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The Effect of Maize Germ on the Presence of Aflatoxins in Corn Flours Treated with a Thermo-Alkaline Process

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Abstract

Background: Aflatoxins are important and frequent teratogens, mutagens and carcinogens of maize, and the germ of the maize seed has linoleic acid, which can control aflatoxins. An aflatoxin analysis of maize flours with and without germ can show the role of the germ in the plant control of these toxins.

Methods: The samples were cooked with the traditional nixtamalization process with varying calcium hydroxide content (from 0 to 2.1 w/w of corn) and steeping times of 0 and 9 h. The aflatoxin purification was performed with immunoaffinity columns, and the quantification was performed using HPLC.

Results: It was found that the presence of the germ and the concentrations of 1.4 and 2.1% w/w of $Ca(OH)_2$ had significant effects ($p \leq 0.05$) on the decrease in the AFB_1 and AFG_1 content.

Conclusion: The linoleic acid of the germ inhibited AFB_1 and AFG_1. However, the aflatoxin content of the experimental samples was higher than 12 μg kg^{-1}, which is the tolerance limit permitted by NOM-247-SSA1-2008.

Keywords: Aflatoxin; Corn; Germ; Nixtamalization

Abbreviations

CAN: Acetonitrile; AFB_1: Aflatoxin B_1; AFB_2: Aflatoxin B_2; AFG_1: Aflatoxin G_1; AFG_2: Aflatoxin G_2; AFs: Aflatoxins; ANOVA: Analysis of variance; $Ca(OH)_2$: Calcium hydroxide; CaO: Calcium oxide; HCl: Hydrogen Chloride; pH: Concentration of hydrogen ions in units of moles per liter; R^2: Correlation coefficient; DNA: Deoxyribonucleic acid; H_2O_d: Distilled water; ≤: Equal or less than; ≥: Equal or more than; EC: European Community; g: Gram; HPLC: High Performance Liquid Chromatography; h: Hours; kg: Kilogram; LOD: Limit of detection; LOQ: Limit of quantification; w/w: Mass/mass; MeOH: Methanol; μg kg^{-1}: Micrograms per kilogram; μL: Microliters; mL(s): Milliliter(s); mL/min: Milliliters per minute; mm: Millimeter; min: Minutes; ng g^{-1}: Nanograms per gram; ng mL^{-1}: Nanograms per milliliter; nm: Nanometers; PBS: Phosphate buffer saline; p: Probability; Rec %: Recovery percentage; RT: Retention Time; rpm: Revolutions per minute; NaCl: Sodium Chloride; NaOH: Sodium hydroxide; TFA: Trifluoroacetic Acid; UV-Vis: Ultraviolet-Visual

Introduction

Maize grains, in their different cooking forms, are the staple food in the diet of the Mexican population, with an average per capita intake of 342 g/day [1]. In Mexico and some countries in Central and North America, most corn-based products are subjected to a thermo-alkaline process called nixtamalization [2]. Most of the maize designated for the miller-tortilla industry and flour is frequently contaminated by insects, mites, bacteria, and fungi, which attack the grain in the field and in storage [3]. Aflatoxins (AFs) are secondary metabolites that chemically correspond to the bis-dihydro-furanocoumarins produced mainly by the fungi *Aspergillus flavus*, *A. parasiticus* and *A. nomius*, which are associated with the stress of the plant in the development stage and stress associated with drought, competition with weeds, poor fertility, and insect pests [4]. The contamination and propagation of the fungi occur during tillage, growth and especially in the post-harvest period [4,5]. Aflatoxins are the most frequently ingested mutagens in food, and the International Agency for Research on Cancer (IARC), in 2002, classified them as Group I carcinogens for humans [6]. The fungal colonization of the grain starts in the tip cap continues in the embryo and finishes in the endosperm. The fungal spores enter the maize flower instead of a pollen grain, and contamination occurs in the maize grain from the inside [7]. *A. flavus* and *A. parasiticus* are capable of invading seeds, are pathogenic to insects, and grow as saprophytes on crop debris in the field and soil. The sclerotia of *A. flavus* can be another source of primary inoculum, which forms in damaged and intact maize kernels and may overwinter in kernels dispersed onto the soil [7]. Insect wounds facilitate the entrance of the fungi directly to the pericarp and endosperm, facilitating AF contamination, although these tissues are not preferred. The presence of *A. flavus* in the germ was detected after 72 h of inoculation with the fungus [8,9]. The colonization of the grain with *A. flavus* and *A. parasiticus* starts in the tissues rich in oil, from the embryo to the endosperm [10]. The germ of

