Limiting Temperature and Relative Humidity for Aflatoxin Production by *Aspergillus flavus* in Stored Peanuts

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Abstract

Sound mature kernels, broken mature kernels, immature kernels and unshelled cured Early Runner peanuts were inoculated with spores of *Aspergillus flavus* and incubated up to 84 days in controlled environment cabinets. In a series of experiments temperatures ranged from 8 to 49 C in combination with 98 ± 1% relative humidity (RH); in others RH was varied from 70% to 99% at 30 ± ½ C and from 83% to 99% at 20 ± ½ C. Samples were removed after 7, 21, 42 and 84 days of incubation and assayed for aflatoxin, free fatty acids and peanut kernel moisture. Aflatoxin was formed in sound mature kernels at 40 C and 14 C and in broken mature kernels at 13 C, but none was formed at 41 C after 21 days or at 12 C after 84 days in 98 ± 1% RH. The limiting temperatures for aflatoxin formation in peanut kernels with intact shells were 41 C for 21 days and 16 C for 84 days of incubation. The limiting RH at 30 C for aflatoxin production in sound mature kernels was 84%, whereas in broken mature and immature kernels it was 83% and in kernels from unshelled peanuts the limiting RH was 86% for 84 days of incubation. The limiting RH at 20 C for sound and broken mature kernels was 83%, whereas it was 86% RH for immature kernels and 92% for kernels from unshelled peanuts. Free fatty acid formation was correlated with visible growth of fungi rather than with aflatoxin production. Aflatoxin formation was generally correlated with kernel moisture contents of 10% or higher.

Introduction

The significance of aflatoxin and other mycotoxins in animal feeds and agricultural commodities as a potential public health hazard is well documented (1–3). Diener et al. (4) demonstrated the presence of aflatoxin-producing strains of *Aspergillus flavus* Link ex Fr. in domestic peanuts and later in corn and other crops (5). Diener and Davis (5,6) reported the effects of temperature and time on aflatoxin production by *A. flavus* and *A. parasiticus* Speare in vitro in sterilized peanut kernels and in artificial media. Later, they determined the limiting temperatures and relative humidity for aflatoxin production in sterile (heat-treated) shelled and unshelled peanuts as well as in immature and broken mature or damaged kernels (7). Recently they reported the limiting temperatures and relative humidities for aflatoxin production in kernels of unshelled, freshly-dug peanuts of the Early Runner and Florigiant varieties (8). This paper reports the results of similar studies conducted with living, cured Early Runner peanuts stored up to 12 weeks after inoculation with *A. flavus*. A preliminary report of this research has been published (9).

TABLE I

Kernel Moisture Content and Production of Free Fatty Acids and Aflatoxin in Sound Mature Kernels Inoculated With *Aspergillus flavus* and Incubated at 99% RH With Varying Temperature

Temp., C[a]	Time, days	KMC, %	FFA, %	Aflatoxin μg/g	
				$B_1 + B_2$	$G_1 + G_2$
12	84	28	51	0	0
13	84	34	25	+[b]	+
14	21			+	+
14	42	25	7	0.6	1.4
14	84	28	23	0.4	0.5
16	21			0.6	1.4
16	42	26	51	6.1	14.3
16	84	49	27	1.7	2.0
18	21			1.4	1.6
18	42	29	46	17.0	40.0
18	84	49	43	1.7	2.0
20	21	20	29	2.8	4.2
20	42	38	30	19.3	45.8
20	84	63	29	1.7	2.0
25	21	29	52	52.4	86.4
30	21	18	51	83.3	50.0
35	21	20	56	38.0	16.0
40	21	19	58	0.1	0
41	21	27	40	0	0
Check	0	7	0.2	0	0

[a] Data at 8, 10, 12 and 15 C for 21, 42 and 84 days were similar to those presented. Data at 43, 45, 46 and 49 C for 21 days were similar to those at 41 C.
[b] + = .001–.049 μg/g aflatoxin (less than 50 ppb).

