Revising the Role of pH and Thermal Treatments in Aflatoxin Content Reduction During the Tortilla and Deep Frying Processes

Patricia Torres,* Martín Guzmán-Ortiz, and Benjamín Ramírez-Wong

Departamento de Investigación y Posgrado en Alimentos, Universidad de Sonora, Apdo. Postal 11-49, Hermosillo, Sonora, México

Naturally aflatoxin-contaminated corn (*Zea mays* L.) was made into tortillas, tortilla chips, and corn chips by the traditional and commercial alkaline cooking processes. The traditional nixtamalization (alkaline-cooking) process involved cooking and steeping the corn, whereas the commercial nixtamalization process only steeps the corn in a hot alkaline solution (initially boiling). A pilot plant that includes the cooker, stone grinder, celorio cutter, and oven was used for the experiments. The traditional process eliminated 51.7, 84.5, and 78.8% of the aflatoxins content in tortilla, tortilla chips, and corn chips, respectively. The commercial process was less effective: it removed 29.5, 71.2, and 71.2 of the aflatoxin in the same products. Intermediate and final products did not reach a high enough pH to allow permanent aflatoxin reduction during thermal processing. The cooking or steeping liquor (nejayote) is the only component of the system with a sufficiently high pH (10.2−10.7) to allow modification and detoxification of aflatoxins present in the corn grain. The importance of removal of tip, pericarp, and germ during nixtamalization for aflatoxin reduction in tortilla is evident.

Keywords: *Aflatoxin; tortilla; masa; nixtamal; corn; Zea mays.*

INTRODUCTION

Corn tortillas are a very important staple food in Mexico. Per capita consumption of this food is 120 kg per year. In rural zones it accounts for 70% of the caloric intake and 50% of the protein (*1*). Tortilla production in Mexico is 11 million metric tons yearly. About 11% of the production is based in dry flour, prepared from corn subjected to the thermal and alkaline process called nixtamalization. The rest of the production is based in freshly ground masa (*1*). Surveys performed at several regions in Mexico have shown that corn tortillas consumed in this country can be contaminated with aflatoxins (*2*−*4*).

Aflatoxins are toxic and hepatocarcinogenic agents (*5*), produced by most of the strains of the *Aspergillus parasiticus* (Speare) and some of the *A. flavus* (Link) fungi (*6*). Although infection of corn by *A. flavus* has been reported since 1920, corn contamination with aflatoxins was considered a storage problem until 1970 (*7*). Contamination of corn with aflatoxins has been associated with high temperatures and stress of the plant during development; stress that can be caused by water deprivation, inadequate fertility, or weed competition (*8*). The role of insects in the infection and spread of the fungus has been recognized for long time (*7*).

Colonization of corn kernels is most extensive in the germ and tip of the kernel, and the route of entrance of *A. flavus* into a developing grain is through the stylar canal (*7*).

The aflatoxins found in grains are the B 1, B 2, G 1, and G 2. They are compounds formed by oxygenated heterocyclic rings, and they contain a lactone ring that makes them susceptible to alkaline hydrolysis (*9*).

Corn tortillas are made from corn using a thermal and alkaline process called nixtamalization (*10*), which reduces the aflatoxin contents (*11*). However, nixtamalization does not always produce safe products (*12*, *13*). Furthermore, the process does not modify in a permanent way all the aflatoxins originally present in corn. A part of these modified aflatoxins are reconverted to their original form by acidification in the human digestive tract during digestion (*11*, *12*).

Limited work has been done at the laboratory level, using beakers and small amounts of grain (*11*, *12*, *13*). Despite the importance of the information produced by these studies, laboratory conditions are not always representative of actual events in a commercial plant. The main reason for this lack of representation is the difference in the mechanisms of heat and mass transfer between small and large containers (*14*); we expect stronger conditions at the laboratory level. This could also explain the difference between results of the present work and the results of Price and Jorgensen (*12*). They measured pH of 10 and 11 in masa and tortilla whereas we did not find that the pH of masa and tortilla increased significantly.

Since aflatoxin contamination occurs mainly in the field, and *A. flavus* primarily uses natural channels such as the stylar canal as the main route of entrance into the grain (*7*), contaminated corn often lacks visible signs of damage. In laboratory-contaminated corn grain, on the other hand, the mold grows on the grain surface, making the grain, mold, and toxin more accessible to any treatment.

* Corresponding author. Telephone and fax: 52 62 125488. E-mail: pitorres@guayacan.uson.mx.

