

SUPPLEMENT TO SAMPLING DICED ALMONDS FOR AFLATOXINS

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Introduction: Aflatoxins are mold metabolites which are both toxic and carcinogenic to mammals and have toxic effects to other species. Since the molds, Aspergillus flavus and Aspergillus parasiticus, which produce aflatoxins are widely distributed, there is a possibility of finding aflatoxins in a wide variety of foods and feeds. Some of the most likely commodities to have significant amounts of aflatoxins are cereal grains, oilseeds and nuts. Aflatoxins have been found in almonds, and the highest incidence of these toxins is in insect-damaged nuts. Hence there is a safety incentive, as well as an economic incentive to lower the amount of insect damage to the crop.

Control of Aflatoxins: The best way to control aflatoxin is to minimize the chance that it will occur in the first place. A. flavus spores are always present in the field, so that only the right growing conditions--a source of nourishment, the right amount of moisture and a proper temperature range--will allow growth of molds and aflatoxin production. Direct control of mold growth in the field is almost impossible to prevent, although mold growth can be inhibited in storage by keeping the commodity dry and cool and, in some cases by use of fungicides. The molds are most likely to be introduced into nut kernels after hullsplit but before harvest by insects. Not only does the insect produce a hole for mold spores to reach the nut kernel but it also may bring in the spores on its own body. Thus, the most effective ways for the grower to minimize aflatoxin in almonds are to control insect populations and to insure that nuts are kept dry during and after harvest.

Removal of Aflatoxins from Contaminated Commodities: While control of aflatoxin by the grower is indirect, control by the processor can be more direct. The processors generally have procedures in which various combinations of hand and machine sorting to remove kernels which are moldy or damaged by insects. When rigid tolerances for damage are maintained, we have found that aflatoxin incidence is very low. A method of removing every individual aflatoxin-containing kernel has not been discovered, however. Hence a monitoring system is necessary.

Monitoring Aflatoxin in Almonds: Both regulatory agencies (U.S. Food and Drug Administration) and processors monitor the finished product, to the best extent they can. Monitoring involves two operations, sampling and analysis. The second of these, analysis, is very sensitive and precise. Chemical methods allow detection of less than one part per billion of aflatoxin, if it is present in the sample analyzed. However, a representative sample is very difficult to get. This is because the aflatoxins are not widely distributed, but actually occur at high concentrations in only a few kernels out of many thousands. Thus, although the toxins may be present in a lot, many small samples may be taken from the lot which show no contamination. The best way to survey whole kernels is to look for kernel damage caused by insects. Diced nuts may be monitored, though, by sampling and analysis because there are so many more particles in a relatively small sample.

The purpose of the work behind the accompanying paper was to lay out a sampling plan based on statistical sampling of actual contaminated lots. Similar plans may be used by processors to insure safety of product.

The sampling plan is based upon accepting 90 percent of the lots with two parts of aflatoxin or less per billion parts of nuts (2 ppb) and rejecting 95 percent of the lots contaminated with 20 ppb or greater of aflatoxin. Because of the random distribution of aflatoxin-contaminated nutmeats in the lot such decisions on risk have to be made.

Figure 7 in the paper shows a decision-making process to accept or reject a lot of diced almonds based on the above sampling plan parameters. This requires consecutive analysis of ten-lb. samples of meal until the cumulative total aflatoxin equals an amount on either the reject or accept curve. Even at very low aflatoxin levels, the sampling plan requires four or five fairly expensive samples for an acceptance.

Because the plan for direct sampling diced almonds is expensive, we also tried sampling the fine by-product meal removed by screening during the dicing process. Figure 8 in the paper shows decision limits for sequential samples of only 150 grams (about five ounces). Here, the samples are less expensive and a decision to accept or reject often requires only two samples.

Figure 9 in the paper shows the number of samples needed to reach a decision when either the diced or by-product was sampled. It was much easier to reach a decision with the fines, taking two or three samples to reach a decision compared to up to eight for the diced nuts. This sampling plan was devised to solve the problem of getting useful analysis of the amount of aflatoxin in a lot of diced almonds. The lot we studied was well-enough mixed that a few samples could give an accurate estimate of the lot aflatoxin content. The plan is based upon two representative lots of almonds and should be applicable to other processing operations. This work is the first step in development of a sampling plan for in-plant use. Those who are interested in devising such a plan applicable to their own situation may consult the authors.

