

PD-AAW-693

53026

AGENCY FOR INTERNATIONAL DEVELOPMENT  
WASHINGTON, D.C. 20523

DATE: 9/2/87

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18  
FROM: AID/SCI, Victoria Ose *VO*  
SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 3. 77-06  
3d Progress Report

Attachment

*1 ey*



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**THIRD PROGRESS REPORT**

**DESTRUCTION OF AFLATOXIN IN CORN AND  
NUCLEIC ACIDS IN YEAST CORN MIXTURE**

**Presentado por el ICAITI a**

**ROCAP  
Office of the Science Advisor AID/Washington  
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**Guatemala, APRIL 19 85**

1. **BACKGROUND**

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The general objectives of the project were to verify the quantitative degradation of aflatoxins in contaminated corn during nixtamalization and to quantify the nucleic acid reduction in yeast-corn mixtures during the industrial process to prepare corn flour.,

The project began in July 1983. This progress report covers the third semester of the project.

**2. ACTIVITIES**

## 2. **ACTIVITIES**

### 2.1 Research Work:

#### 2.1.1 Trials for aflatoxin degradation during nixtamalization:

Nutricia variety corn was contaminated with a strain of *A. parasiticus* NRRL 2999 and two trials were carried out in order to obtain results in duplicate.

#### 2.1.2 Germ inactivation:

The procedure established before was followed to avoid germination: heating the Nutricia variety corn for one hour at 80-85°C, followed by a period with live steam contact humidification for 2-3 hours.

#### 2.1.3 Corn inoculation and storage:

Treated corn for germ inactivation was placed in wide mouth glass jars, 240 g per jar and inoculated for the first assay with a spore suspension of *A. parasiticus* NRRL 2999 to give a concentration of  $2.2 \times 10^6$  spores per gram of corn on dry basis; the inoculum was prepared as before (see Section 2.2.4 Inoculum preparation, second progress report). At the same time steril water was added to obtain a moisture level of 25%.

Inoculated jars were shaken to distribute spores and then incubated at 35°C; this temperature was chosen according to results from preliminary trials which were done in order that the fungal strain produced the four aflatoxins: B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>.

Moldy samples were taken at 3, 7 and 10 days.

The second assay was carried out following the procedure mentioned before, except that spore concentration was of  $4.86 \times 10^6$  per gram; samples were taken at 4, 7 and 10 days.

#### 2.1.4 Nixtamalization process:

Following the procedure established (see Table 1, second progress report) contaminated corn samples at three days for the first assay and fourth days for the second assay were mixed with lime water solutions (0.6% CaO by weight) and cooked in an open Kettle (94°C) at atmospheric pressure for 40 minutes.

At the same time other portion of the same lot was treated with the amount of CaO mentioned before and cooked at 121°C for 30 minutes at 15 psig in an autoclave.

Other two portions of moldy corn samples of the same lot were mixed with more concentrated lime water solutions (1.87% CaO by weight) and cooked in an open Kettle and autoclaved respectively under conditions mentioned before.

All cooked samples were left overnight at room temperature and then washed with water; in this manner corn dough was obtained and tortillas were made.

The same procedure was followed with samples removed at 7 and 10 days in both assays. See Table 1.

#### 2.1.5 Evaluation of aflatoxin destruction by treatment with the two CaO levels during nixtamalization:

Aflatoxin level contents were determined in each control samples

as well as in the water where corn was cooked, in the dough and tortillas at each CaO level and each cooking process. Using for aflatoxin extraction the selected methods mentioned in the second progress report AOAC method (1975), and the densitometric measurement the procedure cited by Stubblefields et al (1967). Moisture was determined on duplicate samples by drying at 100°C to constant weight (AOAC, 1975b).

#### 2.1.6 Results:

Results obtained in the first assay have been arranged in Tables 2, 3 and 4 and for the second assay in Tables 5, 6 and 7.

At three days in the first assay aflatoxin B<sub>1</sub> and G<sub>1</sub> were positive B<sub>2</sub> and G<sub>2</sub> were absent; in the second assay at four days the four aflatoxins were present. At 7 and 10 days the four aflatoxin contents increased. See Figures 1 and 2. It has been pointed out that G<sub>1</sub> was never biosynthesized without B<sub>1</sub>, and aflatoxins B<sub>2</sub> and G<sub>2</sub> never appeared without B<sub>1</sub> and G<sub>1</sub> respectively (Hesseltine et al, 1966).

Although the general trend for aflatoxin production with time was the same for both tests, they can not be considered true duplicates. In the second one more aflatoxins were formed at all times which could be explained by the higher spore count in the inoculum.

Results obtained this time confirmed the ones obtained when corn was inoculated with *A. flavus* SRRRC 167B. In all cases there was a decrease in aflatoxin levels during the nixtamalization process, the reduction increased during tortilla preparation (see Figure 3). However, in all cases the final total aflatoxin level stayed at values higher than those allowed by the USDA, which are 20 µg/kg (1974).

In the water where corn was cooked no aflatoxins could be detected.

### 2.1.7 Statistical analysis:

A least square analysis of our results were carried out employing a trifactorial design.

