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AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 8/18/87

MEMORANDUM

TO: AID/PPC/CDIE/DI, Room 209 SA-18

FROM: AID/SCI, Victoria Ose

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 3.M-06

Attachment

2 cy

PNAAY-524

DESTRUCTION OF AFLATOXIN IN CORN AND NUCLEIC ACIDS IN YEAST CORN MIXTURES

Final

Grant No. 936-5542-3N-03

3. N-06

INSTITUTO CENTROAMERICANO DE
INVESTIGACION Y TECNOLOGIA INDUSTRIAL
(I C A I T I)

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FINAL REPORT

DESTRUCTION OF AFLATOXIN IN CORN AND NUCLEIC ACIDS IN YEAST CORN MIXTURES

Grant No. 936-5542-3N-06

Presented by ICAITI to:

ROCAP

Office of the Science Advisor AID/Washington
Energy Officer
Bureau of Science and Technology LAC/DR, AID/W

Guatemala, 1987

CONTENTS

I.- SUMMARY

A. Reduction of aflatoxins during preparation of tortillas.

Corn of the Nutricta variety was inoculated with Aspergillus parasiticus NRRL 2999 and aflatoxin production at 35°C was determined as a function of time. Tortillas were then prepared with the contaminated corn using the alkaline cooking process called "nixtamalización". Aflatoxin reduction was studied as a function of initial level of the toxin, cooking temperature and amount of calcium hydroxide added to the corn. Starting with initial toxin levels between 0.9 and 1.6 mg Kg-1 and using the amount of calcium hydroxide required to produce an acceptable tortilla, none of the treatments was capable of reaching the allowable threshold values of 20 µg Kg-1, although a considerable decrease in the toxin did occur reaching a 89% reduction of original values. During alkaline cooking, aflatoxins G_1 and G_2 were more susceptible to degradation than B_1 and B_2, B_1 being more resistant than B_2.

B. Enrichment of tortillas with yeast protein concentrate.

In order to improve the nutritional value of the corn tortillas, a method for enriching them with yeast protein from a local distillery was developed. The procedure for obtaining a protein concentrate with low nucleic acid content was established for distiller's yeast cream (Saccharomyces cerevisiae) from an ethanol production plant. During the process, the nucleic acid content was reduced 91% and protein content increased 55%. Different levels of protein concentrate were added to the Nutricta corn dough and tortillas were prepared and submitted to a taste panel for sensorial evaluation. Results obtained for the different doughs showed that the maximum level of protein concentrate that could be added to the dough without negatively affecting organoleptic properties of the tortillas was 18% (DW). There was an improvement in the nutritive value of the tortillas with protein suplementation; the protein level increased 59.6% compared with the control tortilla. The supplement provided a significant increase in lysine, the amino acid in which corn is deficient. The supplemented tortillas had a low nucleic acid content which is important.

II. BACKGROUND

This research project was carried out in Guatemala by the Applied Research División of the Central American Institute for Research in Industry (ICAITI) under Grant No. 936-5542-3N-06 from the Program in Science and Technology Cooperation of the Agency for International Development.

Since tortillas prepared from corn through an alkaline cooking process are a major staple food for the indigenous and ladino population of the region, we proposed to study two areas related to this important food. First we proposed studying the fate of aflatoxin, a potent mycotoxin found commonly in corn, during the alkaline cooking process, and second we proposed to investigate supplementation of the tortillas with locally available yeast protein in order to improve the nutritional quality and amino acid pattern of the tortillas.

The general objectives were:

a. To verify that during the alkaline cooking method developed by the indian population to prepare corn tortillas destruction of aflatoxin occurrs.

b. To improve the nutritive value of corn by adding yeast biomass prior to or during the industrial process for preparing instant corn flour.

The specific objectives were:

a. To quantify the degradation of aflatoxins in the contaminated corn during the "nixtamalización" process, and

b. To measure the reduction in nucleic acids in the yeast-corn mixtures during the industrial process to prepare instant corn flour.

Hypothesed to be tested were:

a. The use of lime under conditions of high temperature and humidity reduces aflatoxins to low or undetectable levels.

b. The alkaline cooking process is able to reduce the nucleic acid content of the distiller's yeast when this is added to the corn in the "nixtamalización" process.

The parameters considered in the investigation are:

a. Reduction of aflatoxin

- a.1 Level of lime used.
- a.2 Cooking conditions: time-temperature ratio.
- a.3 Initial toxin levels.

b. Yeast protein supplemented tortillas

- b.1 Preparation of yeast protein concentrate
  - b.1.1 Cell rupture technique
  - b.1.2 Protein precipitation technique
  - b.1.3 Nucleic acid inactivation technique
- b.2 Point of addition of protein concentrate to the corn or tortilla dough.
- b.3 Level of protein supplement to add without affecting consumer acceptance.

The present report covers thirty-six months of project activities.

III. ACTIVITIES

Through activities at the laboratory level, we were able to establish the average amount of lime used in home practice to make tortillas. Aflatoxin was produced in corn to contaminate the tortillas. Different cooking methods either long term boiling or a shorter period at 121°C were used to study degradation of the toxin. Toxin levels at each step of the preparation of the tortilla as well as in the wash waters were determined. The average amount of lime used in home practice (1.87% W/V) and the open kettle cooking procedure at 95°C greatly reduced aflatoxins, however, the amount remaining was higher than the level recommended for a product destined for human consumption.

For the enrichment studies, the yeast cream from the local distillery was studied. It was found that the yeast cells had to be treated previously in order to reduce the nucleic acid to acceptable levels, and therefore considerable effort was dedicated to yeast cell rupture and protein concentration. Combining the effects of the yeast pretreatment and the tortilla preparation, the nucleic acid content of the yeast was reduced 91%, protein concentration was increased and the undesirable yeast cell wall material was removed.

This project provided an opportunity for training young scientists in specialized chemical analysis and microbiological techniques.

The activities, results and conclusions obtained during the research period are presented in more detail in the form of three papers that have been submitted for publication. Copies of the manuscripts are enclosed.

IV. CONCLUSIONS

1.- Reduction of aflatoxin during "nixtamalización" process.

1.1 Acceptable tortillas with good color, texture and taste were obtained when levels of lime used in the cooking (nixtamalización) process did not exceed 1.87% w/v (3.0% DW).

1.2 When higher amounts (2-10%) of lime were used, the dough and the tortillas were of a yellow color which increased as the amount of lime used increased. The flavor was also strongly affected by the high lime concentrations and was considered to be objectionable by the taste panel.

1.3 When the dough was prepared with corn naturally contaminated with aflatoxin, the following pattern of toxin reduction was found:

1.3.1 There was a decrease in the aflatoxin levels in the dough and the tortillas at all contamination levels tested.

1.3.2 The decrease in aflatoxin was more pronounced during the preparation of the dough than during the final cooking process when the tortillas themselves were prepared.

1.3.3 There was no significant difference in aflatoxin reduction between the two cooking methods studied in the project: boiling in an open kettle and cooking under pressure in an autoclave.

1.3.4 There was no significant difference in aflatoxin reduction between the two levels of lime used for dough preparation: that found to be the optimum and lower levels commonly used in local homes.

1.3.5 A decrease in aflatoxin was found with all the cooking conditions studied. However, none was able to reduce the toxin to levels permitted for human consumption (20 μg Kg-1)

1.3.6 Aflatoxins G_1 and G_2 were more susceptible to degradation by the alkaline cooking process than aflatoxins B_1 and B_2. Aflatoxin B_1 was more resistant than aflatoxin B_2.

1.3.7 The amount of lime normally used in Guatemala in both rural and urban areas is not enough to reduce aflatoxin levels to those permitted for a product destined for human consumption.

2.- Reduction of nucleic acids in yeast corn mixtures.

2.1 The maximum amount of dry yeast which could be added to the dough and not cause rejection by the taste panel was found to be 3% which represents an increase of only 5% in the protein content of the tortillas.

2.2 The point for adding dry yeast to the process was determined to be the grinding step.

2.3 Because of the limitations imposed by 2.1 and 2.2, it could be seen that further treatment of the yeast was necessary in order to permit higher supplementation levels.

2.4 A yeast cell wall rupture procedure was established. This was necessary to facilitate extraction of the intracellular protein.

2.5 A protein concentration procedure was optimized.

2.5.1 During this process, protein concentration was increased by 55% and nucleic acid content was reduced by 91%.

2.5.2 Yeast cell wall removal improved tortilla color and digestibility by avoiding darkening of the protein concentrate.

2.6 The maximum amount of protein concentrate that could be added to the dough without alteration of organoleptic properties was 18% (DW).

