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Aflatoxin Fate during Alkaline Cooking of Corn for Tortilla Preparation

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Corn Nutricta variety was inoculated with *Aspergillus parasiticus* NRRL 2999, and aflatoxin production at 35 °C was determined as a function of time. The contaminated corn was employed to prepare tortillas by the alkaline cooking process called nixtamalizacion. Aflatoxin reduction was checked as a function of initial levels, cooking temperature, and amount of calcium hydroxide. Starting with initial values between 1360 and 1896 μg/kg and employing the amounts of calcium hydroxide required to produce a good tortilla, although there was an aflatoxin decrease, none of the treatments was capable of reaching the allowable threshold values of 20 μg/kg. When 2–10% CaO was used to prepare the dough, an intense yellow color developed and the tortillas lost their normal organoleptic characteristics. In all cases studied, even at the lowest CaO concentration, a higher decrease of total aflatoxin was found from corn to dough preparation during nixtamalization. During alkaline cooking aflatoxins G1 and G2 were more susceptible to degradation than B1 and B2. B1 was more resistant than B2.

In Central America corn is the cereal with highest production and consumption. The present production is above 2000 metric tons, the majority of which is destined for direct human consumption. However, depending on the country, from 5 to 20% of the corn is used for feeding animals, mainly poultry. Due to the diversity of climatic conditions in the Central American area, corn is harvested there all year. One part of the harvest, perhaps one-third, is carried out during rainy months. In the field, the major damage is due to birds, insects, and molds, the latter generally caused by bad weather, before and during harvest. During storage, especially in small farms, insect infestations and problems due to storage of poorly dried grain often occur. This problems are aggravated in corn harvested from May to November and in zones such as certain areas on the Atlantic coast where the rainfall is scattered throughout the year.

In Guatemala, which produces about half of the total production, corn is mostly consumed as tortillas (hand-formed flat pancakes), which are manufactured by the process nixtamalizacion. The procedure is as follows: corn is cooked in a suspension of water and lime (CaO) and left overnight. The cooked grain is then drained, washed with tap water, and lightly pressed to eliminate the seed coats (epicarp) and excess calcium hydroxide. The cooked grain or nixtamal is milled to prepare the masa (dough), and then small amounts are hand-molded and baked on a comal (hot surface) for a few minutes, in order to prepare the tortillas. Most of this processing is done on a rather small scale, although there is an industrial operation following the same methodology that produces instant tortilla flour.

A high percentage of the corn harvested in Guatemala during the early harvest from May to November shows considerable contamination by several fungi (Martinez et al., 1970). Among these, some species of the genera Aspergillus, well-known as aflatoxin producers, were frequently found (de Campos and Marzys, 1979; de Campos et al, 1980). Of the aflatoxins produced by *Aspergillus flavus* or *Aspergillus parasiticus*, aflatoxin B1 is considered the most active hepatocarcinogenic agent (Newberne et al., 1964; Le Breton et al., 1962, Enomoto and Saito, 1972).

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There is evidence that maximum frequency of aflatoxin contamination of corn in Guatemala is during the rainy season (de Campos et al., 1980). Samples analyzed 20 days after corn was harvested had levels of 130 μg/kg of total aflatoxin. The same samples analyzed 60 days later showed a great increase in aflatoxin content from 100 to 1680 μg/kg.

A great deal of research has been carried out to induce aflatoxin inactivation. Several of the chemical agents that have been tested were calcium (Codifer et al., 1976) and sodium hydroxides (Mann et al., 1970). In addition, lime treatment improves the nutritive value of corn by increasing the calcium content and the availability of lysine (Bressani et al., 1958; Trejo-Gonzalez et al., 1982), as well as promotes favorable changes in the amino acid content (Katz et al., 1974; FAO, 1969; Bressani and Scrimshaw, 1958). The alkaline treatment also causes aflatoxin reduction (Martinez, 1968; Ulloa-Sosa and Schroeder, 1969; Ulloa and Herrera, 1970).

The experiments described in this paper were designed in order to establish the effect of the nixtamalizacion process (alkaline treatment) on corn contaminated by fungal growth and to determine the amount of aflatoxin reduction.

MATERIALS AND METHODS

Raw Materials. Sound white corn, of Nutricta variety, not treated with fungicides, was used. Nutricta is an improved variety containing twice as much lysine and tryptophan relative to standard corns. Conditions were established to avoid corn germination during fungal growth; corn was dried in a pilot plant tray dryer employing warm air for 1 h at 80–85 °C, followed by a period with live steam humidification for 2–3 h to obtain a final 25% moisture level, suitable for fungal growth and aflatoxin production.