Summary: A plan, based on statistical analysis, was devised to monitor diced almonds. This plan is designed to have a 95 percent probability of rejecting a lot which exceeds FDA guidelines for aflatoxin. It allows sampling of by-product meal from the dicing process and requires only a few samples to make a decision.

Work on methyl bromide fumigation with Dr. Stanley has just begun. A study needs to be made of effects on the product of high levels of methyl bromide fumigation. **Referencing project number 81-V8**

6. Publications -

Sampling Diced Almonds for Aflatoxin. J. E. Schade, A. D. King, Jr., B. E. Mackey, W. U. Halbrook and G. Fuller. J. Am. Oil Chemists' Soc. 58(9), 852-857 (1981).

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Sampling Diced Almonds for Aflatoxin

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ABSTRACT

To ensure that diced almonds meet the current FDA guideline limit for total aflatoxin, it is necessary to have a sampling plan that will allow representative sampling with defined precision—i.e., with confidence limits on the average aflatoxin found. A sequential sampling plan using 4.54-kg samples of diced almonds or 150-g samples of meal by-product (fines screened from diced nuts during production) was constructed with data from a study of aflatoxin distribution among samples of 2 selected lots of almonds. These 2 lots of whole nuts, estimated to have 400 and 25 ppb aflatoxin, were diced and boxed with normal processing equipment and procedures to approximate the distribution of aflatoxin in the product during commercial production. With a square root transformation of the data from 4.54-kg samples of diced nuts, the aflatoxin in samples of both lots approximated a normal distribution and the within-lot variances were not significantly different, which allowed the statistical plan described. A supplemental study was made of aflatoxin distribution in the meal by-product. The lack of a significant difference between the results for diced nuts and those for the corresponding meal suggests that diced almonds can be monitored for aflatoxin indirectly by sampling the meal, which will allow the use of fewer analyses of 150-g samples of less expensive product to reach a decision.

INTRODUCTION

An accurate determination of aflatoxins in a commodity depends on both representative sampling and precise analysis. At present, the precision of chemically analyzing for aflatoxins far exceeds that of sampling particulate products for these toxins. Analytical techniques for aflatoxins, using thin layer chromatography (TLC) or high performance liquid chromatography (HPLC) with fluorescence detection, are not only precise but are very sensitive and allow quantitation of aflatoxin at levels as low as 1 ppb or less. Representative sampling of particulate commodities for aflatoxin is difficult, because aflatoxin contamination is not uniform but is largely confined to a small proportion of the particles. The ability to ensure that a product truly meets the current FDA guideline limit for total aflatoxin (currently 20 ppb for most foods) depends as much, or more, on representative sampling and sample preparation as on precise chemical analysis.

Sampling various particulate products for aflatoxin has been recognized by various workers as a difficult statistical problem worthy of study. Whitaker et al. (1,2) found the negative binomial distribution to be a reasonable model for the observed distribution of aflatoxin in shelled peanuts. The negative binomial distribution is appropriate for cases of high probabilities of zero counts along with low probabilities of very large counts (e.g., high contamination). It approximates the distribution of aflatoxin in particulate products, especially if there is contagion, i.e., if the level of contamination of one particle influences the contamination of adjacent particles. Whitaker and Dickens (3) also used the negative binomial distribution to study the variability associated with analyzing aflatoxin in corn, and Valasco et al. (4) used it to describe aflatoxin distribution in cottonseed.

Diced almonds were chosen for this study because there appears to be much higher incidence of aflatoxin and, therefore, a greater health hazard with diced almonds than

with whole, select almonds. In previous studies on the incidence of aflatoxin in almonds (5,6) the authors found that the overall proportion of individual whole almonds contaminated was especially low and estimated that, on the average, about one nut in 26,500 unsorted in-shell nuts from the field contained aflatoxin. Sorting shelled almonds to remove physically damaged nutmeats tends to concentrate the aflatoxin in the damaged nutmeats and leave the whole nutmeats, which represent most of almonds marketed, nearly free of aflatoxin. Hence, aflatoxin is rarely detected in select whole almonds and, if detected, would likely not be representative of the mean concentration in the lot sampled. The damaged nutmeats go either to oil-stock for inedible use or to manufacturing stock for use in such products as diced almonds, depending on the type and degree of damage evident. Obviously, poor manufacturing stock will tend to increase the incidence of aflatoxin-containing samples in the manufactured product.

