Limiting Temperature and Relative Humidity for Growth and Production of Aflatoxin and Free Fatty Acids by *Aspergillus flavus* in Sterile Peanuts

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Abstract

Sound mature kernels, broken mature kernels, immature kernels, and unshelled Early Runner peanuts were heat-treated in controlled environment cabinets and inoculated with spores of *Aspergillus flavus*. Treatments were incubated at 97–99% relative humidity at different temperatures ranging from 5 to 55C and also at 30C with relative humidities ranging from 55 to 99%. Samples were removed after 7 and 21 days and assayed for aflatoxin, free fatty acids, and peanut kernel moisture. The limiting relative humidity for aflatoxin production by *A. flavus* was 85 ± 1% relative humidity for 21 days at 30C. The limiting low temperature for visible growth and aflatoxin production by the fungus was 13 ± 1C for 21 days at 97–99% relative humidity. Damaged kernels, however, developed some afflatoxin in 21 days at 12C. The maximum temperature for aflatoxin production was 41.5 ± 1.5C for 21 days at 97–99% relative humidity. Fungus growth and sporulation at 43C were equal to that at 40C, but no aflatoxin was produced. Moisture content of immature kernels was higher at equilibrium with the same relative humidity than the moisture content of sound mature kernels, damaged kernels, or kernels from unshelled peanuts. There appeared to be no proportional quantitative correlation between synthesis of aflatoxin and production of free fatty acids in nonliving peanuts, but no aflatoxin was produced without a simultaneous increase in free fatty acids.

Introduction

ASPERGILLUS FLAVUS Link ex Fries may invade the developing fruit of the peanut (*Arachis hypogaea* L.) prior to digging time (1,6,9), but contamination of peanuts by the fungus and its associated toxin, aflatoxin, probably occurs primarily during curing and subsequent storage (2). The main factors controlling invasion of stored seed by fungi are moisture and temperature (3,11). Diener (5) found that initial storage moisture was correlated with high mold counts in peanuts. Austwick and Ayerst (2) observed good growth by toxin-producing isolates of *A. flavus* in pure culture at relative humidities (RH) of 85% or above at 25–38C; minimal growth was recorded at 80% RH and 30C. Moisture contents of kernels and meal in equilibrium with 85% RH were 11.3 and 19%, respectively. *A. flavus* has been classified as a mesophilic fungus with cardinal growth temperatures of 6–8, 36–38, and 44–46C (11). Diener and Davis (8) reported the optimal time and temperature for aflatoxin production by *A. flavus* and *A. parasiticus* Speare on sterilized peanut kernels and in liquid medium in culture flasks to be 7–15 days and 25–30C, respectively.

This paper reports the effect of temperature and RH on growth and on production of aflatoxin and free fatty acids by *A. flavus* in heat-treated sound and broken mature kernels, immature kernels, and unshelled Early Runner peanuts. A preliminary report of this research has been published (7).

Materials and Methods

Aspergillus flavus, strain Ala-6, used in these experiments was isolated from Alabama peanuts in 1964 (4).

One ton of peanuts (*Arachis hypogaea* L. var. Early Runner) obtained from the Wiregrass Substation, Headland, Ala., was shelled, cleaned, and segregated into lots that consisted of: (a) sound mature kernels, b) broken mature kernels, c) immature kernels, and d) unshelled peanuts with intact shells.

Environmental studies were conducted in Blue M Power-O-Matic 60 (model CFR-7752C) saturable reactor, proportionally controlled, refrigerated humidity cabinets having 10 ft^3 working chambers, 7-day recording psychrometers, and self-contained water purification systems. Temperature range available was 5–90C ± 0.5C, and RH range was 40–90% ± 1%. Two round-rod stainless steel shelves supported two perforated stainless steel trays (16 in. square by 1 in. deep) totaling 4 trays per cabinet.

