African Journal of Biotechnology Vol. 3 (9), pp. 451-455, September 2004
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2004 Academic Journals

Full Length Research Paper

Effect of roasting on the aflatoxin contents of Nigerian peanut seeds

OGUNSANWO, B.M.*[1], FABOYA, O.O.P.[2], IDOWU O.R.[3], LAWAL O.S.[1] and BANKOLE S.A.[4]

*[1] Department of Chemical Sciences, Olabisi Onabanjo University, Ago-Iwoye. Nigeria.
[2] Department of Chemistry, Ladoke Akintola University, Ogbomosho, Nigeria.
[3]Department of Chemistry University of Ibadan, Nigeria.
[4]Department of Biological sciences, Olabisi Onabanjo University, Ago-Iwoye. Nigeria.

Accepted 12 April, 2004.

Peanut seeds were prepared with variations in roasting conditions. Positive correlations were obtained between loss of aflatoxins in the products and the roasting conditions. Seeds dry- roasted at 140^{O}C for 40 min resulted in 58.8% and 64.5% reductions in AFB_1 and AFG_1, those roasted at 150^{0}C for 25 minutes resulted in 68.5% and 73.3% reductions in AFB_1 and AFG_1, respectively. Roasting at150^{O}C for 30mins led to 70.0% and 79.8% reductions in AFB_1 and AFG_1 respectively.

Keywords: Peanut, aflatoxin, roasting.

INTRODUCTION

Prior to the advent of 'oil boom' era of the seventies in Nigeria, a substantial part of the foreign earnings of the country accrued from peanuts (Aradus hypogea). This formed the basis of the famous peanut pyramids in the Northern part of Nigeria at that time.

Locally, consumption of peanut is very popular among the populace. It is mostly eaten in the roasted form throughout the year. A substantial part is also eaten in the cooked form, while fewer people indulge in the eating of the raw seeds.

Attempts have been made by Lee et al. (1968), to apply dry roasting on a means of detoxifying aflatoxin-contaminated peanuts. The work however did not attempt to predict the variation in temperature and time.

In the present work therefore, reduction in the level of aflatoxin as a result of either variation in time of roasting at specific temperatures, or variation in temperature of roasting at specific time, were monitored.

*Correspondingauthor. E-Mail: tundeogunsanwo@yahoo.co.uk.

MATERIALS AND METHODS

Aspergillus flavus species obtained from the Departments of Botany and Agricultural Biology at University of Ibadan, Nigeria, were cultured on a large scale on loaves of bread.

The matured culture was then used to spike fresh peanut seed purchased in a local market. The culture was covered and allowed to stay in the laboratory for 10 days at the end of which the greenish-yellow sporulation of the mould was widespread in the culture.

Cucullu and co-workers et al. (1968), had earlier indicated that aflatoxin contamination of the mould in mould-infested peanut was non-uniformly distributed. To ensure uniform distribution of the toxins in the seeds used for this study therefore, contaminated seeds were whipped and thoroughly mixed before roasting.

Known quantities of contaminated seeds were roasted in the oven at specific temperatures for specific lengths of time. Testas of statistically selected roasted seeds were then removed with the aid of clean gloves previously rinsed with methanol, acetone and chloroform in that order.

For completeness, roasting of seeds was sometimes carried out under conditions too mild to effect production of acceptable roasted seeds, as well as at under very severe conditions where the seeds became scorched.

Extraction of Aflatoxins from each sample was carried out according to the method of Velasco (1975) and the extracts purified with basic cupric carbonate as described by Wiesman et al., (1967). The purified extracts were chromatographed on silica gel 60G plates using diethyl ether-methanol-water (96:3:1, v/v/v) as eluent. The aflatoxin spots were confirmed by comparison with standard spots and by method of Smith et al. (1962). Aflatoxin quantitation was carried out as described by Nabney and Nesbitt (1965).

RESULTS

In virtually all the samples, only AFB_1 and AFB_2 were obtained even though the reference standard spots showed four distinct aflatoxin spots corresponding to Aflatoxins B_1, B_2, $G_1$and G_2, on the same plate.

In addition, spots with R_F values higher than that of AFB_1 (the fastest moving aflatoxin) were obtained on the TLC plates of most of the roasted seeds. These spots did not respond positively to the confirmatory text for aflatoxins. This implies that they were non-aflatoxin spots. They probably must have been products of either thermal decomposition of the aflatoxins or reaction between the toxins and some of the components of the peanuts, especially at high temperatures.

The effect of time on the loss of the aflatoxins in peanut seeds during dry roasting at various temperatures is shown in Tables 1-5 below.

Table 1. Percentage reduction of aflatoxins in peanuts roasted at 100^{o}C.