The higher incidence of aflatoxin in diced almonds than in whole almonds is due not only to the lesser quality of the nuts used but also to the increase in number and dispersion of the contaminated particles, which increases the likelihood of obtaining a sample representative of the true mean aflatoxin concentration. The chance of obtaining a 4.54-kg sample with a contaminated particle increases rapidly with the fineness of dicing, from a very poor chance of getting a positive sample with whole kernels to a very good chance of obtaining a positive sample when each kernel has been cut into 60 pieces (7). The diced nuts used in this study were estimated to contain 60 pieces from each kernel.

A plan for representative sampling with defined precision is necessary to ensure that manufactured almond products meet the current FDA guideline limit for aflatoxin. That is, it is necessary to set up a plan with known risks and decision limits, so that one can decide to accept or reject a particular lot on the basis of 1, 2, or several samples of a stated size. This study represents the first step necessary to develop such a sampling plan for aflatoxin in diced almonds. For this study, 2 lots of damaged almonds, selected on the basis of being naturally contaminated with widely different levels of aflatoxin, were diced and boxed with normal processing equipment and procedures to approximate the usual distribution of aflatoxin in the product during commercial production. Two sampling plans are considered: the first monitors diced nuts directly; the second monitors diced nuts indirectly by sampling the meal (fines) screened from the diced product during its production. In both cases, a sequential sampling plan is developed to illustrate the efficiency of such a plan, i.e., the ability to reach a decision with fewer sample analyses than with a single-stage sampling plan.

EXPERIMENTAL PROCEDURES

Almond Stock

Two lots of almonds that were naturally contaminated with aflatoxin were diced to pass through 14/64 in. screens and over 8/64 in. screens, resulting in nut pieces between 3.2 and 5.6 mm diameter. Lot 1 was prepared from about

454 kg (about half of a normal bin) of reject almonds designated as oil stock. This particular lot of oil stock was selected because of an apparent high level of aflatoxin found in it by the processor. Lot 2 was prepared from about 227 kg (about 1/4 of a normal bin) of damaged almonds that had a minimal amount of serious defects such as insect and mold damage. Normally, this stock of whole and broken or chipped almonds would have been used to manufacture diced nuts. However, a sample from this bin of damaged almonds was found by the processor to contain aflatoxin, so the bin of nuts was rejected and used for this study. Each of the two lots of nuts was diced, sized (screened) to remove the fines, and boxed on a normal processing line. After dicing, lot 1 consisted of 30 boxes (11.35 kg each) of diced nuts and 2-2/3 sacks (120 kg total) of meal as a by-product. Lot 2 consisted of 13 boxes (11.35 kg each) of diced nuts and 1-1/2 sacks (68 kg total) of meal by-product. All boxes of diced nuts and sacks of meal were stored at 0-1 C.

Sampling

A 4.54-kg sample of diced nuts was taken from every other box in lot 1 (15 samples) and from every box in lot 2 (13 samples). Two 150-200-g samples of almond meal were removed from each sack of meal by inserting a Seedburo Quality Sampler (Seedburo Equipment Co., Chicago, IL) to the bottom of the sack at 2 locations; thus, there were 6 meal subsamples of lot 1 and 4 meal subsamples of lot 2.

Sample Preparation

Diced. The 4.54-kg samples of diced nuts were removed from cold storage one or more days before being prepared for assay. Each sample was cut and blended in a Hobart vertical cutter-mixer (25 qt VCM, Hobart Manufacturing Co., Troy, OH). A fine, homogeneous meal was prepared by intermittent cutting (i.e., 15 sec at a time) at the slow speed setting for a total of 1.5 min, using sharp wave-cut blades. Allowing samples to cool between the intermittent cuttings minimized problems of over-heating, oiling-out, and compacting of the blended sample. A single, 50-g subsample of each comminuted sample was analyzed for aflatoxin.

Meal. Each 150-200-g subsample of meal by-product was mixed thoroughly for 2 to 3 min in its plastic bag before a 50-g subsample was removed for analysis. Further size reduction was considered unnecessary for this small supplementary study because over 90% of the meal would pass through a number 12 sieve.

Analysis

Aflatoxins B₁, B₂, G₁, and G₂ were determined by reverse phase HPLC with fluorescence detection, using 2 injections of each sample extract. To increase fluorescence, aflatoxins B₁ and G₁ were hydrated to B_{2a} and G_{2a} by treatment with trifluoroacetic acid, as described by Beebe (8) and by Takahashi (9). Fluorescence detection not only is more sensitive than ultraviolet detection, but it is also more selective and less susceptible to background interference (10). A simple extraction and clean-up procedure similar to that used by Thean et al. (11) for aflatoxins in corn was found suitable for fluorescence detection of aflatoxin in these almonds. This procedure is analogous to the clean-up procedure that Lansden (12) found adequate for HPLC analysis of aflatoxins in peanuts, rice and corn. Lansden suggested that the sorption (alumina column) purification step in his procedure could be eliminated if the aflatoxin/interference ratio were high. Similarly, with these highly contaminated lots of almonds, a sorption purification step using a Sep-Pak silica cartridge (Waters Associates, Milford,

MA) was eliminated from the present procedure without increased interference.