Inoculum Preparation. For the aflatoxin production, a strain of *A. parasiticus* NRRL 2999 maintained on potato dextrose agar at room temperature was used. Spore preparation for corn inoculation was carried out according to the procedures of Hayne et al. (1955). Petri dishes containing potato dextrose agar were inoculated with 0.1 mL of spore suspension and incubated for 10–30 days at

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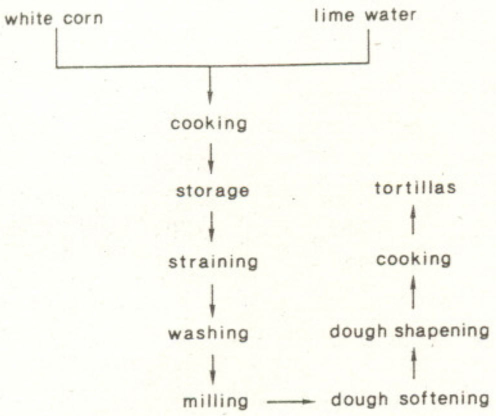

Figure 1. Nixtamalization process flow diagram.

30 °C; a heavy spore crop could then be harvested. The Petri dishes were always isolated in metal containers and then in plastic bags to avoid contamination of the environment. To harvest the spores, the agar surface of each Petri dish was washed twice with 5 mL of 0.1 M phosphate buffer solution at pH 7.0 with 0.1% Tween 80. Both washings were combined.

Corn Contamination for Tortilla Preparation. Germ-inactivated corn (240 g) was placed in wide-mouth glass jars and inoculated with a spore suspension of *A. parasiticus* NRRL 2999. Concentrations of 2.2×10^6 and 4.86×10^6 spores/g of corn (dry basis) were used for the first and second assays, respectively. At the same time, sterile water was added to obtain a moisture level of 25%. Inoculated jars were shaken to distribute spores and then incubated at 35 °C. Controls were prepared without spore suspension. Moldy samples were taken at 3, 7, and 10 days and were submitted to the nixtamalization process, identified as samples 1–3, respectively.

Levels of Lime Employed in the Nixtamalization Process. Taking as a base case the average amount of lime found to be commonly used in home practice, equal to 1.87% w/v (3.00%, w/w), several lower and upper lime concentrations (0.03–10.0%, w/w) were selected for further studies. This was done with the objective of finding a concentration in which the outer seed coat could be removed easily by hand pressure and the organoleptic characteristics of dough and tortilla were not altered. Lime of industrial quality was used and was pulverized and classified through a 14-mesh sieve.

Nixtamalization Process. Following the flow diagram shown in Figure 1, four 250-g contaminated corn samples were mixed with 3.7 L of water and the following proportions of lime: two with 0.6% w/v (1.0%, w/w) (optimum level found in the initial trials); the other two with 1.87% w/v (3.0%, w/w) (commonly used in home practice). One sample of each concentration was cooked by one of the two methods: at atmospheric pressure in an open kettle for 40 min at 95 °C and in an autoclave at 121 °C (15 psig) for 30 min (optimum times). All cooked samples were left overnight at room temperature and then washed several times with tap water and milled. The corn dough was softened, and tortillas were made. The cooking time for tortillas was 1.5 min in a comal (hot plate at a temperature between 180 and 250 °C). While cooking, the tortilla internal temperature was 94 °C. The pH of the corn–water mixture was 6.0–6.5 but increased to 12.0 as lime was added. The pH decreased to 11.2 after the corn was cooked and decreased even more to a value of 9.2 after three washings. The procedure was similar to processes described in the literature; related to Guatemala's practice, the differences rest on the amount of lime used, 0.23% w/v (0.49 w/w) (Bressani et al., 1958).

Table I. Organoleptic Characteristics of Nixtamal Dough and Tortillas at Different CaO Levels

CaO	dough		tortilla	
(w/w), %	texture	color	texture	flavor
2.00	fine	yellow	slightly breakable	slightly alkaline
4.00	coarse	yellow	breakable	alkaline
8.00	coarse	intense yellow	breakable	alkaline
10.00	coarse	intense yellow	breakable	alkaline

CHEMICAL ANALYSIS

Moisture. Moisture was determined on duplicate samples of contaminated corn, dough, and tortillas by drying at 100 °C to constant weight (AOAC, 1984a).