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Table 1. Moisture Content and pH of Corn and Intermediate and Final Products of the Tortilla and Chips Processes

	Traditional Process				Commercial Process			
product	pH[a]	RSD[b]	moisture (%)[c]	RSD	pH[a]	RSD	moisture (%)[c]	RSD
corn	5.83	0.070	13.22	0.044	5.36	0.007	12.07	0.028
nixtamal	6.79	0.067	50.31	0.027	5.84	0.015	48.26	0.005
masa	6.70	0.068	56.91	0.001	5.88	0.030	54.72	0.008
tortilla	6.73	0.060	49.59	0.030	5.86	0.023	48.13	0.027
nejayote	10.28	0.045	93.16	0.001	10.72	0.007	98.60	0.001
tortilla chips	6.47	0.086	2.10	0.138	5.77	0.008	2.86	0.146
corn chips	6.46	0.051	9.29	0.082	5.76	0.010	15.64	0.067

[a] Least significant difference among pH of the products ($p < 0.05$) = 0.172. [b] RSD, relative standard deviation. [c] Least significant difference among moisture content of the products ($p < 0.05$) = 0.819.

The efficiency in reducing aflatoxins in contaminated corn, either in industrial or pilot plant conditions, has not been reported. Besides, there are some questions that need to be answered: Are the thermal and alkaline effects on the aflatoxin molecule responsible for the reduction of the aflatoxin content? Or, is the physical removal during the cooking and washing steps more significant? It is also important to know the contribution of the different processing operations to the overall effectiveness.

The purpose of this study was to determine the role of pH and thermal treatments on the effectiveness of nixtamalization, tortilla baking, and masa deep-frying in reducing the aflatoxin content of naturally contaminated corn.

MATERIALS AND METHODS

Table tortillas were made using naturally contaminated white corn (*Zea mays*). Physical examination of the grain showed 0% insect damage and 2% mold damaged grain, 5.1% broken kernels, and 5.1% foreign materials. The corn contained 8.27% protein, 4.5% fat, 1.86% ash (the previous values expressed as dry basis), and 9.58% moisture content.

Aflatoxins content was determined before each experiment. Corn samples of 25 kg were taken and homogenized manually, using protective clothes, gloves, and respirators to avoid contact with the grain and dust. Subsamples of 5 kg were coarsely ground, and this coarse material was homogenized again. Another subsample of 1 kg was taken from the coarse material and ground finely. This finely ground material was homogenized and samples were taken for the aflatoxin analysis. The remaining 20 kg of corn was used to prepare tortillas.

Corn, intermediate, and final products were sampled, and pH, aflatoxin, and moisture content were determined. Percent aflatoxin reduction was calculated at each of the major processing steps.

Safety. Aflatoxins are poisons: powerful experimental tumorigens, neoplastigens, carcinogens, and teratogens (*27*). Aflatoxin extracts should be handled as very toxic substances (*28*). Procedures for swabbing accidental spills and decontamination of materials used can be found in the chapter 49 of the Official Methods of Analysis of AOAC (*28*). Respiration of dust from grain, and skin contact with dust, grain, and contaminated products should be avoided.

Tortilla Preparation. For each process, 20 kg of the contaminated corn was added to the kettle with 60 L of water and 200 g of lime ($Ca(OH)_2$). The water was heated to boiling (98 °C) before adding lime and corn. For the traditional process, a cooking time of 40 min was given. Corn was then steeped in the cooking liquor for 14 h. For the commercial process, corn and lime were added to boiling water, agitated, and steeped for 14 h, without further heating the corn. Next, nejayote (cooking liquor) was drained and collected. Nixtamal (cooked corn) was washed twice with 60 L of water. This water was later blended with the nejayote and used to determine percent solids removal.

Nixtamal was stone ground to produce masa (corn dough). The separation between milling stones was adjusted to obtain masa with a fine particle size that would be adequate for table tortillas. Corn masa was mixed in a horizontal mixer and water was added to obtain an appropriate texture as subjectively evaluated by the operator.

Tortillas were formed in a celorio cutter machine (*15*) and then baked for 39 s in a three-tiered, gas-fired oven at average temperatures of 177 °C in the upper level, 233 °C in the middle, and 453 °C in the lower level. Tortillas were cooled in a screen conveyor and then they were packed. Samples of corn, nixtamal, masa, tortilla, and nejayote were collected for pH, aflatoxin, and moisture analysis.

Tortilla and Corn Chips Preparation. Samples of 150 g of tortilla and masa strips from the commercial and traditional processes were fried with 1.5 L of safflower oil at 190 °C for 3 min and at 190 °C for 2 min to obtain corn and tortilla chips, respectively (*15*). Corn chips and tortilla chips were analyzed for pH, aflatoxins, and moisture contents.

Product Acidification. Finely ground tortilla and chips (50 g) were homogenized with 100 mL of acidified methanol (pH2) using a blender. The pH of the mixture of sample and methanol was adjusted to a pH of 2 again. Concentrated HCL was used to acidify and to adjust the pH. The mixture was allowed to stand for 30 min, then it was filtered. The pH of the filtrate was adjusted to 5.6, and the aflatoxin content was determined.