Aflatoxins were extracted from 50.0-g samples of the finely ground almonds or meal by mixing with 200 mL of methanol/water (80 + 20) in a Waring blender for 1 min on low speed plus 2 min on high speed. The extract was filtered through E & D 515 fluted paper. About 5 g Celite Hyflo Super Cel was added to a mixture of 75 mL filtrate, 60 mL water and 15 mL saturated ammonium sulfate. The mixture was allowed to stand for 10 min and then filtered through Whatman No. 4 paper. The filtrate (120 mL) was extracted twice with 5 mL chloroform. The extracts (10 mL total) were combined in a small vial and evaporated just to dryness under a stream of nitrogen without heat. After the residue was mixed with a small amount (0.5 mL) of hexane to dissolve any lipid materials which might otherwise occlude the aflatoxins, it was thoroughly mixed with 0.1 mL trifluoroacetic acid to derivatize any aflatoxins B₁ and G₁ present (8). This mixture was diluted with an appropriate volume (e.g., 2 mL) of water/acetonitrile (90 + 10), and if necessary, the lower phase was filtered through a 0.5- μ Millipore FH 01300 filter (Millipore Corp., Bedford, MA). Duplicate aliquots (e.g., 10 μ L) of the derivatized extract were injected successively into the HPLC instrument. Triplicate injections were made with the meal extracts of lot 2. Prior to analyzing each sample, 10 μ L of a derivatized standard mixture containing 1.0 ng/ μ L of each aflatoxin was injected. This derivatized standard solution (B_{2a}, B₂, G_{2a}, G₂) was prepared in a manner similar to that of Takahashi (9) by adding 0.3 mL trifluoroacetic acid to a mixture of 4 aflatoxin standards (10 μ g each of B₁, B₂, G₁, and G₂) and diluting with water/acetonitrile (90 + 10) to 10 mL. The aflatoxins were eluted with a mobile solvent of water/acetonitrile/methanol (71 + 19 + 10) at a flow rate of 1.5 mL/min. All 4 aflatoxins eluted with baseline resolution within 30 min in the following order: G_{2a}, B_{2a}, G₂, and B₂. Each aflatoxin was calculated from the area of the peak relative to that of the standard.

In accordance with the procedure of Takahashi (9) and Beebe (8), a nonderivatized portion of each sample should also be analyzed, and any peak with the retention of B_{2a} or G_{2a} in non-TFA-treated sample should be subtracted from the B_{2a} or G_{2a} found in the TFA-treated sample. Since there was no evidence of such artifacts in the non-derivatized portions of several samples of each lot, non-TFA-treated samples were not run routinely in this study.

Apparatus

The HPLC system consisted of the following: Waters Model 600A solvent pump equipped with a Waters Model U6K injector with a 2-mL loop (Waters Associates, Milford, MA); Excalibur HPLC column, 4.6 mm id \times 250 mm stainless steel, packed with 5- μ Spherisorb ODS (Applied Science Labs, Inc., State College, PA 16801); Schoeffel FS970 LC Fluoromonitor and a GM970 monochromator (Schoeffel Instruments, Div. of Kratos, Inc., Westwood, NJ) with excitation wavelength set at 360 nm and with a 430 nm cutoff interference filter; and an Esterline Angus Model L1102S recorder/integrator (Esterline Corp., Indianapolis, IN).

RESULTS AND DISCUSSION

To facilitate statistical treatment of the data, it is desirable to achieve two conditions: (a) normal distribution within lots, and, (b) variances not significantly different between lots. Both of these conditions were obtained by using a square root transformation of the data. On a square root scale, the distribution of total aflatoxin among the 4.54-