Aflatoxin Analysis. Aflatoxin content was determined in contaminated corn, dough, and tortillas samples prepared at each lime level and cooking process. The same procedure was followed with all samples removed for the two assays. The methods employed for aflatoxin extraction were those described on the AOAC method (1984b). Quantitative thin-layer chromatography was carried out in the above samples on a densitometer (Kontes, Model 800); measurements were done following the procedure cited by Stubblefield et al. (1976). All tests were performed in duplicate.

RESULTS

Recovery of 92% of the aflatoxin added to corn indicated that the method used was applicable.

Levels of Lime Used during the Nixtamalization Process. Results obtained from the organoleptic characteristics of dough and tortillas prepared with different lime levels, up to 1.87% w/v (3.0 w/w) showed the dough to be normally good (in color, texture, and taste). When higher amounts (2–10%) of lime were tested, the nixtamal, dough, and tortillas had a yellow color, which was enhanced by increasing the amount of lime used. Their taste and flavor were extremely alkaline, being completely objectionable for human consumption (Table I). This result was of great importance as it indicated the maximum permissible level of lime to be used for further experiments to obtain an acceptable product for consumers.

Aflatoxin Reduction during Nixtamalization. The evaluation of aflatoxin reduction in contaminated corn, treated with the two selected lime levels, gave the results shown in Table II: (a) There was a decrease in the total aflatoxin contents in the dough and the tortilla in all samples. (b) The decrease was higher from corn to dough than from dough to tortilla. The differences between lime concentrations employed and between cooking methods were not statistically different at a significance level of 0.05. Aflatoxin reduction from corn to dough was higher in sample 3 and equivalent to 96.67% of the original value on a dry basis. From corn to tortilla it was higher for samples 2 and 3, 97.90 and 97.65%, respectively. Although we found that during the nixtamalizacion process there was a decrease of aflatoxin content, none of the treatments presented total levels lower than that, allowed by FAO/WHO/PAG/UNICEF (1969) and United Nations Environmental Program (1979) which is 20 μg/kg on wet basis. This experimental evidence is important because our lower levels of aflatoxin contamination (sample 1) are comparable to those found in stored corn in Guatemala.

Regarding the effect of lime on the four aflatoxins, it was found that aflatoxins G1 and G2 were more susceptible to alkaline hydrolysis than B1 and B2, being reduced in some cases by 100%, samples 4–6 (Table III).

Table II. Aflatoxin Content during Nixtamalization Process: Dough and Tortilla Preparation

	total aflatoxin content, μg/kg					
			open-kettle cooking		autoclave	
	WB[a]	DB[b]	WB[a]	DB[b]	WB[a]	DB[b]
			Sample 1			
moldy corn	1603.51	5244.56				
dough, 0.60% CaO			227.18	473.00	229.38	386.03
dough, 1.87% CaO			446.03	771.98	154.70	241.92
tortilla 0.60% CaO			232.66	405.80	177.95	357.93
tortilla 1.87% CaO			142.93	267.41	146.85	236.46
			Sample 2			
moldy corn	5089.73	24887.80				
dough 0.60% CaO			781.14	1574.24	702.54	1472.97
dough 1.87% CaO			486.57	1086.64	302.10	1270.00
tortilla 0.60 CaO			370.71	705.16	251.79	998.99
tortilla 1.87% CaO			128.74	280.84	68.41	127.62
			Sample 3			
moldy corn	14699.28	60477.67				
dough, 0.60% CaO			1463.51	2405.58	1652.50	2471.57
dough 1.87% CaO			1201.19	1883.90	998.87	1180.49
tortilla 0.60% CaO			1182.11	2215.41	1247.28	1556.80
tortilla 1.87% CaO			712.09	1139.15	498.04	807.19

[a] WB = wet basis. [b] DB = dry basis.

Table III. Reduction of Individual Aflatoxin Content during Nixtamalization Process: Dough and Tortilla Preparation[a]

	aflatoxin content, μg/kg							
	B1		B2		G1		G2	
	WB[a]	DB[b]	WB[a]	DB[b]	WB[a]	DB[b]	WB[a]	DB[b]
				Sample 4				
moldy corn	504.00	3265.01	143.62	512.12	337.21	1236.09	79.77	226.34
dough	193.65	345.62	49.82	87.72	20.25	40.49	6.13	15.75
tortilla	117.59	192.16	40.17	61.61	18.77	40.08	4.38	8.95
				Sample 5				
moldy corn	3193.45	19275.10	509.63	2521.65	1259.58	5490.72	127.08	570.62
dough	394.58	843.86	90.30	233.11	30.62	56.79	6.25	13.20
tortilla	179.30	378.98	32.15	71.97	5.97	12.52	0.00	0.00
				Sample 6				
moldy corn	9094.36	37695.60	2286.11	9513.18	2775.48	11130.62	543.33	1639.27
dough	1094.25	1747.54	201.27	309.76	41.39	63.79	2.39	3.18
tortilla	314.63	902.22	164.81	229.25	35.60	56.65	1.96	2.83

[a] Average values from duplicate assays. [b] WB = wet basis. [c] DB = dry basis.

