Destruction of Aflatoxins in Peanuts during Dry and Oil Roasting

Louise S. Lee, Alva F. Cucullu, A. O. Franz, Jr., and W. A. Pons, Jr.

Blanched whole peanut kernels inoculated with a toxigenic strain of *Aspergillus flavus* and subjected to continuous shaking during incubation for 40, 46, 64, and 72 hours produced peanuts virtually clear of visible mold and containing four graded levels of total aflatoxins—130, 260, 2560, and 6300 p.p.b. (μg. per kg.). Although individual kernels differed in aflatoxin content, replicate assays of 50-gram aliquots of contaminated whole kernels were subjected to oil and dry roasting under five time-temperature conditions simulating those used in industry to effect a normal roast for high quality peanuts. Average reduction in aflatoxin content ranged from 45 to 83%, depending on roasting conditions and the level of toxins in the raw kernels. There was an over-all reduction of 65% in B_1 and 62% in G_1 for oil roasting, and 69% in B_1 and 67% in G_1 for dry roasting. The degree of reduction in aflatoxin content was greatest at the highest aflatoxin contamination levels, for both oil- and dry-roasted peanuts.

In the last decade the problem of mold contamination in peanuts and other foodstuffs has received considerable attention. Under unfavorable conditions of harvesting and storage, peanuts and other agricultural products may be invaded by toxigenic strains of the common mold *Aspergillus flavus* and this could result in the elaboration of a highly toxic class of mycotoxins, commonly referred to as aflatoxins (Wogan, 1965). The chemical and biological effects of the four principal members of this group of mycotoxins, aflatoxins B_1, B_2, G_1, and G_2, have been reviewed by Wogan (1966). Since 70% of the peanut crop is utilized in roasted products (U. S. D. A. Crop Reporting Board, 1967), the effect of roasting on peanut kernels containing aflatoxins is of immediate interest.

Recent studies by Lee *et al.* (1968) suggest that dry roasting of selected naturally mold-damaged peanut kernels leads to an apparent loss or destruction of as much as 80% of the aflatoxin B_1 content, as determined by physiocochemical analysis. These studies were performed on high aflatoxin content kernels, hand-segregated from a large lot of peanuts, since previous data by Cucullu *et al.* (1966) showed that aflatoxin contamination in mold-damaged lots of peanuts is nonuniformly distributed in only a relatively small number of individual kernels. Because of the extreme variation in the aflatoxin content of these selected kernels, 250 to 750,000 p.p.b. of aflatoxin B_1, the roasting studies were performed on individual half kernels, while the other half of each kernel was used to determine the aflatoxin level prior to roasting.

To evaluate the effect of roasting on larger samples of intact kernels, it was necessary to prepare samples of peanuts with more uniform and graded levels of aflatoxin contamination. Techniques were developed in the present study to inoculate and incubate peanuts under constant agitation to provide four graded comparatively low levels of total aflatoxin contamination ranging from 130 to 6000 p.p.b. (μg. per kg.). The samples free of visible mold spores resembled naturally contaminated peanuts and were convenient to handle. Fifty-gram portions of peanuts from each contamination level were then subjected to oil and dry roasting under conditions simulating conventional peanut roasting.

Southern Regional Research Laboratory, New Orleans, La. 70119

EXPERIMENTAL

Preparation of Raw Peanut Kernels with Graded Levels of Aflatoxins. Approximately 4 kg. of Spanish peanuts containing 5.75% moisture were hand-blanched, and all splits discarded. For each of the four graded levels of aflatoxin contamination, 600 grams of kernels were divided into eight 75-gram portions and placed in 1-liter Erlenmeyer flasks. Fifteen milliliters of sterile tap water were added to each flask, and the flasks were stoppered and agitated on a standard Burrell Wrist Action shaker at low speed for 6 hours until all of the added moisture was uniformly absorbed to a final moisture content of 21%. Three milliliters of a suspension of spores of a toxigenic strain of *A. flavus* group (NRRL 3386) known to produce low levels of aflatoxins B_1 and G_1 were added to each sample by pipet. The flasks were capped with porous paper to allow free access to atmospheric oxygen, and agitation was resumed for 40, 46, 64, and 72 hours to produce the four contamination levels recorded in Table I.

