Effect of Storage upon Aflatoxin Levels in Peanut Materials[1]

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ABSTRACT

The effect of storage upon aflatoxin levels in naturally contaminated peanut materials has been studied. The materials were selected to have levels in excess of those found in the trade to emphasize the possible loss during storage. The stimuli for this effort were: (A) a late 1969 report of the possible regeneration of aflatoxins in a commercial meal, and (B) the general lack of information on the long term stability of aflatoxins in commercial peanut products. Samples of deoiled peanut meal, natural peanut meal, raw peanut butter, and roasted peanut butter were held at 73 F and 50% relative humidity exposed to air for 2 years. Three of the four samples were identical to those used in the first International Aflatoxin Check Series. The samples were withdrawn from storage at 0, 6, 12, and 24 months and analyzed in comparison to materials retained at 0 F. Analysis was by the Contaminants Branch procedure (Association of Official Analytical Chemists Method 26.015-020) with the six participating Laboratories (four U.S., one Canadian, and one Danish) using visual and densitometric measurement. A single mixed aflatoxin standard was used throughout the program via retention at 0 F. No statistically significant changes in aflatoxin levels were noted in any of the commodities as a function of either time or temperature of storage throughout the 2 years of the study.

INTRODUCTION

Aflatoxins are the most important mycotoxins, or toxic compounds associated with mold contamination of foods and food raw materials, now known to man. They are the most toxic of the mycotoxins and are proven liver carcinogens in most experimental animals tested. Their prominence dates back to 1960 when the deaths of over 100,000 turkey poults in England were traced to aflatoxin contaminated peanut meal imported from Brazil. Aflatoxins may be produced by *Aspergillus flavus* and *Aspergillus parasiticus*. Their natural occurrence in foods has been demonstrated for peanuts, cottonseed, various tree nuts, and corn and other grains.

The control of aflatoxins in peanuts and peanut products has received considerable attention from technical personnel in many disciplines, including representatives from industry and regulatory agencies in the U.S. Comparatively little effort has been expended, however, to determine what the chemical stabilities of the aflatoxins are in commercial materials. In late 1969, there was a report of aflatoxin regeneration in commercial peanut meal. This report stimulated the only published study on aflatoxin stability, by Waltking (1), and this present effort. Waltking stored raw and roasted pilot plant prepared peanut butters for six months at room temperature (75 F) and reported a significant decrease for the raw butter but not the roasted product. Waltking also listed data on experimental and foreign butters stored 18 and 54 months, respectively, in which significant decreases were noted. Waltking's study involved a single laboratory, his own; not all of the samples were replicated; and he reported no data on commercial butters between the initial and final values. It seemed wise to expand on this effort using more participating laboratories on additional materials and for longer periods of time.

MATERIALS

Four materials were selected for study, commercial peanut butter, pilot plant prepared raw peanut butter, commercial peanut meal, and laboratory deoiled commercial peanut meal. The first three materials were identical to those submitted in 1971 to 150 laboratories throughout the world as part of the initial International Aflatoxin Check Sample Series (2). The levels of aflatoxins in the commercial materials were all higher than what are found in the trade.

PREPARATION OF MATERIALS

An important requirement to this study was to ensure sample-to-sample homogeneity in preparing the materials.

Commercial peanut butter is a homogeneous product. Nonetheless, additional mixing was performed by placing the plant plasticized product in a 50 lb Hobart mixer, heating to ca. 160 F, and then mixing at low speed for 15 min. Slightly over 100 g material was placed in 4 oz glass jars. The filled jars were heated to ca. 160 F, capped, and the contents vigorously shaken. The jars then were placed at 40 F to firm the butter.

To prepare the raw peanut butter, a laboratory blend of 1 lb contaminated peanuts or pickouts was added to 10 lb aflatoxin-free nuts and then put through a Quaker mill followed by blending in a small Hobart mixer for 1 hr. One lb of this product was added to 90 lb of uncontaminated nuts at the stage of the final grind in a Bauer mill with the mill set to give the texture of commercial peanut butter. The material was blended in a jacketed kettle at 150 F with a hydrogenated fat stabilizer for 2 hr. It then was votated and packaged in 4 oz glass jars.