2.7 The protein content of the tortillas prepared with 18% yeast protein concentrate showed an increase of 60% in the protein level when compared to the control tortilla. Lysine, the amino acid in which corn is deficient, was increased significantly with the addition of the yeast protein concentrate. Nucleic acid levels were held to only 0.06 g 100g-1.

2.8 The chemical PER was 2.05, an improvement over normal corn values.

2.9 The nutritive value of tortillas supplemented with protein concentrate obtained from dried distiller's yeast increased and the tortillas can be used for human consumption.

V. FINANCIAL STATEMENT

INSTITUTO CENTROAMERICANO DE INVESTIGACION Y TECNOLOGIA INDUSTRIAL
DIVISION DE CONTROL DE PRESUPUESTOS Y COSTOS

PROJECT: DESTRUCTION OF AFLATOXINS IN CORN AND NUCLEIC ACIDS
ICAITI 82/4.6.1800 AID 936-5542-G-3066-00
PERIOD: LIFE OF PROJECT
EXPENSE REPORT IN C.A. PESOS (1 $CA=1)

	BUDGET	ACCUMULATED TO DATE	AVAILABLE BALANCE
STAFF			
Salaries	68 848.00	69 721.33	(873.33)
TRAVEL & PERDIEM	1 700.00	1 824.14	(124.14)
MATERIALS & SUPPLIES			
Reagents & Mat.	8 700.00	7 526.69	1 173.31
Glassware & Sup.	9 000.00	10 821.99	(1 821.99)
Photocopies & Stat.	400.00	196.01	203.99
Communications	1 152.00	246.60	905.40
Subscriptions	600.00	124.63	475.37
Books	1 400.00	1 721.24	(321.24)
Equipment	24 900.00	25 707.95	(807.95)
Assorted O.D.C.	1 400.00	183.30	1 216.70
TOTAL	**47 552.00**	**46 528.41**	**1 023.59**
TOTAL COST	**118 100.00**	**118.073.88**	**26.12**

"The undersigned hereby certifies" (1) that payments claimed on vouchers were proper and due under the terms of the Agreement; and (2) that the information on the fiscal report is correct, and such detailed supporting information as ROCAP may require will be furnished promptly on request.

COST CONTROL & BUDGET OFFICER

PROJECT PRINCIPAL

APPENDIX I

AFLATOXIN FATE DURING ALKALINE COOKING OF CORN FOR TORTILLA PREPARATION

Submitted for publication in:

Journal of Agricultural and Food Chemistry

AFLATOXIN FATE DURING ALKALINE COOKING OF CORN FOR TORTILLA PREPARATION

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SUMMARY

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 "nixtamalization". Aflatoxin reduction was checked as a function of initial levels, cooking temperature and amount of calcium hydroxide. Starting with initial values between 1.34 and 1.09 x 10^3 µg Kg-1 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 ng Kg-1. When 2-10% CaO were used to prepare the dough an intense yellow color developed and the tortillas lost their normal organoleptic characteristics. In all ones 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 aflatoxin G1 and G2 were more susceptible to degradation than B1 and B2. B1 was more resistant than B2.

INTRODUCTION

Corn is the cereal with the highest production and consumption in Central America. Present production is above 2 000 thousand metric tons the majority of which is destined towards direct human consumption: however, depending on the country, from 5 to 20% is used for animal feeding, mainly poultry. Due to the diversity of climatic conditions where corn is produced in the Central American area there are harvests all year. However, only one part of the harvest perhaps a third is carried out during rainy months. In the field, the major damage occurs due to birds, insects and molds. the latter generally caused by bad weather, before and during harvest. During storage, specially in small farms, insect infestations and problems due to storage of poorly dried grain often occur. Of course, the latter is aggravated in the harvest taking place from May to November, and in zones whose rainfall is scattered throughout the year as in certain areas on the Atlantic coast.

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 called "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 and washed with tap water, lightly pressed to eliminate the seed coats (epicarp) and excess of calcium hydroxide. The cooked grains or "nixtamal" is milled to prepare the "masa" (douch) 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 in a rather small scale, although there is an industrial operation, following the same methodology, that produces

instant tortilla flour.

Studies have revealed that a higher percentage of the harvested corn in Guatemala during the early harvest from May to November shows considerable contamination by several fungi. Among these, species of the genera Aspergillus, wellknown as aflatoxin producers, were frequently found (Martinez et al, 1970; de Campos and Marzys, 1979; de Campos et al, 1980). It has been established that the more harmful mycotoxins, due to their toxicity and damage, are the aflatoxins produced by species of Aspergillus; being aflatoxin B1 the most active hepatocarcinogenic agent (Newberne et al, 1964; Le Breton et al, 1962; Enomoto and Saito, 1972).

There is evidence that maximum frequency of corn aflatoxin contamination in Guatemala, is during the rainy season. Samples analyzed 20 days after corn was harvested, presented levels of 0.13 10^{3} ug Kg-1 of total aflatoxin. The same samples analyzed 60 days later showed a great increase in aflatoxin content form 0.10 to 1.68, 10^{3} ug Kg-1 (de Campos et al, 1980).

A great deal of research has been carried out in the past in order to induce aflatoxin inactivation by different methods, among several chemical agents that have been tested were calcium (Codifer et al, 1976) and sodium hydroxides (Mann et al, 1970). On the other hand, it is known that lime treatment improves the nutritive value of corn by increasing the calcium contents and the availability of lysine (Bressani et al, 1958; Trejo-Gonzalez et al, 1982), as well as promote some favorable changes in the aminoacid content (Katz et al, 1974; FAO, 1969; Bressani and Scrimshaw, 1958). Also such an alkaline treatment causes aflatoxin reduction as has been already informed (Martinez, 1968; Ulloa-Sosa, 1969; Ulloa and Herrera,

1970).

The experiments described in this paper were designed in order to establish the effect that the "nixtamalizacion" process (alkaline treatment) has on contaminated corn due to fungal growth and to quantify the amounts of aflatoxin reduction.

MATERIALS AND METHODS

Raw Material

Sound white corn, of Nutricta variety, not treated with fungicides, was used. Nutricta is an improved variety which contains 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 one hour at 80-85°C, followed by a period with live steam humidification for 2-3 hours to obtain a final 25% moisture level, suitable for fungal growth and aflatoxin production.

Inoculum Preparation

For the aflatoxin production, a strain of Aspergillus 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 to 30 days at 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 N 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 4.06×10^6 spores per gram of corn (dry basis), were used for the first and second assay, respectively. At the same time, sterile water was added to obtain a moisture level of 25%. Inoculated jars were shaked to distribute spores and then incubated at 35°C (temperature which was selected according to the results of the preliminary trials). Controls were prepared without spore suspension. Moldy samples were taken at 3, 7 and 10 days and were submitted to the "nixtamalization" process. These will be identified as samples 1, 2 and 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 practices and which was equal to 1.97% 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 mesh 10 sieve.

Nixtamalization Process

Following the flow diagram shown in Figure 1, four, 250 g, contaminated corn samples were mixed, with 3.7L water and the following proportions of lime: two with 0.6% W/V (1.0% W/W) (optimum

level found in the initial trials), and the other two, with 1.87% W/V (3.0% W/W) (commonly home practice). One sample of each concentration was cooked by one of the two methods: at atmospheric pressure in an open kettle for 40 minutes at 95°C and in an autoclave at 121°C (15 psig) for 30 minutes (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 100°-250°C). While cooking, the "tortilla" internal temperature was 94°C. The pH of corn-water mixture was 6.0-6.5, which increased to 12.0 as lime was added. The pH decreased to 11.9 after the corn was cooked and even more after three washings to a value of 9.2. The procedure followed above was similar to those processes described in the literature related to Guatemala's practice. The differences rest on the amount of lime used (Bressani and Scrimshaw, 1958).

CHEMICAL ANALYSIS

Moisture

Moisture was determined on duplicate samples of contaminated corn, dough and tortillas, by drying at 100°C to constant weight (AOAC, 1975a).

Aflatoxin Analysis

Aflatoxins content were 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 from the two assays. The methods employed for aflatoxin extraction were those

described on the AOAC method (1975b). Employing such technique corn contaminated with a known amount of pure aflatoxin was analyzed obtaining a 92% recovery. Quantitative thin layer chromatography was carried out in the above samples using a densitometer (Kontes, Model 800). Measurements were done following the procedure cited by Stublefield et al, (1976). Filters excitation and emission of 365 nm and 436 nm were respectively used. All tests were performed in duplicate.

