of the parent fumonisin molecule (6) by removing the carboxylic acid moieties. Rat-feeding bioassays have aimed to evaluate the detoxifying effectiveness of nixtamalization. In contrast to FB_1, hydrolyzed FB_1 (HFB_1) did not

cause neural tube defects (30). During food processing, fumonisins may bind to various components within the food matrix (e.g. nixtamal) or react with other ingredients such as reducing sugars. Bound mycotoxins may be an issue as they can be masked and remain undetected by conventional methods resulting in an underestimation of the potential toxicity of the contaminated products. Recent findings (4) indicate that fumonisin quantification in the steeping water (*nejayote*) and masa dough, during nixtamalization, accounted for more than what was initially quantified in the corn kernels, evidencing forms of matrix-associated fumonisins that were released during processing.

Fumonisins are water-soluble and nixtamalization may lower the fumonisin content of food products if the nejayote is discarded (23), thereby reducing the hepatotoxic and nephrotoxic potential of contaminated masa and derived products made from contaminated corn (28). Moreover, partially hydrolyzed FB_1 ($PHFB_1$) and HFB_1 formed during nixtamalization tend to be found mainly in the cooking/steeping liquid and solid waste. Also, the amount of FB_1 in the masa and tortillas tends to decrease with relative increases in lime, although boiling time has no apparent effect (29). At higher lime concentrations more pericarp is removed resulting in reduced fumonisin levels. Nixtamalization, coupled with rinsing, is crucial to reducing the presence of fumonisin in the final product, whereas grinding, sheeting, baking, and cooking the masa seems to have little impact. Loss of fumonisins throughout processing may indicate that they are extracted or otherwise removed from products, destroyed, chemically modified, bound to matrix components, or largely unextractable (11).

Aflatoxin

Aflatoxins (Figure 3A) are toxic and hepatocarcinogenic compounds produced by most of the strains of the *Aspergillus parasiticus* and some of the *A. flavus* fungi. Aflatoxins (AF) found in grains include B_1, B_2, G_1, and G_2 (16), where AFB_1 is the most potent, naturally-occurring liver carcinogen known (15). Aflatoxins are resistant to thermal inactivation, with a decomposition temperature ranging from 237–306°C, although this can vary depending on water availability and pH (16, 26). It has been suggested that the presence of moisture in food matrices facilitates the opening of the lactone ring in AFB_1, allowing the formation of a terminal carboxylic acid which enables its degradation via heat-induced decarboxylation (13). Alkaline conditions encourage thermal degradation through increased aflatoxin solubility (Table 2). However, the pH inside of the kernels does not increase significantly when

Table 1. Examples of reported effects of nixtamalization processing conditions on fumonisin reduction. Parts per million (ppm) = milligrams of fumonisin per kilogram (mg/kg) of corn masa.

Type of fumonisin (FB)	Process parameters	Quantification method	Fumonisin decrease or increase *in masa*	Remarks	Reference
FB_2, 5 ppm	pH 10, 175–200°C, 60 min heating	HPLC	Up to 90% FB_2 decrease	Reduction regardless of pH 4–10. Minimum recommended processing: 150°C, 60 min.	(12)
FB_1, 8.8 ppm	pH 10, 100–125°C, 5 min steaming	HPLC LOD: 0.01 ppm	89.5% FB_1 decrease / 8.4% HFB_1 increase	Dry basis. Hydrolyzed fumonisins (HFB) were the major decomposition compounds detected.	(6)
FB_1, 34.6 ppm / $PHFB_1$, 1.34 ppm / HFB_1, 0.95 ppm	Alkaline pH. Proprietary.	HPLC, LC-MS	86.4% FB_1 decrease / 32.8% $PHFB_1$ decrease / 5.5% HFB_1 decrease	Partially hydrolyzed FB_1 ($PHFB_1$) found predominantly in the liquid and solid waste.	(29)
FB_1, 0.7–1.65 ppm	pH 10.6–11.1. 15–60 min boiling	HPLC LOD: 0.025 ppm	80–100% FB_1 decrease	FB_1 decreased as lime increased. Boiling time had no apparent effect.	(5)
FB_1, 38.1 ppm	105 min boiling	HPLC, LC-MS LOD: 0.025 ppm	50% total fumonisins decrease	Total FB_1/HFB_1 in residual lime water and water washes accounted for 50% of the total FB_1 in the uncooked maize.	(21)

Table 2. Examples of reported effects of nixtamalization processing conditions on aflatoxin reduction. Parts per billion (ppb) = micrograms of aflatoxin per kilogram (μg/kg) of corn masa.