For commercial meal, 1 lb of a highly contaminated commercial meal was mixed with 66 lb of a lightly contaminated commercial meal and the mixture passed through a Bauer mill at close setting. The blend then was mixed for 1 hr in a 50 qt Hobart planetary mixer with a flat blade at low speed. The material used for this study was placed on a polyethylene sheet and mixed further by continuously lifting, in order, the four corners of the sheet for a period of 10 min. At least 25 g was taken from each quadrant to give at least 100 g sample in 8 oz jars.

To make the deoiled meal, contaminated commercial meal was deoiled by extracting with hexane for 2 min in a high speed blender. The resultant slurry was centrifuged for 10 min at ca. 1800 rpm, decanted, and then transferred to a no. 10 can and dried 2 days on a steam bath. The dried meal then was sieved through an 8 mesh screen and mixed

[1]Presented at the AOCS Spring Meeting, Mexico City, April 1974.

TABLE I

Aflatoxins in Contaminated[a] Peanut Materials Stored at 0 and 73 F, μg Aflatoxin/Kg Sample[b]

Storage conditions		Roasted peanut butter					Natural peanut meal	Deoiled peanut meal	Raw peanut butter
Months	Temperature, F	B_1	B_2	G_1	G_2	Total	Total	Total	Total
0	---	29.1 ± 6.1[c] (6)	6.7 ± 2.9[c] (6)	12.6 ± 6.9[c] (6)	3.5 ± 2.3[c] (6)	51.8 ± 16.1[c] (6)	149.6 ± 38.1[c] (5)	53.3 ± 12.3[c] (6)	9.0 ± 2.8[c] (6)
6	0	26.7 ± 3.7 (6)	7.0 ± 2.9 (6)	15.0 ± 5.2 (6)	5.1 ± 2.8 (6)	50.2 ± 7.6 (6)	140.5 ± 40.6 (6)	58.2 ± 17.9 (6)	---
6	73	28.1 ± 9.3 (5)	7.9 ± 3.2 (6)	13.9 ± 4.7 (6)	5.0 ± 2.3 (6)	50.1 ± 12.4 (5)	141.2 ± 47.4 (6)	53.2 ± 24.2 (6)	6.9 ± 5.1 (6)
12	0	27.5 ± 5.9 (7)[d]	8.6 ± 3.1 (7)	16.3 ± 11.6 (7)	6.0 ± 3.0 (7)	55.6 ± 18.6 (7)	119.7 ± 61.4 (7)	46.2 ± 19.2 (5)	---
12	73	22.2 ± 8.7 (6)	6.8 ± 3.0 (6)	9.7 ± 6.9 (6)	3.9 ± 1.4 (6)	42.2 ± 18.5 (6)	114.9 ± 64.6 (7)	46.7 ± 24.1 (7)	6.5 ± 4.7 (7)
24	0	29.7 ± 6.7 (7)	7.4 ± 3.9 (7)	14.9 ± 7.6 (7)	4.3 ± 1.7 (7)	57.8 ± 16.1 (7)	142.8 ± 37.4 (6)	48.3 ± 16.0 (7)	---
24	73	23.3 ± 6.1 (7)	6.0 ± 4.2 (7)	11.0 ± 9.3 (7)	3.0 ± 1.9 (7)	44.0 ± 18.8 (7)	131.5 ± 22.8 (6)	59.0 ± 20.9 (6)	7.1 ± 2.3 (6)
Initial data from International Check Series		26.9 ± 13.9 (45)	6.3 ± 3.1 (41)	18.1 ± 13.7 (43)	4.8 ± 4.1 (40)	57.0 ± 29.6 (39)	136.8 ± 72.2 (33)	---	9.6 ± 7.6 (38)

[a]Materials selected with atypically high values to accentuate possible losses.

[b]Values from visual and densitometric measurements averaged for the statistical analysis. Numbers in parentheses = sample size after elimination of outliers.

[c]± Figures are standard deviations.

[d]A seventh set of data reflects the additional values received from one laboratory who also ran the cellulose column cleanup.

in a twin shell mixer for 2 hr. It was mixed further on a polyethylene sheet as above and then placed in 8 oz glass jars with ca. 80 g material/jar.