RESULTS

Levels of Lime Used During the Nixtamalization Process

Results obtained from the organoleptic characteristics of dough and "tortillas", prepared with different lime levels, showed to be normally good (in color, texture and taste), up to 1.87% W/V (3.0 W/W) lime used. 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 strong to lime, being completely objectionable for human consumption, see Table 1. Hence, this result was of great importance as it gave us the maximum permissible level of lime to be used for our further experiments in order to still obtain a product of acceptance by the consumers.

Aflatoxin Reduction During "Nixtamalization"

The evaluation of aflatoxin reduction in contaminated corn, treated with the two selected lime levels, gave the following results as shown in Table 2: 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. The aflatoxin reduction from corn to dough was higher in sample 3 and equivalent to 96.67% of the original value on dry basis. From corn to tortilla it was higher for samples 2 and 3, 97.90 and 97.55% 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 those allowed by FAO/WHO/FAO/UNICEF (1969) and USDA (1979) which are 20 ug/Kg on wet basis. This experimental evidence is important because our lower levels of aflatoxin contamination (sample 1) are comparable to those found during stored corn in Guatemala.

Regarding the effect of lime on the four aflatoxins, it was found that aflatoxin G1 and G2 were more susceptible to alkaline hydrolysis than B1 and B2, being reduced in some cases in a 100%, see tables 3 and 4.

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, 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 93.36% on wet basis. The equivalent value for the reduction from the contaminated corn to the "tortillas" was 80.58%. These average values were obtained from the two assays carried out at 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 one hour. 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 followed by Ulloa and Herrera (1970), differing in the amount of lime (CaO) used: 7.5% (by volume). We were surprised of the high amounts of calcium employed by these authors.

DISCUSSION

Our data with individual afflatoxins confirm results cited by Codifor et al (1976).

Price and Jorgenson (1985) gave a decrease of 29-74% on dry weight basis of total aflatoxin when 0.75% w/w CaO was used to cook naturally (in the field) contaminated corn to prepare "nixtamal". The corn was cooked for 75 min and allowed to soak for 24 hours. The percentage of aflatoxin decrease was not as great as those already commented and our own findings. The difference might be attributed to the use of naturally contaminated corn vs corn which has been subjected to a controlled surface growth of a pure fungal strain. They also checked the amount of aflatoxin in tortillas after neutrification 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 molecule reformation due to the acid pH conditions, which meant that the lactone ring was closed again. This important suggestion needs to be checked preferably employing a biological test.

Our data confirms previous results by Ulloa and Herrera (1970); Ulloa and Schroeder (1969), and Price et al (1985) which showed the same tendency induced by alkali, which as explained by Dedwith 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.

In summary, it can be concluded than when 2-10% lime (CaO) concentration were used to prepare "nixtamal", dought and tortillas got a yellow color and broke easily. The taste and flavor were strong to lime and completely objectionable for human consumption. The maximum permisible level require to produce good tortillas was 1.07% w/v. The amounts of lime normally used in Guatemala in rural and urban areas are not enough to reduce aflatoxin levels to lower values than ones permitted for a product destined to human consumption.

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 "nixtamalization".

During alkaline cooking aflatoxin G1 and G2 in corn were more susceptible than B1 and B2; and B1 was more resistant than B2.

Considering the corn contamination during harvest and storage found by de Campos et al (1980) and the aflatoxin reformation which may occur in the acid aqueous stomach cited by Price and Jorgenson (1985) it is imperative to take every possible measure to avoid corn

contamination during its growth, harvest and posterior storage.

ACKNOWLEDGEMENT

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FIGURE 1

NIXTAMALIZATION PROCESS FLOW DIAGRAM

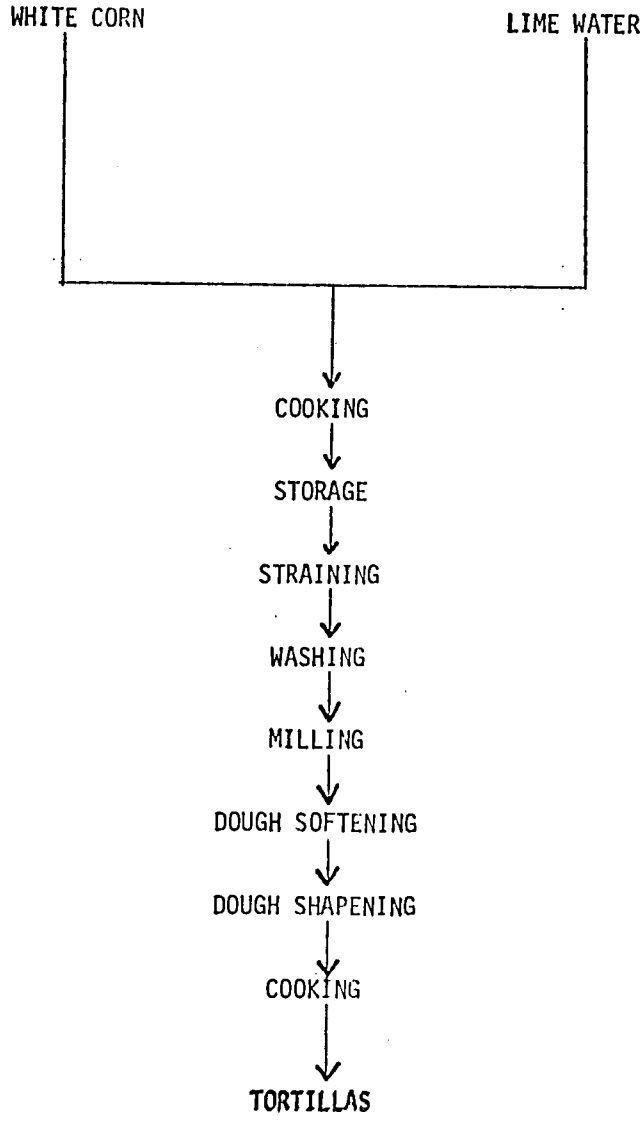
WHITE CORN
LIME WATER
COOKING
STORAGE
STRAINING
WASHING
MILLING
DOUGH SOFTENING
DOUGH SHAPENING
COOKING
TORTILLAS

TABLE No. 1

ORGANOLEPTIC CHARACTERISTICS OF NIXTAMAL DOUGH

AND TORTILLAS AT DIFFERENT CaO LEVELS

CaO (W/W) %	Dough		Tortilla	
	Texture	Color	Texture	Flavor
2.00	Fine	Yellow	Slight brekeable	Slight limy
4.00	Gross	Yellow	Brekeable	Limy
8.00	Gross	Intensive Yellow	Brekeable	Limy
10.00	Gross	Intensive Yellow	Brekeable	Limy

TABLE No. 2

AFLATOXIN CONTENT DURING NIXTAMALIZATION PROCESS

DOUGH AND TORTILLA PREPARATION

Total Aflatoxin Content 10^3 µg/Kg-1			Open Ketle cooking		Autoclare	
	WB	DB	WB	DB	WB	DB
SAMPLE 1						
Moldy Corn	1.63	5.24				
Dough 0.60% CaO			0.28	0.47	0.23	0.39
Dough 1.87% CaO			0.44	0.77	0.20	0.24
Tortilla 0.60% CaO			0.23	0.40	0.20	0.36
Tortilla 1.87% CaO			0.14	0.28	0.15	0.24
SAMPLE 2						
Moldy Corn	5.09	24.88				
Dough 0.60% CaO			0.78	1.57	0.70	1.47
Dough 1.87% CaO			0.48	1.14	0.30	1.27
Tortilla 0.60% CaO			0.37	0.70	0.25	0.99
Tortilla 1.87% CaO			0.13	0.28	0.07	0.12
SAMPLE 3						
Moldy Corn	14.69	60.47				
Dough 0.60% CaO			1.46	2.40	1.65	2.49
Dough 1.87% CaO			1.20	1.99	0.99	1.18
Tortilla 0.60% CaO			0.68	2.21	1.25	1.52
Tortilla 1.87% CaO			0.71	1.14	0.50	0.81