After the specified time, each 600-gram sample of peanuts from each contamination level was thoroughly mixed and partially dried by moving air from a fan for 6 hours, followed by a 24-hour final drying period over a silica gel desiccant in a vacuum oven at room temperature. An additional 600-gram lot of peanuts was used to establish the uniformity in aflatoxin content of 50-gram subsamples. It was inoculated with another toxigenic strain of *A. flavus* group (English strain 3734/10) and agitated for 80 hours.

Moisture determinations were made on the original samples and on the final dried product of each level of contaminated peanuts produced. Uniformity of moisture uptake and drying techniques was established by a series of micro- and macro moisture determinations on another sample of the same peanuts.

ROASTING PROCEDURES. Oil roasting studies were conducted in an 1150-watt thermostatically controlled (± 1° F.) deep fat fryer equipped with a mechanical stirrer. The fryer was filled with a commercial peanut oil and raised to the desired temperature, and 50-gram samples of contaminated peanuts contained in a perforated aluminum container were immersed in the oil,

Table I. Aflatoxin Content (P. P. B.) of Inoculated Raw Peanuts Incubated on Shaker at 25° C.

Level	Incubation Time, Hr.	50-G. Sample		Individual Kernels			
				Range		Average	
		B_1	G_1	B_1	G_1	B_1	G_1
A	72	2200	4100	800–5000[a]	1100–8700	2100	3700
B	64	960	1600	N.D.–3800[b]	N.D.–9400	680	1200
C	46	90	150	N.D.–300[c]	N.D.–500	50	80
D	40	50	80	N.D.–260[a]	N.D.–520	32	50

[a] 24 kernels assayed. [b] 68 kernels assayed. [c] 30 kernels assayed.
N. D. None detectable at sensitivity limit of micromethod, 25 p.p.b.

Table II. Per Cent Reduction of Aflatoxins in Inoculated Peanuts after Oil and Dry Roasting

Roasting Conditions			Level A[a] Reduction		Level B[b] Reduction		Level C[c] Reduction		Level D[d] Reduction	
Time, min.	Temp.		B_1	G_1	B_1	G_1	B_1	G_1	B_1	G_1
	±1° F.				OIL ROASTING					
7	325		83	76	76	68	60	63	78	63
6	330		73	63	70	74	43	37	62	70
5	335		75	66	66	68	51	55	44	33
4	340		78	78	72	72	43	50	48	36
3	345		78	80	71	74	62	70	56	35
		Mean	77	73	71	71	52	55	58	47
	±5°F.				DRY ROASTING					
30	250		84	85	79	76	73	76	78	63
18	275		79	80	78	79	46	59	62	70
10	300		61	61	78	81	46	48	44	33
6	350		73	78	78	83	51	46	48	35
5	400		96	98	92	94	69	64	56	24
		Mean	79	80	81	83	57	59	58	45

[a] 2200 p.p.b. B_1; 4100 p.p.b. G_1 before roasting. [b] 960 p.p.b. B_1; 1600 p.p.b. G_1 before roasting. [c] 90 p.p.b. B_1; 150 p.p.b. G_1 before roasting. [d] 50 p.p.b. B_1; 80 p.p.b. G_1 before roasting.

and roasted under the time and temperature conditions outlined in Table II. These conditions were selected as typical of commercial practice, and on 50-gram samples of control noninoculated peanuts produced a normal and acceptable roast as judged by appearance and taste.

Dry roasting studies were conducted with an electrically heated and thermostatically controlled (± 5° F.) rotisserie fitted with a stainless steel sample drum 10 5/16 inches long with a diameter of 1 11/16 inches and 3/16-inch perforations. After equilibration of the system to the desired temperature, 50-gram samples of contaminated peanuts were introduced and tumbled freely. The five different times and temperatures used (Table II) were selected as representative of commercial practice and produced normal roasts on noninoculated control 50-gram samples.