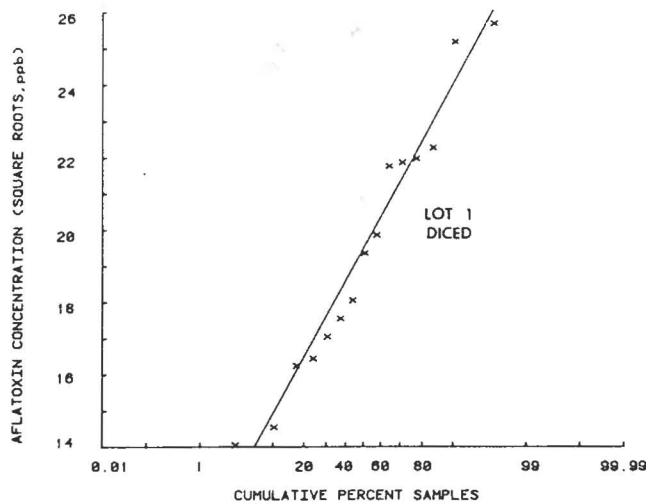


FIG. 1. Normal probability plot for aflatoxin in diced samples from lot 1, using average of 2 injections/4.54-kg sample.

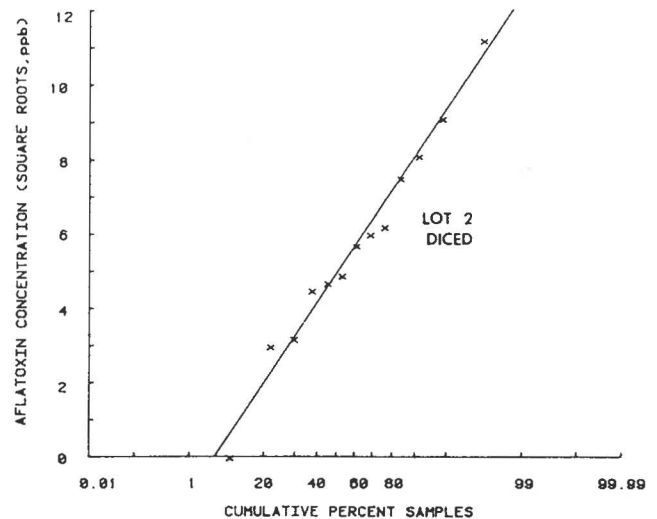


FIG. 2. Normal probability plot for aflatoxin in diced almond samples from lot 2, using average of 2 injections/4.54-kg sample.

kg samples of diced nuts in both lots in this study approximated a normal distribution, as shown by the probability plots in Figures 1 and 2. The variances among the 4.54-kg samples within each lot of diced nuts were calculated and compared by the F test after an analysis of variance was made of the square roots of the data for total aflatoxin. The variance ratio ($F = 1.47$, $p = 0.25$) shows that the variances of the transformed data for the 2 lots were not significantly different. Thus, both conditions a and b are satisfied.

With adoption of the hypothesis of equal variances (transformed data), the variances between samples within any lot, regardless of level of aflatoxin, is constant. An obvious contradiction to this hypothesis occurs at the lower limit of detection, at which the variance becomes zero; so, in effect, it is assumed that the lower limit of detection is not in the range of interest.

With the assumption of equal variance, the transformed data for the 2 lots can be pooled for an analysis of variance for diced nuts. An analysis of variance for the meal by-product was also made in the transformed scale and compared with that for the diced nuts. The variance component estimates (13) for both are shown in Table I. (For this analysis of variance the subsamples of meal were combined with the injections since the subsamples were not a significant source of variability. Thus, for lot 1, the six 150-g subsamples were combined with the 2 injections of each, and for lot 2, the four 150-g subsamples were combined with the 3 injections of each, so that the degrees of freedom (df) for injections including 5 df for subsamples was 19. Samples of meal in Table I represent sacks of meal.) It is not surprising that the largest component of variance was that contributed by lots, since the almonds used for dicing were selected with a desire of obtaining lots having

large differences in total aflatoxin levels. With both products, the smallest component of variance was that contributed by the injections. As might be expected, there was less variability between samples (and more between lots) for nut meal than for diced nuts. These estimates of variance components on the square root scale are the basis of the statistical comparisons and graphs that follow.

A comparison was made between the transformed means of the diced and meal samples of each lot (Table II). Squaring these means give average contamination levels of about 400 ppb and 25 ppb for lot 1 and lot 2, respectively. With both lots, there was no significant difference between the means of the meal samples and those of the diced samples. If this correlation between aflatoxin in diced almonds and aflatoxin in meal were to hold for all lots, one could sample the meal to monitor diced nuts without biasing the estimated average contamination level. Since sampling variation is less for meal, this approach would result in savings in required numbers of samples. Furthermore, the meal is less expensive to sample than the diced product. Additional studies should be made to confirm the correlation of aflatoxin concentrations in meal and diced nuts. For such a study, it is envisioned that samples of meal would be removed from a collecting bin rather than sacks, since this would be a logical quality assurance procedure for almond processors. Samples of diced nuts would be collected in a manner similar to that used in this study.

