PHILIPP AGRIC SCIENTIST
Vol. 99 No. 1, 42–49
March 2016

ISSN 0031-7454

Fate of Aflatoxins from a Novel Procedure for Tortilla Making Based on Infrared Heating

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The fate of aflatoxins was studied during nixtamalization by using two tortilla making processes. Maize contaminated with two aflatoxin contents (AC) [AC1=173 ng g^{-1} and AC2=370 ng g^{-1}] was processed by the traditional (TNP) as well as an innovative nixtamalization procedure based on infrared heating (IRNP). In the case of tortillas from TNP, the aflatoxin contents were 17 ng g^{-1} and 61 ng g^{-1}, achieved higher degradation rates of 90% and 84%, corresponding to AC1 and AC2, respectively. In contrast, in tortillas obtained from IRNP, the aflatoxin contents were 50 ng g^{-1} and 100 ng g^{-1}, with degradation rates of 71% and 73%, respectively. Acidification of extracts prior to mycotoxin quantification did not result in a rebuilding of the aflatoxin structure; on the contrary, an extra reduction in the aflatoxin content was observed, up to 15% and up to 25% in tortillas produced with TNP and IRNP, respectively. A quadratic function and a linear function were fitted to evaluate the aflatoxin content in tortillas; these mathematical functions indicated that the initial aflatoxin content in the maize used to produce tortillas within the maximum limit allowed in Mexico are 163 ng g^{-1} for TNP and 44 ng g^{-1} for IRNP, respectively. Based on these results, IRNP seems to be safe and effective for aflatoxin reduction during tortilla manufacture.

Key Words: *Aspergillus flavus,* infrared nixtamalization, maize, mycotoxins, tortillas

Abbreviations: AC1 – aflatoxin content 1 (173 ng g^{-1}), AC2 – aflatoxin content 2 (370 ng g^{-1}), AF – aflatoxins, AFB1 – aflatoxin B1, AFB2 – aflatoxin B2, AFG1 – aflatoxin G1, AFG2 – aflatoxin G2, CTL – control, $Ca(OH)_2$ – calcium hydroxide, IR – infrared, IRNP – infrared nixtamalization process, MC – moisture content, MSA – malt extract-sodium chloride-agar medium, TNP – traditional nixtamalization process

INTRODUCTION

Aflatoxins (AF) are a group of acutely toxic metabolites produced by toxigenic strains of *Aspergillus flavus* Link, *Aspergillus parasiticus* Speare, and *Aspergillus nomius* Kurtzman et al. (Feibelman et al. 1998; Nesci et al. 2007). These toxins have closely similar molecular structures and form a unique group of naturally occurring highly oxygenated and heterocyclic compounds. Four principal AF are produced: aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), and aflatoxin G_2 (AFG_2). AFB_1 —the most toxic compound in this group— is known to be hepatotoxic, mutagenic, and hepatocarcinogenic (Busby and Wogan 1984); as a consequence, the International Agency for Research on Cancer stated that AF represent a highly potential risk for cancer in humans (IARC 2002). The presence of aflatoxins is also a serious health problem associated with commodities such as peanuts, cotton meal, and copra. As far as grains are concerned, however, it is primarily a problem in maize, since aflatoxins are produced both before and after harvest (Pitt et al. 2013).

Mexico, with an estimated population of 120.4 million people (INEGI 2014), has the highest world per capita consumption of maize, primarily as tortillas (136.2 kg per year). These are traditionally made utilizing the ancient alkaline-cooking process called nixtamalization. The traditional nixtamalization process (TNP) is a practice where maize is boiled in a water suspension containing lime [$Ca(OH)_2$], steeped overnight, washed and ground into maize dough (masa), and finally shaped into tortillas. In many countries, TNP is commonly used to produce Mexican maize-based products such as table tortillas, maize and tortilla chips, tostadas, taco shells, among others. Even with the increasing popularity of these maize-based products, little improvements have been made to this ancient maize processing method practiced historically by the Toltec and Aztec civilizations.