The factors and levels used were the following:

A: Incubation time:

First assay

A<sub>1</sub> = 3 days

A<sub>2</sub> = 7 days

A<sub>3</sub> = 10 days

Second assay

A<sub>1-1</sub> = 4 days

A<sub>2-2</sub> = 7 days

A<sub>3-3</sub> = 10 days

B: CaO concentration

B<sub>1</sub> = 0.6% (W.W.)

B<sub>2</sub> = 1.87% (W.W.)

C: Cooking method

C<sub>1</sub> = Open Kettle

C<sub>2</sub> = Autoclave

The following results at a level of 0.05 and 0.01 grades of probability were obtained:

- There was not a significant difference between treatments: two cooking methods and two CaO levels.
- There was a highly significant difference between % of reduction in the three incubation periods.
- There was not a significant difference between the different interactions:
  - A-B = Time and CaO concentration
  - A-C = Time and cooking method

B-C = CaO concentration and cooking method  
ABC = Time, CaO concentration and cooking method.  
The variance coefficient obtained was of 37.11%.

The levels of total aflatoxin production reported here can not be compared directly to other data reported in the literature because growing conditions were not comparable. However figures are within the same order of magnitude. See Table 8.

Regarding the results on the fate of aflatoxins during nixtamalization, the following comment can be made when a comparison is made with the few references in the literature.

In the work carried out by Ulloa and Herrera (1970) corn was inoculated with a spore suspension of *A. flavus* Link strain number XVI-I (no mention was made of the concentration used) and incubated at 25°C and 100% relative humidity for 3 weeks.

To prepare nixtamal the contaminated corn samples were mixed with lime water solution (10% CaO by weight) and cooked for one hour.

They reported a total aflatoxin content of 436.8 µg/kg (325.8 B<sub>1</sub> and 111 G<sub>1</sub>) in the contaminated corn. A reduction of 84.44% during nixtamalization and 91.21% in the dough.

In the work carried out by Ulloa-Sosa and Schroeder (1969) white corn was inoculated with spores of *A. flavus* XVI-I (no mention of the concentration used) and incubated at 30°C and 90% relative humidity for 2 weeks. Moldy kernels were washed with water, dried at room temperature and mixed with sound white corn at a 3% ratio. The procedure followed to prepare nixtamal was the same followed by Ulloa-Herrera, differing in the amount of CaO used: 7.5% by weight.

They found a 66.67% reduction in aflatoxin content during tortilla preparation.

Note in both cases the higher amounts of CaO employed by the mexican researchers. This variability is expected as tortilla eating countries in Mexico and Central America employ different CaO concentrations which give tortillas a different appearance and taste. Also note that the mexican researchers did not experiment with *A. parasiticus*.

**3. WORK PLAN FOR FIRST SEMESTER 1985**

### 3. WORK PLAN FOR FIRST SEMESTER 1985

#### 3.1 Nucleic Acid Reduction:

Nucleic acids and amino-acids determination will be quantified in corn and yeast (*Saccharomyces cerevisiae*).

Microbiological methods for some amino-acids such as tryptophan, histidine, cistin and cistein are being developed.

Trials looking for nucleic acid degradation will be conducted employing the conditions established for nixtamalization. Also amino-acids will be quantified and a chemical PER will be also determined. The values obtained will be compared with those of the starting materials.

#### 3.2 Optimum Yeast-Corn Mixture:

Employing healthy corn, Nutrieta variety and a strain of yeast *Saccharomyces cerevisiae* different yeast-corn mixtures will be tested and the optimum will be identified.