Analysis of Intermediate and Final Products. Moisture and pH were determined with the AACC 44-15A (*16*) and the 943.2 AOAC (*17*) methods, respectively. Total aflatoxin was measured with the immunochemical AFLATEST method (Vicam, Watertown, MA). The P chromatographic columns were used to purify the samples, and a Sequoia Turner model 451-100 (Mountain View, CA) fluorometer was used to detect and quantify total aflatoxin. Solid losses were determined by drying three aliquots of the homogenized nejayote and wash water at 105 °C in a convection oven.

Statistical Analysis. Factorial design experiments were used to evaluate the effects of processing, and the steps of processing, in aflatoxin reduction. Experiments were run in duplicate. Analysis of variance was performed, and the Fisher least-significance-differences were used to evaluate the effects and separate means. Both tests were done using the SAS statistical analysis software (SAS Institute Inc., Cary, NC) (*18*).

RESULTS AND DISCUSSION

pH of the Products. The pHs of the products made with the traditional process were higher than those for products from the commercial process (Table 1). The pHs of masa, tortilla, tortilla chips, and corn chips were not significantly different ($p < 0.05$) within each processing method. pHs reached by the intermediate and final products were not high enough to effectively detoxify aflatoxins. pH greater than 9.5 is necessary to avoid reformation of the aflatoxin molecule in an acidic medium (*19*). A high pH was found only in nejayote.

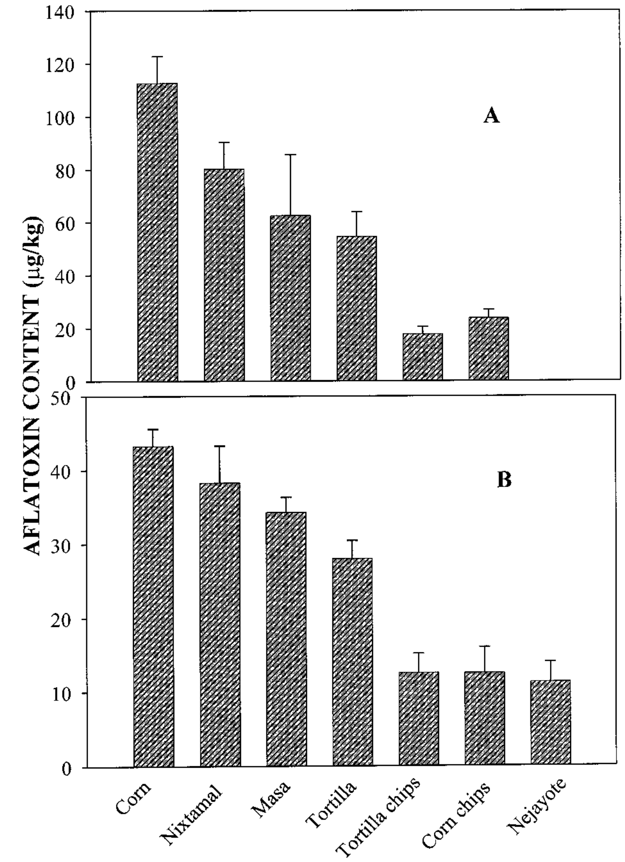

Figure 1. Aflatoxin content (µg/kg, dry basis) of the intermediate and final products of the (A) traditional and (B) commercial tortilla and chips processes. Error bars stand for standard deviation.

Moisture Content. Moisture contents of the products were different, and differences were also observed between the same products of the two processes (Table 1). Corn masa had higher moisture content than nixtamal because water was added during grinding of nixtamal into masa. Masa lost moisture during baking into tortilla, and masa and tortilla were dehydrated during frying to obtain corn chips and tortilla chips, respectively. Intermediate and final products made with the traditional process, except the chips, had higher moisture contents than those obtained with the commercial process. The increased moisture content enhances the modification of aflatoxins during cooking and baking. Aflatoxins are stable to dry heat up to their melting point (260 °C) (*20*). Moisture is necessary to hydrolyze the lactone ring of the aflatoxins at 85−95 °C, temperatures that are commonly used in food processing (*20*, *21*).

Aflatoxin Reduction. Figure 1 shows the aflatoxin content for corn and intermediate and final products of the traditional and commercial processes. Residual aflatoxins were still present in the final products. Conditions normally used in the processing of tortillas are not strong enough to completely detoxify contaminated corn (*11*−*13*).