Materials and Methods

Aspergillus flavus, strain Ala-6, was used as in previous investigations (7,8). Peanuts (*Arachis hypogaea L.* 'Early Runner') of the 1966 crop obtained from the Wiregrass Substation, Headland, Ala., were shelled, cleaned and segregated into four categories: (a) sound mature kernels, (b) broken mature or damaged kernels, (c) immature kernels and (d) unshelled peanuts with visibly intact shells.

Equipment and procedures were similar to those previously reported (7) except that tray lots were increased to 1000 g of sound mature, broken mature, and immature kernels and 1400 g of unshelled peanuts. Peanuts were field-run farmers stock peanuts placed in cold storage at 2 C about 30 days after harvest. The peanuts were inoculated immediately after the trays were placed in environmental cabinets that had been previously adjusted to specific temperatures or relative humidities (RH) or both. Random samples were removed after incubation periods of 7, 21, 42 and 84 days. The occurrence of species of fungi other than *A. flavus* was noted. Determinations of kernel moisture content (KMC) volatile matter, aflatoxin, and free fatty acids (FFA) were made by methods previously reported (7,10,11).

TABLE II

Kernel Moisture Content and Production of Free Fatty Acids and Aflatoxin in Immature Kernels Inoculated With *Aspergillus flavus* and Incubated at 99% RH With Varying Temperature

Temp., C[a]	Time, days	KMC, %	FFA, %	Aflatoxin μg/g	
				$B_1 + B_2$	$G_1 + G_2$
14	84	25	42	0	0
15	21	25	4	+[b]	+
20	21	20	15	3.4	6.1
25	21	29	53	30.0	49.4
30	21	24	36	64.0	49.4
35	21	20	39	19.0	16.0
40	21	25	41	0.3	0
43	21	23	16	0	0
Check	0	7	0.6	+	+

[a] Data at 8, 10 and 12 C for 21, 42 and 84 days and 14 C for 42 days were similar to those at 14 C for 84 days. Data at 45, 46 and 49 C for 21 days were similar to those at 43 C.
[b] See Table I.

TABLE III

Kernel Moisture Content and Production of Free Fatty Acids and Aflatoxin in Damaged or Broken Mature Kernels Inoculated With *Aspergillus flavus* and Incubated at 99% RH With Varying Temperature

Temp., C[a]	Time, days	KMC, %	FFA, %	Aflatoxin μg/g	
				$B_1 + B_2$	$G_1 + G_2$
12	84	24	52	0	0
14	21			+[b]	+
14	42	21	27	0.6	1.4
14	84	22	36	0.1	0.2
16	21			0.6	0.8
16	42	23	33	3.0	7.2
16	84	25	59	0.3	0.4
18	21			2.1	2.5
18	42	28	32	4.9	11.4
18	84	57	46	0.8	1.0
20	21	15	29	0.8	1.2
20	42	28	32	4.9	11.4
20	84	44	34	1.7	2.0
25	21	24	33	22.5	37.0
30	21	17	33	86.0	74.0
35	21	17	36	6.8	12.1
40	21	19	37	0.3	0
41	21	20	26	0	0
Check	0	7	0.3	0	0

[a] Data at 8 and 10 C for 21, 42 and 84 days and at 12C for 21 and 42 days were similar to those at 12 C for 84 days. Data at 43, 45, 46 and 49 C for 21 days were similar to those at 41 C.
[b] See Table I.

Results

Relation of Temperature to Aflatoxin Production at 99% RH

Data on aflatoxin, KMC and FFA for sound mature kernels inoculated with *A. flavus* and incubated at 99% RH and several temperatures are presented in Table I. High total aflatoxin production (54–139 μg/g) occurred at temperatures of 25–35 C in 21 days. Moderate to large amounts (20–65 μg/g) were formed in 42 days at 16, 18 and 20 C followed by a noticeable drop in aflatoxin level after 84 days incubation. Minute amounts (0.1 μg/g or less) were formed in sound mature kernels at 13 and 40 C, but aflatoxin production was limited at constant temperatures of 12 C for 84 days and 41 C for 21 days. Moderate amounts of aflatoxin (9–28 μg/g) were found in seven-day samples (data not presented) incubated at 25–35 C with small amounts occurring at 20 and 40 C.