Samples must assay considerably lower than the aflatoxin guideline limit to have a reasonable assurance that the true concentration in a lot will be less than or equal to this limit. The curves in Figure 3 show the maximal average aflatoxin allowed in relation to the number of samples assayed to be 95% certain that the lot has less than 20 ppb

TABLE I

Variance Components of Transformed (Square Root) Data

Source of variation	Diced			Meal		
	df	Variance component	% of total variance	df	Variance component	% of total variance
Lots	1	94.20	89.2	1	158.51	97.7
Samples	26	10.24	9.7	3	2.77	1.7
Injections	28	1.16	1.1	19	0.95	0.6

TABLE II

Comparison of Means (Square Root) between Diced Almonds and Almond Meal

Lot	Diced			Meal			Comparison	
	n ^a	Mean X	Std. error S _{X̄}	n ^a	Mean X	Std. error S _{X̄}	t ^b calc.	Significance level
#1	30	19.48	0.85	12	21.94	1.08	-1.80	0.15
#2	26	5.70	0.91	12	4.06	1.08	1.16	0.31

^an = number of assays.

^bSee Snedecor and Cochran, p. 115.

aflatoxin. Curves for using 2 injections and 5 injections (calculated from data on 2 injections) in the HPLC analysis are shown. The curves for 5 injections of a sample extract were constructed to illustrate that the variability between injections is small so that there is little advantage to increasing the number of injections per sample. The lower set of

curves in Figure 3 is for 4.54-kg samples of diced nuts, whereas the upper set is for 150-g samples of meal; thus, one must obtain much lower levels of aflatoxin in diced nuts than in meal to attain the same assurance of meeting the guideline limit. For example, two 4.54-kg samples of diced nuts must be essentially negative to be 95% certain the lot contains less than 20 ppb aflatoxin, whereas 2 samples of meal must average less than 6 ppb to have the same assurance.

The curves developed in Figures 4 and 5 show the 95% confidence limits for the average aflatoxin found with 2 or 5 samples per lot of diced nuts (4.54-kg samples) and nut meal (150-g samples). For example, finding an average of 10 ppb with five 150-g meal samples (Fig. 5) would give one 95% certainty that the lot contained between 3 and 22 ppb aflatoxin. Similar results with 4.54-kg samples of diced nuts (Fig. 4), on the other hand, would give 95% certainty that the lot contained between 0 and 37 ppb aflatoxin. Of course, the confidence intervals are even broader with only 2 samples.

In view of the broad confidence intervals obtained for aflatoxin in both diced almonds and meal, it seemed best to consider a sequential sampling plan rather than a single-stage sampling plan. Sequential sampling plans, on the average, use fewer samples than single-stage sampling plans to reach a decision (14,15). As an example, a sequential sampling plan was constructed for diced nuts or nut meal that would accept 90% of the lots at 2 ppb and 5% of the lots at 20 ppb aflatoxin. The acceptance probabilities at other lot concentrations are shown by the operating characteristic curve in Figure 6 (15,16). The decision limits for

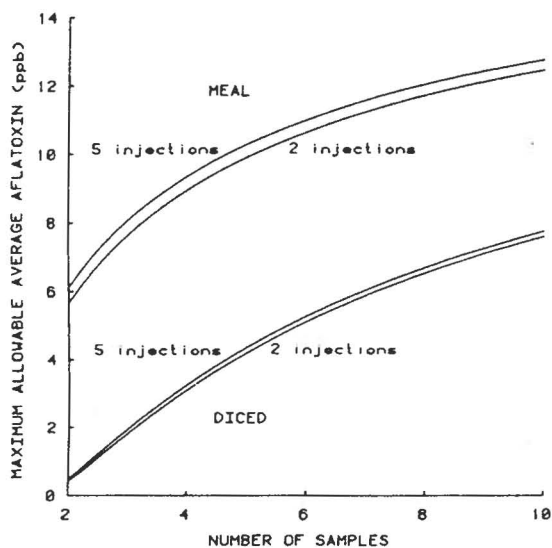


FIG. 3. Maximum average aflatoxin levels permissible in samples of diced almonds, or meal by-product, to be 95% certain the lot contains less than 20 ppb aflatoxin. Sample sizes: 4.54 kg diced nuts; 150 g meal.