Sound mature, broken mature, and immature kernels in lots of 900 g were distributed on each of 3 trays in a layer 1–2 peanuts deep. Unshelled peanuts (1300 g) were placed 2–3 pods deep in the fourth tray. One tray of each of the four treatments was placed randomly on a different shelf location in each cabinet. Peanuts in the 8 cabinets were heat-treated by exposure to 12–14 hr of wet heat (95–99% RH) at 80–85C. Cabinets were allowed to cool for 2 hr and adjusted to predetermined settings for specific temperatures and RH. The moist peanuts were then inoculated by spraying with a suspension of *A. flavus* spores calculated to give 12–15 million spores per tray. Control lots (400–500 g) of each treatment of peanuts were taken randomly from the supply while loading the trays. Other lots were removed immediately after the wet heat treatment and before inoculation to be processed as sterilized controls.

Samples of peanuts (400–450 g) were removed at random from each treatment tray by removing an x-shaped area of peanuts with a small plastic scoop in each quadrat of a tray and compositing them on a balance until the required weight for analyses was accumulated. Remaining seeds were redispersed over the trays uniformly and removed 21 days after inoculation. Safety precautions of personnel during sampling included wearing surgical masks, rubber gloves, and long laboratory coats. Sample baskets were covered until placed in the drying oven. Air was exhausted from the room for several hours and the work areas mopped with 5% NaOCl (12).

TABLE I

Kernel Moisture Content, Free Fatty Acids, and Aflatoxin Production in Sound Mature Kernels Inoculated with *Aspergillus flavus*, and Incubated at 30C at Several Relative Humidities for 21 Days

RH %	KMC %	FFA %	Aflatoxin, μg/kg				
			B_1	B_2	G_1	G_2	Total
55	9.39	.20	5	0	6	0	11
75	7.09	.15	0	0	0	0	0
80	7.03	.25	13	5	11	2	31
82	8.62	.15	4	0	3	0	7
85	9.31	.20	0	0	0	0	0
86	11.93	2.28	405	66	600	50	1121
87	10.93	.85	7	6	18	2	33
88	12.57	3.16	1100	208	1500	250	3058
89	14.82	17.01	15700	4700	39700	8000	68100
90	11.94	15.70	11400	18000	9600	10000	49000
95	12.70	37.35	9500	20000	30400	10000	69900
99	14.23	31.90	26600	20000	25700	10000	82300
Check, raw	5.83	0.26	3	0	3	0	6
Check, sterile	15.71	.17	4	0	3	0	7

Samples (400–500 g) were taken from the environmental cabinets, weighed in wire baskets, and placed in a forced draft oven at 135C for 20 min. Samples were dried to near contant weight at 70C and then ground in a Universal model No. 71 food chopper with a 16-point cutter. The ground peanuts were again dried to near constant weight at 70C and stored in screw-cap amber bottles in a cold room at 1–2C until analyzed chemically.

Peanut kernel moisture content (KMC) was determined by the oven-dray method described in AOCS Method Ab 2-49 on duplicate 25-g samples of seed taken directly from the environmental cabinets. The following modification was required because of the high moisture content of some of the samples. After weighing the samples in aluminum moisture dishes, peanuts and fungus were exposed in a forced draft oven to 135C for 20 min, brought to near constant weight at 70C, and finally dried for 3 hr at 130C.

Aflatoxin analyses were made of duplicate 50-g samples by the aqueous-acetone method of Pons and Goldblatt (10). Free fatty acids (FFA) were determined on duplicate samples by AOCS Method Ab 5-49 and Aa 6-38, Section C, paragraphs 2, 3, 4, except that samples were first ground with a Universal model No. 71 food chopper rather than a Henry nut slicer.

Results

Relation of Relative Humidity to Aflatoxin Production by A. flavus at 30C

Relation of RH to aflatoxin production in sound mature kernels is shown in Table I. Total aflatoxin up to 82,300 μg/kg formed in 21 days at 89% RH and higher. Total aflatoxin of 1,121 and 3,058 μg/kg was found at 86 and 88% RH, respectively. Control raw and sterilized peanut samples run in quadruplicate averaged 6–7 μg/kg. Little aflatoxin was found in sound mature kernels held at 85% RH and below.