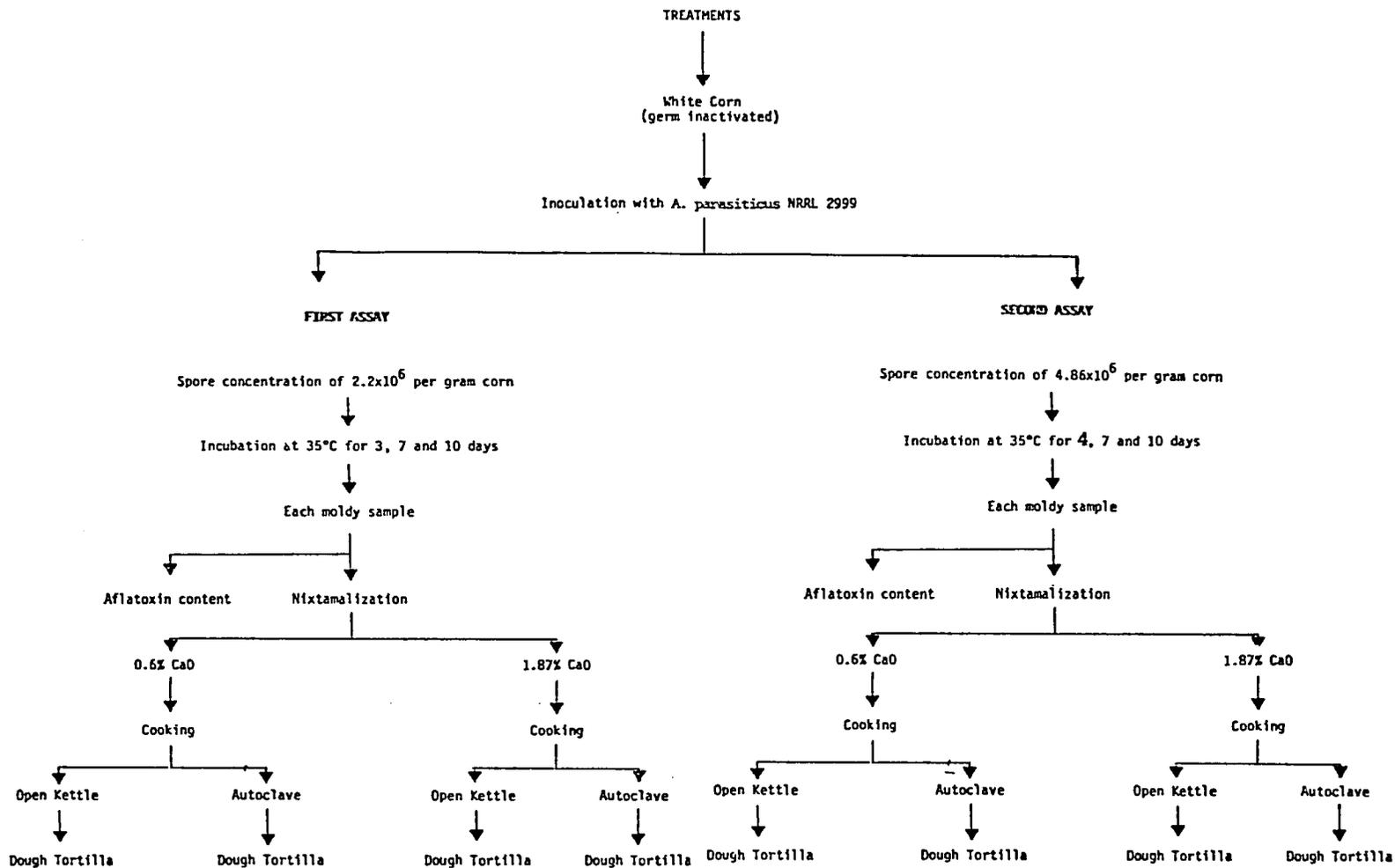
#### 4. REFERENCES

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## TABLES

TABLE 1



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TABLE 2

AFLATOXIN REDUCTION IN CONTAMINATED CORN  
(Nutricia variety) A. parasiticus NRRL 2999  
FIRST ASSAY (3 days)

	CaO Concentration 0.6% (H.W.)								CaO Concentration 1.84% (H.W.)								
	O.K.				A.				O.K.				A.				
	Aflatoxin ug/kg		Reduction %		Aflatoxin ug/kg		Reduction %		Aflatoxin ug/kg		Reduction %		Aflatoxin ug/kg		Reduction %		
	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	
CONTAMINATED CORN																	
Aflatoxin B <sub>1</sub>	886.57	1 830.24															
Aflatoxin B <sub>2</sub>	80.91	167.03															
Aflatoxin G <sub>1</sub>	197.43	407.58															
Aflatoxin G <sub>2</sub>	95.55	197.25															
Total AFLATOXIN:	1 260.46	2 602.10															
DOUGH																	
Aflatoxin B <sub>1</sub>		158.60	284.43	82.11	84.46	137.43	274.14	84.49	95.36	160.33	332.50	81.91	81.85	114.31	186.72	87.11	89.80
Aflatoxin B <sub>2</sub>		54.24	49.82	32.96	70.17	32.28	64.39	60.10	61.45	44.40	92.08	45.12	44.87	42.35	69.18	47.66	59.18
Aflatoxin G <sub>1</sub>		27.78	90.10	85.93	77.89	24.10	18.08	87.79	95.56	22.20	46.03	88.75	78.22	12.12	19.80	93.86	95.14
Aflatoxin G <sub>2</sub>		10.70	19.19	80.80	90.27	20.01	39.32	79.05	59.92	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00
Total		247.32	443.54	80.38	82.95	213.82	426.53	83.03	83.61	226.93	470.61	81.99	81.91	168.78	275.70	86.61	89.40
TORTILLAS																	
Aflatoxin B <sub>1</sub>		154.56	247.68	82.56	86.46	49.52	158.26	94.41	91.35	71.71	185.74	91.91	94.98	96.69	147.62	89.09	91.91
Aflatoxin B <sub>2</sub>		47.76	76.54	40.97	54.17	44.34	39.86	45.20	76.14	54.15	102.50	33.08	38.63	66.74	101.89	38.99	72.74
Aflatoxin G <sub>1</sub>		22.09	35.40	88.81	91.31	39.77	53.41	79.86	86.89	18.37	34.77	90.69	91.47	11.54	17.62	94.15	91.06
Aflatoxin G <sub>2</sub>		10.31	16.52	82.71	91.62	27.21	64.96	71.52	67.07	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00
Total		234.71	376.14	81.38	85.54	160.84	335.63	87.23	87.10	144.23	273.01	88.55	86.51	174.94	267.13	86.12	89.73

O.K. = Open Vessel  
A. = Autoclave  
W.W. = Wet Weight  
D.W. = Dry Weight

TABLE 3

AFLATOXIN REDUCTION IN CONTAMINATED CORN  
(Nutricia variety) A. parasiticus NRRL 2999  
FIRST ASSAY (7 days)

