Survey of Aflatoxins in California Tree Nuts

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ABSTRACT AND SUMMARY

Surveys of the California walnut and almond crops to determine incidence of aflatoxins are reported. Average proportions of contaminated nuts from the field were one in 28,250 for walnuts and one in 26,500 for almonds. It was shown that there is a high correlation of contamination with damaged nuts which are removed by standard sorting procedures. Statistical treatment of data from the surveys indicates some of the problems in sampling tree nuts for analysis.

INTRODUCTION

Aflatoxins, produced by certain species of Aspergillus, especially *A. flavus*, have been found in many food products. Widespread incidence of aflatoxins in corn, peanuts, cottonseed, and a variety of oilseeds and oilseed meals has led to extensive investigation of procedures for sampling and analysis for these toxins. The problem of determining whether a given lot of a particulate commodity is contaminated with aflatoxin and to what extent such contamination has occurred, results from the fact that only one seed in many thousands may be contaminated, but individual seeds may contain very high levels of toxin. Hence, contamination is not uniform throughout a lot and sampling is difficult. The problems of sampling and analyzing for mycotoxins in peanuts and cottonseed have been addressed by Tiemstra (9) and by Whitaker and coworkers (10,12-14), who have concluded that the major cause of large variations in analysis is the sampling procedure.

In 1971 the U.S. Food and Drug Administration reported to industry that they had found aflatoxins in commercial samples of walnuts and almonds. As a result, both the walnut and almond industries financed cooperative projects between the handlers and the Western Regional Research Center to survey the 1972-73 crops of these nuts. The objectives of these surveys were (a) to determine the occurrence and geographical distribution of aflatoxins in tree nuts, (b) to evaluate the effectiveness of sorting procedures used by the handlers as means of removing contaminated nuts, and (c) to provide a statistical basis for sampling and analysis. The results of the initial almond survey have been published (7). This paper reports the survey of the walnut crop for 1972-73 and provides followup data for subsequent crops of California almonds.

EXPERIMENTAL PROCEDURES

Collection of Walnuts

Sampling included in-shell walnuts from grower and processor, shelled nuts from process streams, finished grade walnuts, and inedible oil stock. At three different times during the harvest season (late September through November) samples of incoming nuts, as received, were taken from six different receiving stations at various locations throughout California. At the same time collections were made from five separate processors. Finally, collections were made from the same five processors during the post-harvest period, February through May, 1973. A total of 155 samples were collected for analysis (Table I). We attempted to obtain 45-lb samples of unshelled nuts and inedible oil stock and 30-lb samples of shelled products. Actual average sample sizes were 43.3 lbs and 27.7 lbs respectively. Each separate sample was wrapped in polyethylene and stored at 34 F within 24 hours of collection until the day prior to preparation for analysis. Walnuts from receiving stations were unfumigated and not dried. Shelled unsorted nuts, partially processed (hand sorted or electronically sorted) and processed nuts (finished product ready for sale), as well as oil stock (inedible rejects), were taken from the processors to reflect the end products from a specific day's processing. Only oil stock and finished products were taken during the post-harvest sampling. Ten different varieties of walnuts were represented, but no correlations were found either with variety or with geographical location from which the samples were taken.

Analysis of Walnuts (Fig. 1)

Sample reduction was accomplished in a Hobart Vertical Cutter-Mixer (VCM) capable of grinding 15-18 lb of sample at a time. A modification of AOAC Method I (2) was used for grinding and subsequent analysis. Samples were first divided into 15-lb portions. Three pounds of Hyflo Super Cel (Johns-Manville Corp.) was added to each 15-lb portion, which was then cut in the VCM for 30 seconds at low speed, followed by 1 min. at high speed. A 2-lb sample was taken from the ground mixture and mixed with similar 2-lb portions from the other 15-lb increments of the sample. The combined samples were then stored at 34 F for analysis. This procedure was checked periodically with added aflatoxin standard samples. All subsequent analytical procedures were carried out under gold fluorescent lighting.