The relationship of RH to aflatoxin in immature kernels is given in Table II. KMC's of immature kernels were about 3–4% higher at the same RH than sound mature kernels. However, aflatoxin levels were not generally greater except at 86, 87 and 88% RH. The highest aflatoxin level below 85% RH was 70 μg/kg. Control lots averaged 11 μg/kg.

Relation of RH to aflatoxin level in broken mature kernels is presented in Table III. Total aflatoxin up to 115,400 μg/kg formed in 21 days at 89% RH and higher. From 662 to 29,800 μg/kg of aflatoxin were produced at the 86–88% RH range. Raw and sterile control samples averaged 48 and 3 μg/kg, respectively, as compared with 11 μg/kg or less in sound mature and immature kernels.

Relation of RH to aflatoxin in kernels of unshelled peanuts is given in Table IV. From 13,600 to 92,400 μg/kg of aflatoxin were found in 21 days at 89–99% RH, but only 245 to 528 μg/kg formed at 87 and 88% RH. Unshelled peanuts contained little aflatoxin at 86% RH and lower when compared with initial aflatoxin levels of raw and sterile controls that were 27 and 5 μg/kg, respectively.

Analyses of 7-day samples (unpublished data) show the relationship of time and RH to aflatoxin formation. *A. flavus* produced 644 to 3,300 μg/kg of total aflatoxin in sound mature, broken mature, and immature kernels at 89% RH in comparison with 16,600 to 27,000 μg/kg at 99% RH at 30C in 7 days. These aflatoxin levels were approximately 8–10% of those found in 21-day samples. Less than 300 μg/kg of aflatoxin were produced in 7 days at 86–88% RH, whereas 30 to 100 times this amount formed in 21 days. Broken mature kernels contained 50–60% more aflatoxin in 7 days than sound mature kernels, immature kernels or kernels from unshelled peanuts, but there was little difference between treatments after 21 days. With unshelled peanuts, less than 200 μg/kg aflatoxins were found at 89–95% RH. No aflatoxin occurred at 86–88% RH in 7-day samples.

TABLE II

Kernel Moisture Content, Free Fatty Acids, and Aflatoxin Production in Immature Kernels Inoculated with *Aspergillus flavus*, and Incubated at 30C at Several Relative Humidities for 21 Days

RH %	KMC %	FFA %	Aflatoxin, μg/kg				
			B_1	B_2	G_1	G_2	Total
55	9.55	.30	5	0	7	0	12
75	10.35	.35	5	0	7	1	13
80	11.43	.40	27	5	30	7	69
82	11.83	.25	T[a]	0	T	0	T
85	13.10	.65	T	0	0	0	T
86	15.42	4.95	3800	1000	3200	500	8500
87	16.28	4.58	4300	1100	7600	1400	14400
88	15.34	4.09	1500	400	1700	500	4100
89	16.63	6.41	12200	1900	39700	8000	61800
90	15.73	7.25	3800	4000	4800	6000	18600
95	13.79	26.85	15200	18000	32000	10000	75200
99	17.97	38.70	10500	12000	8800	6700	38000
Check, raw	8.19	.36	3	0	2	0	5
Check, sterile	20.68	.33	7	0	4	0	11

[a] T = trace (less than 1 μg/kg).

TABLE III

Kernel Moisture Content, Free Fatty Acids and Aflatoxin Production in Damaged Kernels Inoculated with *Aspergillus flavus*, and Incubated at 30C at Several Relative Humidities for 21 Days

RH %	KMC %	FFA %	Aflatoxin, μg/kg				
			B_1	B_2	G_1	G_2	Total
55	5.30	.25	25	0	32	0	57
75	7.18	.35	5	0	T[a]	0	5
80	7.39	.30	0	0	0	0	0
82	8.50	.90	8	0	8	2	18
85	8.53	.45	30	22	19	6	77
86	12.15	12.03	3000	400	2600	200	6200
87	12.00	14.49	9700	8300	10200	1600	29800
88	12.62	11.92	317	83	213	49	662
89	13.19	22.77	36500	5800	51200	8000	101500
90	12.07	23.83	25600	16000	21600	8000	71200
95	10.74	34.60	50000	18000	38400	9000	115400
99	17.46	52.55	34200	20000	28800	10000	93000
Check, raw	5.70	.31	19	0	29	0	48
Check, sterile	13.48	.15	2	0	1	0	3

[a] T = trace (less than 1 μg/kg).