	CaO Concentration 0.6% (W.W.)										CaO Concentration 1.87% (W.W.)									
	Aflatoxin ug/kg		O.K.		Reduction %		Aflatoxin ug/kg		A.		Aflatoxin ug/kg		O.K.		Reduction %		Aflatoxin ug/kg		A.	
	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.
CONTAMINATED CORN																				
Aflatoxin B <sub>1</sub>	2 780.49	18 992.24																		
Aflatoxin B <sub>2</sub>	413.97	2 827.66																		
Aflatoxin G <sub>1</sub>	491.97	3 360.45																		
Aflatoxin G <sub>2</sub>	60.52	413.39																		
Total AFLATOXIN:	3 746.95	25 593.92																		
DOUGH																				
Aflatoxin B <sub>1</sub>		527.59	1 431.72	81.02	92.46	271.17	1 139.37	90.25	94.00	424.58	1 092.31	84.73	94.25	63.87	225.53	97.70	98.81			
Aflatoxin B <sub>2</sub>		168.28	385.48	59.35	86.37	43.30	181.93	89.54	93.57	164.98	424.44	60.15	84.99	22.80	80.51	94.49	97.15			
Aflatoxin G <sub>1</sub>		142.05	157.32	71.13	95.32	0.00	0.00	100.00	100.00	51.51	132.54	89.53	96.05	11.60	41.17	97.64	98.77			
Aflatoxin G <sub>2</sub>		46.46	100.30	23.23	75.74	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00			
Total		884.38	2 074.82	76.40	91.89	314.47	1 321.30	91.61	94.84	641.07	1 649.25	82.89	93.55	98.33	347.21	97.37	98.64			
TORTILLAS																				
Aflatoxin B <sub>1</sub>		253.25	512.06	90.53	97.30	126.69	284.63	95.44	98.50	87.50	177.48	96.85	99.06	49.62	121.98	98.81	99.47			
Aflatoxin B <sub>2</sub>		65.86	128.11	84.09	95.47	18.89	42.44	95.43	98.49	15.04	30.51	96.37	98.92	11.96	29.40	97.57	97.93			
Aflatoxin G <sub>1</sub>		40.64	79.05	33.54	97.65	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00			
Aflatoxin G <sub>2</sub>		0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00			
Total		369.75	719.22	90.13	97.19	145.59	327.07	96.11	98.72	102.54	207.94	97.26	99.19	61.58	151.38	98.36	99.41			

O.K. = Open Kettle  
A. = Autoclave  
W.W. = Wet Weight  
D.W. = Dry Weight

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TABLE 4

AFLATOXIN REDUCTION IN CONTAMINATED CORN  
(Nutricia variety) A. parasiticus NRRL 2999  
FIRST ASSAY (10 days)

	CaO Concentration 0.6% (W.W.)								CaO Concentration 1.87% (W.W.)								
	Aflatoxin ug/kg		O.K.		Aflatoxin ug/kg		A.		Aflatoxin ug/kg		O.K.		Aflatoxin ug/kg		A.		
	W.W.	O.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	
<b>CONTAMINATED CORN</b>																	
Aflatoxin B <sub>1</sub>	7 863.69	36 575.30															
Aflatoxin B <sub>2</sub>	2 060.61	9 584.23															
Aflatoxin G <sub>1</sub>	1 562.03	7 265.26															
Aflatoxin G <sub>2</sub>	214.57	3 278.54															
Total AFLATOXIN:	<u>11 700.90</u>	<u>54 422.74</u>															
<b>DOUGH</b>																	
Aflatoxin B <sub>1</sub>		1 081.02	1 886.27	86.25	94.84	983.36	1 663.93	87.49	95.45	1 086.63	1 709.08	86.18	95.33	1 280.41	2 258.22	83.72	93.82
Aflatoxin B <sub>2</sub>		213.35	372.27	89.65	96.58	242.30	403.83	88.24	95.79	169.42	266.47	91.78	92.22	135.53	239.03	93.42	97.51
Aflatoxin G <sub>1</sub>		0.00	0.00	100.00	100.00	21.10	35.17	98.65	99.52	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00
Aflatoxin G <sub>2</sub>		0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00
Total		<u>1 294.37</u>	<u>2 258.54</u>	<u>89.94</u>	<u>95.85</u>	<u>1 261.76</u>	<u>2 102.93</u>	<u>89.22</u>	<u>96.14</u>	<u>1 256.05</u>	<u>1 975.55</u>	<u>89.26</u>	<u>96.37</u>	<u>1 415.94</u>	<u>2 497.25</u>	<u>87.89</u>	<u>95.41</u>
<b>TORTILLAS</b>																	
Aflatoxin B <sub>1</sub>		622.05	1 602.81	92.08	95.62	272.77	1 164.20	96.53	96.82	542.23	980.35	93.10	97.32	607.01	1 023.80	92.28	97.20
Aflatoxin B <sub>2</sub>		160.60	413.81	92.21	95.68	575.23	552.05	72.08	94.24	93.33	168.74	95.47	98.24	134.28	226.48	93.48	97.64
Aflatoxin G <sub>1</sub>		0.00	0.00	100.00	100.00	45.67	92.43	97.08	98.73	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00
Aflatoxin G <sub>2</sub>		0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00
Total		<u>782.65</u>	<u>2 016.62</u>	<u>93.31</u>	<u>96.29</u>	<u>893.67</u>	<u>808.68</u>	<u>92.36</u>	<u>96.67</u>	<u>635.54</u>	<u>1 149.09</u>	<u>94.57</u>	<u>97.89</u>	<u>741.29</u>	<u>1 250.28</u>	<u>93.66</u>	<u>97.76</u>