Data on aflatoxin, KMC and FFA for immature kernels incubated at 99% RH and several temperatures are presented in Table II. Large amounts of total aflatoxin formed in 21 days at 25–35 C, but only a small amount formed at 40 C and a minute amount at 15 C. Limiting temperatures for aflatoxin formation were 14 and 43 C for 84 and 21 days, respectively.

Data on aflatoxin, KMC and FFA for damaged or broken mature kernels incubated at 99% RH and several temperatures are presented in Table III. Large amounts (60–170 μg/g) of aflatoxin formed at 25 and 30 C in 21 days. Moderate amounts (10–19 μg/g) formed at 35 C in 21 days and at 16, 18 and 20 C in 42 days, but the latter was followed by a drop in 84 days. Moderate amounts (21–35 μg/g) formed at 25–35 C in seven days (data not presented). Limiting temperatures were 12 C for 84 days and 41 C for 21 days with only minute amounts being formed by *A. flavus* at 14 and 40 C in 21 days.

TABLE IV

Kernel Moisture Content and Production of Free Fatty Acids and Aflatoxin in Kernels From Intact Pods Inoculated With *Aspergillus flavus* and Incubated In-the-Shell at 99% RH With Varying Temperature

Temp., C[a]	Time, days	KMC, %	FFA, %	Aflatoxin μg/g	
				$B_1 + B_2$	$G_1 + G_2$
16	84	25	11	0	0
18	42	28	10	1.2	2.9
18	84	37	11	0.3	0.4
20	21	15	2	+[b]	+
20	42	30	6	2.4	5.7
20	84	43	5	0.3	0.4
25	21	20	10	4.5	7.4
30	21	17	9	8.0	6.1
35	21	17	14	4.5	2.4
40	21	19	36	0.5	0
41	21	26	14	0	0
Check	0	6	0.2	0	0

[a] Data at 8, 10, 12, 14 and 15 C for 21, 42 and 84 days and at 16 C for 42 days were similar to those at 16 C for 84 days. Data at 43, 45, 46 and 49 C for 21 days were similar to those at 41 C.
[b] See Table I.

TABLE V

Kernel Moisture Content and Production of Free Fatty Acids and Aflatoxin in Sound Mature Kernels Inoculated With *Aspergillus flavus* and Incubated at 30 C With Varying Relative Humidity

RH, %[a]	Time, days	KMC, %	FFA, %	Aflatoxin μg/g	
				$B_1 + B_2$	$G_1 + G_2$
83	84	9	3	0	0
84	84	9	3	+[b]	+
85	21	10	4	0.9	2
85	42	14	26	12.4	17.9
85	84	13	35	0.9	1
87	21	11	11	1.9	3.5
87	42	11	13	6.1	14.3
87	84	13	28	0.8	1
92	21	15	28	90.7	161.3
99	21	18	45	288	243
Check	0	7	0.2	0	0

[a] Data at 70%, 75%, 80%, 83% and 84% RH for 21 and 42 days, and at 80% for 84 days were similar to those at 83% RH for 84 days. Data at 86% RH for 21, 42 and 84 days were similar to those at 85% and 87% RH.
[b] See Table I.

Data on aflatoxin, KMC and FFA for peanut kernels from intact shells incubated in the pod at 99% RH and several temperatures are presented in Table IV. Moderate amounts of aflatoxin (7–14 μg/g) formed at 25–35 C in 21 days and at 20 C in 42 days. A minute amount of aflatoxin formed at 20 C in 21 days with a moderate amount (8 μg/g) developing in 42 days. This decreased to 10% of the 42 day value by the 84th day. Some aflatoxin also developed at 18 C and 42 days and decreased to smaller amounts in 84 days. No aflatoxin formed at 16 C in 84 days or at 41 C in 21 days.

