Project No 95-ARS-Reduction of Aflatoxin in Manufactured Almonds by Means of Sorting

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Objectives:

1. To establish the extent and location [in terms of process streams] of aflatoxin contamination in almonds

2. To find methods of identifying unblanched almonds containing insect pinhole damage.

Results:

1. The analysis of the total distribution by grade for the 1993 crop has been computed, based on DFA and individual processor in-house data, covering 78% of the 1993 handle. Aflatoxin is concentrated virtually entirely in the natural almonds of unknown grade and in fine chopped and ground almonds. Overall aflatoxin level amounts to 0.67 ppb, or 0.17 ppb when the above mentioned process streams are removed. This material has been prepared as a manuscript which has been submitted to the J. Food Agric. Chem. A copy of this manuscript, currently in review at this journal, is submitted herewith as part of the final report.

2. Pinhole damage due to attack by small worms occurs in almonds and is nearly impossible to detect in natural almonds (unblanched). It is possible that similar to other types of insect damage, pinhole damage carries with it potential for mold introduction and toxin production. We have found that pinhole damage in almonds can be revealed in film x-ray images. An image database consisting of 522 almonds containing pinholes and 1565 control almonds has been created. This image set is being used to develop recognition algorithms. Preliminary results indicate that we can easily detect 65% of the pinhole almonds with 4% of the controls being misclassified. Other algorithms will detect approximately 90% of the infested nuts but misclassification of the controls increases to 10 to 15%. We are currently working on subroutines which will eliminate the primary cause of misclassified controls, cracks between the germ and cotyledons of the nut.

Distribution of Aflatoxin in Almonds

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Abstract

The aflatoxin levels in whole and/or broken natural almonds and in manufacturing stock almonds has been surveyed for the 1993 crop. Data was based on results for samples submitted to a non-profit analysis laboratory serving the industry as well as in-house laboratory data of several large processors. The survey thus included data from processors accounting for 78% of total almond production in 1993. The overall aflatoxin level amounted to 0.67 ng/g, of which 33% came from diced fine and ground almonds, 11% from slivered and sliced almonds, while 49% was due to natural almonds of unknown grade. However, substantially all of the latter resulted from samples submitted by a single processor. 1.7 percent of whole and/or broken natural almonds and 9.7% of manufacturing stock contained in excess of 1 ng/g aflatoxin.

Keywords: *Processing, Tree Nuts, 1993, survey, aflatoxin, DFA, Almond Board*

Running Title: Aflatoxin in Almonds

The ultimate goal of this research is to develop an on-line sorting system, which will require image capture by means of line scanning digital x-ray. We have imaged a set of almonds with insect holes using digital x-ray, using the image capture equipment currently available in this laboratory. This system was state of the art until about two years ago, when a French company offered super high resolution detectors. We find we can detect only the most gross insect damage with the present system. Accordingly, we are presently investigating the French system with almonds and plan to purchase such an array in the near future. In continued work, algorithms will be applied to such super high resolution images.

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INTRODUCTION

The mycotoxin aflatoxin is known to be a potent carcinogen (Palmgren and Hayes, 1987). The Food and Drug Administration of the U.S. (FDA) monitors domestic and imported foods and feeds for this toxin and maintains a control level of 20 ng/g total aflatoxin (Wood, 1989). Similarly, foreign governments test imported foods and demand generally even lower levels, 4 ng/g being typical. In the U.S. four commodity groups are of concern as being subject to such mold contamination: peanuts and its products, tree nuts, corn and corn products and cottonseed (the latter two as feeds). Among the tree nuts pistachios, almonds and walnuts have shown positive aflatoxin results in the FDA program (Wood, loc.cit.). Schatzki recently surveyed freshly harvested U.S. domestic pistachios (Schatzki, 1995) and also measured the aflatoxin content of various process streams of two pistachio processors (Schatzki and Pan, 1996). A correlation was found between product quality and aflatoxin content; aflatoxin content was higher for low quality product. Earlier, Schade et al. (1975) measured aflatoxin content of domestic almond process streams and obtained similar results. While the sample numbers were small, they found clear indication that sorting for quality on the basis of visible appearance concentrated the aflatoxin-containing almonds in the reject streams. As a result, whole select nuts contained no aflatoxin, sliced meats 0.2 ng/g , while diced meats (presumably produced from streams rejected based on their visible appearance) contained

12.7 ng/g on average. The work of Schade et al. had been sponsored in part by the Almond Board of California (ABC). On the basis of this work ABC initiated a survey of aflatoxin levels. 4.54 kg samples of select and manufactured almonds and oil stock (non-edible rejects) were submitted yearly by processors and analyzed for aflatoxin by DFA of California, a on-profit quality control lab serving California producers and processors. Results are shown in Table 1 (Mosebar, 1994). Select refers to a U.S. Standard Grade (USDA, 1987), a high quality almond kernel in the skin. Manufacturing stock refers to lower quality blanched, sliced and/or ground almonds, while oil stock refers to non-edible almonds sold as animal feed or processed into non-aflatoxin containing oils. Results in Table 1 through 1991 are based on fluorescence detection, capable of detecting about 5 ng/g aflatoxin; 1992 and 1993 results were obtained by HPLC, with a detection limit of at least 1 ng/g. These results suggest that high quality almonds are now essentially free of aflatoxin.