O.K. = Open Kettle  
A. = Autoclave  
W.W. = Wet Weight  
D. = Dry Weight

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TABLE 5

AFLATOXIN REDUCTION IN CONTAMINATED CORN  
(Nutricia variety) A. parasiticus NRRL 2999  
SECOND ASSAY (4 days)

	CaO Concentration 0.6% (W.W.)										CaO Concentration 1.87% (W.W.)							
	Aflatoxin ug/kg		O.K.		Reduction %		Aflatoxin ug/kg		A.		O.K.		Reduction %		Aflatoxin ug/kg		A.	
	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.
CONTAMINATED CORN																		
Aflatoxin B <sub>1</sub>	1 129.43	4 699.79																
Aflatoxin B <sub>2</sub>	206.33	857.21																
Aflatoxin G <sub>1</sub>	476.99	2 064.60																
Aflatoxin G <sub>2</sub>	63.89	255.43																
Total AFLATOXIN:	<u>1 896.60</u>	<u>7 887.03</u>																
DOUGH																		
Aflatoxin B <sub>1</sub>		240.58	392.40	78.70	91.65	105.81	290.16	90.63	93.83	525.31	846.46	53.49	81.99	106.86	158.14	90.54	96.63	
Aflatoxin B <sub>2</sub>		35.77	58.34	82.66	93.19	20.29	28.61	90.16	95.66	111.14	179.08	46.13	79.54	33.76	49.97	83.83	94.17	
Aflatoxin G <sub>1</sub>		29.59	48.26	93.79	97.66	16.52	23.29	96.53	98.27	29.68	47.82	93.77	95.46	0.00	0.00	100.00	100.00	
Aflatoxin G <sub>2</sub>		2.10	1.43	96.71	98.66	2.32	3.27	96.27	98.72	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00	
Total		308.04	502.43	<u>83.76</u>	<u>93.63</u>	244.94	345.53	<u>87.09</u>	<u>95.62</u>	666.13	1 073.36	<u>64.87</u>	<u>86.39</u>	140.62	208.14	<u>92.58</u>	<u>97.36</u>	
TORTILLAS																		
Aflatoxin B <sub>1</sub>		155.19	293.03	89.80	93.76	129.00	251.46	99.88	91.65	107.62	198.93	90.47	95.77	87.23	54.59	92.28	98.84	
Aflatoxin B <sub>2</sub>		39.45	74.49	80.88	90.56	32.03	72.25	84.47	91.57	34.02	62.38	83.51	90.26	31.54	51.00	84.71	94.05	
Aflatoxin G <sub>1</sub>		31.03	58.59	93.49	95.47	27.40	62.43	94.25	96.97	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00	
Aflatoxin G <sub>2</sub>		4.95	9.35	92.25	96.34	6.63	12.93	89.63	94.94	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00	
Total		230.62	435.46	<u>87.84</u>	<u>94.48</u>	195.06	380.23	<u>89.72</u>	<u>95.18</u>	141.64	251.81	<u>92.53</u>	<u>96.68</u>	118.77	205.89	<u>93.74</u>	<u>97.39</u>	

O.K. = Open Kettle  
A. = Autoclave  
W.W. = Wet Weight  
D.W. = Dry Weight

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TABLE 6

AFLATOXIN REDUCTION IN CONTAMINATED CORN  
(Nutricia variety) A. parasiticus NRRL 2999  
SECOND ASSAY (7 days)