From the combined analytical sample a 60-g portion (representing 50 g of ground meats) was combined with 250 ml of chloroform, 25 ml of water, and an additional 15 g of Hyflo Super Cel in a 500-ml flask. The flask was shaken vigorously on a Burrell wrist-action shaker for 30 min and the contents rapidly filtered through a sintered glass funnel which had been wet-packed with 25 g of anhydrous magnesium sulfate in chloroform. A 25-ml aliquot of the filtrate was chromatographed on a silica gel column and analyzed by thin layer chromatography on

TABLE I

Sampling of California Walnuts

Processor[a]	Number of samples taken
A	29
B	22
C	29
D	23
E	27
Total from processors	130
Receiving station[b]	
I	4
II	3
III	3
IV	4
V	5
VI	5
VII	1
Total from receiving stations	25
Grand total	155

[a]30-lb samples shelled, except for fifteen 45-lb samples in-shell.
[b]45-lb samples in-shell.

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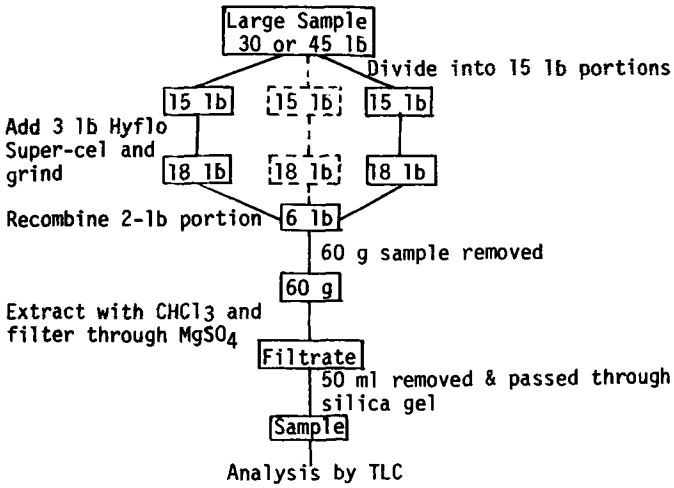

FIG. 1. Analytical scheme for walnut samples.

TABLE II

Aflatoxin-Contaminated Samples of Unsorted In-shell Walnuts[a]

Sample	Aflatoxin (ppb), total wt. basis[b]				
	B_1	B_2	G_1	G_2	Total
1	0	10	0	0	10
2	1.3	1	0	0	2.3
3	0	2	0	0	2
4	← Traces →				1
5	← Traces →				1
6	← Traces →				1
7	← Traces →				1
8	← Traces →				1
9	← Traces →				1
10	0	<1	0	0	1
11	0	<1	0	0	1
12	0	<1	0	0	1
13	0	<1	0	0	1
14	0	<1	0	0	1

[a]14 samples of 40 contained aflatoxin.

[b]It was assumed that kernel weight was approximately 1/2 of total weight.

Absorbasil-1 by developing with 20% acetone:chloroform containing 0.25% water. Aflatoxins were estimated quantitatively by comparison of their fluorescence to that of a known standard, according to AOAC method I (2).

Analysis of Almonds

Follow-up analyses of the almond crop were taken in two subsequent years in order to check sorting procedures, to ascertain the variability of the crop from year to year, and to attempt to find less expensive procedures for sampling than those now used. During the 1973-74 season, 15-lb samples of Nonpareil almond rejects were obtained from several processors and analyzed at WRRC in the same manner as reported earlier (7). During the 1974-75 crop year, 30-lb samples of finished whole almonds, manufacturing stock and inedible oil stock were collected from two processors by Mr. George Stanley of the DFA (Dried Fruit Association) of California and screened by the AOAC millicolumn technique (2). Results were provided to the authors by the DFA for statistical analysis.

