76-N3

ANNUAL REPORT

TITLE: Almond Diseases (76-N3)
Aflatoxin Analytical Methods and Monitoring Programs

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1. Objectives:

Project 76-N3 has four objectives: (1) to obtain official status for the minicolumn method by conducting a collaborative study with the AOAC, (2) to participate in an aflatoxin analytical check program in cooperation with USDA and FDA, (3) to monitor almonds and almond products for aflatoxin, and (4) to work with USDA Market Quality Research on a concealed damage project.

2. Interpretive Summary:

There is a very high positive correlation between certain visible damage to almond kernels and the incidence of aflatoxin. Research has shown that almonds which have visible evidence of navel orange-worm damage in the field are ten times more likely to have A. flavus contamination than undamaged nuts. Consequently, removal of insect and mold damaged kernels, by sorting procedures (electronic and hand sorting), can place over 90 percent of contaminated kernels into inedible oil stock. The balance will be in the lower grade manufacturing stock. Thus, careful sorting and maintenance of strict removal practices for damaged kernels are essential to the prevention of aflatoxin in shipments of almonds.

Detection of aflatoxin is extremely difficult. Since in contaminated lots, an average of one almond in 66 pounds contains aflatoxin, sample sizes on most shipping lots should be considerably larger than 66 pounds, to have a better than 50-50 chance of finding the toxin. However, because of the high cost of almond samples, FDA checks 30 pound samples and rejects those lots in which the 30 pound sample has more than 20 parts per billion of aflatoxin. The almond industry should follow the FDA sampling procedures. Even then handlers must recognize the statistical uncertainty and realize that the recommended control program is not a guarantee that all products are in compliance.

Removal of mold and insect damaged kernels should be strictly maintained for whole finished almonds. Where this is done, monitoring whole almonds, other than an occasional spot check, is probably unnecessary.

Aflatoxin monitoring should be concentrated on manufacturing stock, for two reasons. First, the normal tolerances for damage tend to be greater and secondly, when almonds are sliced or diced, the aflatoxin may be spread through the lot. Thus samples from final sliced or diced almonds are more likely to reveal aflatoxin to users or FDA, if it is present. If a lot is found to be contaminated after slicing or dicing, it may not be possible to further sort or process to remove the contamination. Hence, prior to usage handlers should monitor their manufacturing stock by frequent sampling and analyses to determine insofar as possible, that it will meet FDA guidelines.

3. Experimental Procedure:

Sampling for aflatoxin was done according to the following schedule:

Sample Schedule - Whole Almond Meats

Lot Size (Number of 100# bags, 50# containers, etc.)	Sub Sample Size	Total Sample Size
10-100	3 lb. taken from 10 cont.	30 lbs.
Over 100 to 200	1-1/2 lb. taken from 20 cont.	30 lbs.
Over 200 to 500	1 lb. taken from 30 cont.	30 lbs.
Over 500 to 1,000	3/4 lb. taken from 40 cont.	30 lbs.
Over 1,000	1/2 lb. taken from 60 cont.	30 lbs.

The 30 lb. samples were thoroughly ground and mixed in a Hobart vertical cutter mixer. The aflatoxin analysis was done according to Chapter 26 of the Official Methods of Analysis of the Association of Official Analytical Chemists (26.AO1-26.A08)
Two analytical procedures were used for the concealed damage project: 1. Free fatty acid values of the almonds will be determined as an index of quality (rancidity) 2. Roasting of the almonds will be done to determine the % black kernels which is also an index of concealed damage. The free fatty acid analysis was done according to Chapter 16 of the Official Methods of Analysis of the Association of Official Analytical Chemists (16.193-16.195).

The roasting procedure was done according to suggestions given by the California Almond Growers Exchange:

1. A 6 quart deep fat fryer with a screen and adjustable thermostat was purchased.

2. Oil was pre-heated to 350° F.

3. The almonds (100 kernels to 1 or 2 pounds) were covered with oil and roasted for 2 to 3 minutes.

4. Then the almonds were removed, drained and placed on paper toweling to cool. The almonds continue to "cook" after they are removed from the oil.

5. The black nuts are then counted and the % concealed damage is thus determined.

4. Results:

Plan #1:

AOAC collaborative study on minicolumn - On November 18 letters to 21 potential collaborators were sent throughout the United States asking for their participation in an AOAC sanctioned collaborative study of the minicolumn screening method for the detection of aflatoxin in almonds. Return letters indicate that all laboratories but one will participate. Almond samples containing differing levels of aflatoxin have been sent to FDA New Orleans for processing to almond paste. Samples will be sent out shortly after the New Year.

Plan #2:

Aflatoxin analytical check program - This is an ongoing project. The last sample analyzed by this laboratory was found to contain 31 ppb. The mean of the analytical results was 36.8 ppb and the range was 25-52 ppb. Twenty-three laboratories participated in the check analysis.

Plan #3:

Aflatoxin monitoring program (51 samples of reject material and 51 samples of manufacturing stock). Fifty-three samples have been processed. Twenty-four have been reject material and twenty-nine have been manufacturing stock. Six positive samples have been found and they were all reject material. The range was 14-171 ppb. Only one was under the FDA guideline of 20 ppb.

15 tests on almond hulls - Twelve samples have been processed and all were negative for aflatoxin.

15 tests on almond press-cake meal - Fifteen samples have been processed and all were positive for aflatoxin. The range was 19-129 ppb. Fourteen were over the FDA guideline of 20 ppb.

13 samples of almonds with soil and irrigation conditions (in cooperation with WRRL) - thirteen samples were processed and four were positive. Range was 26-130 ppb. Two were over the FDA guideline.

5 samples of rained on and dry nuts - Five were processed and all were negative for aflatoxin.

Plan #4:

Concealed damage project - 50 aflatoxin analysis - Fifty were processed and 10 were positive. Range 30-20,000 ppb (approx.).

50 free fatty acid analyses - None complete. Received 12/23/76.

50 roasting tests - None complete. Received 12/23/76.

Final compilation of last years (75-N) 100 sample program. Samples were manufacturing stock. Thirteen percent (13/100) were positive. Range 10-83 ppb. Six were over FDA guideline of 20 ppb. Breakdown was:

whole nuts	(positive)	- 8
diced nuts	(positive)	- 4
sliced nuts	(positive)	- 1

5. Discussion:

The industry is effectively concentrating the aflatoxin in the reject material. Manufacturing stock should be monitored as it generally runs 13% positive. Diced and sliced almonds should be especially well checked. Blanched whole almonds are less of a problem. Top quality almonds very rarely contain aflatoxin. Almond hulls did not have any aflatoxin but such a small number of samples were analyzed, no conclusions should be drawn. Aspergillus flavus is grown on almond hulls regularly in the laboratory. Almond press-cake meal contains quite a bit of aflatoxin and should be ammoniated before being added to cattle feed as is cotton-seed meal.

Future work could center on: 1. Modifications of the mini-column procedure (which is already a reality) to make it faster and cheaper. 2. Quantitation of the aflatoxins by High Pressure Liquid Chromatography. 3. A reanalysis of hull samples for aflatoxin. 4. A search for the cause of concealed damage. 5. More detailed work on the effects of roasting and blanching on almonds. 6. Testing the effects of ammoniation on almond press-cake meal.

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