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maize is the reproductive part that germinates to grow into a plant [11]; it is the embryo of the seed. A survey of the AFs in maize tortillas from shops and supermarkets in Mexico City was carried out and 13% of the samples had higher amounts of AFs than the tolerance limit allowed by Mexican legislation (12 μg kg^{-1}) [12]. However, the analysis of other nixtamalized products such as flours has not been carried out.

Maize germ oil [13] may be used as a source for oil extraction or used directly as a food ingredient. The germ is retained as an integral part of whole-grain foods. However, in countries such as Colombia and Venezuela, the grain of maize used to make arepas, empanadas and snacks is fractioned by a mechanical process that intentionally removes the pericarp and the major part of the germ; this product is known as threshed maize. The flours obtained from threshed maize have lower fiber and fat contents and are therefore enriched to improve their nutritional value [14]. *Milling with the removal of the cereal germ is the method most used today to ensure better preservation* [15].

Non-whole grain methods of milling are intended to isolate the endosperm, which is ground into flour, with the removal of both the husk (bran) and the germ. The removal of the bran is aimed at producing flour with a white rather than a brown color and eliminating fiber; neither of these objectives is desirable from a nutritional perspective.

The germ is rich in polyunsaturated fats, primarily omega-6 fatty acids such as linoleic acid, which have a tendency to oxidize and become rancid in storage. The germ removal therefore improves the storage qualities of the flour. Linoleic acid is an essential fatty acid that must be consumed for proper health. During the thermo-alkaline processing of the corn grain with 1 to 3% CaO, 97% of the AFs in the dough are degraded and in the case of tortillas, they are apparently reduced by 98% [5,16].

The aim of the present study is to evaluate the effect of the presence of germ on the aflatoxin content (AFB_1, AFB_2, AFG_1 and AFG_2) in nixtamalized corn flours obtained by the traditional method with different concentrations of calcium hydroxide and steeping times.

Materials and Methods

Sample preparation

Ten kg of corn kernels harvested in 2014 (Puma hybrid-Monsanto) used for tortilla making in Mexico were chosen for this experiment. Three hundred grams of corn kernels and 600 mL of distilled water were cooked at 92°C with different $Ca(OH)_2$ (Fermont, Monterrey, NL, Mexico) concentrations (0, 0.7, 1.4, and 2.1 w/w) with 3 or 9 h of steeping [17].

Two samples, each of 300 g, were lime treated or "nixtamalized" at the same time and with the same process conditions. After cooking and steeping, the germ of one of these samples was manually removed, while the germ was left on in the second sample. The corn samples with and without germ was ground into corn dough and then dehydrated in a furnace (40°C for 8 h). The samples were re-milled using a 0.8-mm hammer mill (Pulvex 200, México D.F. México). Finally, different types of corn flour were obtained.

Aflatoxin extraction from corn flours

Steeping times of 3 and 9 h were chosen for this analysis because these times correspond to the industrial and traditional lime treatment ("nixtamalization") processes, respectively. Three different concentrations of $Ca(OH)_2$ (0.7, 1.4 and 2.1) were used [5] to obtain nixtamalized corn flour with and without germ and raw corn flour and cooked corn flours with and without germ.