The analysis files of DFA, going back some 20 years, provide another source of information in almonds in commercial channels (aside from the very limited FDA data). DFA conducts around 1500 almond aflatoxin analyses per year at the request of processors and of buyers. A preliminary survey of the results of these analyses (Mosebar, loc. cit.) suggested that aflatoxin in commercial almonds was low, but not as low as the ABC survey suggested. Whether this difference was real and whether it might be caused by a bias in selecting samples for the ABC survey was of some concern. In any event, an independent check of the amount of aflatoxin currently in commercial channels was of interest. The present work addresses this problem.

MATERIALS AND METHODS

DFA results obtained during 1 October 1993 - 30 September 1994 were used, corresponding roughly to the 1993 crop year. During this time period DFA analyzed a total of 1547 almond samples (not including the survey samples). For each of these the following information was available: a) processor, b) in 97% of all samples, the grade or information from which the grade can be deduced, c) whether the sample was certified or not and d) the aflatoxin level. Grade descriptions and related information were taken directly from the description given by the submitter of the sample, without visual re-grading. Grades were estimated as one of 10 levels, listed in Table 2 in decreasing order of quality, the first five corresponding to USDA grades for shelled almonds (USDA, 1987), the latter five manufacturing stock in order of particle size. Processors may submit samples to monitor their operations, particularly if they have no in-house analysis labs, much in the way they submit samples for the annual ABC survey. Buyers, on the other hand, typically request certified analyses, which are not issued unless DFA does the sampling to avoid possible sampling bias (even when buyer initiated, samples are identified by producing processor). Only certified results were used. Analysis was carried out on 4.54 kg samples by grinding, homogenizing, extracting, passage through an affinity column, HPLC and post column derivatization. Only a single sample was drawn for each request, so that sampling error was not determined. For present purposes, actual aflatoxin level measured was used, although DFA reports levels below 1 μg/g as zero because of uncertainty of HPLC peak size.

In addition to the DFA data, data were obtained from several large processors not included in the DFA data base, covering in each case all their in-house almond results over the same time period. Grades were again assigned from description of the samples. Only data corresponding to samples ready for sale were used (i.e. in-house process control data was discarded). Analysis method matched that of DFA in each case. The fraction of total 1993 crop production accounted for by each processor (referred to as the "handle") was available from ABC as well. The results of all these processors were combined with the DFA results to obtain a weighted result corresponding to 78% of the total 1993 handle.

All data were entered into a spreadsheet (Lotus 1-2-3, Lotus Development Co., Cambridge, MA) and analyzed using the spreadsheet software. The DFA analyses contained results from processors having a total handle of 36%. However, the fraction of samples corresponding to a given processor generally did not match that processors's handle. In order to have the final results represent overall U.S. production it was necessary to weight the sample results. The weighting used was given by handle/(total number of certified samples from that processor). In the case of computing the average value over all certified samples this amounts to computing the average aflatoxin value for all samples from that processor and taking a weighted sum, where the weighting is the handle. When only a subset of results is computed (as e.g. the average value of all select almond samples) one should strictly use "total number of select almond samples from that processor" in the weighting factor. However, when subsets were considered the number of samples from a given processor became too small in many cases to make this approach practical. Instead, the total number of samples from a given processor was kept in the weighting. This amounts to correcting for weighting between processors, but not within processors, or, in effect, assuming that each processor had the same mix of grades within the samples submitted. Even with this simplification small sample numbers occasionally caused problems. Thus there were several relatively large processors who submitted but a few samples to DFA (and presumably had in-house facilities for the rest of their production). In this case a single high aflatoxin sample can bias the result. One such case will be pointed out below.

RESULTS AND DISCUSSION

Results are shown in Table 2. The second column gives the fraction of samples which fell into a particular grade or range of grades, weighted by each processor's handle, as described above. It is not clear whether this truly represents actual market volumes for the industry, since this breakdown is not known independently. In the next four columns the fraction of samples within each grade which fall into aflatoxin ranges of interest is shown, followed by the average aflatoxin value for that grade (both subject to the described weighting). Cursory inspection of Table 2 shows that aflatoxin contamination level and frequency increases with decreasing quality. With the exception of the natural material of unknown grade, most of the aflatoxin is contained in the ground product. Since ground product is generally produced from kernels showing major damage due to various causes, it appears that aflatoxin content and damage are related. This result is in agreement with the results of Schade et al. (1975). When the 1993 results of Table 1 are compared with Table 2 some differences are noted. In the latter 1.0% of Select and 1.7% of all natural nuts exceed 1 ng/g aflatoxin, rather than 0%, while 9.7% of manufacturing stock exceeds that level rather than 2.5%. Neither the Select nut nor the natural nut difference is statistically significant, although in the case of the manufacturing stock the difference is significant at the p=10% level. It thus appears that there may have been some bias by producers in choosing nuts to submit for the ABC survey, although the effect is not large. To prove bias would take a much larger number of samples.