WB = Wet Basis
DB = Dry Basis

TABLE No. 3

REDUCTION OF INDIVIDUAL AFLATOXIN CONTENT DURING

NIXTAMALIZATION PROCESS, DOUGH AND TORTILLA PREPARATION

FIRST ASSAY

SAMPLE 1

AFLATOXIN CONTENT 10^3 µg Kg-1	B_1		B_2		G_1		G_2	
	WD	DB	WB	DB	WB	DB	WB	DB
Moldy Corn	0.88	1.83	0.08	0.16	0.19	0.40	0.09	0.19
Dough	0.14	0.26	0.04	0.09	0.02	0.05	0.00	0.02
Tortilla	0.11	0.18	0.04	0.05	0.02	0.03	0.00	0.01
SAMPLE 2								
Moldy Corn	2.78	18.99	0.41	2.82	0.49	3.36	0.06	0.41
Dough	0.32	0.97	0.09	0.26	0.05	0.08	0.01	0.02
Tortilla	0.15	0.27	0.02	0.05	0.01	0.02	0.00	0.00
SAMPLE 3								
Moldy Corn	7.86	36.57	2.06	9.58	1.56	7.26	0.21	3.27
Dough	1.10	1.87	0.19	0.32	0.02	0.03	0.00	0.00
Tortilla	0.51	1.19	0.12	0.26	0.01	0.02	0.00	0.00

WB = Wet Basis
DB = Dry Basis

TABLE No 4

REDUCTION OF INDIVIDUAL AFLATOXIN CONTENT DURING NIXTAMALIZATION PROCESS, DOUGH AND TORTILLA PREPARATION

SECOND ASSAY

AFLATOXIN CONTENT 10^3 µg Kg-1	B_1		B_2		G_1		G_2	
	WD	DB	WD	DB	WB	DB	WB	DB
SAMPLE 1								
Moldy Corn	1.12	4.69	0.20	0.85	0.47	2.06	0.06	0.25
Dough	0.24	0.42	0.05	0.07	0.01	0.02	0.00	0.01
Tortilla	0.11	0.19	0.03	0.06	0.01	0.04	0.00	0.00
SAMPLE 2								
Moldy Corn	3.60	19.55	0.60	2.21	2.02	7.62	0.19	0.72
Dough	0.46	0.71	0.08	0.19	0.00	0.03	0.00	0.00
Tortilla	0.20	0.48	0.03	0.08	0.00	0.00	0.00	0.00
SAMPLE 3								
Moldy Corn	10.32	38.81	2.51	9.44	3.98	14.99	0.87	3.27
Dough	1.08	1.61	0.21	0.29	0.06	0.09	0.00	0.00
Tortilla	0.11	0.61	0.20	0.18	0.05	0.09	0.00	0.00

WB = Wet Basis
DB = Dry Basis

APPENDIX 2

AFLATOXIN AND TORTILLA PREPARATION IN GUATEMALA

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AFLATOXIN IN MAIZE

Aflatoxin in Maize

A Proceedings of the Workshop

El Batan, Mexico, April 7-11, 1986

Sponsored by: CIMMYT, UNDP and USAID

Aflatoxin and *Tortilla* Preparation in Guatemala

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Maize is the cereal that has both the highest production and the highest consumption in Central America. Present production in the region is above two million tons, most of which is used for human consumption; from 5 to 20% of the crop, depending on the area, is used for livestock feed, especially for poultry. About one-third of the crop is harvested during the rainy months. In the field, major maize damage is caused by insects, birds and molds, the latter often aggravated by bad weather before and during harvest. During storage, especially on small farms, the grain is subject to insect infestations and storage problems because grain is poorly dried. The storage problem is especially severe with early harvested maize (May to November) and in areas where rainfall is scattered throughout the year, as is the case in certain parts of the Atlantic coast.

In Guatemala, where about half of the maize in Central America is produced, maize is mostly consumed as *tortillas* (flat, unleavened maize cakes), which are made by the process called nixtamalization (Figure 1). With this procedure, maize grain is boiled in lime water (CaO) and then left to soak overnight. After draining and washing, the grain is lightly pressed to remove the seed coats (pericarp) and excess lime. The resulting *nixtamal* is then ground to prepare the *masa* (dough) and small amounts are shaped and baked on a *comal* (flat clay or metal plate) on top of the stove for a few minutes. Most of the processing of *tortillas* is done on a small scale, although there is an industrial operation that follows the same procedure for producing instant *tortilla* flour.

Fungal Contamination of Guatemalan Maize

Studies have shown that maize harvested in Guatemala during the early harvest period (May to November) has considerable fungal contamination. Among the fungi are species of the genus *Aspergillus* (8,9,36), which produce some of the most harmful mycotoxins, the hepatoxic aflatoxins (7,20,30,37). Maximum contamination with aflatoxin occurs during the rainy season and in storage. Samples analyzed 20 days after harvest had aflatoxin levels of 130 ppb, and levels up to 1680 ppb when analyzed 60 days later (8).

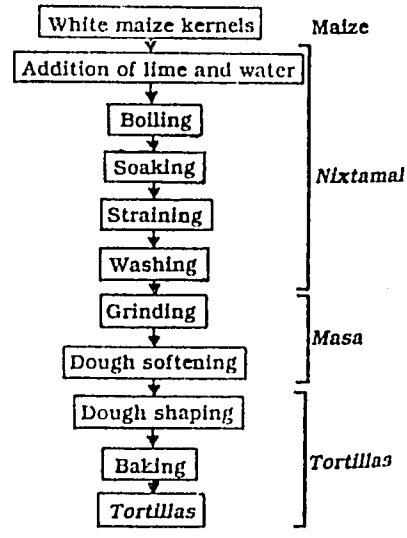

Figure 1. Flow chart, *tortilla* making, Guatemala

Aflatoxin Deactivation

Much research has been carried out on the deactivation of aflatoxin by various means, including insecticides (26,43), chemicals (4,12,14,18,19,24,25,31,34, 41,45,46,49), radiation (19,49), biological inactivation by acid-producing fungi (10) and by physical methods (4,13,14,21,33,38,42,46, 47,54). Chemical agents that have been tested include the bases calcium hydroxide (12) and sodium hydroxide (18,24,25,34).

In addition to reducing aflatoxin contamination (35,51,52), treatment with lime has been found to improve the nutritional value of maize by increasing calcium values and lysine availability in the glutelin fraction of the protein (50). It also promotes some favorable changes in amino acid content (5,22,29).

The experiments described in this paper were designed to determine the effect of the nixtamalization process (alkaline treatment) on aflatoxin-contaminated maize, quantify aflatoxin reduction, and determine whether treated material was safe for human consumption. Two different assays were made, differing in spore concentration of the inoculum, incubation time and temperature.

Materials and Methods

Intact white maize grain of the improved variety Nutricta was used in the two studies discussed here; it was not treated with fungicides. Nutricta has a high nutritional value, with lysine and tryptophane levels twice that of normal maize. The grain was first dried in a pilot plant-tray dryer using warm air for one hour at 80° to 85°C. This was followed by a period of live steam humidification for two to three hours, until the grain reached a final moisture level of 25%, suitable for fungal growth and aflatoxin production.

Inoculation

Spore preparation for inoculation was carried out according to the procedures of Hayne *et al.* (26). Petri dishes containing potato dextrose agar (PDA) were inoculated with 0.1 ml spore suspension of *Aspergillus parasiticus* strain NRRL 2999 and incubated for 10 to 30 days at 30°C for spore production. The petri dishes were isolated in metal containers and then in plastic bags to avoid contamination. To harvest the spores, the agar surface of each petri dish was washed with 5 ml of 0.1 M phosphate buffer solution at pH 7.0 with 0.1% Tween 80.

To avoid germination, the maize grain was treated to inactivate the germ and inoculated with the spore suspension. The inoculum was distributed in petri dishes containing 25 g of grain and incubated at 21°, 28° and 35°C. The grain was sampled after 10, 17 and 24 days and analyzed to determine aflatoxin levels at different temperatures.

The germ-inactivated maize grain (240 g) was placed in wide-mouthed glass jars and inoculated with a spore suspension of *A. parasiticus*. Concentrations of 2.2×10^6 spores per gram of dry maize were used for the first assay, and 4.86×10^6 spores for the second. Sterile water was added to obtain 25% grain moisture. The jars were shaken to distribute the spores and incubated at 35°C. Controls were prepared without spore suspension. Samples were taken at three, seven and ten days (first assay) and four, seven and ten days (second assay) to be submitted to the nixtamalization process.

Nixtamalization

In the first trial, the amount of lime commonly used in the nixtamalization process in home *tortilla* making in Guatemala (1.87% W/V, 3.00% W/W) was tested as well as several higher

and lower lime concentrations (0.03 to 10.0% W/W). The objective was to find a concentration level that would permit easy hand removal of the pericarp, without alteration of the organoleptic characteristics of the dough and the *tortilla*. Lime of industrial quality was pulverized and sifted through a no. 14 mesh screen. Cooking times from 20 to 40 minutes were studied with an open kettle (domestic) process and an autoclave (industrial) process. Data were recorded on pH changes, the amount of water added to the cooked grain to soften the dough, and the time and temperature necessary to bake the *tortillas*.

