Relationship Between *Aspergillus flavus* Growth Fat Acidity, and Aflatoxin Content in Peanuts[1]

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Abstract

The influence of fungal growth, under standardized conditions, on fat acidity in large-seeded Virginia-type peanuts inoculated with *Aspergillus flavus* and relationships between fat acidity and aflatoxin, a toxic metabolite produced by *A. flavus* were studied. Fat acidity increased quadratically and was highly correlated with visible fungal growth. A lag in aflatoxin production in relation to fat acidity increase was noted; fat acidity reached 60 mg KOH per 100 g kernels before aflatoxin became detectable. This relationship suggests that a rapid method of determining fat acidity might be used to screen peanut samples for the possible presence of aflatoxin. A rapid method of determining fat acidity is cited and compared with the official A.O.A.C. method.

Introduction

DAMAGE IN PEANUT kernels often originates from either a pathological activity, such as fungal growth, or from some physiological disorder (1). Pathological damage is an important factor in lowering the quality of grain and oilseeds crops. In Federal and Federal-State inspection of these commodities the degree of pathological damage is estimated visually. The discovery of the toxic fungal metabolite, aflatoxin, has brought about a new awareness that visual judgment for the amount of pathological damage in a sample is insufficient and that a more objective method is needed for use during marketing of farmers' stock peanuts.

The fat acidity test was developed (2,3) as an objective index of pathological damage in grains. Fat acidity is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids from 100 g of seeds calculated on a moisture-free basis. Baker et al. (4), developed a rapid method for the determination of fat acidity in grains and showed a relationship between grain deterioration due to fungal damage and fat acidity. Recently Bains and co-workers (5) adapted a colorimetric procedure, developed by Baker (6), to the estimation of fat acidity in peanuts and peanut meal but did not relate their findings to fungal damage. Eldridge et al. (7), studied aflatoxin content and fatty-acid composition of peanuts inoculated with *Aspergillus flavus*. They reported aflatoxin contents up to 100 mg/kg kernels and that palmitic, stearic, oleic, linoleic, and arachidic acids increased with incubation time.

This paper reports the application of the rapid method developed by Baker et al. (4), to the determination of fat acidity in peanut kernels. Investigations were made of the influence of *Aspergillus flavus* growth, under standardized conditions, on fat acidity in peanuts and possible relationships between fat acidity and aflatoxin, a toxic metabolite produced by *A. flavus*.

Experimental

Growth Conditions

One hundred grams of air-dried, large-seeded Virginia-type (Var. NC2) peanut kernels with a 5% moisture content (wet basis) and 26.7 ml distilled water, calculated to yield 25% moisture content (wet basis), were placed in glass jars. The kernels were maintained in the containers at 4C for 24 hr and were rotated periodically to equalize moisture distribution. Each sample was then inoculated with 5 ml of a spore suspension, containing 6–7 million spores per milliliter, from a toxin-producing strain of *A. flavus* (NRRL 3000) obtained from C. W. Hesseltine, NURRD, USDA, Peoria, Illinois. The glass jars were covered with two layers of cheesecloth to prevent contamination. The samples were maintained at constant conditions of 32 ±1C and 90 ±2% relative humidity in a thermostatically controlled room throughout the experiment. The relative humidity was determined using a direct reading hygrometer and checked daily. Individual samples were withdrawn at prespecified time intervals, ranging from 6 to 12 hr in length. Fungal growth in each sample was arrested by drying at 82C for 12 hr. After drying, total fungal growth on the samples was scored visually on a scale from 0 to 4 against a set of standard samples. In samples with a fungal score of 4 approximately 10% of the kernels showed visible fungal growth; all samples scored above 4 were discarded as damaged beyond market acceptance. This study was repeated three times and a total of 67 samples were acceptable.

Analyses

Fat acidity was determined by the official A.O.A.C. method (8) and by the rapid method as described by Baker et al. (4).

Aflatoxin was determined by the method of Robertson et al. (9).

Results and Discussion

Comparison of Methods

Fat acidity of 12 individual samples of peanut

TABLE I
Fat Acidity of Peanut Kernels by the Rapid and Official Method

Sample	Fat acidity (mg KOH per 100 g Kernels[a])	
	A.O.A.C.	Rapid
1	29	22
2	18	25
3	25	30
4	20	21
5	18	24
6	21	18
7	17	18
8	17	17
9	21	19
10	18	17
11	26	24
12	25	29
Mean	21.25	22.00

[a] Average of three determinations.
LSD.05 (Mean for A.O.A.C. vs. Rapid) = 3.609.

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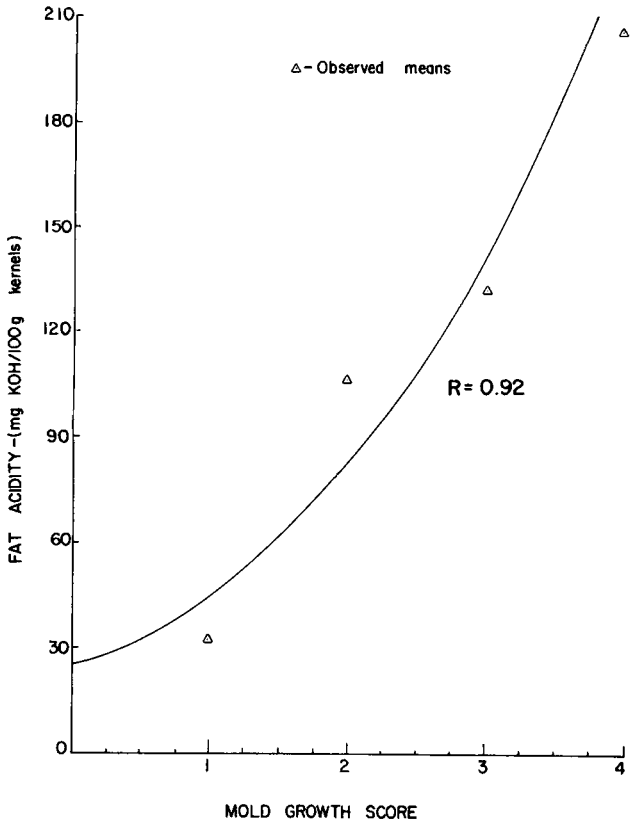

FIG. 1. Relationship between fat acidity and mold growth score of inoculated peanut kernels.