Amount in raw seeds (ppm)		Roasting period	Percentage reduction of aflatoxin			
			seeds with testa		seeds without testa	
B_1	G_1	(min)	B_1	G_1	B_1	G_1
1.80	1.24	30.0	0	0	11.1	20.2
1.80	1.24	45.0	4.4	4.8	15.0	24.2
1.80	1.24	60.0	4.4	4.8	15.0	24.0
1.80	1.24	75.0	15.0	24.2	26.1	42.7
1.80	1.24	90.0	33.3	60.5	44.4	60.5
1.80	1.24	105.0	68.3	81.5	70.6	82.3
1.80	1.24	120.0	70.6	82.3	70.6	82.3
2.24	2.58	20.0	7.1	7.8	7.1	7.8
2.24	2.58	30.0	15.7	38.8	42.9	46.5
2.24	2.58	45.0	42.9	46.5	42.9	46.5
2.24	2.58	60.0	42.9	46.5	42.9	46.5
1.44	1.58	20.0	0	0	0	0
1.44	1.58	30.0	0	0	0	0
1.44	1.58	45.0	22.2	24.7	22.2	24.7
1.44	1.58	60.0	33.3	37.3	33.3	37.3

Table 2. Percentage reduction of aflatoxins in peanuts roasted at 120^{0}C.

Amount in raw seeds (ppm)		Roasting period	Percentage reduction of aflatoxins			
			seeds with testa		seeds without testa	
B_1	G_1	(min)	B_1	G_1	B_1	G_1
1.78	1.10	0.0	14.6	2.7	29.2	29.1
1.78	1.10	45.0	19.1	7.3	29.2	29.1
1.78	1.10	60.0	29.2	29.1	43.8	32.7
1.78	1.10	75.0	52.8	52.7	50.0	50.0
1.78	1.10	90.0	52.8	76.4	64.6	76.4
1.78	1.10	105.0	64.6	76.4	76.4	76.4
1.78	1.10	120.0	64.6	76.4	76.4	76.4
2.24	2.58	20.0	7.1	7.8	14.3	15.5
2.24	2.58	30.0	42.9	46.1	50.0	53.9
2.24	2.58	45.0	42.9	46.1	50.0	53.9
2.24	2.58	60.0	50.0	53.9	57.1	53.9
1.44	1.58	20.0	0	0	0	0
1.44	1.58	30.0	0	0	0	0
1.44	1.58	45.0	33.3	37.3	33.3	37.3
1.44	1.58	60.0	33.3	37.3	33.3	37.3

Table 3. Percentage reduction of aflatoxins in peanut seeds Roasted at 130°C.

Amount in raw Seeds (ppm)		Roasting period	Percentage reduction of aflatoxins			
			seeds with testa		seeds without testa	
B_1	G_1	(min)	B_1	G_1	B_1	G_1
2.24	2.58	20.0	21.4	15.5	28.6	23.3
2.24	2.58	25.0	21.4	23.3	28.6	23.3
2.24	2.58	30.0	42.9	53.9	50.0	53.9
2.24	2.58	35.0	50.0	53.9	57.1	53.9
2.24	2.58	40.0	57.1	53.9	57.1	53.9
1.44	1.58	20.0	22.2	24.7	22.2	24.7
1.44	1.58	25.0	22.2	24.7	22.2	24.7
1.44	1.58	30.0	33.3	37.3	33.3	37.3
1.44	1.58	35.0	44.4	50.0	44.4	50.0
1.44	1.58	40.0	44.4	50.0	44.4	50.0
6.51	7.79	20.0	15.7	24.3	32.1	50.1
6.51	7.79	30.0	39.5	55.6	48.2	59.4
6.51	7.79	35.0	54.7	69.3	58.8	66.4
6.51	7.79	40.0	59.3	71.5	65.3	75.4

Table 4. Percentage reduction of aflatoxins in peanut seeds roasted at 140°C.

Amount in raw seeds (ppm)		Roasting period	Percentage reduction of toxins			
			seeds with testa		seeds without testa	
B_1	G_1	(min)	B_1	G_1	B_1	G_1
2.24	2.58	20.0	21.4	23.3	28.6	31.0
2.24	2.58	25.0	35.7	38.4	42.9	46.1
2.24	2.58	30.0	50.0	53.9	50.0	53.9
2.24	2.58	35.0	57.1	61.6	57.1	61.6
2.24	2.58	40.0	64.3	61.6	64.3	61.6
6.63	7.46	20.0	22.5	17.7	31.2	29.2
6.63	7.46	30.0	55.8	57.2	56.6	57.5
6.63	7.46	35.0	62.3	64.3	62.3	64.3
6.63	7.46	40.0	66.2	68.0	67.7	69.3

Table 5. Percentage reduction of aflatoxin in peanut seeds roasted at 150°C.