Type of aflatoxin (AF)	Process parameters	Quantification method	Aflatoxin decrease or increase *in masa*	Remarks	Reference
Total aflatoxin	~pH 10 (nejayote) 98°C *Traditional*: 40 min boiling *Commercial*: Directly steeping	Fluorometry	*Traditional*: 52–85% total aflatoxin decrease *Commercial*: 30–71% total aflatoxin decrease	Most aflatoxin modification and detoxification occur in the cooking liquor due to high pH.	(26)
Total aflatoxin, 678.3 ppb	pH 8.24, 35 min at 85°C	HPLC LOD: 0.5 ppb	93.2% decrease / 57.2% increase	Acidification caused reformation of the original aflatoxin on corn masa.	(18)
Total aflatoxin, Level 1: 29 ppb, Level 2: 93 ppb	*Traditional*: 0.99% lime (w/v), 70 min at 85°C *Ecological*: 0.37% lime (w/v), 10 min at 92°C	HPLC LOD: 0.5 ppb	*Traditional*: Level 1: 89% decrease, Level 2: 87% decrease *Ecological*: Level 1: 25% decrease, Level 2: 13% decrease	Ecological nixtamalization uses less water and uses the whole grain.	(17)
AFB_1, 495 ppb, AFM_1, 402 ppb, AFB_1 dihydro-diol, 30.4 ppb	0.3% lime (w/w), 30 min at 90–96°C	HPLC LOD: 5 ppb AFB1, 15 ppb total AF	94.2% AFB_1 decrease / 94.5% AFM_1 decrease / 92.8% AFB_1 di-hydrodiol decrease	Addition of 3% H_2O_2 resulted in a higher reduction of AFB_1	(7)

compared to the surrounding lime solution. Therefore, it is likely that only the toxin located on the outer layers of the kernels may be solubilized into the *nejayote* fraction, potentially increasing the safety of the masa. The opening of the lactone ring can be, however, a reversible phenomenon. Modified aflatoxins on the masa-derived products can be converted back to their original form by acidification in the human digestive tract during digestion (23, 26). Elias-Orozco et al (7) showed that the addition of 3% hydrogen peroxide (H_2O_2) to the corn-water mixture resulted in a higher reduction of AFB_1 than when each treatment, lime or H_2O_2, was applied independently. This phenomenon is possibly due to the reaction of lime with the lactone ring and the interaction between H_2O_2 and the double bond in the furan ring of the aflatoxin molecule. Despite the potential of this treatment this addition compromises the palatability of the masa and derived products.

Other Toxins

Aside from fumonisins and aflatoxins, there are other mycotoxins that can be found in corn. Deoxynivalenol (vomitoxin, DON) and its derivatives are mycotoxins produced by certain *Fusarium* mold species that frequently

infect corn, wheat, and other grains. DON has been reported to be significantly reduced after nixtamalization due to its instability in alkaline conditions. Similarly to what is observed with fumonisins, the outer layer of contaminated corn kernels contains a high amount of DON, therefore the removal of the pericarp during the nixtamalization process further reduces this contaminant (*14*). Abbas et al (*1*) reported that zearalenone, an estrogenic mycotoxin produced by some *Fusarium* species, can undergo a reduction of 59–100% in contaminated corn following nixtamalization. The same group also reported a loss of 72–82% of DON, as well as a total (100%) reduction of 15-acetyl-DON in corn samples after alkaline cooking. Reduction of moniliformin via alkaline cooking of corn has also been evaluated (*22*), and a 71% reduction of the toxin was observed during the nixtamalization process.

Concluding Remarks

Reduction of mycotoxins in processes such as nixtamalization is variable, as it depends of several parameters not limited to cooking time, temperature, pH, and other food ingredients. During nixtamalization, both the removal of the pericarp, as well as mycotoxin solubility during steeping seem to be directly related to the contamination levels found in the final corn-based product.

While mycotoxins can potentially be controlled to some degree with nixtamalization through removal, chemical modification to less toxic compounds, or degradation, food processors are recommended to always use good quality starting materials, in accordance to regional mycotoxin regulations in place, to prevent mycotoxin contamination of corn-based products. Even though processing methods may assist in reducing contamination, ensuring the safety of corn-based products should primarily rely on the use of grain, that is as much as possible, free of mycotoxins.

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