In the second study, four 250-g samples of contaminated grain were mixed with lime, two with 0.6% W/V (1.0% W/W), the optimum found in the first trial, and the other two with the home level of 1.87% W/V (3.0% W/W). One sample of each concentration was cooked by one of the two methods, in an open kettle at 94°C for 40 minutes and in an autoclave at 121°C (15 psig) for 30 minutes. All of the cooked samples were left a. room temperature overnight and then washed several times with tap water and ground. Water was added to the dough to soften it, and the *tortillas* were made.

Chemical analysis

Moisture was determined for duplicate samples of contaminated grain, dough and *tortillas*, by drying at 100°C to constant weight (1). Aflatoxin levels were determined for the samples for lime level and cooking method; aflatoxin content was determined by the AOAC method (2). Grain contaminated with a known amount of pure aflatoxin was extracted (92%) and analyzed. Quantitative thin-layer chromatography was carried out using a densitometer (Kontes, model 800), and measurements made following the procedure cited by Stubblefield *et al.* (48). Filter excitation and emissions of 365 nm and 436 nm were used.

Results and Discussion

Aflatoxin levels according to temperature and incubation time

Production of aflatoxins B_1, B_2, G_1 and G_2 was determined for three temperatures sampled at 10, 17 and 24 days (Figure 2). Diener and Davis have reported that the optimum temperature range for aflatoxin production of *A. parasiticus* on natural and semisynthetic media is 25° to 30°C for 7 to 21 days of incubation (16,17); maximum production of aflatoxin B_1 is obtained at 30° to 35°C, and G_1, 25° to 30°C (15). The ratios between G_1 and B_1 and between G_2 and B_1 have been reported to vary with respect to temperature (17) and incubation time (44) (Figure 2).

Results of the first of the studies demonstrated similar patterns. Most aflatoxins increased with incubation time, although some leveled off after 17 days. In only one case, G_2 at 35°C, a sharp and unexplained decrease occurred. In general, more B_1 and B_2 were produced at higher temperatures, and more G_1 and G_2 at lower temperatures. Routinely, a higher production of the four aflatoxins was observed at 35°C (Figure 3). For this reason, 35°C was used in the second experiment.

Lime levels in the nixtamalization process

The pH of the original maize-water mixture was found to be 6.0 to 6.5, which increased to 12.0 when lime was added. Due to the buffering capacity of maize grain, the pH did not change significantly as more lime was added. The pH decreased to 11.2 after the maize was cooked and to 9.2 after three washings.

Organoleptic characteristics (color, texture and flavor) of the *nixtamal*, dough and *tortillas* were normally good with lime levels up to 1.87% W/V (3.0 W/W). When higher amounts, 2 to

10%, were used, the *nixtamal*, dough and *tortillas* were yellow, becoming darker as lime increased. The flavor of lime also become objectionable. With the optimum lime level (0.6% W/V, 1.0% W/W), kernels were soft and easy to peel and had a normal color, the dough and *tortillas* were fine textured, and the *tortillas* were elastic and did not break easily.

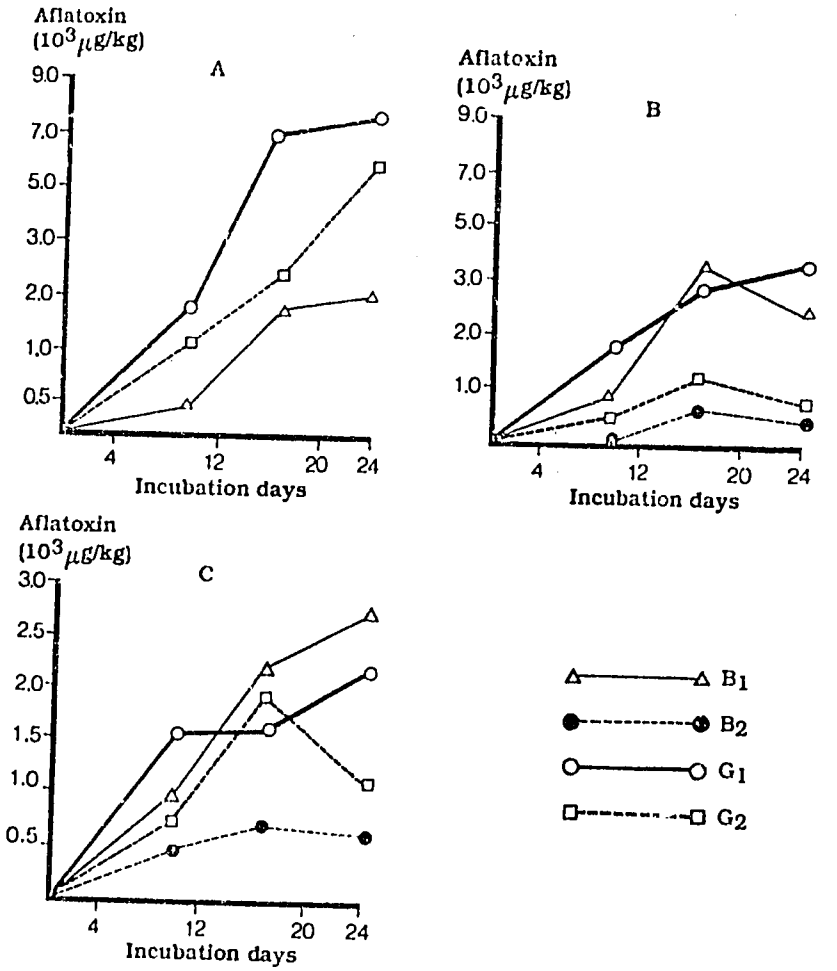

Figure 2. Aflatoxin production in maize grain at three different temperatures, study no. 1, Central American Industrial Research Institute, Guatemala[a/]

[a/] Aflatoxin production at 21° (A), 28° (B) and 35°C (C)

Cooking and baking

Optimum autoclave cooking time and temperature were found to be 30 minutes at 121°C (15 psig); a longer time was necessary when the maize was cooked under atmospheric conditions, the optimum being 40 minutes at 95°C. The baking time for *tortillas* ranged from 1.5 to 3 minutes on a *comal* at temperatures of 180° to 250°C. During baking, the internal temperature of the *tortillas* reached 94°C.

Aflatoxin Levels in the Two Studies

Aflatoxin levels were determined at 35°C for three, seven and ten days (Figure 4). The levels of toxin produced were higher in the second experiment, when a higher spore concentration was used in the inoculum. Comparison of these data with those of the first study (Figure 3) shows striking differences. In the second study, aflatoxin B_1 was predominant, while G_1 was highest in the first. Practically all aflatoxins were produced in much greater amounts in the second study, undoubtedly because of better fungal growth caused by the higher spore concentration. Aflatoxin B_1 had a seven-fold increase, B_2 had a three-fold increase, G_1 increased about 1.4 times, and G_2 remained almost the same. Hesseltine *et al.* (29) have pointed out that G_1 is always biosynthesized along with B_1; if neither is present, B_2 and G_2 are not found.

Aflatoxin Destruction During Nixtamalization

Aflatoxin destruction in contaminated maize treated with the two lime levels was determined (Figure 5). A decrease in aflatoxin levels in dough and

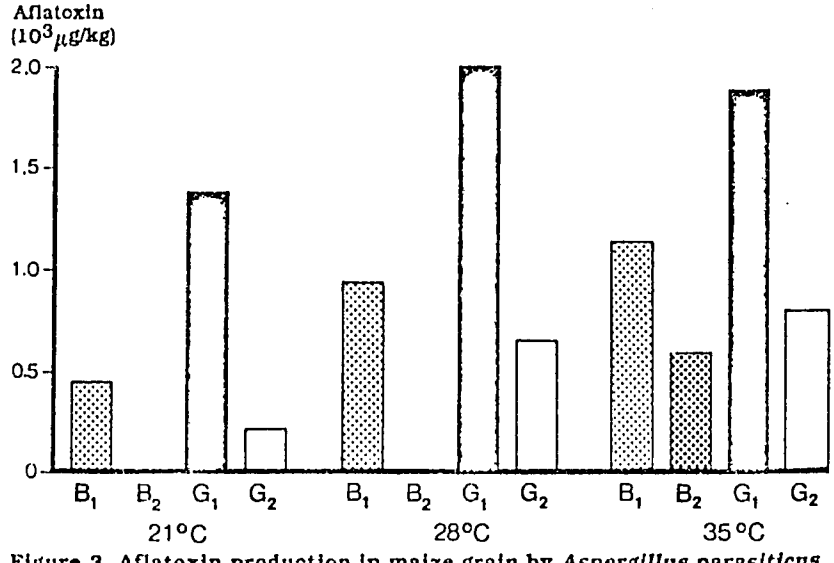

Figure 3. Aflatoxin production in maize grain by *Aspergillus parasiticus* at three different temperatures, study no. 1, Central American Industrial Research Institute, Guatemala

tortillas was found for all levels of contamination, the decrease being greater from maize to dough than from dough to *tortillas*. The reduction was more pronounced with the higher level of lime. The effect of alkalinity was more important than that of temperature, although temperature has also been reported as important (22,34,48,54).