Recently, a tortilla-making process based on infrared (IR) heating was proposed in which maize grains are mixed with water and lime, cooked in an IR radiation field, steeped, and finally stone-milled to obtain fresh masa. IR heating offers multiple advantages not found in

other methods including: energy efficiency, reduced heating time, uniform product temperature, reduced quality losses, versatility, space savings (simple and compact equipment), precise process control, high heat transfer coefficient, selective heating and the outcome of a food product endowed with improved physicochemical, compositional, nutritional and viscoamylographic properties (Méndez-Albores et al. 2014). IR heating has been applied in drying (Pan et al. 2008), cooking (Rastogi 2012) and surface decontamination processes (Hamanaka et al. 2011). However, the use of this type of energy for the production of tortillas elaborated with aflatoxin-contaminated maize has not been evaluated. Consequently, the present research was conducted to determine the effect that the infrared nixtamalization process (IRNP) could have on aflatoxin reduction in tortillas compared with those obtained by means of the TNP, when using two batches of maize contaminated with relatively high levels of aflatoxins.

MATERIALS AND METHODS

Safety Precautions
Procedures used for handling aflatoxin-contaminated materials were adopted from recommendations published by the International Agency for Research on Cancer (Castegnaro et al. 1981).

Maize Grain
Regular maize of the commercial hybrid AS-900 (Aspros, Mexico) grown and harvested in 2014 at Celaya-Guanajuato, Mexico, with 11.4% moisture content (MC) was utilized. This material has a thousand-kernel weight and test weight of 344.34 ± 2.45 g and 73.18 ± 0.10 kg hL^{-1}, respectively. MC was determined by drying replicate portions of 5 to 7 g each of whole grain at 103 °C for 72 h, with percentages calculated on a wet-weight basis. The aflatoxin content of the maize grain was below the limit of detection (0.5 ng g^{-1}) of the 991.31 AOAC method described subsequently.

Fungal Isolate
The fungus *A. flavus* Link (strain UNIGRAS-1231) was obtained from the Culture Collection of the Grain and Seed Research Unit of the National Autonomous University of Mexico. The fungus was plated into Petri dishes containing MSA medium (%: malt extract, 2; sodium chloride, 6; agar, 2) at 25 °C for 7 d. This strain produces both AFB_1 and AFB_2 (Pérez-Flores et al. 2011). AFB_1 is the most abundant toxin produced by this strain and usually accounts for up to 97% of the total aflatoxin content found in inoculated maize grains (Méndez-Albores et al. 2007).

Fungal Inoculation Technique
To inoculate the grain, fungal spores were removed from the Petri dishes with a spatula. A sterile-water spore suspension (1.7 L) was prepared with approximately 100,000 spores per milliliter, and used to increase the MC of the grain. The amount of inoculum (approximately 10,000 spores per gram of maize) was determined to eliminate competition with other storage fungi that can potentially grow under the incubation conditions. The MC of the maize grains was adjusted to 18% and stored in plastic bottles (10 kg of maize per container). Bottles were covered with thin polyethylene film to minimize the loss of humidity from the grain; however, ten perforations with a pin were made in the film to avoid the accumulation of carbon dioxide. The bottles were incubated at 27 °C during 15 and 21 d for the fungus-inoculated experimental units, and 21 d for the control (the experimental unit received the same treatment during incubation in the absence of spores of the fungus). These incubation periods were chosen to obtain two different aflatoxin contents (AC1 and AC2) in the inoculated grain. After the incubation periods, the experimental units were put under a 1000 mg L^{-1} ethylene oxide gas atmosphere for 5 h, to stop further development of the toxigenic fungus and to avoid the dispersal of viable spores (Méndez-Albores et al. 2004). Finally, the grain was oven-dried to approximately 11% MC, transferred to plastic bags and stored at 4 °C.