	CaO Concentration 0.6% (W.W.)										CaO Concentration 1.87% (W.W.)							
	O.K.					A.					O.K.				A.			
	Aflatoxin ug/kg		Reduction %		Aflatoxin ug/kg		Reduction %		Aflatoxin ug/kg		Reduction %		Aflatoxin ug/kg		Reduction %			
	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.		
CONTAMINATED CORN																		
Aflatoxin B <sub>1</sub>	3 606.42	19 557.96																
Aflatoxin B <sub>2</sub>	605.30	2 215.55																
Aflatoxin G <sub>1</sub>	2 027.19	7 621.00																
Aflatoxin G <sub>2</sub>	193.64	727.86																
Total AFLATOXIN:	<u>6 4432.52</u>	<u>24 182.37</u>																
DOUGH																		
Aflatoxin B <sub>1</sub>		553.63	876.83	84.65	95.51	903.12	1 345.34	74.96	93.12	298.59	471.19	91.72	97.59	114.16	168.65	96.83	99.14	
Aflatoxin B <sub>2</sub>		99.65	456.66	83.54	79.39	168.72	251.33	72.13	88.66	33.49	52.87	94.46	97.61	21.46	31.70	96.45	98.57	
Aflatoxin G <sub>1</sub>		24.63	39.01	98.78	99.49	15.20	22.64	99.25	99.70	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00	
Aflatoxin G <sub>2</sub>		0.00	0.00	100.00	100.00	3.58	5.33	98.05	99.26	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00	
Total		677.91	1 073.66	<u>89.46</u>	<u>95.56</u>	1 090.62	1 624.64	<u>83.04</u>	<u>93.28</u>	332.08	524.04	<u>94.84</u>	<u>97.83</u>	135.62	200.35	<u>97.89</u>	<u>99.17</u>	
TORTILLAS																		
Aflatoxin B <sub>1</sub>		311.51	579.23	91.36	97.03	298.62	886.64	91.72	95.47	130.26	297.40	96.39	98.48	66.91	172.40	98.14	99.12	
Aflatoxin B <sub>2</sub>		60.17	111.88	90.06	99.46	52.26	155.17	91.37	92.99	24.68	56.34	95.92	97.46	8.34	21.49	98.62	99.03	
Aflatoxin G <sub>1</sub>		0.00	0.00	100.00	100.00	7.11	21.11	99.65	99.72	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00	
Aflatoxin G <sub>2</sub>		0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00	
Total		371.68	691.11	<u>94.22</u>	<u>97.14</u>	357.99	1 062.92	<u>94.43</u>	<u>95.60</u>	154.94	353.74	<u>97.59</u>	<u>98.54</u>	75.25	193.89	<u>98.83</u>	<u>99.20</u>	

O.K. = Open Kettle  
A. = Autoclave  
W.W. = Wet Weight  
D.W. = Dry Weight

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TABLE 7

AFLATOXIN REDUCTION IN CONTAMINATED CORN  
(Nutricia variety) A. parasiticus NRRL 2999  
SECOND ASSAY (10 days)

	CaO Concentration 0.6% (W.W.)										CaO Concentration 1.87% (W.W.)								
	Aflatoxin ug/kg		O.K.		Reduction %		Aflatoxin ug/kg		A.		Reduction %		O.K.		Reduction %		A.		
	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	W.W.	D.W.	
CONTAMINATED CORN																			
Aflatoxin B <sub>1</sub>	10 325.03	38 815.90																	
Aflatoxin B <sub>2</sub>	2 511.61	9 442.14																	
Aflatoxin G <sub>1</sub>	3 998.93	14 995.98																	
Aflatoxin G <sub>2</sub>	872.09	3 278.54																	
Total AFLATOXIN:	<u>17 697.66</u>	<u>66 532.56</u>																	
DOUGH																			
Aflatoxin B <sub>1</sub>		1 238.24	1 935.96	88.00	95.01	1 631.71	2 268.15	84.19	94.16	979.49	1 531.33	90.51	60.59	473.21	727.57	95.42	98.17		
Aflatoxin B <sub>2</sub>		240.56	376.11	90.42	96.01	304.90	423.23	87.86	95.52	166.89	260.93	93.35	97.24	88.59	136.21	96.47	99.54		
Aflatoxin G <sub>1</sub>		153.86	240.56	96.14	98.39	92.89	129.12	97.67	99.14	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00		
Aflatoxin G <sub>2</sub>		0.00	0.00	100.00	100.00	13.75	19.11	98.42	99.42	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00		
Total		<u>1 632.66</u>	<u>2 552.63</u>	<u>90.77</u>	<u>96.16</u>	<u>2 043.25</u>	<u>2 840.21</u>	<u>88.45</u>	<u>95.73</u>	<u>1 146.33</u>	<u>1 792.26</u>	<u>93.52</u>	<u>97.31</u>	<u>561.80</u>	<u>863.74</u>	<u>96.82</u>	<u>98.70</u>		
TORTILLAS																			
Aflatoxin B <sub>1</sub>		947.43	1 448.89	90.82	99.62	1 299.85	1 860.12	92.70	95.21	604.73	365.88	94.14	99.75	208.18	297.48	97.98	99.71		
Aflatoxin B <sub>2</sub>		381.46	583.92	84.81	93.82	237.57	342.07	98.63	96.38	183.91	263.33	92.68	97.21	46.62	66.62	98.14	99.70		
Aflatoxin G <sub>1</sub>		183.45	280.55	95.40	98.13	55.74	80.26	99.68	99.46	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00		
Aflatoxin G <sub>2</sub>		68.93	105.40	92.10	99.97	15.73	22.65	99.91	99.31	0.00	0.00	100.00	100.00	0.00	0.00	100.00	100.00		
Total		<u>1 581.57</u>	<u>2 413.67</u>	<u>91.06</u>	<u>96.37</u>	<u>1 600.89</u>	<u>2 305.10</u>	<u>90.95</u>	<u>96.53</u>	<u>788.64</u>	<u>1 129.21</u>	<u>95.54</u>	<u>98.30</u>	<u>254.80</u>	<u>364.10</u>	<u>98.56</u>	<u>99.45</u>		

O.K. = Open Kettle  
A. = Autoclave  
W.W. = Wet Weight  
D.W. = Dry Weight

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TABLE 8

LEVELS OF TOTAL AFLATOXIN PRODUCTION REPORTED BY  
A. parasiticus AND A. flavus IN CORN