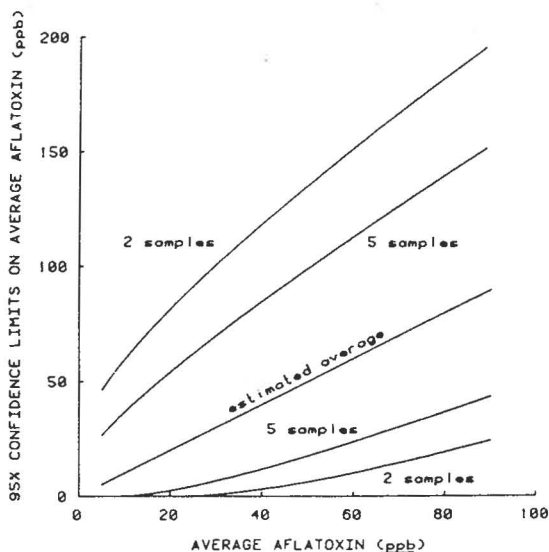


FIG. 4. Confidence intervals for average aflatoxin found in diced almonds using 2 or 5 samples of 4.54 kg each.

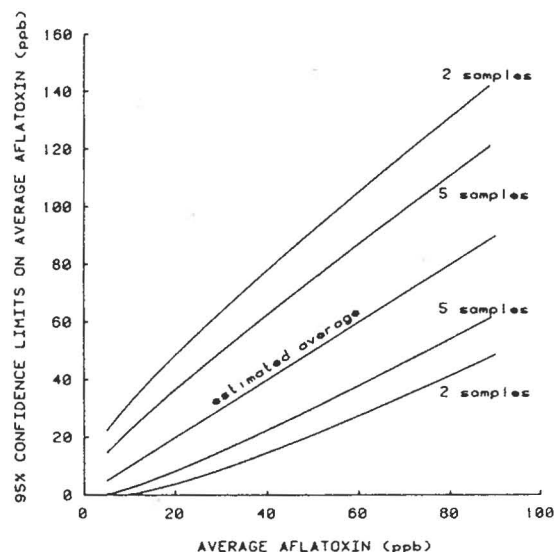


FIG. 5. Confidence intervals for average aflatoxin found in meal by-product using 2 or 5 samples of 150 g each.

this sequential sampling plan are shown in Figure 7 for diced nuts and Figure 8 for meal (15,16). With this plan, 4.54-kg samples of diced nuts or 150-g samples of meal are drawn and analyzed randomly until the cumulative total aflatoxin of the samples (average of 2 injections each) falls in the accept or reject regions. Actual sampling should be done by groups of two or more, depending on the minimal number of samples required for lot acceptance.

With the assumption that the correlation between diced almonds and meal by-product indicated in this study is true in general, it would be more efficient (i.e., require fewer samples of smaller size) to monitor diced nuts indirectly by sampling meal than directly by sampling diced nuts (cf. Figs. 7 and 8). The expected number of 4.54-kg samples of diced nuts, or 150-g samples of meal, required for reaching a decision is graphed in Figure 9 (15,16). For diced nuts, a sequential sampling plan with the same operating characteristic requires 2-3 times as many samples on the average as the comparable plan for nut meal. Furthermore, each 4.54-kg sample of diced nuts must be about

30 times larger than the 150-g sample of meal used.

For comparison, a t-test for the difference between 2 means (17) was used to estimate the number of samples required to reach a decision with a single-stage sampling plan having the same risks. With the same size samples (4.54 kg diced or 150 g meal) and the average of 2 injections per sample, it would be necessary to use 21 samples of diced nuts or 7 samples of meal to reach a decision with the same limits on lot acceptance ($\alpha = 0.05$ at 20 ppb; $\beta = 0.10$ at 2 ppb).

The results of this study suggest that sequential sampling is a worthwhile alternative to using larger or more numerous samples of almonds in single-stage sampling. The sequential sampling plan presented for monitoring diced almonds, either directly or indirectly, was developed on the basis of a limited study. It is presented as an illustration of the efficiency of sequential sampling plans in reaching a decision. Using more extensive data of their own, almond processors could derive similar plans, with their own limits on lot acceptance, for diced almonds and other products.