The AF extraction consisted of blending 25 g of flour with 100 mL of a mixture of MeOH: H_2O_d (80:20 v/v) and 1 g NaCl to clarify. Later, the blended mixture was centrifuged (ALC 4235 Working Cool System, USA) at 4300 rpm for 10 min and the supernatant retained. Four mL of the supernatant was diluted with 16 mL of PBS pH 7.4, and this dilution was passed through an immunoaffinity column (Easi-Extract Aflatoxin R-Biopharm Rhone Ltd., Glasgow, Scotland, UK) previously balanced with 20 mL of PBS [18,19] for the detection of total AF (AFt). After receiving the sample filtrate, the immunoaffinity column was washed with 20 mL of H_2O_d at a flux of 5 mL per min.

The immunoaffinity column was dried by passing air through the agarose gel, and it was eluted with 1.5 mL of HPLC grade MeOH by gravity at one drop per second. Afterwards, 1.5 mL of H_2O_d was applied with refluxing to denature the gel with AFt antibodies and release the pure AF into the 3 mL eluate. Finally, the eluate was dried at 40°C and derivatized [20,21].

Aflatoxin (AF) standards

The AF standards AFB_1, AFB_2, AFG_1 and AFG_2 (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in a benzene:acetonitrile (ACN) mixture (98:2 v/v) and stored in amber vials at 4°C to prepare 1000 ng/mL solutions. A UV-Vis spectrophotometer (Genesys 10 UV-Vis, Thermo Electron Corporation, Madison, Wisconsin, USA) was calibrated for the correction factor (Method 971.22B, AOAC 2006) [22]. The absorbance in the spectrophotometer was adjusted to 0 with a blank of HPLC methanol in a quartz cell, and the wavelength of maximum absorbance (360-362 nm) for the four AFs was determined [22].

A stock solution of 1000 ng/mL of each of AFB_1, AFB_2, AFG_1 and AFG_2 was used to make 15 dilutions to determine the linearity (calibration curves) of the validation assay method.

Derivatization

The dry AF standards were resuspended in 200 μL acetonitrile (ACN) and 800 μL of derivatizing solution to increase the fluorescence. The derivatizing solution consisted of 5 mL of trifluoroacetic acid (TFA) (Sigma-Aldrich, St. Louis, MO, USA), 2.5 mL of glacial acetic acid (Merck, Naucalpan, Edo Mex, México) and 17.5 mL of deionized water to obtain a final concentration of 20% TFA (v/v). The mixture was vortexed (Vortex G-560, Bohemia, NY, EEUU) for 30 s and the vials were placed in a water bath (Equipos de Laboratorio BG Mod. BM 40T, Mexico) at 65°C for 10 min [20,21]. Finally, 60 μL of the derivatized AFs were injected into the HPLC for chemical quantification.

AF quantification by HPLC

The standard AF HPLC analysis [23] and the eluted samples were quantified with an Agilent Technologies HPLC (Series 1200) with an isocratic pump (G1310A Series DE62957044), fluorescence detector (G1321A Series DE60456380) and autosampler (G1329A Series DE64761666), a chromatographic Agilent Eclipse XDS-C18 column (4.6 × 250 mm, 5 μm of particle size) and the HPLC program ChemStation 32. The analytical conditions were a H_2O/ACN/MeOH (65:15:20 v/v/v) mobile phase, 60 μL injection volume per triplicate, 1 mL/min fluid speed for 20 min, excitation at 362 nm and emission at 425 nm for AFB_1 and AFB_2 and 450 nm for AFG_1 and AFG_2.

Validation of the HPLC quantification method for AFs in corn flour

The validation requirements for the HPLC quantification method for AFs in corn flour have been previously developed [24,25].

Selectivity

This assay was performed to determine the degree to which the method can determine the analyte (AF) without matrix interference. A mixture of the 4 AF standards was analyzed (blank), as were the two matrixes (corn flour with germ and corn flour without germ) independently enriched with the four AFs. The blank and the two corn flour sources with AFs were extracted, concentrated and purified with immunoaffinity total anti-aflatoxin columns. They were then eluted and derivatized, and the resulting chromatograms were compared.