Aflatoxin Quantification
Aflatoxin content was determined according to the 991.31 AOAC method (AOAC 2000) using monoclonal antibody columns for aflatoxin B1 and B2 (VICAM, Milford, MA). Samples (50 g) were extracted by blending with 100 mL methanol-water (80:20, v/v) using a laboratory blender (Mod 51BL30; Waring, New Hartford, CT, USA). The mixture was filtered through a Whatman 1 filter paper and a 5 mL portion was diluted with 20 mL of distilled water. The diluted preparation was filtered through a microfiber filter, and 10 mL were applied to an immunoaffinity column (Afla B; VICAM Science Technology, Watertown, MA, USA). Subsequently, the column was washed twice with 10 mL of distilled water and dried with sterile air flow. The toxins were then eluted with 1 mL of high-performance liquid chromatography (HPLC) methanol and quantified in a fluorometer VICAM Series-4EX (VICAM Source Scientific, Irvine, CA, USA) after reaction with 1 mL of 0.002% aqueous bromine. The detection limit for aflatoxins via fluorescence measurement is approximately 0.5 ng g^{-1}. When the total aflatoxin content was greater than 25 ng g^{-1}, dilutions from the extracts were made before they were passed through the immunoaffinity columns.

Recovery Checks
The performance of the 991.31 AOAC method was tested by measuring the percentage of aflatoxin recovery using the HPLC method on spiked masa and tortilla flours, injecting four replicates of six different aflatoxin contents (from 0.78, 1.56, 3.13, 6.25, 12.50 to 25 ng g^{-1}). A Waters HPLC equipment with two pumps (Model 510. Waters Associates, Milford, MA), and a Waters nova-pak C_{18} reverse phase column (5 µm, 3.9 mm, 150 mm) was used. Samples collected from the immunoaffinity columns (20 µL) were injected into a HPLC and eluted

isocratically with a mobile phase of 12.5 mN acetic acid:acetonitrile (1:1, v/v) at a flow rate of 1 mL min^{-1}. Aflatoxins were fluorometrically detected using a fluorescence detector (Waters model 470); the excitation and emission wavelengths were 338 and 425 nm, respectively. Aflatoxins were identified by their retention time (Rt), compared with those for a pure aflatoxin standard solution under identical conditions. The aflatoxin recovery for the immunoaffinity column method was 92%, with a standard error of 1.2, and a coefficient of variation value of 4.4%. These results indicated that the method used was reliable.

Extract Acidification Procedure

Maize, masa (maize dough), and tortilla extracts from the aflatoxin assay were adjusted to a pH of 3 by means of a commercial mixture of hydrochloric acid-potassium acid phthalate (J.T. Baker, Mallinckrodt Baker, Mexico) to simulate stomach pH as occurs during digestion (Méndez-Albores et al. 2004). Samples were incubated in an agitated water bath (Bellco Glass Inc. Vineland, NJ, USA) at 37 °C for 30 min. The pH was determined using a pH meter model PC45 (Conductronic, Puebla, Mexico). Aflatoxins were quantified using the 991.31 AOAC method previously described.

Tortilla Making Processes

Traditional Nixtamalization Process (TNP): Three 1000 g whole contaminated maize samples of each aflatoxin content (AC1 and AC2), as well as the control (CTL), were mixed with 3 L of tap water and 15 g of lime (99% of calcium hydroxide). The maize was cooked in a kettle at 90 °C for 43 min and steeped for 18 h at room temperature (Martínez-Bustos et al. 2000).

Infrared Nixtamalization Process (IRNP): Infrared nixtamalization was carried out according to the method of Méndez-Albores et al. (2014) with minimal modifications. Maize was cooked using the same maize-tap water input ratio, time-temperature, and lime content as in the case of TNP. The maize was cooked in an IR-resistant container, and the cooking stage was carried out in a commercial convention IR oven (model AX–767MH; Thane International, Zhejiang, China). The power output of the IR lamp was 1300 W, and the operating frequency was 60 Hz.

After nixtamalization processes, the cooked grain was steeped in closed plastic containers at room temperature for 18 h, and the nejayote (maize processing water or steep liquor) removed. The cooked maize (nixtamal) was washed with 3 L of tap water to remove lime excess and pericarp tissue. Finally, the nixtamal was stone-ground (FUMASA model MN-400, Puebla, Mexico) to provide masas with MC of about 60% and 53% for TNP and IRNP, respectively.