However, in 21 days the aflatoxin levels in unshelled peanuts were as high as other lots. Thus, the shell appeared to delay *A. flavus* invasion and aflatoxin production in the kernel for a few days at the lower RH.

Relation of Temperature to Aflatoxin Production by A. flavus at 97-99% RH

Relation of temperature to aflatoxin in sound mature kernels is presented in Table V. From 229,800 to 364,000 μg/kg of aflatoxin were produced in 21 days in the 20 to 35C range, whereas levels of 29,800, 5,066, and 4,666 μg/kg formed at 15, 14 and 40C, respectively. Little or no aflatoxin was found at 12C and lower or at 43C and higher in 21 days as compared with raw and sterile control samples that produced no measurable aflatoxin.

Relation of temperature to aflatoxin in immature kernels is shown in Table VI. Levels of aflatoxin of 40,100 to 218,500 μg/kg occurred in the 15–35C range, whereas only 2,699 μg/kg formed at 40C. Little aflatoxin was found in 21 days in immature kernels at 14C and lower or at 43C and higher when compared wtih raw and sterile control samples.

Table VII gives the relation of temperature to aflatoxin in damaged kernels. Total aflatoxin from 58,500 to 204,800 μg/kg was produced in the 20–35C range in 21 days, whereas levels of only 2,025 to 5,962 μg/kg occurred at 14, 15, and 40C. Nearly 100 μg/kg formed in 21 days at 12C in damaged kernels. Only a trace of aflatoxin was found in control samples.

Relation of temperature to aflatoxin in kernels of unshelled peanuts is given in Table VIII. Total aflatoxin from 127,900 to 321,000 μg/kg was found in kernels in the 20 to 35C range, whereas levels of 13,200 and 10,300 μg/kg formed at 15 and 40C, respectively. At 14C, 593 μg/kg were produced in kernels from unshelled peanuts. Aflatoxin in trace amounts was found only in the control samples.

Analyses of 7-day data (unpublished) show the relationship of time and temperature to aflatoxin formation. Little or no aflatoxin formed in 7 days at 15C, whereas levels of 593 to 5,066 μg/kg were produced in 21 days in sound and broken mature kernels and unshelled peanuts at 14C. However, no aflatoxin formed in immature kernels at 14C. Aflatoxin (95 μg/kg) was also found in damaged kernels at 12C after 21 days, which was 2C lower than the other treatments.

Growth and Sporulation by Aspergillus flavus

Mycelial growth of *A. flavus* on broken mature and immature kernels was visible in 24–30 hr at favorable temperatures and RH. Heavy sporulation was typically noted in 48–72 hr. Growth and sporulation apparently were inhibited or temporarily restricted by the seed coats of sound mature kernels. The fungus first appeared as a tuft of hyphae and conidiophores on areas of kernels damaged in shelling, although extensively damaged peanuts had been removed by hand from sound mature kernel lots before incubation. In all cases, visible mycelial mats developed later on sound mature kernels than on broken mature and immature kernels, but the level of aflatoxin was similar. Heavy mycelial mats seldom formed on the surface of pods of unshelled peanuts. Except at temperatures above 40C, visible growth and sporulation by *A. flavus* was associated with aflatoxin production. Fungus growth was restricted at temperatures below 14C and at RH below 86% during the 21-day storage period. Sporulation occurred at 43C but not at 46C. *A. flavus* could still be isolated at the end of the incubation period in either temperature.

