Relationship Between Aflatoxin Content and Buoyancy of Florunner Peanut Kernels

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ABSTRACT

A water flotation method was used to study the distribution of aflatoxin relative to kernel density in naturally contaminated samples of shelled farmers stock peanuts. Five-hundred gram samples of visibly undamaged, contaminated peanuts were added to 2000 mL of tapwater, and approximately 15-30% of the kernels rose to the surface as buoyant kernels. These buoyant kernels contained an average of 95+% of the total sample aflatoxin content. Buoyant kernels, when examined internally, all had a hollow space or "lumen" inside the kernel between the two cotyledons. Data showed an association between aflatoxin content, kernel lumen volume, and the propensity of kernels to float. The lumen may provide a reservoir of air for flotation, fungal growth, and aflatoxin production. The positive association between the presence of a lumen and aflatoxin contamination may provide a possible resistance strategy, if the presence or absence of a lumen is genetically controlled or if it can be manipulated physiologically.

Key Words: Density segregation, buoyancy, non-buoyancy, aflatoxin, farmers stock peanuts, kernel lumen.

The conditions under which aflatoxin contamination occurs have been well documented in a series of studies utilizing some novel environmental control plots (1,2,6,8). A hot and droughty situation during the pod maturing process is conducive to aflatoxin contamination. However, it has been noted that contamination is not uniformly distributed throughout all the kernels in a lot (1,2,6,8). Some kernels become physically injured in the soil, thus predisposing them to invasion by *Aspergillus flavus* and/or *A. parasiticus* with subsequent aflatoxin contamination. A more perplexing problem, however, is that some kernels become contaminated in a given population of stressed kernels but show no obvious signs of damage or any visible evidence of fungal invasion.

A number of techniques have been used to separate contaminated kernels from noncontaminated kernels during the various milling operations which have led to mixed and incomplete success. These techniques are separation of light from heavy kernels in the shelling plant with gravity tables (3), color sorting and handpicking to remove discolored and damaged kernels (4), and blanching followed by color sorting (9).

Density segregation by water flotation has been used for many years in Europe to remove ergot-infested grain. In the United States, Haslam (5) recommended "floating" to remove toxic kernels of corn from sound kernels to prevent equine blind staggers.

A technique based on density segregation using water flotation to remove moldy and mycotoxin contaminated components from corn and grain has been studied with varied success. Huff and Hagler (7) used the water flotation method to reduce mycotoxin levels of corn samples naturally contaminated with aflatoxin or deoxynivalenol, and wheat samples naturally contaminated with deoxynivalenol or zearalenone. The use of

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density segregation may provide a useful adjunct to existing techniques for removing aflatoxin contaminated peanut kernels from a contaminated lot.

Materials and Methods

Seventy-three samples of shelled Florunner peanuts (*Arachis hypogaea* L.), most of which came from lots known to contain aflatoxin, were used in this study. Samples contained all grade sizes with only splits and fragments removed because they always sank. The samples, which averaged about 500 g, were immersed in two liters of tapwater for about 15 seconds. Gentle stirring was necessary to prevent buoyant kernels from being trapped underneath the kernels that sank. Buoyant and non-buoyant kernels were separated and each fraction was extracted and analyzed for aflatoxin by reversed phase high performance liquid chromatography (HPLC) using post-column derivatization with iodine (Dorner & Cole, 1988).

To determine the lumen size of kernels or presence of fungal growth in the lumen, individual kernels were split in half perpendicular to the cotyledons with a scalpel. To determine lumen size, a microliter syringe containing colored water was placed into the lumen of each kernel half and colored water injected to fill the cavities. The volume of each lumen could then be determined directly from the microliter syringe. Each kernel was also weighed on a micro-balance (Mettler M-5) to determine kernel weight. Twenty-five kernels selected randomly from each jumbo, medium and No. 1 size categories that floated and sank were examined in this manner.

Results and Discussion

Results showed a direct relationship between buoyancy characteristics of peanut kernels and aflatoxin content (Table 1). Twenty-nine out of seventy-three samples were contaminated with aflatoxin ranging from 1-1818 ppb. Twenty-one of these samples had 97 + % of the total aflatoxin contained in the floating kernels. The mean aflatoxin was reduced from 301 ppb initially to 20 ppb after the flotation procedure which resulted in an 88% total aflatoxin removal. The floating kernels represented 27.2% of the total sample weight.

In a comparison of lumen size of buoyant kernels to lumen size of the non-buoyant kernels, there was a direct relationship between the presence and size of the lumen to the propensity of these kernels to float (Table 2; Fig. 1A). The average mass of the different commercial categories was essentially the same (0.55 vs 0.57); however, the average volume of the lumen of the buoyant kernels was almost 2.5 times greater than that of the non-buoyant kernels. The average ratio of lumen volume to kernel mass was 3.8 times greater in the buoyant kernels than in non-buoyant kernels. In a few instances this distinction was less well defined. This could be due to tiny air pockets of trapped air sometimes observed on loose-skinned kernels, physical damage to the kernels integument, or occasional density differences in the cotyledons themselves. Further evidence that the presence of a lumen was primarily responsible for the flotation properties of peanut kernels was indicated when buoyant kernels were split and both halves placed back into water. These half kernels always sank. The flotation characteristics of kernels could also be destroyed by removing the integument (seed coat). Removal of the integument persumably allowed water to displace the air in the lumen, which destroyed the buoyancy properties of the kernel. In both of the above cases, the buoyancy properties resulting from the air present in the lumen was destroyed.