MECHANICS OF THE STUDY

Six laboratories, (The Procter & Gamble Co.; Southern Regional Research Center, ARS, USDA; Food and Drug Administration; Canadian Health Protection Branch; Royal Veterinary and Agricultural University, Denmark; and Best Foods Research Center) well qualified to conduct aflatoxin assays, agreed to participate in the study. They were asked to use the CB method (3) using visual and densitometric measurement as was reasonably possible. Since the intent of this study was to determine most accurately the actual levels of aflatoxins present, the participants were permitted their choice of solvent systems, absorbents, etc., to ensure optimum quantitation.

The study was started in September 1971 with shipment of samples of all four materials for analysis. This represented the 0 months time.

The retained samples were stored at either 0 or 73 F, 50% relative humidity. The 0 F capped samples represented the controls, or samples wherein no significant changes in levels of aflatoxins were expected. There was an insufficient supply of raw butter to permit storage at 0 F. The 73 F, 50% relative humidity samples were the experimental or test samples. They were stored uncapped but protected by cheesecloth to permit ready exchange of air.

Samples were withdrawn from storage at 6, 12, and 24 month intervals. Shipments packed in dry ice were made directly to the participants via air freight. The laboratories were asked to analyze promptly, but, if unable to, they were requested to store the samples at 0 F while awaiting analysis.

A key aspect of the study was the steps taken to ensure standards of identical quality. There were three. First and most importantly, a supply of vials of standards for the entire program was obtained from the Southern Regional Research Center, ARS, USDA. These are pure crystalline aflatoxins dissolved in 98:2 v/v of benzene/acetonitrite. They were held at 0 F prior to distribution. The particular lot number was SA 14-4. It assayed 99.5% pure based upon molar absorptivity. The concentration of the standard as measured by UV was 12.96 μg/ml as compared to the actual amounts totaling 13.01 μg/ml. Secondly, the participating laboratories were asked to check, each time samples were run, the UV absorbance at 355 mu using a 1 cm cell and calculate the molar absorptivity. This enabled the laboratories to dilute to the proper concentration. As additional checks on the condition of the standards, the participants calculated the total aflatoxins present and closely observed the absence of extraneous spots on the thin layer chromatographic (TLC) plates. Thirdly, standard SA 14-4 was checked at the 24 month interval against the then current, SA 14-8, standard being supplied by the Southern Regional Research Center. They were found to be essentially identical.

RESULTS AND DISCUSSION

Statistical analyses of the data from the study are given in Table I. The results on the roasted butter are presented in greater detail to show the type of information obtained and the variability in aflatoxin analyses using TLC.

There were no significant differences encountered in the data as analyzed by the least significant differences approach with a 5% risk in the level of any aflatoxins or the total aflatoxins as a function of either time of storage or temperature of storage. In this study, therefore, aflatoxins were found to be stable at either 0 or 73 F storage in peanut materials for as long as 2 years. M. Whitten in a private communication has indicated stability of aflatoxins in cottonseed meal for as long as 4-5 years. No explanation is known for the different findings of this work and the Waltking (1) report. A contributing factor, however, on the Waltking peanut butters toward a decrease is that the samples taken at the two time periods were from the same container. Hence, at the initial or zero time sampling, the remaining material was disturbed with considerably more surface exposed to oxidation. On the other hand, the average shelf-life of peanut butter is less than 1 year, so that not much decrease can be anticipated from a market-consumer standpoint.

The laboratories were invited to run both densitometric and visual measurements of the TLC plates. The purposes were twofold: (A) to obtain additional data for the statistical analysis and (B) to gain an additional comparison of the two means of measurement. In three instances, statistical differences were seen, all with the visual values being higher than densitometry. On only one total, for roasted butter, was this the case. Higher values from visual measurement are attributed to analyst compensation for interfering fluorescers.

It is concluded that: (A) aflatoxins are, for all practical purposes, stable in peanut commodities when stored at either 0 or 73 F exposed to air; (B) mixed aflatoxin standards prepared from pure crystalline individual aflatoxins dissolved in benzene/acetonitrite may be kept at 0 F without deterioration; and (C) the densitometric and visual measurements of the TLC plates are essentially equivalent in the readings obtained.

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