Results of the current study confirm reports by Ulloa and Herrera (52), Ulloa-Sosa and Schroeder (53) and Price *et al.* (41) regarding aflatoxin reduction with alkalinity. This is mainly caused by the opening of the lactone ring and its irreversible binding with the protein (3,33).

Data analysis

The data were submitted to least square analysis employing a trifactorial design (11,39). The parameters and levels used included incubation times of three, seven and ten days for the first assay and four, seven and ten days for the second, two lime (CaO) concentrations, 0.6% W/V (1.0% W/W) and 1.87 W/V (3.0 W/W), and two cooking methods, open kettle and autoclave.

At significance levels of 0.05 and 0.01, no significant difference in amount of aflatoxin reduction was found between the two cooking methods or the two lime levels; however, the difference was highly significant for the three incubation periods. There were no significant differences among the various interactions of incubation time and lime level, incubation time and cooking method, lime level and cooking method, and time, lime level and cooking method. Although there was a decrease in aflatoxin content during the nixtamalization process, none of the treatments lowered it sufficiently to meet the 20 μg/kg standard suggested as safe by FAO/WHO/PAG/UNICEF (22) and the US Food and Drug Administration (23).

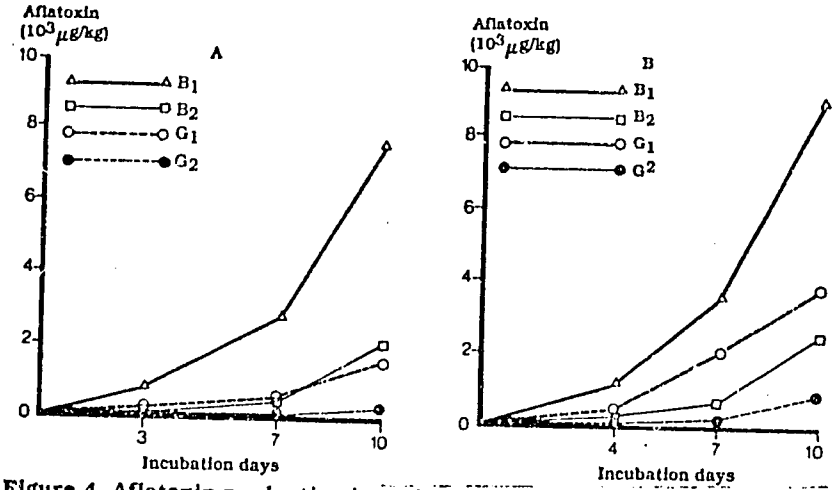

Figure 4. Aflatoxin production in maize grain at 35°C, Central American Industrial Research Institute, Guatemala[a/]

[a/] A = study no. 1, B = study no. 2

Aflatoxins G_1 and G_2 were lowered more by alkaline hydrolysis than were B_1 and B_2; in most cases, they were reduced by as much as 100%. These results confirm those of Codifer *et al.* (12). Through the nixtamalization process, aflatoxin reduction from contaminated grain to dough stage was 82.5% on a wet-weight basis (three days of incubation), 89.2% (seven days) and 89.6% (ten days of incubation). On a dry-weight basis, it was 86.1% (three days of incubation), 95.6% (seven days) and 97.3% (ten days). The equivalent values for the reduction from contaminated grain to *tortillas* were 89.6% (three of days incubation), 89.2% (seven days) and 93.5% (ten days) on a wet basis, and 91.8% (three days), 95.6% (seven days) and 97.1% (ten days) on a dry-weight basis. These results are an average of the two assays at the two lime levels, two cooking methods and different numbers of days of incubation.

Conclusions

The levels of lime normally used to prepare *nixtamal* in rural and urban areas of Guatemala do not reduce aflatoxin levels in contaminated grain sufficiently to make it safe for human consumption. When lime levels above 2% are used, high aflatoxin reduction

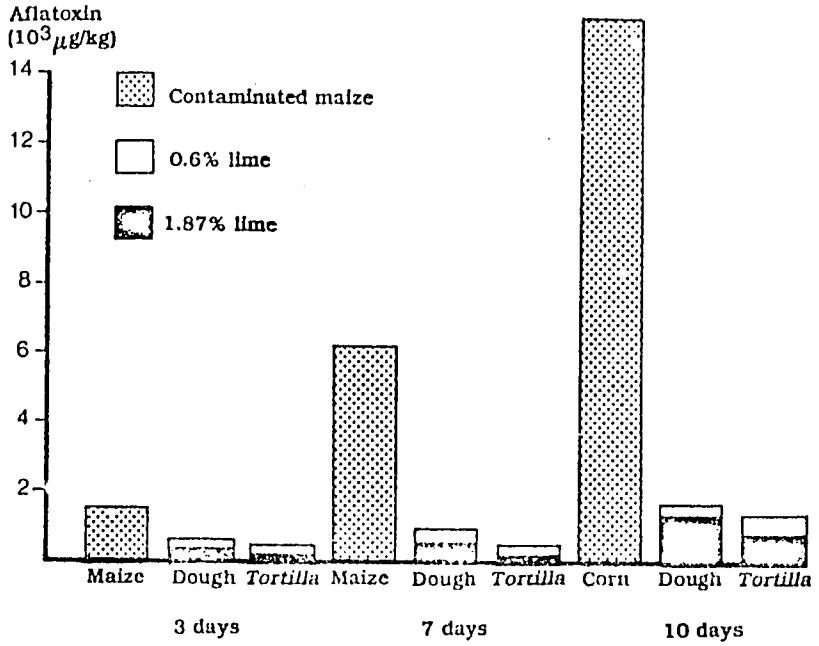

Figure 5. Aflatoxin content of dough and *tortillas* made with contaminated maize after nixtamalization with two different lime levels, studies no. 1 and 2, Central American Industrial Research Institute, Guatemala[a/]

[a/] Determined at 35°C

is achieved; however, the resulting *tortillas* are undesirable. During alkaline cooking, aflatoxins G_1 and G_2 are reduced more than B_1 and B_2; B_1 is reduced the least. In all cases, even at the lowest lime levels, a decrease is shown for total aflatoxins during the nixtamalization process.

Acknowledgements

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APPENDIX 3

SUPPLEMENTATION OF TORTILLAS WITH PROTEIN CONCENTRATE FROM DISTILLERY YEAST

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SUPPLEMENTATION OF TORTILLA WITH PROTEIN CONCENTRATE FROM DISTILLERY YEAST

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SUMMARY

The purpose of this study was to increase the protein quality of tortillas, supplementing them with protein concentrate obtained from the yeast (Saccharomyces cerevisiae) cream from an alcohol distillery

Rupture of the yeast cell wall was studied, and a procedure for obtaining a high percentage of broken cells from the yeast was developed. A protein concentrate was obtained from the cell wall free portion. Techniques were developed to obtain the protein concentrate with a low level of nucleic acids.

Different levels of protein concentrate were added to dough made from corn of the Nutricta variety. The resulting tortillas were submitted to a taste panel for sensory evaluation and the results were analyzed statistically. The highest level of supplementation that did not negatively affect acceptation by the panel was 12% (DW).

The nucleic acids were reduced 91% and the protein increased 65% with respect to the initial yeast.

In the tortillas with the highest acceptable level of supplementation, protein content increased 60% when compared to the control tortillas, and a significant improvement in lysine content was observed. Nucleic acid content was low having been reduced to levels which did not represent a limiting factor for the use of yeast in a product destined for human consumption.

RESUMEN

El objetivo de este estudio fue el de aumentar la calidad protéica de las tortillas, suplementándolas con concentrado protéico obtenido a partir de crema de levadura (*Saccharomyces cerevisiae*) proveniente de una planta procesadora de alcohol y ron.

Se determinó la forma más eficiente para lograr un alto porcentaje de rompimiento de la pared celular de la levadura seca, y a partir del sobrenadante libre de pared celular, se estableció el procedimiento para la obtención de un concentrado protéico con bajo nivel de ácidos nucléicos.