Making a detailed comparison of our results concerning aflatoxin reduction in corn with the scarce literature existent employing the nixtamalization process, the following comments can be made. First, we should only compare sample 1 (Table II) because in the other two samples aflatoxin contamination levels are extremely high relative to what has been found in actual practice in Guatemala. The average values of aflatoxin reduction from the contaminated corn to dough were 82.36% on a wet basis. The equivalent value for the reduction from the contaminated corn to the tortillas was 88.58%. These average values were obtained from the duplicate assays carried out on products from two lime concentrations for the two cooking methods. In the work carried out by Ulloa and Herrera (1970), corn was inoculated with a spore suspension of *A. flavus* strains number XVI-1 (no mention was made of the concentration used) and incubated at 25 °C and 100% relative humidity for 3 weeks. To prepare nixtamal, contaminated corn samples were mixed with lime water solution (10% lime CaO by volume) and cooked for 1 h. The authors reported a total aflatoxin reduction of 91.2% in the dough on a wet weight basis.

Our reduction results during tortilla preparation were higher than the 66.7% (w/w) reported by Ulloa-Sosa and Schroeder (1969). The procedure followed by them to prepare nixtamal was the same as that followed by Ulloa and Herrera (1970), differing in the amount of lime (CaO) used; 7.5% (by volume). The levels of lime used by these were surprisingly high causing an extremely alkaline tortilla flavor.

DISCUSSION

Our data with individual aflatoxins confirm results cited by Codifer et al. (1976) that treatment with alkali reduces aflatoxins, with G1 and G2 more susceptible than B1 and B2. Price and Jorgensen (1985) gave a decrease of 20–46% on a dry weight basis of total aflatoxin when 0.75% w/w CaO was used to cook naturally contaminated corn to prepare nixtamal. The corn was cooked for 75 min and allowed to soak for 24 h. The percentage of aflatoxin decrease was not as great as those already reported and our own findings. The difference might be attributed to the use of naturally contaminated corn vs corn subjected to a controlled surface growth of a pure fungal strain. They also checked the amount of aflatoxin in tortillas after acidification of the product to pH 6 and found that in this case they could recover practically all the initial amounts of aflatoxin. To explain this fact, the authors proposed a reversible reaction in which acidic conditions caused the lactone ring to close again. This important suggestion needs to be checked, preferably by a biological test.

Our data confirm previous results by Ulloa and Herrera (1970), Ulloa-Sosa and Schroeder (1969), and Price et al. (1985) that showed the same tendency induced by alkali, which as explained by Beckwith et al. (1975) is mainly caused by the opening of the lactone ring in basic media and can lead to electrostatic and/or hydrogen-bonding interaction with the substrate.

During alkaline cooking aflatoxins G1 and G2 in corn were more susceptible than B1 and B2, and B1 was more resistant than B2. Corn is generally contaminated with *A. flavus*, which produces the B toxins meanly.

Considering the corn contamination during harvest and storage found by de Campos et al. (1980) and the aflatoxin re-formation occurring in the acid monogastric stomach cited by Price and Jorgensen (1985), it is imperative to take every possible measure to avoid corn contamination during its growth, harvest, and posterior storage.

In summary, it can be concluded that when 2–10% lime (CaO) concentration was used to prepare nixtamal, dough and tortillas developed a yellow color and broke easily. The strong alkaline flavor was undesirable and made the product completely objectionable for human consumption. The maximum permissible level of lime required to produce good tortillas was 1.87% w/v. The amounts of lime normally used in Guatemala in rural and urban areas are not enough to reduce aflatoxin levels to values lower than those permitted for a product destined for human consumption. Consequently, rural inhabitants are probably consuming aflatoxins; therefore, further experiments should be carried out in order to test the effect of digestive juices on tortillas obtained with the nixtamalizacion process.

In all cases studied, even at the lowest CaO concentration, a higher decrease of total aflatoxins was found from corn to dough preparation during the nixtamalizacion.

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Registry No. CaO, 1305-78-8; aflatoxin B_1, 1162-65-8; aflatoxin B_2, 7220-81-7; aflatoxin G_1, 1165-39-5; aflatoxin G_2, 7241-98-7.

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