Relation of Free Fatty Acids (FFA) to Aflatoxin Production by A. flavus in Sterile Peanuts

The development of high percentages of FFA at 30C was generally correlated with high RH and high total aflatoxin (Tables I–IV). FFA were relatively

TABLE IV

Kernel Moisture Content, Free Fatty Acids, and Aflatoxin Production in Unshelled Peanuts Inoculated with *Aspergillus flavus*, and Incubated at 30C at Several Relative Humidities for 21 Days

RH %	KMC %	FFA %	Aflatoxin, μg/kg				
			B_1	B_2	G_1	G_2	Total
55	6.48	.25	0	0	0	0	0
75	7.23	.25	28	5	24	7	64
80	7.85	.20	T[a]	0	T	0	T
82	8.90	.15	0	0	0	0	0
85	10.64	.25	0	0	0	0	0
86	10.79	.57	5	3	8	1	17
87	10.76	.57	194	3	176	128	528
88	10.81	.93	95	12	112	26	245
89	14.09	11.49	12200	1900	23000	3800	40900
90	10.97	3.70	3400	4000	3200	2000	13600
95	10.60	19.38	30400	16000	25600	9000	81000
99	17.80	41.28	30400	20000	32000	10000	92400
Check, raw	5.96	.13	22	0	5	0	27
Check, sterile	16.60	.15	3	0	2	0	5

[a] T = trace (less than 1 μg/kg).

TABLE V

Kernel Moisture Content, Free Fatty Acids, and Aflatoxin Production in Sound Mature Kernels Inoculated with *Aspergillus flavus*, and Incubated at 97–99% Relative Humidity and at Various Temperatures for 21 Days

Temp C[a]	KMC %	FFA %	Aflatoxin, μg/kg				
			B_1	B_2	G_1	G_2	Total
12	16.95	.14	0	0	0	0	0
14	16.39	11.61	1700	1300	1400	666	5066
15	18.57	17.50	6900	1800	17600	3500	29800
20	19.15	31.74	84200	19900	213300	46600	364000
25	17.06	[b]	83300	13300	159900	55000	311500
30	14.71	41.51	94900	33300	106600	20800	255600
35	12.64	38.14	126600	33300	53300	16600	229800
40	13.19	38.93	2500	1000	1000	166	4666
43	19.47	29.51	T[c]	T	T	T	T
46	12.30	1.27	0	0	0	0	0
Check, raw	5.60	.16	0	0	0	0	0
Check, sterile	16.84	.12	0	0	0	0	0

[a] Data for 5, 10, and 49, 52, 55C were similar to those at 12 and 4C, respectively.
[b] Sample lost.
[c] T = trace (less than 1 μg/kg).

low at RH below 86% as was total aflatoxin. At high RH and moderate temperatures (15–40C) that permitted good fungus growth, percentage FFA was correlated with total aflatoxin (Tables V–VIII). However, aflatoxin and FFA levels were not proportionally correlated at temperature extremes, e.g., percentage FFA was high at 40C, but aflatoxin level was relatively low. Likewise, percentage FFA was high and aflatoxin low at 14C. Also, percentage FFA was high at 43C, although aflatoxin was not produced during the 21-day storage period. FFA production, growth, and sporulation of *A. flavus* were extensive at 43C, but little or no toxin was present. Thus, percentage FFA was closely related to fungus growth in all cases, but was not directly proportional to aflatoxin production.

Discussion

From these data, it appears that RH is a better criterion for safe storage levels than peanut kernel moisture. Aflatoxin was not produced in either sound mature or immature kernels stored at 85% RH, even though immature kernels contained an average KMC of 13% as compared with 9–10% in sound mature kernels. It appeared that a minimum kernel moisture in sound mature kernels of 11–12% was required for aflatoxin to form, whereas approximately 15% was required in immature kernels.

RH data agree with those of Austwick and Ayerst (2), who reported that *A. flavus* grew at the rate of 1 mm/day at 85% RH and 30C in pure culture. However, at 80% RH the growth rate was only 0.1 mm/day. In our experiments, no significant amount of aflatoxin formed in 21 days at 85% RH and lower. Thus, the limiting RH for aflatoxin production by *A. flavus* in nonliving peanuts for a 21-day storage period was 85 ± 1% RH.