A la masa de maíz variedad "Nutricta" se agregaron diferentes niveles de concentrado protéico. Las tortillas preparadas fueron sometidas a evaluación sensorial y los resultados obtenidos se analizaron estadísticamente. Se estableció que el nivel máximo de suplementación que no alteró las propiedades organolépticas de las tortillas, fue 18% (base seca).

Los ácidos nucleicos durante la obtención del concentrado protéico fueron reducidos 91% y la proteína aumentó 55% con respecto a la levadura inicial.

En las tortillas con el máximo nivel de concentrado protéico establecido, se incrementó el contenido de proteína 60% con respecto a la tortilla control. Se obtuvo además un aumento significativo de lisina. El contenido de ácidos nucléicos fue bajo por lo que ya no es factor limitante para el uso de la levadura en un producto destinado a consumo humano.

INTRODUCTION

In some areas of Latin America there exists a protein shortage and defficiency in the human diet mainly due to nutritional habits, availability and price of food. In northern Central America and Mexico, corn is the principal source of food energy and vegetable protein in low-income groups of the population, being consumed essentially in the form of tortillas.

Although corn protein is defficient in lysine and tryptophan (1), new improved varieties have been developed through plant breeding techniques.

Another approach followed has been to complement and enrich the nutritional value of the protein through mixing with other food materials and developing processed food products (2). Many vegetable protein sources have been used as supplements in the past; however, rather few efforts have been directed towards the possibility of employing microbial biomass from different microorganisms.

The industrial processing technology for producing microbial biomass from a series of different raw materials has favored the development of the continuous system of submerged cultivation in stirred tank bioreactors and the separation of the microbial biomass solid. Among microorganisms, yeast have been the most studied and several

industrial factories in the world produce such products (3,4). The major limitation in their future expansion is economic : its selling price must be competitive with alternative sources.

The protein content of yeast varies according to the strain, the substrate and growth conditions and is approximately 50%; nutritionally it is low in sulfur amino acids as is typical for all microorganisms (5). Its major drawbacks are a cell wall which interferes with protein digestion (6,7), the presence of antigenic and allergic compounds (8) and a rather high (6-16%) nucleic acid content (5).

Hence, in order for yeast to be incorporated into human food products, certain processing steps are needed to circumvent the mentioned problems. Cell wall rupture and protein concentration can be done by mechanical, chemical and enzymatic methods, alone or in combination (9,10) and nucleic acid separation can also be incorporated into such processing schemes.

The objective of the present work was to obtain a yeast protein concentrate, low in nucleic acid, suitable to be mixed with corn dough for tortilla preparation. The maximum amount that could be added without impairing the organoleptic characteristics of the finished product was established, also.

MATERIALS AND METHODS

Yeast biomass: The yeast employed in the experiments was obtained directly from a local rum factory fermenting a mixture of virgin and blackstrap cane molasses. It was a ethanol efficient *Saccharomyces cerevisiae* strain. In the factory it had been acid treated, washed and centrifuged. The paste was brought to the laboratory and was freeze-dried.

Cell wall breakage: all the tests for breaking the yeast cell wall were done by mechanical friction employing a laboratory glass bead disintegrator operated at 4 C. The bead diameter was 0.3-0.5 mm.

Protein concentration: basically the methodology of Hedenskog and Mogren (10) was followed. The cell suspension from the mechanical cell wall disintegrator was centrifuged until a clear supernatant was obtained. The pH was adjusted to 9.5, a volume of a 4.2% NaCl solution was added and the mixture was heated rapidly to 60C and kept at this temperature for only 3 s. The precipitate was centrifuged at 10 000 rpm and 4 C washed several times with water and freeze dried.

Tortilla manufacture: the detailed description of this operation has been published elsewhere (11). Corn from the improved Nutricta variety was used.

Organoleptic evaluation: tortillas made with corn and corn-yeast and corn-protein concentrate were evaluated for flavor, color, aroma and texture with a previously trained panel following standard procedures (12). The results were statistically evaluated at 1 and

5% of significance (13).

Chemical analysis: moisture and nitrogen were determined by the AOAC methods (14,15); protein hydrolysis , for amino acid determination according to Kaiser et al (16); amino acid analysis by liquid chromatography in an autoanalyzer; nucleic acids according to Sarwar et al (17).

Protein efficiency: it was determined from the amino acid analysis employing the equations of Alsmeyer et al (18).

RESULTS AND DISCUSSION

Cell wall breakage was studied as a function of biomass concentration, pH and time which are the main importante variables (19). Results are presented in Table 1 where it is seen that with a 20% yeast suspension at pH 9.5 a cell wall breakage of 97% was obtained after a disruption time of 25 min. A successful mechanical treatment, as the one obtained here, has its advantages over alternative procedures, either chemical or enzymatic, as it can be scaled up and its operation optimized (9,10). Cell wall separation has many advantages: it increases nitrogen extraction (7), eliminates coloring reactions and compounds that might originate gastrointestinal problems (4,21) and improves digestibility (22). It also presents processing disadvantages: it reduces the protein yield and adds another unit operation to the process (13,21). In our case it was a necessary procedure in order to improve product

acceptability.

Figure 1 illustrates the experimental procedure followed to obtain the protein concentrate once the cell wall was separated. Noreen-skog and Mogren (10) recommended a pH of 11.5, however we found that at 9.5 the protein precipitated in a more firm way which favored its ultimate recovery. Less drastic alkaline conditions improve the membrane permeability, increasing protein solubility and reducing nucleic acids (25,26). A low NaCl concentration was used as no further improvements were obtained at higher values. The presence of salt improved the coagulation of protein precipitate as reported elsewhere (23,25). Heat was needed to activate the endogenous yeast ribonucleases (27,28) and the rapid heat shock eliminates an enzymatic inhibitor (29). Cell wall washings improved protein recoveries by about 10%.

A protein yield of 65.3% was obtained after cell wall breakage. The precipitated protein represented 97.2% of the amount in solution.

The protein content of the concentrate was 53.0 % on dry basis. The fresh yeast had 34.3%, hence a protein increase of 54.6% was obtained when comparing the two products. In the literature a concentrate with 55.6% was obtained from a yeast with an initial protein value between 40 and 45% (10).

The nucleic acid content of the concentrate was rather low, as shown in Table 2. It was reduced by 91.2% from the initial value, again when comparing the products. Its value is acceptable to fit the

recommendations of the daily human nucleic acid intake (30,31) when mixed with a human food.

In Figure 2 the alkaline corn cooking method for tortilla preparation denominated locally as nixtamalizado is described. It is a standarized procedure already described (32) with the only difference to the one used commercially being the amount of calcium employed. The corn dough moisture in the process was 63.2% and for the tortilla 49.8%, hence some water is lost during tortilla cooking.

Fresh yeast corn mixtures were used to prepare tortillas employing the procedure described. The amount of yeast was varied between 2.4, 4.8, 7.2, 12 and 24% of the corn on dry weight basis. The resulting products were compared to an only corn control in organoleptic tests. The data showed that 4.8% was the maximum amount of yeast that could be added to the product without impairing its organoleptic. Similar results had been obtained previously by Bressani et al (33).

Another set of data was obtained employing the protein concentrate at levels of 6, 12, 18 and 19% on dry weight basis. The same type of organoleptic evaluations showed in this case that it was possible to incorporate up to 18% without establishing differences between the enriched products and the control.

In Table 2 it is easily seen that this consumer acceptable tortilla had 18.9% of protein, figure which is 59.7% higher than the control

product. The amount of nucleic acids in this product is quite low.

Table 3 shows the amino acid composition for the different materials and products. The higher lysine content of the enriched tortilla over the control is statistically significant at 0.05 level. For different commercial corn varieties an average value for this amino acid of 3.1g/100g of protein has been reported previously (34). The enriched tortilla is higher than this value. The protein efficiency index (chemical PER) for the tortilla control was 1.86; this value is higher than that reported for commercial varieties equal to 1.55±0.25 (34). The difference seems logical as the Huamicta corn employed here was an improved variety. The yeast protein enriched product have a value equal to 2.05. This is a very attractive result and is equivalent to 71% the value for casein.

FINAL COMMENTS AND CONCLUSIONS

The experimental results show clearly that in order to incorporate yeast biomass as a source of protein in indigenous corn-based food products, like tortilla, it is necessary to use a protein concentrate preparation. Fresh yeast can be employed only at very low levels. During preparation of the protein concentrate, yeast cell walls are removed, and more important, the nucleic acid content is lowered drastically. The process conditions to accomplish this have been fixed during the experimentation on yeast biomass from the production of ethanol. This is important to point out, as most of the published work in the literature has been done with yeast

biomass obtained in aerobic processes employing yeast strains which have a low fermentative metabolic capacity.