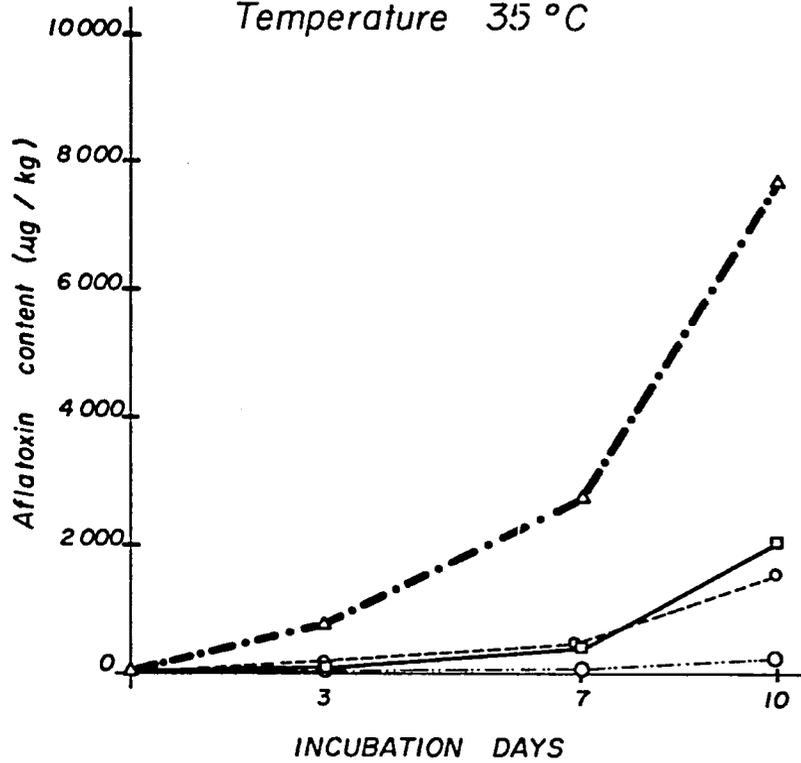
	<u>Production of total aflatoxin</u>		
	<u>NRRL 3000</u>	<u>µg/kg</u> <u>NRRL 2999</u>	<u>NRRL 3145</u>
Autoclaved corn inoculated with A. parasiticus strains in a solid fermentation at 28°C in 6 days (Hesseltine et al, 1974).	53 000	47 000	60
		<u>µg/100 ml</u> <u>NRRL 2999</u>	
Fermentation in a liquid medium chemically defined for 5 days (Shih and Marth, 1973).		4 526	
		<u>µg/kg</u> <u>NRRL 2999</u>	
A. parasiticus inoculated to cracked corn at 28°C for 6 days (Hesseltine et al, 1966).		B <sub>1</sub> 19 000	
		B <sub>2</sub> 5 000	
		G <sub>1</sub> 21 000	
		G <sub>2</sub> 2 000	
	TOTAL =	47 000	
		<u>µg/kg</u>	
A. flavus in shelled corn stored one week at 30°C with 13% moisture (Trenk and Keruman, 1970)	24°C 300	30°C 2 000	35°C 2 000