Linearity

This assay was performed to obtain results proportional to the analyte (AF) concentration. Fifteen standard dilutions (0.01, 0.05, 0.5, 1, 2, 4, 5, 8, 10, 16, 30, 32, 64, 100, 128 ng mL^{-1}) of each of the four AFs were tested separately to obtain the calibration curves.

Limits of detection (LOD) and quantification (LOQ)

The LOD was determined by observing the minimal concentration that displayed a chromatographic peak signal on the HPLC equipment. The LOQ was calculated as the LOD multiplied by five, taking into account the sample results.

Recovery percentage (R %)

The recovery percentage is the efficiency of the method in detecting all of the analyte (AFs) present in a sample. Each matrix of cooked corn flour with or without germ was fortified with 100 ng g^{-1} of each one of the four AFs independently before the analysis. Later, the extraction and derivatization were performed, and the recovery percentage was used for the adjustment of the AF concentrations in the samples.

Statistical analysis

Three factors of variation were considered: the presence of the germ, the concentration of $Ca(OH)_2$ and the steeping time. A three-way ANOVA and a post hoc Bonferroni procedure for multiple comparisons with a confidence index of 95% were calculated. The tolerance limit for AFt in maize tortillas according to the Mexican Official Rule [26] is 12 µg kg^{-1}.

Results and Discussion

Validation of the method to quantify AF selectivity

The chromatograms after the AF addition to the three matrices showed that the elution order of the analytes and the retention times (RT) were not modified by the different matrices and the four AF analytes did not overlap.

Linearity, LOD and LOQ

The LOD and the coefficient of correlation (R^2) of the AF calibration curves for the 4 AFs were AFB_1 (LOD=0.1 ng mL^{-1}, R^2 = 0.9973); AFB_2 (LOD=0.01 ng mL^{-1}, R^2=0.9908); AFG_1 (LOD=0.01 ng mL^{-1}, R_2=0.9969); and AFG_2 (LOD=0.5 ng mL^{-1} R^2=0.9908). The LOD of the equipment was highly efficient and facilitated accurate results.

The RTs were adjusted according to the selectivity and linearity for the two treatments of corn flour with and without germ, and the ranges were for AFB_1, RT=7.195 to 8.983 min; for AFB_2, RT=18.965 to 23.272 min; for AFG_1, RT=7.681 to 9.541 min; and for AFG_2, RT=12.214 to 14.492 min.

Recovery percentages

The recovery percentages were for AFB_1, 91%; for AFB_2, 88%; for AFG_1, 90%; and for AFG_2, 90%.

AF quantification of corn with and without germ samples by HPLC

The AFt contents of the nixtamalized corn flour with germ and the nixtamalized corn flours without germ were higher than 12 µg kg^{-1}, which is the tolerance limit established in NOM-247-SSA1-2008 [26] Figures 1-5.

The germ, which contains linoleic acid, diminishes the amount AFB_1 (Figure 1) and AFG_1 (Figure 3) but does not control AFB_2 (Figure 2) or AFG_2 (Figure 4). The deduction is that the effect of the linoleic acid is on the double bond of the first furan ring, which is the place where AFB_1 attaches to the DNA and this place is related to the mutagenic and carcinogenic effects of AFB_1 and AFG_1. The linoleic acid of the germ had no effect on AFB_2 or AFG_2 because neither have a double bond in the first furan. The effect of the linoleic acid of the germ on the opening of the lactone ring would produce a loss of fluorescence, but in all cases, the AFs were detected due to the presence of their fluorescence. The

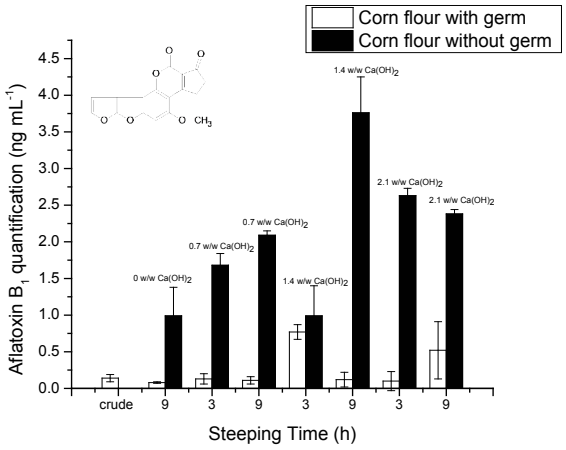

Figure 1: Effect of the steeping time and different $Ca(OH)_2$ concentrations on the amount of Aflatoxin B_1 of corn flour with and without germ.