RESULTS AND DISCUSSION

Handling of almonds and walnuts is generally similar. The trees are shaken by machines and the ripe nuts drop to the ground, from which they are usually collected the same day. The outer hulls are removed near the point of harvest and walnuts are dried in-shell to a moisture content of 7% or less. Almonds are already at this low moisture content unless there is contact with water while they are on the ground. Tree nuts are then taken to the processing plant where they may be stored for a period of a few days to several months before processing. However, the majority of tree nuts are processed in time for the Christmas trade. Processing begins with cracking the nuts in mechanical crackers and removing the shells from the kernels. The shelled nuts are sorted by electronic sorters, hand sorting, or (most usually) by a combination of these methods. Walnuts are separated into light and dark fractions, which are further sorted by hand on moving belts to remove damaged kernels. Almonds are electronically sorted for damage; breakage of the skin presents a white surface causing rejection of the nut. "Rejects" from electronic separation are sorted further by hand to remove kernels which may have been damaged by insects or which are otherwise undesirable from those which were broken mechanically. Almonds accepted by the electronic sorters are also hand sorted to remove undeisrable kernels which escaped electronic detection. Almonds which have suffered only mechanical damage are often diverted to manufacturing stock, which is made into sliced or diced products. The heavily damaged walnuts and almonds are used as oil stock to produce oil for nonedible purposes. Processors establish tolerances for damaged kernels to meet specifications for grade.

After nuts are hulled and dried, there is relatively little likelihood of mold growth and proliferation. However, *A. flavus* spores are present in almost all lots of nuts (6). Intact shells appear to protect nuts from mold (8) but damage to hull and shell may allow the mold to proliferate on the kernels while the nuts are still moist. Hence, insect damage, split shells, or sunburned hulls may result in aflatoxin production in kernels. If so, the sorting procedures used by the processors should remove aflatoxin contaminated, damaged nuts. Part of the purpose of our survey was to test the effectiveness of sorting, as done by industry, and if possible, to suggest improvements.

Walnut Survey

Analyses of in-shell walnuts are summarized in Table II. Only three 45-lb samples of the forty analyzed had aflatoxin contents higher than one ppb. The other eleven positive samples showed a slight fluorescence, but in the application of statistical analysis they were treated as zeroes. Several lots of walnuts, both in-shell and shelled, were unusual in that aflatoxin B_2 was present without B_1. Positive identification of B_2 was made in a few, but not in all cases by comparison of the mass spectrum with that of a standard sample. Possibly, aflatoxin B_1 was converted to B_{2a} or another compound not identified. Such conversion did not occur during sample preparation and analysis, since added amounts of B_1, B_2, G_1, and G_2 were always detected. Table III summarizes similar results for walnuts at various stages of sorting. Processing concentrated aflatoxin in the dark walnuts and especially in the rejects (oil stock).

Analysis of Data from the Walnut Survey

In the entire study there were so few positive samples there can be no correlation of aflatoxin positives with source, variety, time of harvest, or other variables prior to processing. The potential problem appears to be widespread geographically, but at least in one harvest year, aflatoxin contamination was not an acute problem. The sorting process, especially hand sorting, appears to be effective in removing the most highly contaminated nuts. With electronic sorting, a higher proportion of contaminated kernels is in the dark fraction. Therefore it seems that current processing is relatively effective in removing aflatoxin contamination with dark and damaged nuts. The assumption that contamination occurs at a high level in only a few nuts was supported by analysis of a lot in which a 45-lb sample

TABLE III

Aflatoxin in Contaminated Samples of Processed Walnuts

Product type	Proportion of positive samples X/n	No. of samples less than 1 ppb	Total aflatoxin (ppb)		Aflatoxin B_1 (ppb)	
			Range	Average	Range	Average
Nut meats, finished halves	7/41	6	0-12.6	0.343	0-6.3	1.0
After electronic sort-lights	4/13	4	0-<1	–	0-<1	–
After electronic sort-darks	4/12	0	0-23.7	4.15	0-8.1	2.8
Before electronic sort-shelled	4/20	1	0-7	0.628	0-5	1.2
Oil stock (rejects)	14/29	5	0-474	32.5	0-412	24.6

TABLE IV

Almond Survey Three-Year Results

Sample source[a]	$\bar{X}_L$ (ppb)[b]	$\bar{P}$[c]	$\bar{X}_{cn}$ (ppb)[d]
		($\times 10^{-5}$)	($\times 10^5$)
1972-73			
In-shell	1.28	3.78	0.337
Unsorted	3.53	2.23	1.58
Rejects	67.7	16.3	4.15
1973-74			
Rejects	69.0	26.8	2.57
1974-75 Processor A			
Manufacturing stock	4.33	1.52	2.85
Rejects (hand sort)	47.8	18.3	2.61
1974-75 Processor B			
Manufacturing stock	6.72	1.11	6.05
Rejects (hand sort)	30.4	3.92	7.76

[a]Finished whole nuts showed no samples with positive aflatoxin values. Hence $\bar{P}$, and $\bar{X}_L$ and $\bar{X}_{cn}$ could not be calculated.