Tortilla Preparation

Masa was flattened into thin discs of approximately 12.5 cm diameter, 1.2 mm thickness and 28 g weight, using a commercial tortilla roll machine (Model TM–G, Casa Gonzalez, Monterrey, Mexico). Tortillas were baked 17 s on one side (first side), 55 s on the other side, and again 17 s on the first side on a griddle at 270 °C (temperature commonly used in Mexico to bake tortillas). The temperature was measured with a non-contact portable infrared thermometer Fluke-572 (Fluke, Melrose, MA, USA). Finally, masa (500 g) and tortillas from each treatment (n=20) were oven-dried at 40 °C for 48 h, then milled and stored at 4 °C in polyethylene bags for further analysis.

Physicochemical Properties of the Nixtamalized Products

Two analyses were performed in masa, tortilla and nejayote: MC (drying at 105 °C for 24 h) and pH (potentiometer method), following the AOAC official methods 925.10 and 943.02, respectively (AOAC 2000).

Experimental Design and Statistical Analysis

The experiment was conducted as a completely randomized design; the six experimental conditions were carried out with three replicates. Data were assessed by a single ANOVA incorporating the two nixtamalization processes (TNP and IRNP) and the three levels of aflatoxins in the maize grain (AC1, AC2 and CTL). Means comparisons were performed according to the Tukey test using the Statistical Analysis System (SAS 2004). To evaluate the relationship between the initial aflatoxin content in the maize grain and the remnant in tortillas elaborated with the two nixtamalization processes, a quadratic and a linear regression models were fitted and its significances were assessed by confidence intervals.

RESULTS AND DISCUSSION

Aflatoxin Content in Maize

The aflatoxin contents quantified in the maize grain are shown in Table 1. In the case of the control (CTL), the experimental grain received the same treatment during incubation (27 °C, 18% MC, 21 d) in the absence of spores of the aflatoxin-producing fungus; consequently, no aflatoxins were detected. For aflatoxin content 1 (AC1) and aflatoxin content 2 (AC2), the values were 173 and 370 ng g^{-1}, respectively (Table 1). The *A. flavus* strain used in this research mainly produced AFB1, as previously reported (Pérez-Flores et al. 2011).

The technique used here (*A. flavus* strain, spore load, grain MC, incubation temperature time) worked quite well to obtain two different aflatoxin contents. These total aflatoxin values (up to 370 ng g^{-1}) represent contents that may be found in commercial maize used to produce tortillas in several regions of Mexico (Torres-Espinosa et al. 1995). Table 1 also shows some physicochemical properties of the incubated maize grain. Statistical differences were not observed for MC and pH, the average values for these parameters were 11.47% and 6.45, respectively. Results on these physicochemical properties are quite similar to those reported previously

Table 1. Physicochemical properties and aflatoxin content of the incubated maize grain (commercial hybrid AS-900).

Sample	MC (%)	pH	Aflatoxin (ng g^{-1})
CTL	11.43[a]	6.45[a]	ND[a]
AC1	11.50[a]	6.46[a]	173[b]
AC2	11.49[a]	6.44[a]	370[c]
Mean (± SEM)	11.47 ± 0.02	6.45 ± 0.01	271.5 ± 98.50

For each response, means not sharing a common superscript differ significantly.

CTL – Control, AC – aflatoxin content, MC – moisture content, ND – Not detectable (below immunoaffinity column detection limit 0.5 ng g^{-1}).

Aflatoxin content expressed on dry basis.

by other researchers for maize grain intended for use in the tortilla industry (Elias-Orozco et al. 2002; Méndez-Albores et al. 2003; Reyes-Moreno et al. 2003).

Physicochemical Properties

Table 2 shows some physicochemical properties of the nejayote (steep liquor). TNP produced nejayote with the highest content of organic solids (3.14%), meanwhile IRNP registered the lowest average value in this parameter with 1.38%. Campechano-Carrera et al. (2012) reported 3.2% organic matter content in nejayote from TNP and values of up to 1.4% from ecological variants (the lime was replaced by calcium salts). These values are in close agreement with our results.