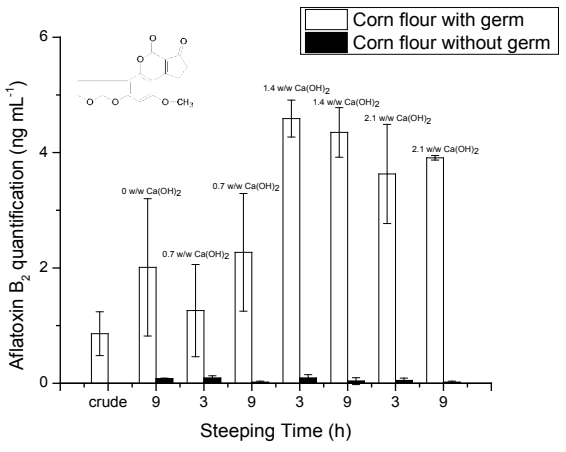

Figure 2: Effect of the steeping time and different $Ca(OH)_2$ concentrations on the amount of Aflatoxin B_2 in corn flour with and without germ.

conclusion is that the effect of the linoleic acid of the germ is on the first furan ring and not on the lactone ring. In fact, there were only traces <1 ng of AFG_2, which was more related to the steeping time than to the presence of the germ with linoleic acid (Figure 4). It is very important to note that AFG_2 and AFB_2 have to be converted to AFG_1 and AFB_1 to recover their mutagenicity.

Of all the AFs, aflatoxin G_1 had the highest concentrations (>40 ng) in the corn flour without germ for 1.4% $Ca(OH)_2$ and a 9 h steeping time, and the linoleic acid of the germ diminishes the AF content (0.54 ng) (Figure 3). It is important to recall that AFG_1 had the highest concentration in all the studied samples. It is important to emphasize that the intake of maize contaminated with AFs for a prolonged time can cause accumulation in the DNA and contribute to AFB_1-DNA adduct formation that can trigger mutation and possibly the development of cancer. The toxicity of the AFs according to their properties of being teratogenic, mutagenic and carcinogenic follow the decreasing order of AFB_1>AFG_1>AFG_2>AFB_2 [27-29].

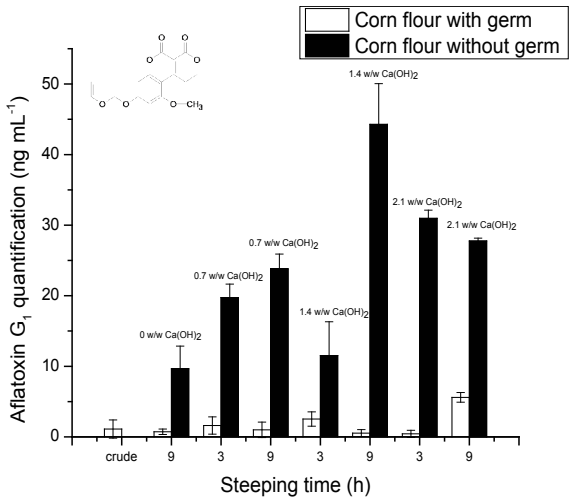

Figure 3: Effect of the steeping time and different $Ca(OH)_2$ concentrations on the amount of Aflatoxin G_1 in corn flour with and without germ.