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**FIGURES**

FIGURE No. 1

FIRST ASSAY  
Temperature 35 °C



----- B1  
————— B2  
----- G1  
..... G2

FIGURE No. 2

SECOND ASSAY  
Temperature 35 °C

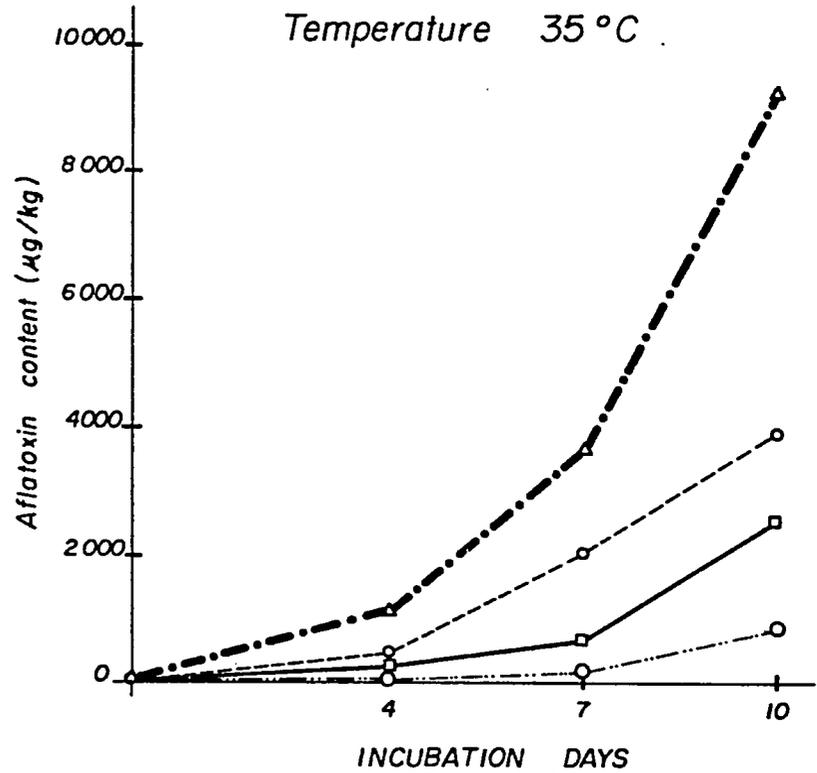
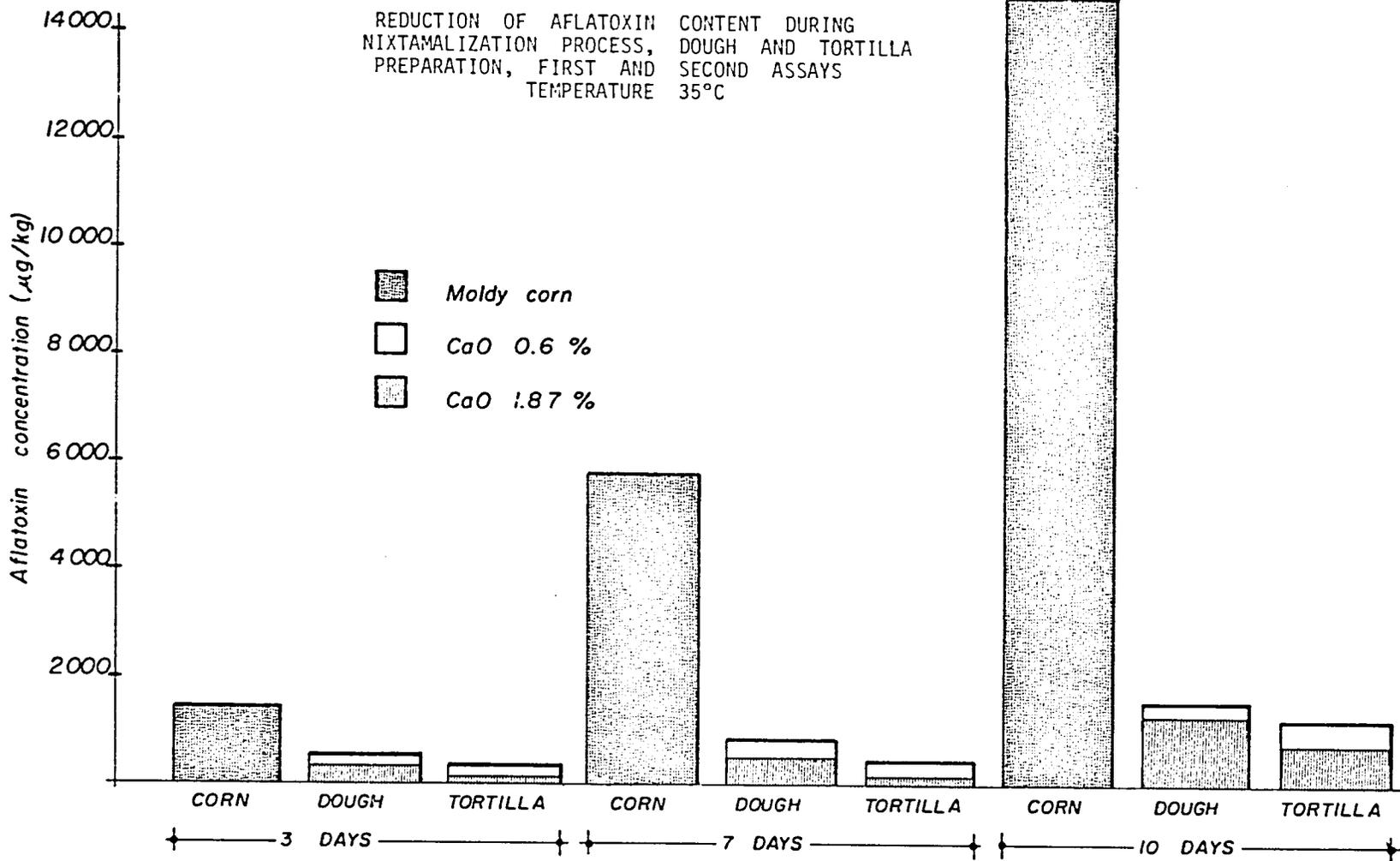


FIGURE NO. 3

REDUCTION OF AFLATOXIN CONTENT DURING  
NIXTAMALIZATION PROCESS, DOUGH AND TORTILLA  
PREPARATION, FIRST AND SECOND ASSAYS  
TEMPERATURE 35°C





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THIRD PROGRESS REPORT

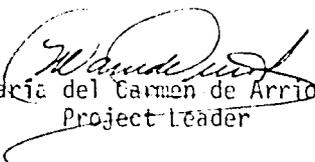
DESTRUCTION OF AFLATOXIN IN CORN AND  
NUCLEIC ACIDS IN YEAST CORN MIXTURE

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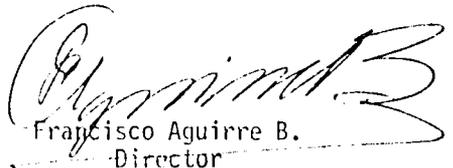
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