The extremely wide range of the data regarding the content of organic solids in the steep liquor may be a consequence of the differences between the processing parameters, considering that dry matter losses are mainly influenced by several factors including maize genotype, endosperm hardness, cooking and steeping times and type of heating (Serna-Saldívar et al. 1991; Méndez-Albores et al. 2014). With IRNP, 56% less of the total solids were lost compared with the TNP. Since nejayote is one of the most difficult to treat waste waters due to the high content of organic soluble and insoluble solids (Salmerón-Alcocer et al. 2003), the reduced solids found in nejayote from IRNP results in a great advantage. The IRNP appears to avoid polluting the nejayote with high content of pericarp residues; consequently, it gives high yield nixtamalized products, as well as improves the nutritional quality, considering that during the cooking and washing of the nixtamal, several nutrients are lost including protein, fat, dietary fiber, vitamins and some minerals (Maya-Cortés et al. 2010). On the contrary, no significant differences in pH values of nejayote were observed. TNP produced nejayote with an average pH value of 11.70, whereas IRNP nejayote presented an average pH value of 11.83 (Table 2). Maya-Cortés et al. (2010) reported a pH value of 11.90 in nejayote from traditional nixtamalization and a pH value close to neutral for ecological variants, while other authors reported pH values ranging from 10.50 to 11.20 (Sefa-Dedeh et al. 2004). On the other hand, significant differences in the calculated MC and pH were determined in masa and tortillas produced with both thermal-alkaline processes, as shown in Table 3. Masa from TNP had the highest MC (59.87% vs. 53.24%) and pH (8.58 vs. 7.65) in comparison with their counterparts in IRNP. The same

Table 2. Physicochemical properties of steep liquor (nejayote) obtained from the two nixtamalization processes.

Sample	Steep Liquor			
	Solids (%)		pH	
	TNP	IRNP	TNP	IRNP
CTL	2.91[a]	1.23[b]	11.65[a]	11.80[a]
AC1	3.26[a]	1.48[b]	11.73[a]	11.81[a]
AC2	3.26[a]	1.44[b]	11.73[a]	11.88[a]
Mean (±SEM)	3.14 ± 0.14	1.38 ± 0.08	11.70 ± 0.03	11.83 ± 0.03

For each response, means not sharing a common superscript differ significantly.

TNP – traditional nixtamalization process, IRNP – infrared nixtamalization process, CTL – control, AC1 – aflatoxin content 1 (173 ng g^{-1}), AC2 – aflatoxin content 2 (370 ng g^{-1}).

behavior was observed in the case of tortillas; tortillas from TNP had the highest MC value (52.60%) in comparison with tortillas from IRNP (45.29%). In general, masa had higher MC values than tortillas, because masa lost MC during the tortilla baking process.

Regarding these physicochemical properties, our research group had reported MC of up to 53.7% in tortillas produced from the commercial instant maize flour MASECA® and 45% in tortillas elaborated with the IRNP (Méndez-Albores et al. 2012; 2014). Those MC values are perfectly in accordance with these results. Besides, pH values of tortillas produced with both thermal-alkaline processes showed significant differences (Table 3), even when nixtamalization was performed with the same lime content (1.5%, wt/wt). Tortillas produced with the TNP presented an average pH value of 8.53 in comparison with 7.60 from tortillas produced with the IRNP. Sefa-Dedeh et al. (2004) reported pH values in the range of 7.01 to 7.88 for lime-treated maize using 0.33–1% (wt/wt) calcium hydroxide. Milán-Carrillo et al. (2004) produced nixtamalized flours from quality protein maize with pH values from 7.39 to 10.07, using 3.3 to 6.7 g $Ca(OH)_2$ L^{-1} water. These results agree with those obtained in this research. The pH is an important quality parameter which affects some physical, sensory and textural properties of the nixtamalized products. However, with some maize genotypes, a high content of lime could be used (up to 3%, wt/wt), which leads to a yellowish end-product extending the shelf-life of the tortillas.