AFLATOXIN DETERMINATIONS. Aflatoxins in both unroasted and roasted 50-gram samples of contaminated peanuts were determined with the fluorodensitometric procedure of Pons *et al.* (1968). This technique utilizes a densitometer equipped for fluorescence measurements to estimate aflatoxins separated by thin-layer chromatography on silica gel–coated plates, and affords a measurement precision of ± 5%. Aflatoxins in individual peanut kernels were estimated by the micromethod of Cucullu *et al.* (1966).

RESULTS AND DISCUSSION

The adaptation of the wrist action shaker-incubation to the production of graded aflatoxin contamination levels in whole peanuts was fairly successful, in that the inoculum was evenly distributed. Under these conditions of agitation-incubation, the kernels swelled appreciably during the incubation period, but returned to a normal kernel appearance and moisture content after vacuum drying. Moreover, the contaminated kernels were free of visible mold spores, and confirmed the observations of Shotwell *et al.* (1966), who noted the absence of sporulation in shaken cultures of *A. flavus* on rice. Six hundred grams at each contamination level (A, B, C, and D in Table I) was the maximum amount of peanuts which could be produced at one time with the equipment used and the maximum amount that could be dried quickly so that there would be no further mold growth.

Even under these laboratory conditions, complete homogeneity was not attained. Table I indicates a variation in the aflatoxin content of individual kernels from each contamination level. However, the average of the values for 24 to 68 individual kernels within a given level was in reasonably good agreement with the assay on a 50-gram sample. This agreement was best at the highest level (A, Table I), where all of the individual kernels assayed contained aflatoxins, and poorer at the lower levels (C and D, Table I). However, individual kernels at levels C and D, designated as nondetectable (N.D.) in Table I, could have up to 25 p.p.b. of aflatoxins, because on whole peanut kernels this level is the sensitivity limit of the micro aflatoxin procedure of Cucullu *et al.* (1966).

The range of aflatoxin content in the individual kernels was not a function of nonuniform moisture uptake prior to inoculation and incubation. Results

outlined in Table III indicate a fairly uniform moisture content in either a 10-gram sample or six samples representing two individual kernels from a lot of 75 grams of peanuts equilibrated with 15 ml. of water for 6 hours prior to inoculation. There was also a uniform moisture level in the individual kernels after air and vacuum drying.

Repetitive assays on 50-gram samples of the peanuts incubated for 80 hours indicated a fair degree of uniformity in aflatoxin content. The average of six analyses was 5400 p.p.b. B_1 and 7000 p.p.b. G_1, with a range of 4000 to 6200 p.p.b. of B_1 and 5700 to 7000 p.p.b. G_1. While these data and those shown in Table I do not suggest absolute uniformity in the level of aflatoxin contamination of laboratory-inoculated and incubated peanut kernels, they are considerably more uniform than lots of naturally contaminated peanuts where aflatoxin contamination is usually limited to a small portion (less than 1%) of the kernels (Cucullu *et al.*, 1966).

AFLATOXIN REDUCTION DURING ROASTING. Average reductions in the contents of aflatoxins B_1 and G_1 during oil and dry roasting of contaminated peanuts ranged from about 45 to 83% (Table II). The averages of all reductions for all four contamination levels were 64.5% of B_1 and 61.5% of G_1 for oil roasting, and 68.8% of B_1 and 66.8% of G_1 for dry roasting (Table II). Thus a similar degree of reduction was produced by either oil or dry roasting, and no appreciable difference in the reduction of B_1 or G_1.

For each type of roasting a consistent pattern can be noted: The percentage reduction of both aflatoxins was highest at the higher aflatoxin contamination levels (A and B, Table II). Since the highest contamination levels were those in which the mold grew for 72 hours, it is logical that these also resulted in the maximum degradation of the peanut substrate by the mold. Probably the presence of increased amounts of substrate degradation products, far in excess of the aflatoxin content, afforded more active materials for chemical reaction with aflatoxins during roasting. Also, the average reduction in aflatoxin B_1 for levels A and B (2200 and 960 p.p.b.) was 71 to 81%, similar to previous data reported by Lee *et al.* (1968). In their study on individual naturally contaminated kernels, ranging from 1500 to 100,000 p.p.b., there was a reduction of 70% in aflatoxin B_1 content after dry roasting.