Amount in raw seeds (ppm)		Roasting period	Percentage reduction of aflatoxins			
			seeds with testa		seeds without testa	
B_1	G_1	(min)	B_1	G_1	B_1	G_1
5.35	6.09	20.0	66.5	67.3	75.7	78.7
5.35	6.09	25.0	77.8	79.6	78.5	80.1
5.35	6.09	30.0	82.4	81.3	83.9	84.2
2.24	2.58	20.0	71.4	69.4	71.4	77.1
2.24	2.58	25.0	71.4	77.1	71.4	77.1
2.24	2.58	30.0	78.6	84.9	78.6	84.9

DISCUSSION

From the results, it is obvious that roasting causes reduction in the levels of aflatoxins in peanuts. This observation is in agreement with earlier reports (Lee et al., 1968; Lee et al., 1969; Waltking, 1971; Hamada and Megalla, 1982; Escher et al., 1973) that heating reduces aflatoxins in agricultural products.

In considering the results displayed above, there are three main possibilities for the aflatoxin loss as a result of application of heat. These are:

(i) Heat liability of the aflatoxins;
(ii) Thermodynamically enhanced reactions between the aflatoxins and other constituents of the peanut seeds; and
(iii) Thermal destruction of other constituent of the peanut seeds and less extractability of the aflatoxins in the presence of these products of heat destruction.

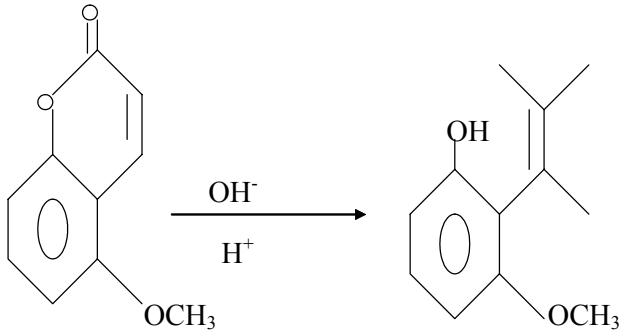
Option (iii) seems unlikely since aflatoxins were detected even after extremely harsh conditions of roasting. Pyrolysis of aflatoxins especially at elevated temperature would lead to their decomposition. It will be difficult to predict the product of this pyrolysis since during this process, which is a very drastic condition of attack on any compound, bond breakage becomes indiscriminate.

Notwithstanding, the products of such decomposition are either simple hydrocarbons or simple organic compounds. This probably explains the presence of spots with R_F values higher than that of AFB_1 on the TLC plates of roasted peanut samples.

In a similar manner, reactions between the aflatoxins and other materials in the peanut seeds could modify the structures of the toxins and possibly give products with R_F values higher than that of AFB_1 on the TLC plates of the roasted peanut samples. Thermolability of the toxins could possibly occur. The presence of the coumarin moiety in the aflatoxins makes these compounds susceptible to acid-base attack of the general form in Figure 1. It is, therefore, possible that this pH dependent decomposition of the aflatoxins could be promoted by the action of heat on some constituents of peanuts.

In the case of chemical reaction involving the aflatoxins, it is anticipated that the G series would be more reactive than their B counterparts. This could be predicted from the structural differences between these compounds. In the B series, there is just one ether linkage while in the G series there are two ether linkages. Ether linkages are susceptible to chemical attacks and therefore the presence of two of such sites in the G toxins makes them more susceptible to chemical attacks when compared to the B series. It is therefore expected that AFG_1will be more thermo labile than AFB_1 and thus expected to suffer higher degradation.

Results obtained in this work seem to support the above prediction with respect to thermolabilities of these



5-methoxy coumarin

2- (3-methylbutenyl)-3-methoxyl phenol

Figure 1. Acid-base conversion of coumarinmoeity.

compounds as there appears to be higher percentage reductions of AFG_1 than AFB_1 in the peanut seeds roasted under the same conditions. Similar observation, have been reported by Hamada and Megalla (1982).

From statistical analysis, a correlation coefficient (r) of 0.76 was obtained between the reduction ofAFB_1in seeds with testa and the period of roasting of the seeds at 100°C. This value indicates an association between these two variables conforming to a linear equation:

$$y = .60x (\pm 0.03) - 7.88$$

Where y = percentage reduction
x = roasting period (min)

AFG_1 in the seeds with testa has r = 0.83 between the reduction of toxin and period of roasting at 100°C and confirms with a linear equation:

$$y = 0.78x (\pm 0.32) - 12.7$$

A comparison of the slopes of the regression lines for AFB_1 and AFG_1 at 100°C for seeds with testa however shows that at this temperature, there appears to be no significant statistical difference in the thermolabilities of these compounds.