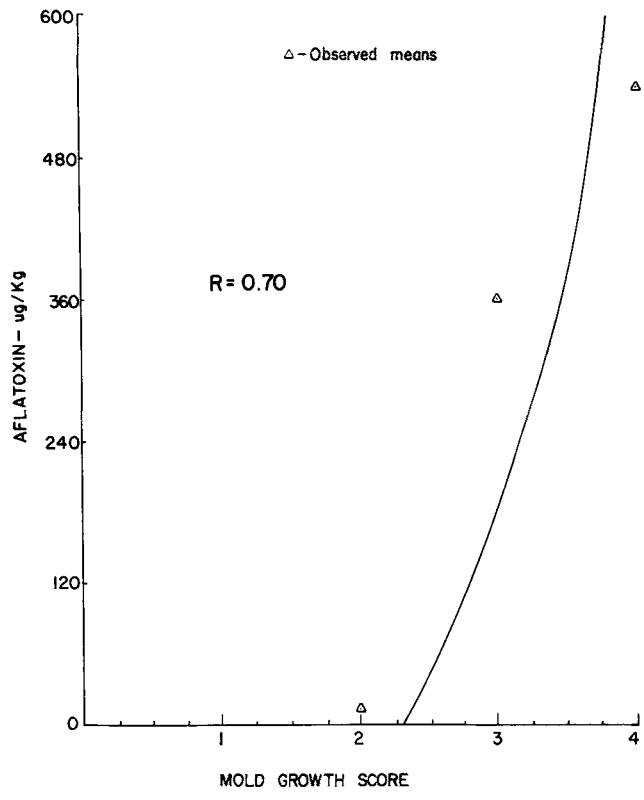

FIG. 2. Relationship between aflatoxin and mold growth score of inoculated peanut kernels.

kernels was determined by the rapid method of Baker et al. (4), and by the official A.O.A.C. method (8). By both methods the samples ranged in fat acidity values from 17 to 29 mg KOH per 100 g kernels (Table I). The mean difference was 0.75 and the standard deviation of the mean difference was 1.7, indicating that the rapid method was satisfactory for determining fat acidity in peanut kernels. Analysis of one sample takes approximately 10 min by the rapid method as compared to 16.5 hr by the official A.O.A.C. method.

Relationship of Fungal Growth, Fat Acidity and Aflatoxin

Uninoculated, large-seeded Virginia-type (Var. NC2) peanuts were found to have a fat acidity value of about 22 when cured under optimum conditions as recommended by Beasley and Dickens (10). With the initiation of mold growth on inoculated peanuts (mold growth score of 1) a detectable change in fat acidity was noted (Fig. 1). This increase in fat acidity was highly correlated with the amount of visible mold growth. The correlation value was obtained using a quadratic equation to fit the curve to the data.

TABLE II

Relationship Between Fat Acidity and Aflatoxin of Inoculated Peanut Kernels

Fat acidity range	Range of mold score	Number of observations	% Samples containing aflatoxin	Aflatoxin range	Average aflatoxin level
				μg/kg Kernels	
16–30	0–1	18	0	0	0
31–60	1–2	4	0	0	0
61–90	1–3	7	71	0–72	18
91–120	2–3	10	90	0–1200	238
121–150	2–4	10	100	6–900	341
151–190	2–4	10	100	6–900	312
191–300	4	8	100	72–900	515

The lipolytic activity of *A. flavus* appears to begin with the onset of fungal growth. Under the conditions of this study the immediate increase in fat acidity appears to be a sensitive indicator of *A. flavus* growth in the initial stages of development. The results of this study (Fig. 1) suggest that in large-seeded Virgina-type peanuts fat acidity values above 30 indicate the possibility of fungal damage.

Although an immediate increase in fat acidity is associated with initial visible mold growth, there is a lag in aflatoxin production by *A. flavus* (Fig. 2). This lag in aflatoxin production confirms the data of Dickens and Pattee (11) which show aflatoxin becomes detectable 48–60 hr after inoculation with *A. flavus* when kernels at 26–31% moisture content were stored at 32C. After the initial lag, i.e., when the mold growth score reached 2 to 3, aflatoxin content increased sharply. This relationship suggests the possibility that the fungus must attain a certain physiological stage before aflatoxin is produced.

Comparison between aflatoxin content and fat acidity shows that fat acidity increases markedly before aflatoxin becomes detectable (Table II). Aflatoxin was not detected in samples having a fat acidity below 60. Only three samples having fat acidity above 60 did not produce detectable aflatoxin. Under the test conditions the rapid method of analysis for fat acidity served as an index of fungal growth and aflatoxin production.

If a similar relationship could be established between fat acidity and fungal growth in samples of peanuts taken for official grading where the fungal population is more heterogenous, the rapid method of determining fat acidity might be used to screen samples for suspicious levels of fungal growth. Suspect samples could then be qualitatively and quantitatively assayed for aflatoxin using the currently available procedures. Attempts to establish such a relationship are currently in progress.

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