In oil roasting, between the 7 minutes at 325° F. and 3 minutes at 345° F., there was no apparent trend in the degree of aflatoxin reduction within any given contamination level. This is probably due to the moderate range in both time and temperature. Conversely, in the case of dry roasting (Table II) there was considerable variation in the degree of reduction, within a given contamination level, over the extremes of treatment ranging from 5 minutes at 400° F. to 30 minutes at 250° F. Although almost complete reduction in aflatoxin content, 92 to 98%, was observed for levels A and B roasted for 5 minutes at 400° F. (Table II), these peanuts were severely scorched and would not be considered an acceptable roast. The wider variation in aflatoxin reduction during dry roasting within a given contamination level, as compared to oil roasting (Table II), is probably due to difficulty in maintaining experimental temperature in the rotisserie during the dry roasting experiments.

Table III. Moisture Content of Peanut Kernels

Samples for Moisture Detn.	Original Kernel, %	Kernels after Water Uptake for 6 Hours, %	Kernels after Air and Vacuum Drying, %
Whole peanuts[a]	5.75	20.32	5.05
Individual peanut kernels[b]	5.47	21.68	5.58
	5.49	19.31	5.63
	5.73	19.54	5.28
	5.71	19.37	5.08
	6.25	21.82	5.32
	6.08	21.62	5.52
Av.	5.79	20.61	5.40

[a] 10 gram sample dried 3 hr. at 130° C.
[b] Two kernels, ca. 1 gram, dried 3 hr. at 130° C.

In both oil and dry roasting studies of contaminated kernels, the presence of a nonaflatoxin greenish fluorescent artifact at the R_f of aflatoxin G_2 was observed on thin-layer chromatograms of purified extracts from levels C and D. The greenish fluorescent artifact was more intense in dry-roasted than in oil-roasted nuts. It was also observed in concentrated extracts of oil- and dry-roasted control high quality peanuts devoid of aflatoxins.

During longer incubation periods metabolites other than aflatoxins were produced along with free fatty acids from the fungal breakdown of the peanut glycerides. These constituents probably caused the darkening of the higher aflatoxin level peanuts as compared with the aflatoxin free controls. This suggests that photoelectric sorting of roasted peanuts, after roasting, may afford an increased assurance of removing the limited number of highly contaminated kernels likely to be present in a lot of normal peanuts. This same observation was made by Lee *et al.* (1968) during dry roasting of individual naturally contaminated peanut kernels.

LITERATURE CITED

Cucullu, A. F., Lee, L. S., Mayne, R. Y., Goldblatt, L. A., *J. Am. Oil Chemists' Soc.* **43,** 89 (1966).

Lee, L. S., Cucullu, A. F., Goldblatt, L. A., *J. Food Technol.* **22,** 1131 (1968).

Pons, W. A., Jr., Cucullu, A. F., Franz, A. O., Jr., Goldblatt, L. A., *J. Am. Oil Chemists' Soc.* **45,** 694 (1968).

Shotwell, O. L., Hesseltine, C. W., Stubblefield, R. D., Sorenson, W. G., *Appl. Microbiol.* **14,** 425 (1966).

U.S.D.A. Crop Reporting Board, Stat. Report. Service, **Gr Lg 11-2-1(67)** (1967).

Wogan, G. N., *Bacteriol. Rev.* **30,** 460 (1966).

Wogan, G. N., "Mycotoxins in Foodstuffs," pp. 1–5. M.I.T. Press, Cambridge, Mass., 1965.

Received for review October 1, 1968. Accepted December 18, 1968. Division of Agricultural and Food Chemistry, 156th Meeting, ACS, Atlantic City, N. J., September 1968. The Southern Regional Research Laboratory is one of the laboratories of the Southern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture. Mention of firm names or trade products does not constitute an endorsement by the USDA over other firms or similar products not mentioned.