Aflatoxin Content

Table 4 shows the aflatoxin content in alkaline and acidified extracts of masas and tortillas elaborated when using both nixtamalization processes. Results indicate that there was a significant reduction in the aflatoxin content due to the thermal-alkaline treatments. Extract acidification of these nixtamalized products, with a pH similar to that of the human stomach indicates that the aflatoxin reduction was not stable; on the contrary, an extra reduction in the aflatoxin content (measured as a loss of fluorescence) was observed. Moreover, no statistical differences in the aflatoxin content were observed for the two batches of contaminated grain (non-nixtamalized), even with the acidification treatment of the extracts (data not shown). These results suggest that

Table 3. Physicochemical properties of products obtained from the two nixtamalization processes.

Sample	Masa				Tortillas			
	MC (%)		pH		MC (%)		pH	
	TNP	IRNP	TNP	IRNP	TNP	IRNP	TNP	IRNP
CTL	60.04[a]	52.27[b]	8.59[a]	7.82[b]	52.60[b]	44.77[c]	8.55[a]	7.60[b]
AC1	59.89[a]	53.39[b]	8.58[a]	7.58[b]	52.51[b]	45.67[c]	8.53[a]	7.59[b]
AC2	59.69[a]	54.06[b]	8.57[a]	7.54[b]	52.70[b]	45.44[c]	8.50[a]	7.60[b]
Mean (±SEM)	59.87 ± 0.10	53.24 ± 0.52	8.58 ± 0.01	7.65 ± 0.09	52.60 ± 0.06	45.29 ± 0.27	8.53 ± 0.02	7.60 ± 0.01

For each response, means not sharing a common superscript differ significantly.
TNP – traditional nixtamalization process, IRNP – infrared nixtamalization process, MC – moisture content, CTL – control, AC1 – aflatoxin content 1 (173 ng g^{-1}), AC2 – aflatoxin content 2 (370 ng g^{-1}).

Table 4. Aflatoxin content of products obtained from the two nixtamalization processes as well as effect of pH on mycotoxin quantification

Sample		Masa				Tortillas			
		Aflatoxin* (ng g^{-1})	Reduction (%)	Aflatoxin** (ng g^{-1})	Extra Reduction (%)	Aflatoxin* (ng g^{-1})	Reduction (%)	Aflatoxin* (ng g^{-1})	Extra Reduction (%)
TNP	CTL	ND	—	ND	—	ND	—	ND	—
	AC1	38 ± 8.95[a]	78	16 ± 1.15[e]	13	17 ± 2.89[e]	90	4 ± 0.30[i]	8
	AC2	116 ± 2.89[b]	69	28 ± 2.31[f]	23	61 ± 6.06[c]	84	6 ± 0.30[i]	15
IRNP	CTL	ND	—	ND	—	ND	—	ND	—
	AC1	73 ± 4.04[c]	58	25 ± 6.35[f]	29	50 ± 1.73[g]	71	7 ± 0.60[i]	25
	AC2	149 ± 5.77[d]	60	31 ± 4.04[a]	32	100 ± 4.91[h]	73	22 ± 1.70[ef]	22

Mean of three replicates ± standard error.
Means not sharing a common superscript differ significantly.
TNP – traditional nixtamalization process, IRNP – infrared nixtamalization process, CTL – control, AC1 – aflatoxin content 1 (173 ng g^{-1}), AC2 – aflatoxin content 2 (370 ng g^{-1})
*Aflatoxin extraction at pH of the nixtamalized products.
**Aflatoxin extraction at pH of 3 (acidification procedure).
Data on aflatoxin content are expressed on dry basis.

aflatoxins extracted from the maize grain were not modified in its fluorescence or a significant portion of the antibodies of the immunoaffinity column were not denatured due to the acidification procedure; thus, the possibility for underestimation of the aflatoxin content in masa and tortilla extracts, giving the appearance of further aflatoxin degradation at low pH, was discarded. In this regard, Méndez-Albores et al. (2004) reported that acidification of maize extracts at a pH of 3 did not cause either reformation or loss of aflatoxin fluorescence, indicating the robustness of the immunoaffinity column method.