Data presented in Tables I–IV on aflatoxin showed no correlation with KMC or FFA or both over the range of temperature-time combinations of these experiments.

Relation of Relative Humidity to Aflatoxin Production at 30 C

When sound mature kernels were incubated at a constant temperature of 30 C at varying RH (Table V), total aflatoxin production was large (252–531 μg/g) at 99% and 92% RH in 21 days. Incubation for 42 and 84 days showed that at decreased RH (85% and 87%) aflatoxin production peaked at 42 days and decreased to 10% of the 42 day amount by the 84th day. No aflatoxin was produced at 83% RH, and only a minute amount at 84% RH after 84 days.

Data on aflatoxin, KMC and FFA in immature kernels incubated at 30 C and varying RH are presented in Table VI. Large amounts of aflatoxin were formed at 92% and 99% RH in 21 days. Aflatoxin production was limited by 83% RH and only a minute amount was formed at 84% after 84 days.

Moderate to large amounts of aflatoxin were formed in damaged (broken mature) kernels incubated at 30 C and 92% and 99% RH for 21 days (Table VII). Only 20% of the amount of aflatoxin present in 42 day samples was noted in 84 day samples from 85% and 87% RH treatments. No aflatoxin was produced at 83% RH, but only a minute amount was found at 84% RH after 84 days.

Data on aflatoxin, KMC and FFA in the kernels of peanuts incubated in-the-shell at 30 C are presented

TABLE VI

Kernel Moisture Content and Production of Free Fatty Acids and Aflatoxin in Immature Kernels Inoculated With *Aspergillus flavus* and Incubated at 30 C With Varying Relative Humidity

RH, %[a]	Time, days	KMC, %	FFA, %	Aflatoxin μg/g	
				$B_1 + B_2$	$G_1 + G_2$
83	84	13	12	0	0
84	84	11	11	0.2	0.1
85	21	13	3	0.8	1.3
85	42	17	13	0.6	1.8
85	84	14	30	0.5	2.2
92	21	20	39	90.7	161.3
99	21	20	38	90.7	62.7
Check	0	9	0.7	0	0

[a] Data at 70%, 75%, 80%, 83% and 84% RH for 21 and 42 days and at 80% for 84 days were similar to those at 83% RH. Data at 86% RH for 21, 42 and 84 days and at 87% for 21 days were similar to those at 85% RH.

in Table VIII. A moderate amount of aflatoxin (12 μg/g) was formed at 99% RH in 21 days. Considerably less was produced by *A. flavus* at 92% RH. A minute amount formed at 87% RH in 21 days, but aflatoxin was not found in 42 and 84 day samples. No aflatoxin was found at 83% RH and only minute amounts were recorded at 84% RH after 84 days and at 85% RH after 21, 42 and 84 days incubation.

Thus, aflatoxin formed at 84% RH after 84 days incubation in sound mature kernels, damaged or broken mature kernels, immature kernels and in kernels incubated with intact shells. No aflatoxin formed at 83% RH in 84 days. In general, the higher the RH the greater was the aflatoxin production by *A. flavus* for 21 days incubation.

Growth and Sporulation by Aspergillus flavus and Other Fungi

Growth and sporulation by *A. flavus*, as visually observed in the environmental cabinets, were correlated with temperature and RH combinations at which aflatoxin was produced. Growth and sporulation by *A. flavus* were also profuse at temperatures of 41–45 C, at which no aflatoxin was formed. Other fungi grew at RH and temperatures favorable and unfavorable for the development of *A. flavus*. Most of the fungi growing at RH below 84–86% were members of the *A. glaucus* group (*A. amstelodami, A. ruber, A. chevalieri* and *A. repens*). Several fungi (*A. niger* and *A. wentii*) competed well with *A. flavus* at high RH (92–99%) and temperatures of 30–40 C, whereas *A. ochraceus, A. tamarii*, and several species of *Penicillium* were prominent at high RH and temperatures of 15–25 C.