The maximum amount of concentrate was 10% of the corn on dry weight basis. The tortilla had the same organoleptic characteristics as the control and showed improved nutritional properties.

The technology that was developed could easily be adapted in Guatemala and other tortilla consuming countries as distillers yeast is available and more important the protein concentrate could be added during the industrial production of instant tortilla flour.

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TABLE 1

CELL WALL BREAKAGE

EFFECT OF BIOMASS CONCENTRATION
(Disruption time, 15 min; pH = 7)

YEAST CONCENTRATION %	BROKEN CELLS %
5	21.55
10	79.60
16	69.85
25	65.05
50	33.00

EFFECT OF pH
(Disruption time, 15 min; yeast concentration, 10%)

pH	BROKEN CELLS %
6.5	79.60
9.5	87.52
11.0	83.17

EFFECT OF DISRUPTION TIME
(Yeast concentration, 10%; pH - 9.5)

TIME, min.	BROKEN CELLS %
20	93.78
25	97.00
30	95.61
35	94.52

TABLE 2

PROTEIN AND NUCLEIC ACID LEVELS DURING THE PROCESS

MATERIALS	PROTEIN g/100g, dry basis	NUCLEIC ACIDS g/100g, dry basis
Nutricta Corn	18.84	0.00
Original Yeast	34.30	4.21
Protein Concentrate	53.04	0.37
Control Tortilla	12.09	0.00
Supplemented Tortilla (18% yeast supplement)	18.90	0.96

TABLE 3

ESSENTIAL AMINO ACIDS

g/100g PROTEIN

	PROTEIN CONCENTRATE	TORTILLA CONTROL	TORTILLA 18% PROTEIN CONCENTRATE	YEAST	NUTRICTA CORN
ISOLEUCINE	5.61	1.48	2.05	2.55	0.81
LEUCINE	8.94	5.29	5.59	4.39	5.71
LYSINE	8.99	2.63	4.15	6.36	2.53
METHIONINE	0.69	0.36	0.50	0.22	1.75
PHENYLALANINE	4.09	1.58	1.65	1.62	1.48
THREONINE	3.87	1.57	1.96	2.70	1.28
VALINE	6.81	3.03	2.52	4.05	3.42

* Mean of 5 determinations

FIGURE 1

PREPARATION OF PROTEIN CONCENTRATE

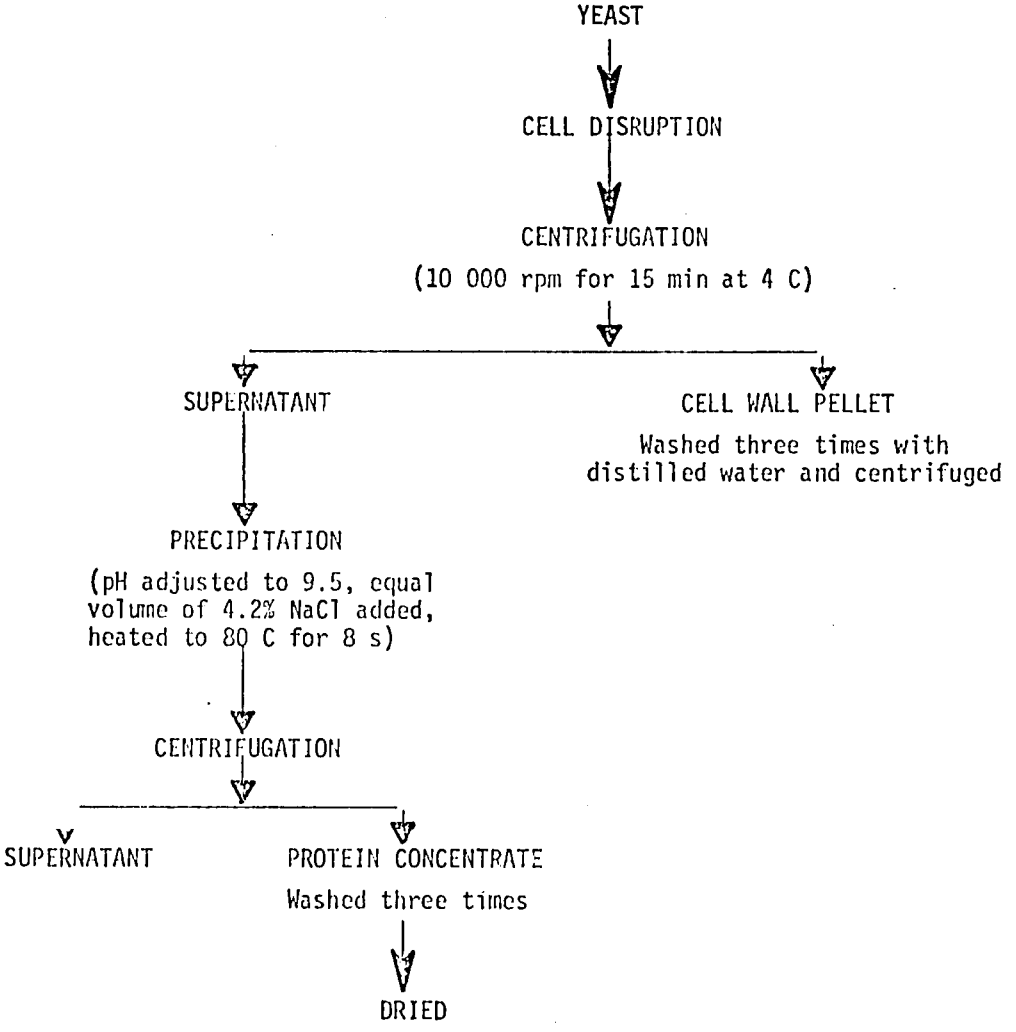
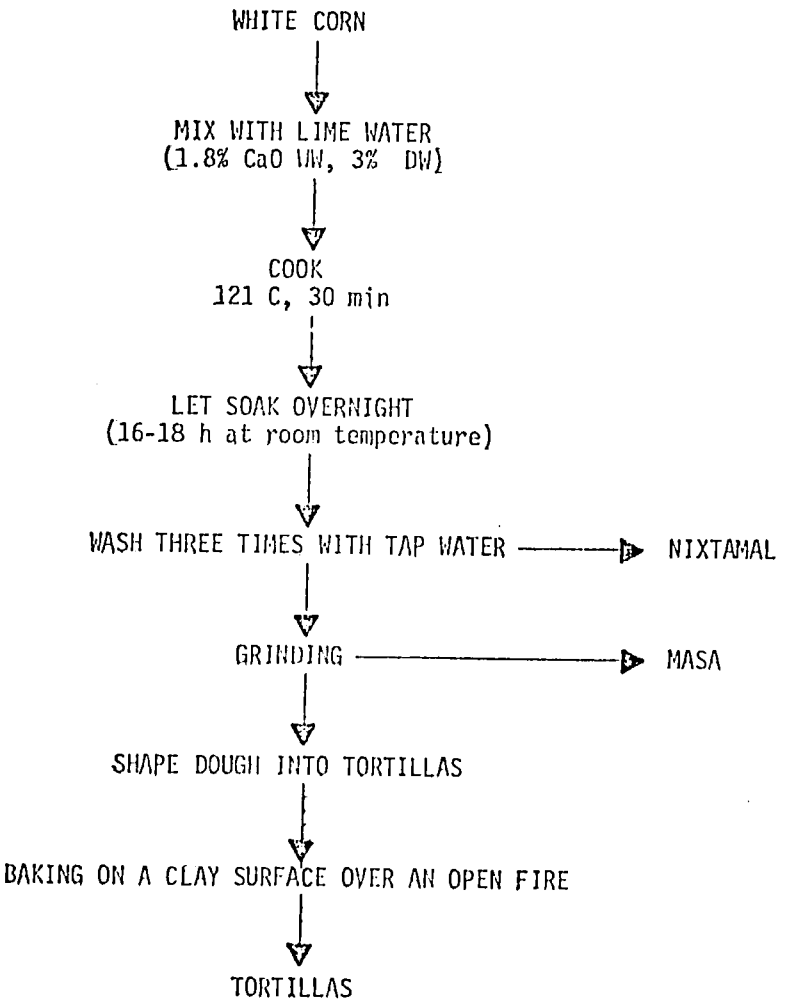


FIGURE 2

NIXTAMALIZATION PROCESS USED



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FINAL REPORT

DESTRUCTION OF AFLATOXIN IN CORN

AND NUCLEIC ACIDS IN YEAST CORN MIXTURES

Presented by ICAITI to:

ROCAP

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