Kernel moistures of peanuts stored at temperatures of 10–25C generally came to equilibrium at higher seed moistures than peanuts stored at 30–40C, except at the extremely high temperatures of 52 and 55C. Aflatoxin levels were restricted at 14–15C, which was somewhat unfavorable for fungus growth despite the higher KMC of the peanuts. Seed moisture relationships are complicated by the fact that fungus growth is a process that creates moisture, thereby making interpretation of moisture data difficult.

Aflatoxin levels of 200,000 μg/kg were produced in 21 days at 97–99% RH in all seed lots at 25C, and more than 300,000 μg/kg were produced in sound mature kernels and unshelled peanuts. The apparent optimum temperature range for aflatoxin production in sound mature, broken mature, and immature kernels was 20–25C in 21 days, whereas it was 25–30C for unshelled peanuts. The optimum temperature was 25C for all lots for a 7-day storage period (unpublished data), although more aflatoxin was present at 30C than at 20C regardless of the kernel type. After 21 days, there was more aflatoxin present at 20C than at 30C in all lots except unshelled peanuts. Peak aflatoxin content was found at 25C in all lots except sound mature kernels in which maximum production was at 20C. Thus, the optimum temperature for aflatoxin production was approximately 25C.

The ratio of aflatoxins B_1 and B_2 to G_1 and G_2 varied with temperature. The change in the ratio of B_1 to G_1 as temperature increased has been previously reported (8), but data on aflatoxins B_2 and G_2 were not included. These data indicate that aflatoxins G_1 and G_2 may be less stable at high temperatures than B_1 and B_2.

In 7 days broken mature kernels contained 50% more aflatoxin than the other three lots at 20, 25, and 30C. At the end of 21 days, broken mature kernels contained 30–50% less aflatoxin than sound mature kernels and unshelled peanuts at all temperatures from 15 to 40C. Some aflatoxin may have been degraded in the broken mature kernels after it was formed since the source and nature of peanut ker-

TABLE VI

Kernel Moisture Content, Free Fatty Acids, and Aflatoxin Production in Immature Kernels Inoculated with *Aspergillus flavus*, and Incubated at 97–99% Relative Humidity and at Various Temperatures for 21 Days

Temp C[a]	KMC %	FFA %	Aflatoxin, μg/kg				
			B_1	B_2	G_1	G_2	Total
12	17.11	.38	T[b]	T	T	T	T
14	15.03	1.36	T	T	T	T	T
15	27.88	23.30	12600	2100	21300	4100	40100
20	22.29	37.45	31000	10000	103400	14000	158400
25	22.46	46.63	101300	5300	85300	26600	218500
30	18.91	32.97	41600	6600	33300	5800	87300
35	18.03	29.65	38000	20000	20000	10000	88000
40	17.72	24.27	1200	333	1000	166	2699
43	22.34	17.90	0	0	0	0	0
46	18.27	1.36	T	T	T	T	T
Check, raw	8.87	.21	0	0	0	0	0
Check, sterile	18.24	.42	T	T	T	T	T

[a] Data for 5, 10, and 49, 52, 55C were similar to those at 12 and 46C, respectively.
[b] T = trace (less than 1 μg/kg).

TABLE VII

Kernel Moisture Content, Free Fatty Acids, and Aflatoxin Production in Damaged Kernels Inoculated with *Aspergillus flavus*, and Incubated at 97–99% Relative Humidity and at Various Temperatures for 21 Days