Relation of FFA Formation to Aflatoxin Production

FFA formation was high at temperatures and RH above, below and near those limiting for aflatoxin production because of the growth of other fungi, previously noted, as well as *A. flavus* that compose the mycoflora of stored peanuts. FFA formation was correlated with aflatoxin production primarily when *A. flavus* was dominant in the mycoflora, i.e., from 25–40 C. FFA level was an index of total fungal growth, whereas aflatoxin production was an index of the growth of *A. flavus* except at temperatures above 40 C. At 41–45 C, FFA were a measure of the growth of *A. flavus* and other fungi, but no aflatoxin was produced. Also, high FFA were formed in peanuts stored below 83% RH and at temperatures below 13 C as a result of the growth of fungi other than *A. flavus*. FFA of control lots of immature kernels were notably higher (0.6–0.7%) than in lots of sound mature and damaged kernels and of kernels from unshelled peanuts (0.2–0.4%).

TABLE VII

Kernel Moisture Content and Production of Free Fatty Acids and Aflatoxin in Damaged Kernels Inoculated With *Aspergillus flavus* and Incubated at 30 C With Varying Relative Humidity

RH, %[a]	Time, days	KMC, %	FFA, %	Aflatoxin μg/g	
				$B_1 + B_2$	$G_1 + G_2$
83	84	11	23	0	0
84	84	11	27	0.1	0.4
85	21	11	23	1.3	1.6
85	42	14	30	2.6	6.2
85	84	13	36	1	1.2
87	21	10	25	6.3	5.2
87	42	13	36	4.9	11.4
87	84	14	48	1.7	2
92	21	12	28	16	12.4
99	21	15	33	32.1	24.6
Check	0	8	0.4	0	0

[a] Data at 70%, 75%, 80%, 83% and 84% RH for 21 and 42 days and at 80% for 84 days were similar to those at 83% RH for 84 days. Data at 86% RH for 21, 42 and 84 days were similar to those at 85% and 87% RH.

Relation of Relative Humidity to Aflatoxin Production at 20 C

Data on aflatoxin production in sound mature kernels incubated at 20 C and several RH are presented in Table IX. Moderate amounts of aflatoxin developed at 92% and 99% RH in 21 and 42 days, but the level decreased more than 50% by 84 days. No aflatoxin was produced at 83% RH. At 86% RH, aflatoxin formed only after 84 days incubation at the reduced temperature (20 C) of this study.

In immature kernels, aflatoxin formed in moderate amounts at 99% RH in 21 and 42 days and at 92% and 99% RH in 84 days (Table X). No aflatoxin was present at 92% RH after 21 days of incubation.

In damaged or broken mature kernels, aflatoxin developed in peanuts stored 84 days at 86% RH, but not in 21 or 42 days at this RH nor at 83% RH (Table XI). Small amounts (6 μg/g) of aflatoxin formed at 92% and 99% RH in 21 days with 21 μg/g being recorded at 92% RH for 42 days, after which it decreased to about 15–18% of that amount in 84 days of storage. The small amount of aflatoxin at 99% RH for 21 days appeared to be an anomaly.

In kernels from unshelled peanuts with intact pods, aflatoxin formed at 99% RH and 20 C for all storage periods (Table XII); none was present in samples stored at 83%, 86% and 92% RH for 21, 42 and 84 days.

Reducing the storage temperature to 20 C (Tables IX-XII) did not greatly change the limiting relative humidity for aflatoxin production in peanuts from that at 30 C, which was a more favorable temperature for growth and aflatoxin production by *A. flavus* (Tables I-VIII). Data for kernel moisture content and FFA followed patterns similar, in general, to those observed at the same RH at 30 C.