[b]$\bar{X}_L$ = Average concentration per lot.

[c]$\bar{P}$ = Estimated proportion of contaminated nuts.

[d]$\bar{X}_{cn}$ = Average concentration per contaminated nut.

TABLE V

Duplicate Thirty-pound Samples of Almonds at Least One of Which Was Positive

Lot No.	Aflatoxin, ppb	
	Sample 1	Sample 2
1	10	9
2	33	0
3	163	0
4	84	143
5	130	0
6	0	84
7	41	0
8	5	0
9	0	26
10	0	9
11	26	7

assayed 474 ppb of aflatoxin. Six 50-g subsamples from the lot were all negative. Hence, a positive sample could result from the presence of only one or a few kernels containing toxin.

Almond Survey

Similar conclusions to those above were found in the initial almond survey (7). The results of that survey and the subsequent two-year samplings are shown in Table IV. The year to year variations of aflatoxin in similar samples have been relatively small, indicating little change in initial concentration or in removal during sorting. In 1974-75 a conservative estimate of aflatoxin concentration ratios was made by dividing average concentration in oil stock by the upper 95% confidence level for finished products. These ratios were 25 for Processor A and 5.3 for Processor B. The attempt during the 1974-75 crop year to correlate concentration in finished products with that in manufacturing stock or rejects taken from the same plant on the same day

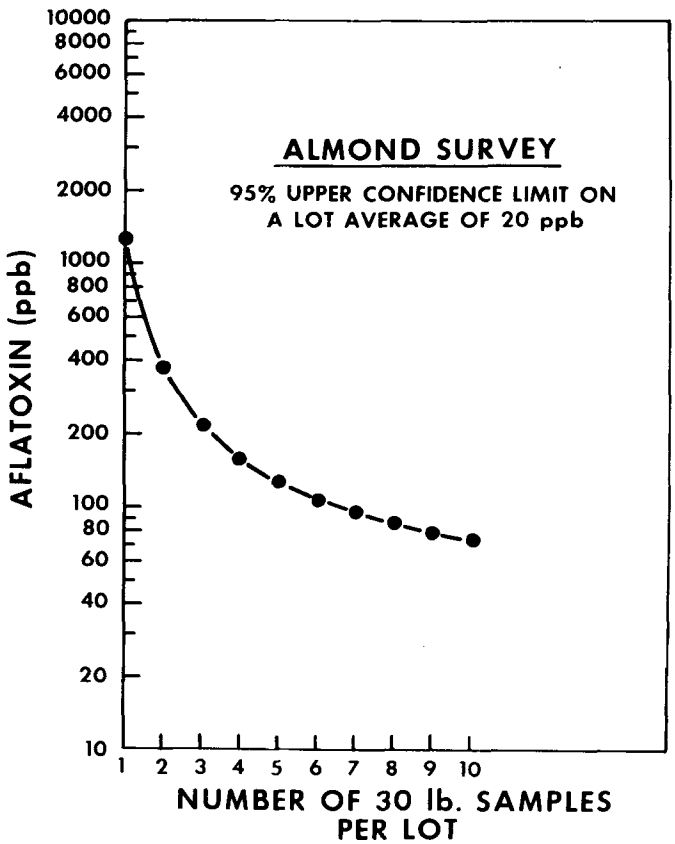

FIG. 2. 95% Confidence levels for lots of almonds in which one or more samples average 20 ppb total aflatoxin.

was unsuccessful, because there were so few positive samples in any product except oil stock. Duplicate 30-lb samples were analyzed from a number of lots. The concentration variation of samples from those lots with at least one positive is shown in Table V. Analysis of variance on these samples allowed calculation of the points in Figure 2, which expresses the upper 95% condifence limits for one to ten samples from a lot which, when analyzed, shows an average of 20 ppb aflatoxin. Thus, with one sample at 20 ppb the lot has a reasonable probability of containing less than 1200 ppb. If ten samples from the same lot average 20 ppb, the upper confidence limit is still almost 80 ppb. This uncertainty illustrates the problem of balancing cost against risk in a sampling plan.

