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Corn "Nixtamalización" and the Fate of Radiolabelled Aflatoxin B1 in the Tortilla Making Process

D. Guzmán-de-Peña,[1] L. Trudel,[2] G. N. Wogan[2]

[1]Centro de Investigación y Estudios Avanzados, IPN-Unidad Irapuato, Apdo Postal 629, Irapuato, Gto. Mexico
[2]Massachusetts Institute of Technology, Division of Toxicology, Massachusetts Avenue, Cambridge, Massachusetts, USA

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"Nixtamalización" is an ancient and traditional process used in México to make the corncakes (tortillas), which constitute the basic Mexican food, using corn exposed to a lime/heat treatment. The physical and chemical effect of the lime over corn has been reported by different authors. Physically, it causes corrugations and partial dissolution of the outermost layer while the aleurone layers keep enclosing the endosperm (Paredes *et al.* 1982). Chemically, there is leaching of proteins. Most of the protein leached seem to consist of albumins and globulins of low molecular weight. This process also increases the rate of release of the most essential amino acids (Bresani 1958). The loss of leucine during the process improves the biological value of tortilla protein by partially correcting the isoleucine to leucine disproportion (Bresani 1958).

Several reports about the deteriorative activity of lime ($Ca(OH)_2$) over aflatoxin in corn have been published, and widely varying estimates of the effectiveness of "nixtamalización" in removing aflatoxin B1 contamination from corn have been published. Ulloa-Sosa and Shroeder (1969), for example, reported that about 70% of the aflatoxin is removed during the process, while Arriola *et al.* (1986), indicated that "nixtamalización" does not reduce aflatoxin levels. Other authors, Rosiles (1979), Machorro and Valdivia (1987), and Price and Jorgensen (1985) report results somewhere in between. However in these studies artificially-contaminated corn was used, the normal nixtamalizacion process was not followed, and they failed to determine the fate of the toxin during the process.

Correspondence to: D. Guzmán-de-Peña

In the present study care was taken to use naturally-contaminated corn, and to follow the traditional process of nixtamalizacion employed in México. The fate of aflatoxin during the process was followed employing radiolabelled aflatoxin B1. In the former case, aflatoxin levels were monitored throughout the different steps of the process using the modification of the method 1 AOAC (CB-method) (Guzman de Peña *et al.* 1992). Radioactivity and AflatestTM. assays were used for the corn to which radiolabelled aflatoxin was added.

MATERIALS AND METHODS

Samples of white and yellow corn naturally contaminated with aflatoxin B1 from the state of Tamaulipas (México), were used in the first part of this study. White corn, Vineyard 42-7 free of aflatoxin B1, was used in the radiolabel assay.

Radioactive aflatoxin solution (^{3}H-AFB1, Moravek, La Brea, CA, final specific activity of 18.6 mCi/mmol) was used to spike the corn. For this purpose, a one-kg sample of the Vineyard corn contained in a 4 L glass vessel was soaked in water during 6 hr at room temperature, and the water was discarded. The corn sample was then transferred to a glass vessel containing enough acetone to cover the corn, radioactive aflatoxin (ca. 30 μCi) was added, the vessel was covered, and left overnight (14 hr). The acetone was discarded according to the procedures for radioactive material (MIT Radiation Protection Committee), and the corn was dried down. Triplicate samples (50 g each) were taken to determine radioactivity as follows: samples of corn or corn dough (masa, see below) obtained by nixtamalización were ground with 100 ml of 80% methanol in a blender, the sample was filtered through filter paper, and aliquots of the extract were mixed with scintillation fluid, and their radioactivity was measured. Alternatively, aflatoxin content in the samples was measured by the Aflatest method.

The levels of aflatoxin contamination throughout the process of nixtamalización in yellow and white corn were determined according to the modification of the method 1 AOAC as published elsewere (Guzman de Peña *et al.* 1992). The initial and final levels of aflatoxin contamination in Vineyard corn during the radiolabel study were determined by Aflatest and dpm equivalents.

The nixtamalización process was performed as it is traditional in Mexico: (i) lime activity (minuteman-lime. MA.) was checked by organoleptic testing; (ii) corn (1 kg) was washed 2 or 3 times to discard floating debris; (iii) corn was covered with water; (iv) 10 g of lime (Minuteman-lime commercial grade) were added; (v) mixture was boiled (94° C) until water was turbid (50 min); (vi) mixture was left soaking overnight (17 hr); (vii) corn was washed 2 or 3 times and mill-ground to obtain the dough ("masa").
Five replicates of corn of 1 kg each were used for the experiments with naturally-contaminated corn.