An exception to the general pattern is seen in the product which could only be identified as natural almonds of uncertain grade. The high aflatoxin average is due to a single sample, which was one of but eight samples submitted by a medium size processor. Elimination of this sample would drop the average for that grade down from 2.97 to 0.26 ng/g, for all natural almonds down to 0.07 ng/g and for all samples down to 0.37 ng/g. There is, however, no basis for dropping this sample from the data base. Five of the eight samples submitted by the processor in question tested positive (in the range of 2-63 ng/g), a far higher fraction than that of other processors. Further, it is specifically known that these samples were being offered for sale. While a lot testing above the allowable level would clearly be withdrawn from sale, the reported values are taken as indicative of the mean level in that type of lot as aflatoxin measurement in almonds is dominated by sampling error (Schade et al. 1975). One is forced to conclude that at least at that time period this processor had a serious aflatoxin problem and appears to have accounted for one half of all the aflatoxin in almonds offered for sale. Inspection of the data showed that there were no other cases where unusual results were obtained from a single or a few samples.

While it is possible to estimate the variance of the data presented, such variances would be very large in most cases because of the small number of samples for a given quality level. Furthermore, even with the explicit assumption, used here, that all samples for a particular grade can be lumped as samples from a combined lot comprising the product of all processors, this sampling is clearly not random. The results presented here can only be viewed as the best industry-wide average which can be obtained from the available data. A better result would require actual sampling from all sold material.

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Reference to a company or product name does not imply approval or recommendation of that product by the U.S. Department of Agriculture to the exclusion of others that might be suitable.

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Table 1. Almond Aflatoxin Survey: Number of positive[a] samples/total number of samples analyzed. From Mosebar (1994)

Crop Year	Select Nuts	Manufacturing Stock	Oil Stock
1973	NS[b]	11/50	NS
1974	0/34	7/50	22/34[c]
1975	NS	13/100	16/16
1976	NS	7/55	30/51[c]
1977	1/100	1/40	10/10
1978	5/100	2/41	10/10
1979	1/100	2/40	10/10
1980	2/100	1/41	10/10
1981	3/114	2/41	10/10
1982	4/93	2/41	6/9
1983	3/100	1/40	9/10
1984	2/107	1/40	8/13
1985	0/104	2/36	2/2
1986	0/95	3/44	6/7
1987	0/105	0/38	9/10
1988	1/97	3/44	19/21
1989	2/130	2/36	8/8
1990	3/98	1/40	9/11
1991	1/99	3/40	3/4
1992	0/100	3/40	10/10
1993	0/100	1/40	5/5

[a] 1973-1991: Positive = approximately ≥ 5 ng/g total aflatoxin.
1992-1993: Positive = approximately ≥ 1 ng/g total aflatoxin.

[b] NS: not sampled [c] Reject nuts, all others press cake meal.

Table 2. **Almonds: Percent[a] of Samples by Grade and Aflatoxin Level**
(Weighted by production of each submitting processor/
total number of samples from that processor in the data base)

Grade	Part of Total	> 0 ng/g	≥ 1 ng/g	≥ 4 ng/g	≥ 20 ng/g	Average ng/g
In Shell	1	0	0	0	0	0
Extra #1	2	2.6	2.6	0	0	0.04
Superior[b]	17	0.9	0.8	0.1	0	0.01
SSR[c]	15	1.0	1.0	0.1	0	0.00
Wh. & brkn., natural	1	0	0	0	0	0
Ungraded, natural	11	9.4	5.5	5.2	4.6	2.97
Total, whole and/or brkn., natural	46	2.7	1.7	1.3	1.0	0.72
Whole, blanched	4	8.9	8.9	3.1	0.2	0.33
Wh. & brk., blanched	2	0.4	0.4	0	0	0.01
Slivered[d]	31	5.4	4.1	1.7	0.2	0.23
Diced, coarse	6	8.7	7.5	1.4	0.1	0.29
Ground[e]	7	48.6	47.5	28.3	3.0	3.13
Total, Manufact.	51	10.7	9.7	4.8	0.4	0.62
Ungraded	3	18.3	18.3	0.3	0	0.64
All samples	100	7.2	6.3	3.0	0.7	0.67

[a] All but last column
[b] Includes Select
[c] Select or Standard Sheller Run
[d] Includes Sliced, all thicknesses
[e] Includes Diced, fine