If kernels that float are predisposed to aflatoxin contamination and the factor that is responsible for the flotation properties of the kernel is the presence of the lumen, then it is likely that the lumen is necessary for aflatoxin contamination to occur in these cases. Therefore, the production of peanut kernels without a lumen may result in a highly resistant peanut kernel. How-

Table 1. Distribution of aflatoxin in contaminated samples

	Initial	Buoyant	Non-buoyant
	Before Treatment		
	(ppb)	(ppb)	(ppb)
	1	0	1.4
	2	0	3
	3	20	0
	4	7	3
	4	15	0
	5	22	0
	11	0	13
	13	76	0
	19	63	2
	24	4	27
	30	105	1
	34	71	2
	43	235	0
	45	132	0
	57	142	20
	62	550	9
	72	568	0
	94	155	57
	94	434	0
	103	205	1
	130	1622	0
	152	249	0
	498	1201	0
	559	2677	59
	636	1836	0
	685	2953	38
	1761	7799	307
	1762	4922	1
	1818	4233	38
Means:	301	1045	20

Table 2. The ratio of lumen volume to kernel mass for buoyant and non-buoyant peanut kernels from a contaminated lot.

Size	Lumen Volume (μL)	Kernel Mass (g)	Ratio (μL/g)
	Buoyant		
Jumbo	56	0.71	79
Medium	45	0.58	77
No. 1	35	0.41	84
Average	45	0.57	80
	Non-Buoyant		
Jumbo	19	0.66	29
Medium	15	0.58	27
No. 1	2.4	0.40	6
Average	12	0.55	21

Each value is the mean of 25 observations.

ever, the basis for the presence or absence of a lumen in a peanut kernel is not known. This resistance strategy would be feasible if the presence or absence of a kernel lumen was a genetically-controlled feature of the peanut kernel. Physiological manipulation may also provide a feasible strategy. Studies are currently under way to provide insight into whether or not this feature is controlled genetically or physiologically.

A possible explanation for the lumen being necessary for aflatoxin contamination to occur in these cases, is that the lumen may provide the fungus with a reservoir of air essential for fungal growth and/or production of aflatoxin.

A careful examination of peanut kernels from a contaminated lot revealed several instances where the only evidence of fungal growth and spore production was located in the lumen (Fig. 1b; Fig. 1c). This latter phenomenon presumably results in hidden contamination that is very difficult to detect or remove during the normal milling processes. It is currently not known to what extent this type of preharvest contamination occurs in nature. The flotation process could compliment other reasonably effective milling processes, such as blanching, followed by color sorting, by removing those kernels that are highly contaminated but not visibly damaged from their external appearance.

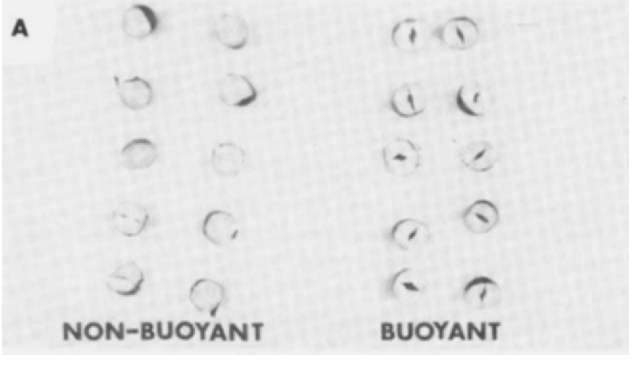

Fig. 1a Comparison of the lumen size of non-buoyant and buoyant peanut kernels.

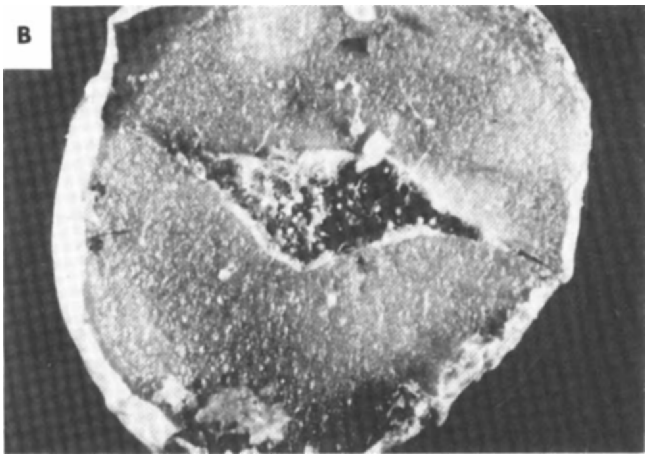

Fig. 1b Cross section of peanut kernel showing *Aspergillus flavus* growth and sporulation within the lumen.

Fig. 1c Split peanut kernel showing growth and sporulation of *A. flavus* in peanut lumen.

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