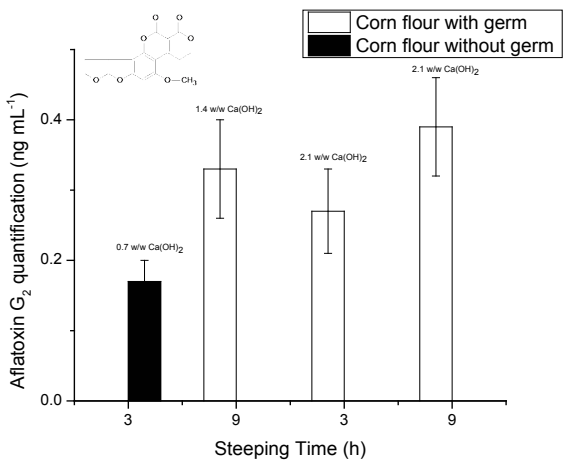

Figure 4: Effect of the steeping time and different $Ca(OH)_2$ concentrations on the amount of Aflatoxin G_2 in corn flour with and without germ.

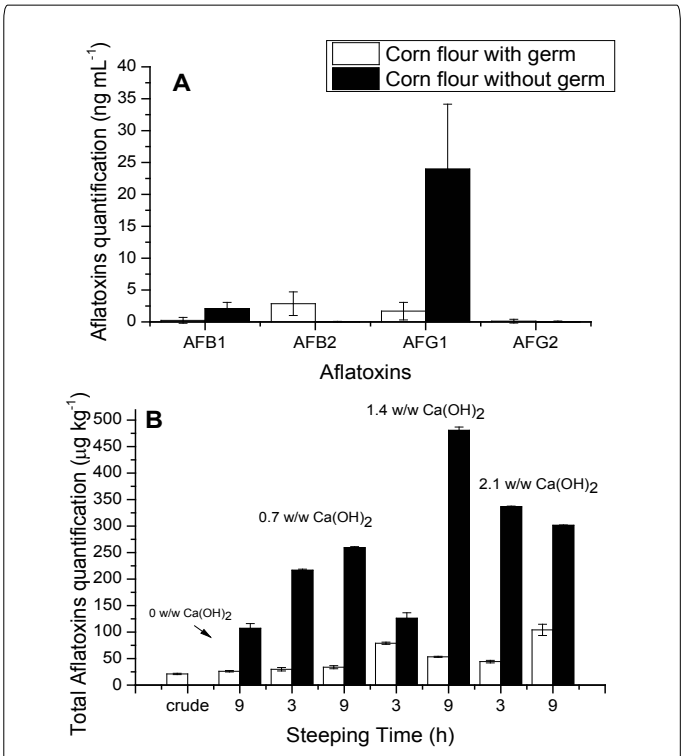

Figure 5: Effect of total AF quantity on the different types of aflatoxin in corn flour with and without germ (5A) and the effect of the steeping time with different $Ca(OH)_2$ concentrations (5B) on the total amount of aflatoxins in corn flour with and without germ.

The AF groups G and B differ in the enzymes involved in their biosynthesis, although they have the same intermediate product; the enzymes implicated in the formation of the AFs of the G group are less stable than those of the B group [30]. The changes in the pH conditions determine the type of metabolites synthesized. Therefore, the AFs of group B are favored at acid pH values below 6, while the AFs of group G are stimulated with neutral to basic pH values above 6 [31]. The different species of fungi produce different types of AFs; *Aspergillus flavus* produces only AFB_1 and AFB_2 and *Aspergillus parasiticus* produces AFB_1, AFB_2, AFG_1 and AFG_2. Therefore, we can deduce that the maize samples were contaminated with both fungi, although they were likely exposed for a longer time to *A. parasiticus.* The biological factors such as the exposure to different species of Aspergillus, the physical factors such as the humidity and substrate and the biochemical and environmental factors favor AF production. In the same way, inadequate storage conditions such as a temperature between 20°C and 40°C, humidity between 10 and 20% and relative moisture in the air between 70°C to 90°C increases the AF levels [32,33].