Temp C[a]	KMC %	FFA %	Aflatoxin, μg/kg				
			B_1	B_2	G_1	G_2	Total
10	18.65	1.04	T[b]	T	T	T	T
12	16.22	6.66	38	17	32	8	95
14	16.21	13.57	1700	1300	1400	666	5066
15	13.84	13.37	2500	666	2130	666	5962
20	17.27	27.54	79900	7600	74600	27000	189100
25	16.13	[c]	41600	6660	106600	50000	204800
30	12.86	45.98	50000	12000	64000	20000	146000
35	12.21	[c]	25300	13300	13300	6600	58500
40	19.63	39.16	1260	499	212	54	2025
43	14.90	26.42	T	T	T	T	T
46	12.96	2.65	0	0	0	0	0
49	12.96	.14	T	T	T	T	T
Check, raw	5.78	.17	0	0	0	0	0
Check, sterile	14.59	.17	T	0	T	0	T

[a] Cabinet breakdown resulted in no data at 5C; data at 52 and 55C similar to those at 49C.
[b] T = trace (less than 1 μg/kg).
[c] Sample lost.

nels were the same. This effect was more apparent at the temperature extremes of 15, 35, and 40C. No measurable aflatoxin was found in peanuts stored at temperatures of 43C and higher. Thus, the lower temperature limit for aflatoxin development was 13 ± 1C for a 21-day incubation period (except for damaged kernels that had some toxin at 12C), and the maximum temperature for aflatoxin production was 41.5 ± 1.5C.

Growth and sporulation of *A. flavus* was correlated with time at RH above 85% and temperatures of 14–43C. Mycelial growth was abundant at 46C but no sporulation occurred in 21 days. However, in environments of 97–99% RH and 43C, fungus growth did not result in aflatoxin production. Thus, temperature and not fungus development was the limiting factor in aflatoxin production at high temperatures, since at 43C growth and sporulation by *A. flavus* was equal to that at 40C, but no aflatoxin was found.

Formation of free fatty acids was correlated with fungus growth. However, percentage of FFA was not necessarily correlated with aflatoxin production.

Aflatoxin production by *A. flavus* varied considerably with the nature of the four peanut substrates. The skin of sound mature kernels was a barrier primarily to the visible aerial development of the fungus rather than to the invasion of peanut kernel itself. Broken mature kernels appeared to be more rapidly invaded by *A. flavus* than the other peanut lots since aflatoxin levels were higher in broken mature kernels than in the other 3 treatments after 7 days, but after 21 days aflatoxin levels in broken mature kernels were similar in all treatments. Immature kernels averaged 3–4% higher kernel moistures than the other lots, but aflatoxin did not develop more rapidly or to greater concentrations than in sound mature, broken mature, and unshelled kernels. In general, immature kernels had the same RH and temperature requirements for aflatoxin development as the other lots. Unshelled peanuts produced less aflatoxin under suboptimal conditions only for short periods of time (unpublished data) since aflatoxin levels were similar in all lots after 21 days. Presence of the intact shell was apparently only a temporary physical barrier to invasion by *A. flavus*.

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TABLE VIII

Kernel Moisture Content, Free Fatty Acids, and Aflatoxin Production in Unshelled Kernels Inoculated with *Aspergillus flavus*, and Incubated at 97–99% Relative Humidity and at Various Temperatures for 21 Days

Temp C[a]	KMC %	FFA %	Aflatoxin, μg/kg				
			B_1	B_2	G_1	G_2	Total
12	16.55	.14	0	0	0	0	0
14	23.18	2.57	152	17	384	40	593
15	18.58	10.16	3800	1000	6400	2000	13200
20	16.33	49.04	33300	5300	85300	4000	127900
25	19.32	56.29	58300	9300	149300	93300	310200
30	14.16	26.38	114000	24000	128000	55000	321000
35	14.34	[b]	76000	60000	32000	10000	178000
40	13.97	42.13	5000	3300	1500	500	10300
43	19.36	14.45	0	0	0	0	0
46	17.40	1.24	T[c]	T	T	T	T
Check, raw	5.77	.14	T	0	T	0	T
Check, sterile	15.32	.18	T	T	T	T	T

[a] Data at 5, 10, and 49, 52, 55C were similar to those at 12 and 46C, respectively.
[b] Sample lost.
[c] T = trace (less than 1 μg/kg).