In the case of TNP, the percentages of aflatoxin reduction in masas from aflatoxin content 1 (AC1) and from aflatoxin content 2 (AC2) were 78% and 69%, respectively. For IRNP, lower degradation percentages were observed, reaching values of 58% and 60% for AC1 and AC2, respectively (Table 4). Extracts acidification caused a notable extra reduction in the aflatoxin content. Based on these results, up to 23% (for TNP) and up to 32% (for IRNP) fluorescence disappearance occurred as a result of acidifying those masa extracts. In contrast, tortillas elaborated using the TNP presented values of 17 ng g^{-1} and 61 ng g^{-1} of total aflatoxins, corresponding to reductions of 90% and 84%, respectively. Moreover, aflatoxin contents of 50 ng g^{-1} and 100 ng g^{-1} were registered in tortillas from AC1 and AC2 prepared with the IRNP, which corresponded to reductions of 71% and 73%, respectively (Table 4). In all cases, tortillas produced with both nixtamalization processes were above the maximum limit allowed in Mexico (12 ng g^{-1}) for aflatoxin contamination (Official Mexican Standard NOM-187-SSA1/SCFI-2002). Furthermore, acidifying tortilla extracts from TNP and IRNP caused up to 15% and up to 25% aflatoxin fluorescence disappearance, respectively (Table 4). Consequently, nixtamalization plus acidification caused notable cumulative reductions in the aflatoxin content in tortillas elaborated with both thermal-alkaline processes, reaching values up to 98% for TNP and up to 96% for IRNP. In general, acidification of masa and tortilla extracts (as occurs during digestion) did not result in an increase in fluorescence, which means that most of the aflatoxins were modified permanently during the thermal-alkaline treatments. These results are perfectly in accordance with those reported previously by other researchers (Anguiano-Ruvalcaba et al. 2005; Torres et al. 2001). It is well known that higher MC and pH enhance the modification of aflatoxins during cooking or baking; thus, high MC in combination with high pH favor hydrolysis of the lactone ring of the aflatoxin molecule (Mann et al. 1967; Samarajewa et al. 1990); consequently, in this research, TNP yields tortillas with higher percentages of aflatoxin reduction (up to 90%). Interestingly, the percentages of aflatoxin reduction in tortillas produced with IRNP were quite similar for both aflatoxin levels tested (72% average). This phenomenon could be due to the fact that non-ionizing radiation (including infrared waves) in sufficient intensity, leads to a rise in temperature, which usually is accompanied by potential changes in the structure of the aflatoxin molecules present in the irradiated matrix (Rustom 1997).

Thermal-alkaline conditions used during both nixtamalization processes were not adequate for aflatoxin reduction at "*safe*" levels, so that a residual content of aflatoxins always remains in the tortillas. Conditions normally found in the processing of tortillas with the TNP are also not adequate to completely detoxify contaminated maize; as a result, several researchers pointed out that TNP degrade 50 to 92% of the initial aflatoxin content in the maize during tortilla production (de Arreola et al. 1988; Elias-Orozco et al. 2002; Guzmán de Peña et al. 1995; Méndez-Albores et al. 2004; Pérez-Flores et al. 2011; Price and Jorgensen 1985; Torres et al. 2001). In relation to the previously cited aflatoxin reductions, it can be said that degradation of aflatoxin-contaminated maize varies considerably, depending on the nixtamalization parameters, such as cooking type–time–temperature, lime concentration, steeping time, as well as the initial aflatoxin content in the maize grain. It is also important to emphasize that masa MC, pH, cooking temperature–time for baking the tortillas and ultimately acidification (as occurs during digestion) are also factors contributing to a greater extent toward obtaining higher cumulative aflatoxin reductions. Moreover, in the case of TNP, solids are lost in the nejayote (up to 3.14%), and they go into the wastewater. These solids contain mainly tip-cap, pericarp, and germ tissue; therefore, aflatoxins present in these anatomic parts of the grain are removed and extracted to the washing water. In consequence, physical removal of the aflatoxins is also a crucial step for higher reductions in the aflatoxin content during TNP.