In seeds without testas, AFB_1 has r = 0.83 for the association between the reduction of the toxins and the period of roasting at 100°C, and conforms to a linear equation:

$$y = 0.61x (\pm 0.26) - 4.35$$

While AFG_1 has r =0 .88 describing a linear relationship:

$$y = 0.75x (\pm 0.24) - 5.09$$

Comparison of the slopes of the regression lines for $AFB_1$and AFG_1at 100°C for peanut seeds without testas

Table 6. Results in the rate of aflaxotins during heating period.

Roasting at 120°C	
seeds with testas	**seeds without testas**
AFB_1: r=0.85; y=0.61x (+0.23)+0.019 AFG_1:r=0.86; y=0.79x (+0.28)-7.54	AFB_1: r=0.85; y=0.67x (+0.25)+3.34 AFG_1: r=0.84; y=0.66x (+0.25)+5.83
Roasting at130°C	
AFB_1: r=0.93; y=1.87x (+0.45)-19.07 AFG_1: r=0.86; y=2.10x (+0.77)-20.29	AFB_1: r=0.84;y=1.63x (+0.65)-7.02 AFG_1: r=0.86; y=2.10x (+0.77)-20.29
Roasting at 140°C	
AFB_1: r=0.97; y=2.21x (+0.51)-19.11 AFG_1: r=0.94; y=2.28x (+0.72)-20.05	AFB_1: r=0.97; y=1.80x (+0.38)-3.72 AFB_1: r=0.97; y=1.80x (+0.38)-3.72
Roasting at 150°C	
AFB_1: r=0.88; y=1.16x (+0.88)+45.81 AFG_1: r=0.96; y=1.47x (+0.63)+39.73	AFB_1: r=0.72; y=0.77x (+1.05) +57.33 AFG_1: r=0.86; y=0.67x (+0.54) +63.73

also show lack of significant difference in the thermolabilities of these compounds. Results in Tables 1-5 also appear to support the view of Lee and co-workers (1969) that the rates of reduction of the aflatoxins during heating depend on the initial level of contamination. By subjecting the values in tables 2-5 to statistical analysis, the values in Table 6 were obtained.

From the statistical results it is obvious that there is no significant difference in the heat labilities of AFB_1 and AFG_1 for roasting at 120°C – 150°C. However the loss of the toxin in seeds with testas is significantly different from the loss in the seeds without testas for each toxin. This confirms the earlier observation that removal of seed testas increases the loss of the aflatoxins.

Also from the results in tables 1-5, it is obvious that AFG_1 is more thermo labile than AFB_1. This is in agreement with the earlier prediction and goes to

strengthen the postulation stated above on the destruction of these toxins as a result of heat enhanced reaction involving them.

Roasting destroys AFB_1 and AFG_1 significantly and positive correlations were obtained between the loss of these toxins and roasting conditions.

REFERENCES

Cucullu AF, Lee LS, Mayne RY, Goldblatt LA (1966). Determinations of aflatoxins in individual peanut and peanut sections J. Am. Oil Chem. Soc. 43: 89.

Escher FE, Koehler PE, Ayres JC (1973). Effect of roasting on aflatoxin content of artificially contaminated pecans J. Food Sci. 38: 889.

Hamada, AS, Megalla SE (1982). Effect of malting and roasting on reduction of aflatoxin in contaminated soybeans. Mycopathology, 79: 3.

Lee LS, Cucullu AF, Franz AO, Pons WA (1969). Destruction of aflatoxins in peanuts during dry and oil roasting. J. Agric. Food Chem. 17:451.

Lee LS, Cucullu AF, Goldblatt LA (1968). Appearance and aflatoxin contents of raw and dry roasted peanut kennels. Food Technol. 22: 1131.

Nabney J, Nesbitt BF (1965). A spectrophotometric method for determining the aflatoxins. Analyst 90: 155.

Smith RH, Mckerman W (1962). Hepatotoxic action of chromatographically separated fractions of A.flavus extracts. Nature 195: 1301.

Velasco J (1975). Fluorometric measurement of Aflatoxins adsorbed on florisil in Mini columns. J.A.O.A.C. 58: 757.

Waltking AE (1971). Fate of aflatoxin during roasting and storage of contaminated peanut products. J..A.O.A.C. 54: 533.

Weisman HG, Jacobson WC, Harmeyer WC (1967). Note on removal of pigments from chloroform extracts of aflatoxin cultures with copper carbonate. J.A.O.A.C. 50: 982.