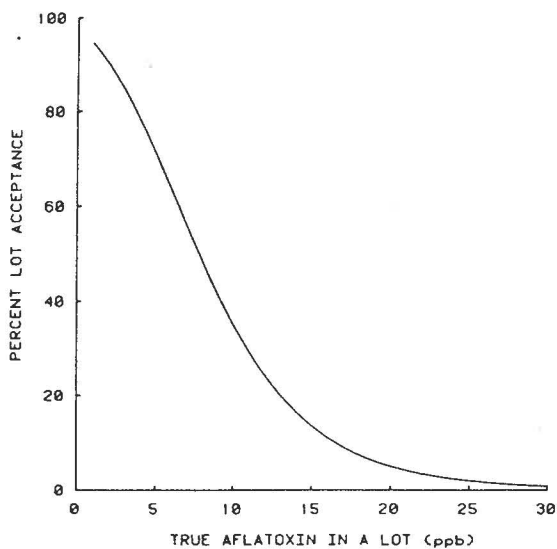


FIG. 6. Operating characteristic curve of sequential sampling plan for diced almonds or meal by-product.

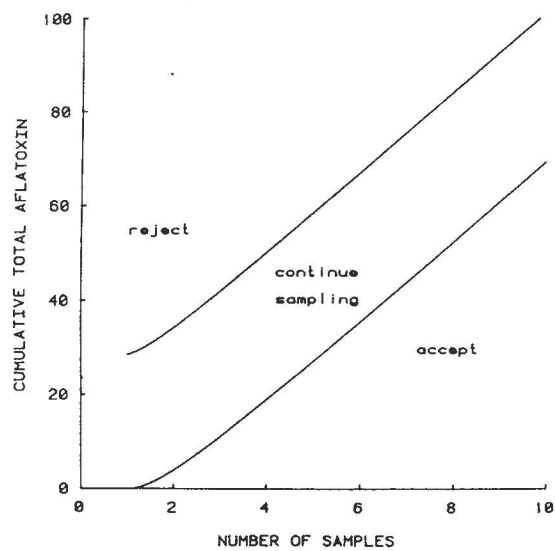


FIG. 8. Decision limits for cumulative total aflatoxin found in meal by-product (average of 2 injections/150-g sample) with the limits on acceptance shown in Fig. 6.

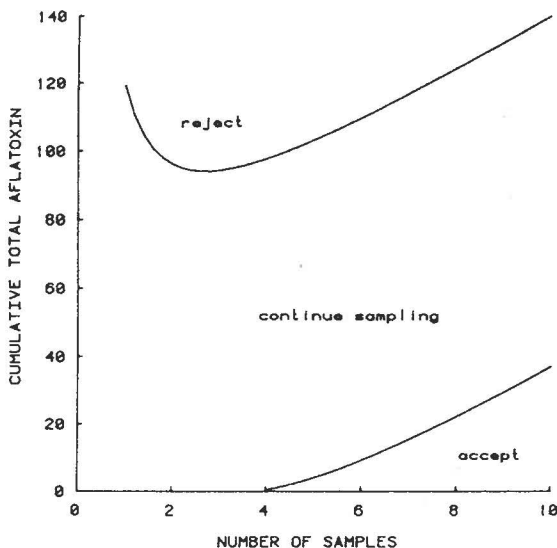


FIG. 7. Decision limits for cumulative total aflatoxin found in diced almonds (average of 2 injections/4.54-kg sample) with the limits on acceptance shown in Fig. 6.

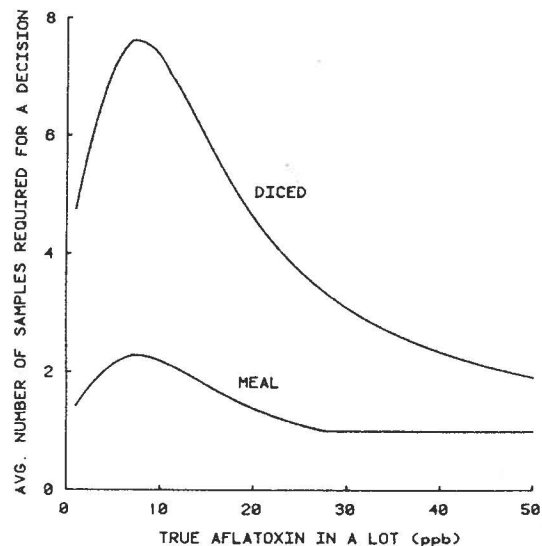


FIG. 9. Expected number of samples of diced almonds (4.54 kg), or meal by-product (150 g), required for reaching a decision.

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ACKNOWLEDGMENTS

The Almond Board of California and the California Almond Growers Exchange supplied diced almonds used.

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