TABLE VIII

Kernel Moisture Content and Production of Free Fatty Acids and Aflatoxin in Unshelled Kernels Inoculated With *Aspergillus flavus* and Incubated at 30 C With Varying Relative Humidity

RH, %[a]	Time, days	KMC, %	FFA, %	Aflatoxin μg/g	
				$B_1 + B_2$	$G_1 + G_2$
84	42	13	0.4	0	0
84	84	9	1	+[b]	+
85	21	11	0.9	+	+
85	42	13	0.4	+	+
85	84	12	9	+	+
87	21	8	2	0.2	0.1
87	42	10	0.7	0	0
87	84	12	4	0	0
92	21	14	5	2.4	2.4
99	21	15	7	5.3	7
Check	0	6	0.3	0	0

[a] Data at 70%, 75%, 80% and 83% RH for 21, 42 and 84 days and 84% RH for 21 days were similar to those at 84% RH for 42 days. Data at 86% RH for 21, 42 and 84 days were similar to those at 85% RH.

[b] See Table I.

TABLE IX

Kernel Moisture Content and Production of Free Fatty Acids and Aflatoxin in Sound Mature Kernels Inoculated With *Aspergillus flavus* and Incubated at 20 C With Varying Relative Humidity

RH, %[a]	Time, days	KMC, %	FFA, %	Aflatoxin μg/g	
				$B_1 + B_2$	$G_1 + G_2$
86	42	9	0.6	0	0
86	84	10	25	0.2	0.8
92	21			2.4	5.7
92	42	19	37	4.5	16 6
92	84	17	36	1.9	6.8
99	21			4.9	12.8
99	42	22	53	8 6	22.5
99	84	42	32	0.9	5.1
Check	0	7	0.2	0	0

[a] Data at 83 RH for 21, 42 and 84 days and at 86% for 21 days were similar to those at 86% for 42 days.

Results and Discussion

Estimation of aflatoxin by the methods used in this investigation (10) has been reported to be accurate to about 1–4 ppb (μg/kg) aflatoxin in cottonseed meats and meals. Modifications of this technique for peanuts and peanut products (11) have been reported to detect as little as 0.3 ppb (μg/kg) of aflatoxin B_1, but our data are considered accurate to 1–2 ppb aflatoxin. Low levels of natural-occurring aflatoxin contamination in the 4 lb. experimental samples of peanuts were occasionally found in the check samples (Table II). The presence of aflatoxin in the original lot of peanuts may not be the only source of aflatoxin contamination (12). Data on aflatoxin in these experiments have been summarized as positive for .001–.049 μg/g (less than 50 ppb) and 0.1 μg/g for 50–149 ppb, and aflatoxin negative for amounts less than .001 μg/g or 1 ppb.

The limiting temperature data for living cured peanuts in this investigation closely parallel those of previous studies using sterile mature and immature peanuts (7) in that aflatoxin was formed at 14 C in sound mature kernels, but not at 12 C. Likewise, aflatoxin formed in immature kernels at 15 C, but not at 14 C. However, in damaged or broken mature kernels, some aflatoxin formed at 12 C in sterile peanuts (7), whereas in cured, living, broken mature kernels aflatoxin formed at 14 C but not at 12 C. The greatest contrast between results obtained here and those previously reported (7) was in the living kernels of unshelled peanuts that showed no aflatoxin at 16 C (even after 84 days), whereas aflatoxin formed in heat-treated unshelled peanuts at 14 C in 21 days. This difference in the limiting temperature is probably associated with the barrier or resistance, or both, of the intact shell and testa of the living kernel to penetration and colonization by *A. flavus* at high RH (99%) as previously noted by Bampton (13). Physiological activity of the living peanut at high RH and 16 C must have afforded resistance adequate to prevent invasion by the fungus, which readily colonized heat-treated kernels with intact shells under those conditions (7).