Both Surveys

The experiments accomplished the first two objectives. We have established that aflatoxin may occur in tree nuts throughout the growing area and that, as received, an average of one walnut in 28,250 contains aflatoxin. Upper and lower 95% confidence limits for this figure are one in 10,000 and one in 146,000. The average probability of contamination in almonds is one kernel in 26,500 with upper and lower confidence limits of one in 14,700 to one in 55,300. Over a three-year period these proportions have

been fairly constant although they may be influenced by inclement weather at harvest or by changes in handling nuts before arrival at the processing plant.

It has also been demonstrated that electronic and hand sorting for damage, used by the industry, is effective in placing contaminated kernels in the inedible rejects. Data were insufficient to quantitate this effectiveness, since so few samples of finished product were aflatoxin-positive, but there is probably at least a ten-fold concentration of toxin in walnut rejects and a somewhat higher concentration in almond oil stock. Insofar as the industry maintains rigid tolerances for damage, especially in manufacturing stock, their products will meet present FDA guidelines of 20 ppb.

The third objective, an effective sampling program, is still a problem. Tree nuts are expensive; the cost to the processor for an analytical sample may exceed $1.00 per pound. Since the kernels are larger than peanuts, and occurrence of aflatoxin is less frequent, very large samples or a number of smaller samples are needed to provide assurance that a lot is aflatoxin-free. Ultraviolet sorting techniques specifically for aflatoxin do not detect all contamination since fluroescence is often quenched. Reducing aflatoxin in a lot already heavily contaminated is also probably not feasible. Stored nuts probably do not increase in toxin content with time (1), but the toxin is stable. Roasting destroys some aflatoxin, but it cannot be counted on to remove all of it (4,11), while chemical treatment with ammonia or hypochlorite (3,5) is impractical for a food product with large pieces or whole kernels. Consequently, our recommendation to industry has been to maintain rigid tolerances for damage in edible products.

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REFERENCES

1. Ashworth, L.J., Jr., J.L. McMeans, B.R. Houston, M.E. Whitten, and C.M. Brown, JAOCS 48:129 (1971).
2. AOAC Official Methods of Analysis, 12th ed. (1975) Chap. 26.
3. Gardner, H.K., Jr., S.P. Koltun, F.G. Dollear, and E.T. Rayner, JAOCS 48:70 (1971).
4. Lee, L.S., A.F. Cucullu, and L. Goldblatt, Food Technol. 22:1131 (1968).
5. Natarajan, K.R., K.C. Rhee, C.M. Cater, and K.F. Mattil, JAOCS 52:160 (1975).
6. Phillips, D.J., M. Uota, D. Monticelli, and C. Curtis, J. Amer. Soc. Hort. Sci. 101:19 (1976).
7. Schade, J.E., K. McGreevy, A.D. King, Jr., B. Mackey, and G. Fuller, Applied Microbiol., 29:48 (1975).
8. Schroeder, H.W., and J.B. Storey, Hortscience 11:53 (1976).
9. Tiemstra, P.J., JAOCS 46:667 (1969).
10. Velasco, J., J.B. Whittaker, and M.E. Whitten, Ibid. 52:191 (1975).
11. Waltking, A.E., JAOAC 54:533 (1971).
12. Whitaker, T.B., J.W. Dickens, and R.J. Monroe, JAOCS 51:214 (1974).
13. Whitaker, T.B., J.W. Dickens, R.J. Monroe, and E.H. Wiser, Ibid. 49:590 (1972).
14. Whitaker, T.B., and E.H. Wiser, ibid. 46:377 (1969).

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