The thermo-alkaline treatment increases the pH of the products and triggers the hydrolysis of the lactone ring of the AFs, forming salts soluble in water. These AF products are lost in the washings, and they can accumulate in the residual water called "nejayote" [5]. The removal of the remaining water could be an alternative for eliminating AFs. Nevertheless, the conditions used in this nixtamalization process did not reduce the levels of aflatoxins B_1, B_2, G_1 or G_2 in the crude, cooking and nixtamalized flours with germ, and there were no significant differences in the conditions. Other studies [34] demonstrated that the AFs zearalenone and deoxynivalenol resist alkaline treatment with a concentration of 2% w/w Ca $(OH)_2$ and a cooking temperature of 110°C.

The concentrations of AFB_1 and AFG_1 in the corn flours without germ were higher than those in the corn flours with germ in the thermo-alkaline process (Figures 1 and 3). This result shows that compounds in the germ, mainly linoleic acid, exert a protective effect against AFs. However, an opposite effect was observed for AFB_2 for all the conditions tested (Figure 2), where the AFs are found mainly in the corn germ, and they accumulate in the section richest in fatty acids [8].

The AFt content in the corn flours with germ was significantly lower ($p \leq 0.05$) in comparison to that in the flours without germ (Figure 5A). This demonstrates that the germ inhibits the AFs due to its high content of fatty acids, mainly linoleic acid, and other components such as flavonoids, monoterpenes and carotenoids that have the capacity of suppressing the metabolic activation of the AFs, specifically AFB_1 and AFG_1.

The Bonferroni test showed that the presence of the germ and the 1.4 and 2.1% w/w concentrations of $Ca(OH)_2$ in the nixtamalization process had a significant effect ($p \leq 0.05$) on the contents of AFB_1, AFB_2, AFG_1 and AFt. Uniquely, the steeping time had a significant effect on the contents of AFB_2 (p=0.0458) and AFG_1 (p=0.0445). The content of AFG_2 is not significantly affected by the three factors analyzed (presence of germ, concentration of $Ca(OH)_2$ and steeping time).

In some cases, the boiling water in the alkaline process can reduce the detected AF levels in the maize grain from 127 μg kg^{-1} to 68.6 μg kg^{-1} in tortillas [35]. The AF reduction was between 20 to 46% and the residue concentration was higher than the tolerance limit (20 μg kg^{-1}).

The AF levels appear to be reduced due to the alkaline pH of the nixtamalization process that opens the lactone ring. The acid pH of the gastric fluid in the stomach during digestion closes the lactone ring and facilitates the reactivation of the AFs in the neutral pH of the pancreatic fluid [36]. A study analyzed the role of pH, temperature, and the frying effect in the AF reduction in tortillas and maize snacks, where only the AFs of the samples treated in pH 9.5 were activated again [5]. The increase in AFs by acidification was much lower, ranging from 0 to 18% higher than the reported values [35]. In alkaline solutions, a slow hydrolysis of the AF lactone ring can be observed. This hydrolysis is reversible, with the reformation of the lactone ring by the environmental acidification [5,35]. In a study of the levels of AFs in peanut beverages artificially contaminated with AFB_1, the role of pH in the inactivation of the AFs was observed at temperatures higher than 100°C. The effects of the pH (5, 8 and 10.2), temperature (121, 130 and 140°C) and heating time (between 5 and 20 s and 15 min) did not significantly ($p<0.001$) reduce the mutagenic activity. Heating the beverage at 130°C for 20 s or 121°C for 15 min at pH 10.2, however, reduced the mutagenic activity by 79.4 ± 5.3% and 86.6 ± 5.2%, respectively [37]. This decrease in the mutagenicity is attributed to the transformation of AFB_1 to AFD_1, which is 450 times less mutagenic. The lactone ring hydrolyzes when NaOH is added to adjust the pH. The reduction of the mutagenic effect is attributed to the partial hydration of the lactone ring in the presence of the chloride added to adjust the pH. The AFB_1 is transformed to AFB_{2a}, which is 1000 times less mutagenic. In peanut beverages, the change of pH from 8 to 5 or 10.2 was not significant for the reduction of the AF mutagenicity if there is no heating [37].