TABLE X

Kernel Moisture Content and Production of Free Fatty Acids and Aflatoxin in Immature Kernels Inoculated With *Aspergillus flavus* and Incubated at 20 C With Varying Relative Humidity

RH, %[a]	Time, days	KMC, %	FFA, %	Aflatoxin μg/g	
				$B_1 + B_2$	$G_1 + G_2$
92	21			0	0
92	42	20	22	1.2	1.6
92	84	21	34	1.6	11.4
99	21			2.4	11.4
99	42	27	27	2.6	9.4
99	84	45		3.6	7.2
Check	0	8	0.3	0	0

[a] Data at 83% and 86% RH for 21, 42 and 84 days were similar to those at 92% RH for 21 days.

TABLE XI

Kernel Moisture Content and Production of Free Fatty Acids and Aflatoxin in Damaged Kernels Inoculated With *Aspergillus flavus* and Incubated at 20 C With Varying Relative Humidity

RH, %[a]	Time, days	KMC, %	FFA, %	Aflatoxin μg/g	
				$B_1 + B_2$	$G_1 + G_2$
86	42	10	16	0	0
86	84	14	22	0.6	1.8
92	21			0.2	5.7
92	42	18	42	7	14.3
92	84	18	24	0.2	0.3
99	21			0.7	5.7
99	42	22	24	1	2.3
99	84	21	34	0.5	0.8
Check	0	8	0.4	0	0

[a] Data at 83% RH for 21, 42 and 84 days and 86% RH for 21 days were similar to those at 86% RH for 42 days.

Data confirmed previous results (5,7,8) in that the ratio of B_1 to G_1 was a function of temperature (Tables I–IV) as well as the strain of fungus (6). Ratios at 18, 20 and 25 C were about 1:2 (B_1 to G_1), whereas at 35 C the ratio was about 1:1–2:1.

Temperature data showed that aflatoxin production peaked at 42 days at temperatures of 16–20 C (Tables I, III, IV) and then dropped sharply with additional time. This was previously noted for shorter periods of time at more favorable temperatures for growth and aflatoxin production in peanuts and synthetic media (5). This decline in aflatoxin concentration after peak yield also occurred in pure culture (14). Since the decline here was greater than that previously noted (5), it was possibly a result of degradation by or competition of the other fungi of the mycoflora. This facet is worthy of further investigation.

The limiting relative humidity data determined with living cured peanuts closely paralleled that previously obtained using sterile peanuts (7) in that 85% was the lowest RH at which aflatoxin formed during a 21 day period. When the storage period was extended to 84 days, aflatoxin was formed in immature kernels and in damaged or broken mature kernels at 84% RH. However, no aflatoxin was present at 84% RH in sound mature kernels after 84 days. In kernels of unshelled peanuts, aflatoxin was present at 87% RH only at 21 days, which is similar to that reported for sterile peanuts (7) and for freshly dug peanuts (8). In the latter case, aflatoxin was present in Early Runner peanuts at 85% RH after 21 days.

FFA formation was generally correlated with growth of the fungi composing the mycoflora of stored cured peanuts and not to the growth of *A. flavus* alone.

Data in Table columns headed kernel moisture content (KMC) probably could be more realistically labeled SMC for substrate moisture content, since under conditions even slightly favorable for fungus growth seeds soon die and deteriorate as they are rapidly metabolized by *A. flavus* and other fungi. Hygroscopic moisture in dead peanut seed supporting extensive fungal growth differs greatly from that of living seed. Obviously, moisture data herein cannot be correlated with data in the literature on sound mature peanuts. Seed carbohydrates are metabolized by the growing fungus in two weeks or less; oil is then utilized for growth by the fungus with the production of large amounts of carbon dioxide and water (15). In fact, fungal mycelium on dead seed is mostly water. Thus, substrate moisture increased with time at 20 C (Tables I, III, IV) because of the continued growth of fungi in the substrate. At high RH, high KMC (substrate moistures) were generally

TABLE XII

Kernel Moisture Content and Production of Free Fatty Acids and Aflatoxin in Unshelled Kernels Inoculated With *Aspergillus flavus* and Incubated at 20 C With Varying Relative Humidity