RESULTS AND DISCUSSION

Fifteen analyses of aflatoxin were performed to determine the initial levels of aflatoxin in the naturally-contaminated corn. A 5-kg corn sample containing 37 µg/kg AFB1 was used as a control. A 5-kg corn sample with 251 µg/kg AFB1 was used as naturally highly contaminated corn. The samples were divided into1-kg subsamples. Both types of corn (the low and highly contaminated) were treated by nixtamalización as described in Material and Methods. 2.0 kg of dough were obtained for each kg of corn. The lime-water in which the corn was cooked, as well as the water used during the corn washings analyzed

Aflatoxin determination by means of the modified-CB method was performed on all of the washing waters and on the dough obtained during the process. Ten analyses of each of the

washing liquids and one hundred and two hundred analysis of aflatoxins were performed on the total dough obtained after the nixtamalización procedure to the low and highly contaminated corn samples, respectively. Our results demonstrated that aflatoxins were completely eliminated from the corn during the nixtamalizacion process in the less contaminated corn, and by more than 90% in the case of the highly contaminated corn (Table 1).

Table 1. Effect of "nixtamalizacion" on aflatoxin B1 in naturally-contaminated corn.@

Yellow corn@ sample*	Level of aflatoxin B1** Before nixtamalizacion	After	Aflatoxin destruction by nixtamalizacion (%)
Less contaminated	37	0	100
Highly contaminated	251	6±1.0	97

@ corn from Tamaulipas harvest 1988-1989
* 5-kg sample was divided into 1-kg subsamples, and treated separately
**Aflatoxin determination by modified CB method (Guzman de Peña *et al.* 1992). One hundred analyses to the control and 200 analyses to the contaminated corn were performed. Data expressed as µg/kg, + SEM.

To determine whether aflatoxins were extracted by the lime treatment, we proceeded to analyze the four types of liquids used during cooking and washing of the corn for aflatoxin content (ten analyses of each sample). For all the samples, no fluorescent spots corresponding to aflatoxins were observed when TLC plates were illuminated with UV light. These results demonstrate that aflatoxin levels in the liquids were below the detection limits of the technique. The pH values of

the liquids ranged from 5 to 12. The highest pH value corresponded to the lime-water solution after cooking. Such a high value could explain the absence of aflatoxins in the final dough and in the liquids. It is well known that pH values above 8 or below 4, can break the difuran ring which confers the typical fluorescence to aflatoxins (Goldblatt 1969).

To determine whether none-fluorescent metabolites from aflatoxins, still contaminated the final dough, we proceeded to contaminate artificially the corn with radioactive aflatoxin, and follow the distribution of the label during the process.

Initial radioactive levels of spiked corn were 12.1 μCi per kg. If we refer to equivalents of aflatoxin in the corn sample, it corresponded to 0.65 mmoles. After nixtamalizacion, radioactive values of the dough were in the order of 2 μCi/kg corresponding to a loss in the order of 80%. These values were confirmed when the aflatoxin content was measured by the Aflatest method (Table 2).

Table 2. ^{3}H-AFB1 Removal from Corn During Nixtamalizacion

Method of analysis	Aflatoxin content*		Aflatoxin removal (%)
	Before treatment	After treatment	
Radiolabel assay	0.650	0.107	84
Aflatest	0.625	0.121	81

*Averages from three different 50 g samples expressed as μmoles per kg.

In order to determine the fate of the radioactivity, this was measured in the cooking liquid and washes. Most of it was

found in the cooking liquid and the first wash (Table 3). These results confirm that most of the aflatoxin byproducts were removed during the cooking process.

Table 3. Distribution of Radioactivity from Aflatoxin B1 During Nixtamalizacion

Sample	Radioactivity*	Per cent of total
Initial	26.8	100
Cooking Liquid	7.9	29.3
1st Wash	9.2	34.2
2nd Wash	4.8	17.8
3rd Wash	0.57	2.1
" Masa " (dough)	4.4	16.3
TOTAL	26.9	100

*Expressed as dpm per kg X 10^{-6}

Our results demonstrate that only a small amount of aflatoxin (less than 17%) survives the nixtamalizacion process. Most of the toxin is destroyed during the alkaline cooking, and is transformed into products which do not display the characteristic fluorescent properties of the original compound. The correspondence existing between the toxin values calculated from the residual radioactivity in the dough, and the analysis by the Aflatest method, are evidence that the aflatoxin which survives the process retain mostly unaffected its property to be recognized by the monoclonal antibodies.

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