The maize grain has developed protection mechanisms against AFs through the synthesis of lipids, flavonoids, and monoterpenes, among other components [32]. Research on corn grains contaminated with AFs showed that linoleic acid (the major fatty acid of the lipid content of the germ) has the capacity to suppress the metabolic activity *in vitro* of AFB_1 [27,38]. The effect of the lipids of the germ on nixtamalized corn flours has not been reported.

The role of linoleic acid is not well known, there are some reports about an antioxidant effect. Linoleic do reduce the aflatoxin content or its fluorescent detection, but it inactivates the mutagenic effect of aflatoxins. Most of the carcinogens are activated by oxidation. The maize plant cannot avoid the presence of the *Aspergillus flavus* or *A. parasiticus* fungi, but it can inhibit its mutagenic effects by avoiding the oxidation of the first furan of the aflatoxin that has the double bond, with the linoleic acid in the germ. The aflatoxin B_1 (Figure 1), AFG_1 (Figure 3) and the hydroxylates AFM_1, AFP_1 and aflatoxicol have a double bond in the first furan where the oxygen links and make the 8,9 exo-epoxide that links to the DNA and can produce a mutagenic effect. In Figures 1 and 3 we can appreciate that the germ (with linoleic and oleic acids) inhibits the aflatoxin B_1 and G_1 production, but the germ (with linoleic and oleic acids) have no effect on Aflatoxin B_2 (Figure 2) and Aflatoxin G_2 (Figure 4) because they have no double bond in the first furan and have to be bio transformed to AFB_1 and AFG_1 respectively to be carcinogenic, so the double bond of the first furan might be the place where the linoleic acid has its effect. Around 90% of the carcinogens are mutagens. Therefore many cereals such as maize, sorghum, rice etc., spices (like pepper and chili peppers), and oilseed plants such as nuts, pistachios, cottonseeds, peanuts, etc. have many antioxidants raised by the presence of the fungi, to inhibit its mutagenicity, but they have also lots of aflatoxins.

It is like a war, the plant produces antioxidants to avoid the oxidation of the first furan of the aflatoxins B_1 and G_1, that is the one that links to N^7 of the guanine DNA, mainly guanine, to form the adduct (active carcinogen), but these seeds with antioxidants such as linoleic acid in maize are full of inactive and not detectable aflatoxins, because many times the coumarin section of the aflatoxin has the lactone opened and lacks fluorescence. In the case of peanut the antioxidant is resveratrol. It is a dialectic (union of opposites) situation: maize has aflatoxins inactivated by linoleic acid, but 17% of them can be stored in the human DNA during many years. Aflatoxins are "unavoidable toxins" because humans normally eat cereals, spices and oilseeds to live, but these aflatoxins are dangerous because they can produce aflatoxin B_1-formamide pyrimidine (AFB_1-FAPY) adducts that is the active carcinogens. The problem is that the physicians recommend the ingestion of oilseeds to cardiac or gastric disease persons, because they have antioxidants, but they are not aware that they also have "inactivated aflatoxins" that in the animal or human livers can be metabolized and reactivated again.

The AFB_1 mutagenicity could be tested with spiked maize tortilla samples using the Ames test but not with naturally contaminated corn, showing the resistance or control of the maize plant against the AFB_1 mutagenicity [36]. The oils from the maize germ can help to reduce the effect of the AFs.

The conclusion of the present work is that the linoleic acid of the germ inhibited AFB_1 and AFG_1, and its mechanism of action possibly involved the first furan ring and not the lactone ring. There were only traces <1 ng of AFG_2.

The AFt in all studied samples was higher than 12 μg kg^{-1}, which is the maximum tolerance limit permitted by NOM-247-SSA1-2008. However, 1.4 and 2.1% w/w concentrations of $Ca(OH)_2$ and the presence of the germ have significant effects ($p \leq 0.05$) in decreasing the content of AFB_1 and AFG_1.

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