Mathematical Models

Additionally, the effect of TNP and IRNP on aflatoxin content in tortillas as a function of the initial aflatoxin content in the maize grain fit with a quadratic function and a linear function, respectively (Fig. 1), as follows:

$$y = 4.51 \times 10^{-4} x^2 + \varepsilon \quad \varepsilon \sim (0, \sigma^2) \qquad (1)$$

$$y = 2.7 \times 10^{-1} x + \varepsilon \quad \varepsilon \sim (0, \sigma^2) \qquad (2)$$

in which y = aflatoxin content in the tortilla (ng g^{-1}), x = initial aflatoxin content in the maize grain (ng g^{-1}), and ε = experimental error.

These mathematical functions could be a useful tool for predicting expected values for the aflatoxin content in tortillas when processing maize with other aflatoxin contents not tested in this research. It is also important to recognize the limitations of the mathematical models; while it is obvious from the graph that for x between 0 and 370 ng g^{-1}, the model values are very close to the observational aflatoxin values, we should not assume that the functions will give an accurate prediction of the expected aflatoxin content in the tortilla for values of x much larger than 370 ng g^{-1}. The mathematical functions indicated that the initial aflatoxin contents in the maize grain used to produce tortillas within the maximum limit allowed in Mexico are 163 ng g^{-1} for TNP and 44 ng g^{-1} for IRNP, respectively. To check these model values,

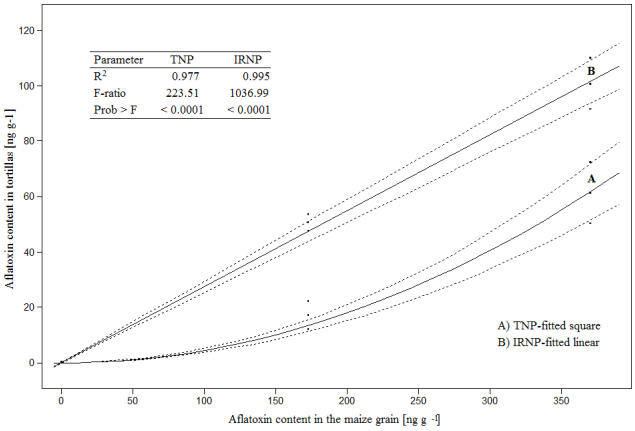

Fig. 1. Effect of traditional nixtamalization process (TNP) (A) and infrared nixtamalization process (IRNP) (B) on aflatoxin content in tortillas as a function of the initial aflatoxin content in the maize grain.

three experimental units of each aflatoxin level were prepared and processed with both nixtamalization processes at identical processing conditions mentioned in the material and methods section. Aflatoxins were only quantified in tortillas. Results indicated that tortillas produced through TNP contained 13 ± 2.56 ng g^{-1}, while tortillas elaborated with IRNP presented an aflatoxin content of 11 ± 1.44 ng g^{-1}. These observational values are very close to the recommended maximum level of 12 ng g^{-1} that Mexico considers for aflatoxin contamination in tortillas.

CONCLUSION

Based on the mathematical models, the IRNP with moderate levels of aflatoxin contamination in the maize grain (up to 44 ng g^{-1}) may have been effective in reducing the aflatoxin content in tortillas. Nevertheless, further studies need to be conducted to verify accuracy of the predicted curves at aflatoxin levels below those calculated as safe for both thermal-alkaline processes. Moreover, acidification of masa and tortilla extracts did not result in a significant increase in aflatoxin fluorescence; on the contrary, an extra reduction in the aflatoxin content was observed, which means that under these particular processing conditions, both nixtamalization processes can yield "*safer*" products for human consumption. More in vitro and in vivo research, however, pertaining to the possible effect of this modified tortilla making process on aflatoxin extra reduction upon acidification, needs to be conducted.

ACKNOWLEDGMENTS

The authors are grateful to UNAM for the financial support for this research through the PAPIIT Grant No. IT203114 and PIAPI Grant No. C-03.

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