Significant differences ($p < 0.01$) in the level of reduction between the traditional and commercial processes were observed. The traditional process that includes boiling of the corn in the lime solution was more effective in reducing aflatoxin content. There were no interaction effects between process and unit operation ($p < 0.01$). Each operation contributed the same

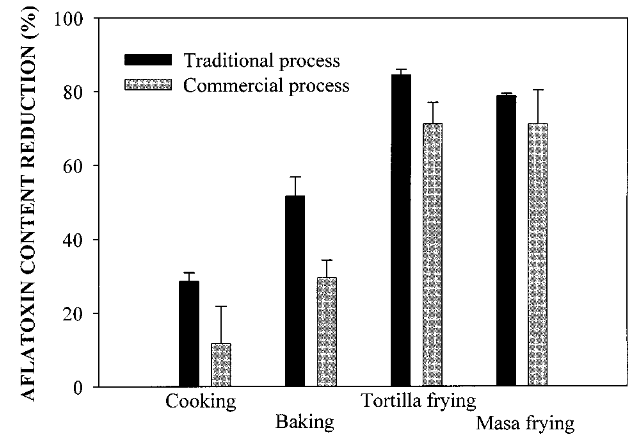

Figure 2. Cumulative aflatoxin content reduction during the operations involved in tortilla and chips production for traditional and commercial processes. Error bars stand for standard deviation.

proportion to the reduction in both processes. Figure 2 shows the cumulative aflatoxin reduction for each major operation during processing. All the operations that include a thermal treatment contributed significantly to the overall reduction. The steps involved in the traditional process produced a greater reduction in aflatoxin content than those of the commercial process. The reduction values expressed on a dry basis provide information related to the effectiveness of the thermal and alkaline treatment. The process modifies the toxin in such a way that cannot be detected by fluorescence. However, reduction in fluorescence does not always mean reduced toxicity. The reconverting of the molecule to its original form in acidic medium (*19*) should be considered especially if the pH of the treated products was not alkaline enough (pH = 9.5).

The reduction in aflatoxin increased during baking and frying. Frying is the most important step, especially when masa is fried directly into corn chips. The high moisture content of masa and the high temperature during a relative long frying time enhance reduction of the aflatoxins.

To explain how the reduction in aflatoxin content of the contaminated corn takes place, it is necessary to consider that the molds *A. flavus* or *A. parasiticus* infect or invade the corn grain and produce aflatoxins mainly in the germ, but they can grow in the pericarp and the endosperm (*7*). During nixtamalization of corn, solids are lost in the nejayote and wash water, and they go into the wastewater (*22*−*24*). In the present experiment, 6.8 and 4.1% of solids were lost during the traditional and commercial processes, respectively. These solids contained mainly tip cap, pericarp, and germ of corn kernel. Chemical composition of the solids and specific grain chemical compounds losses (*22*) agree with the observation of solids composition made during experimentation. Aflatoxins present in pericarp and germ were undoubtedly removed and extracted to the nejayote during cooking and steeping, when these anatomic parts of the grain were partially or sometimes almost completely removed. Extracting or physically moving the aflatoxins to the nejayote, which occur during solid losses, is very important for the reduction of aflatoxin during nixtamalization. Most of the aflatoxins modification and detoxification occurs in the cooking liquor, because it is the only component that has high enough pH.

During nixtamalization, the absorption of alkaline solution increased the pH of the grain from 5.83 to 6.79 and increased the moisture content from 13.22 to 50.13%. Although a considerable amount of the alkaline solution is absorbed, the pH of the grain did not increase in a significant way. The grain components seem to act as natural buffers. The pH of the grain is not alkaline enough to allow the aflatoxins present inside of the grain to be modified permanently.

The thermal and alkaline treatments that corn receives during nixtamalization, cause hydrolysis of the lactone ring of the aflatoxin molecule, yielding a water-soluble salt (*25*, *26*). Thus, the alkali solubilizes accessible aflatoxins, which go to the nejayote where the alkaline and thermal conditions favor degradation, including decarboxilation (*25*, *26*).

Aflatoxin reduction during baking and frying operations is caused by thermal degradation of the hydrolyzed aflatoxin. Part of the open lactone ring may interact mainly with the nonprotein components of the grain (*21*, *26*) and part is thermally degraded. These thermal treatments reduced the aflatoxin content. However, because of the relatively low pH of masa and tortilla, closing of the lactone ring and reconverting of the original aflatoxin can be expected during acidification.

Acidification of the Products. The percentage increase in aflatoxin concentration after acidification of contaminated tortilla varied from 0 to 18%. These values were lower than those reported by Price and Jorgensen (*12*). The zero increments of aflatoxin concentration after acidifiction were in tortillas made with the commercial process (no extra boiling). Aflatoxins percentage increase in tortilla and corn chips varied between 76 and 96% after acidification. Results suggest that part of the aflatoxins modified by thermal treatments revert to the original fluorescent form upon acidification. However, more research is needed in this area.

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