RH, %[a]	Time, days	KMC, %	FFA, %	Aflatoxin μg/g	
				$B_1 + B_2$	$G_1 + G_2$
92	84	14	5	0	0
99	21			0.7	11.4
99	42	26	14	1.4	15.7
99	84	32	13	0.8	2.9
Check	0	4	0.2	0	0

[a] Data at 83% and 86% RH for 21, 42 and 84 days and at 92% RH for 21 and 42 days were similar to those at 92% RH for 84 days.

associated with temperatures of 16–25 C and with storage periods of 42 and 84 days. KMC of immature kernels were generally 2–4% higher than those from sound mature and damaged kernels and those kernels from unshelled peanuts stored at similar RH and temperature as previously noted with sterile peanuts (7). It is doubtful if a state of equilibrium was ever attained, since the nature of the substrate was continuously altered even though temperature and RH were precisely controlled and remained constant throughout the experiments. Another consideration is that numerous fungi besides *A. flavus* make up the normal peanut seed microflora with each fungus frequently having a different temperature optimum, a different RH (substrate moisture) threshold for growth, and a different effect on substrate moisture (16).

KMC or substrate moisture was correlated with fungal growth rather than with aflatoxin or FFA production. Although aflatoxin accumulation was largely a measure of the growth of *A. flavus* as influenced by RH, temperature and time, it was also probably reduced by the competition of other fungi in the microflora. Many of these fungi grew profusely at RH and temperatures unfavorable for *A. flavus,* e.g., the *A. glaucus* group of species visually dominated the microflora at RH of 70–80% and temperatures of 20–25 C.

The AOCS official method Ab 2–49 measures loss in moisture and also any material in the sample that is volatile from exposure to 130 ± 3 C for 3 hr. Samples of peanut kernels randomly collected for moisture determination usually were covered with a heavy mat of spores and mycelium of *A. flavus* or several other fungi, or both, after incubation for 21–84 days in controlled environments of 30 C and 80–99% RH or 20–40 C and 99% RH. With this method samples of badly deteriorated peanuts lost the moisture in fungus mycelia and dead peanuts and possibly also volatile alcohols, acids, aldehydes and ketones produced by fungi metabolizing the organic substrate. However, in these studies we made no attempt to determine or measure individual volatile compounds.

Several inversions in data with respect to moisture content can be noted. In Table I after 21 days, the KMC was determined as 19.6% at 20 C, 28.8% at 25 C, and 18% for 30 C. FFA and aflatoxin levels at 25 and 30 C were almost identical. The 21 day data for KMC at 20, 25 and 30 C in Tables II, III and IV reveal a similar pattern in all cases. However, FFA and aflatoxin levels at 25 and 30 C were variable. When 21 day data at 35 C was included in these comparisons, KMC were similar or slightly lower than those at 30 C, FFA levels were higher than at 30 C in all cases, and aflatoxin lower in all cases. At this time, we have no explanation for this inversion in KMC at 25 C. However, we would point out that there were probably more species of fungi in the peanut microflora with growth optimums near 25 C than at 20, 30 or 35 C (16). *A. flavus* grew profusely at 25 C, although competitively it was more dominant in our experiments at 30 and 35 C, since its optimum is 35–37 C. Interpretation or correlation, or both, of substrate moistures (KMC) with temperature, aflatoxin and FFA production, and other factors or with KMC in living seed in other investigations is difficult and of doubtful validity for these foregoing reasons.

In experiments at 20 C, aflatoxin formed in sound mature and broken mature kernels at 86% RH only after 84 days. In immature kernels no aflatoxin was found below 92% RH. Aflatoxin formed only at the highest RH (99%) in kernels from unshelled peanuts with intact shells. Thus reducing the temperature had a notable effect on the limiting RH for aflatoxin formation in kernels of unshelled peanuts as compared to sound mature kernels.

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