Project No. 80-W7 (Continuation of Project No. 79-X6)

<u>Cooperator:</u>

USDA/SEA Western Regional Research Center 800 Buchanan Street Berkeley, California 94710

Project Leaders: Dr. Glenn Fuller

Dr. Douglas King

Phone (415) 486-3379 or 486-3657 Phone (415) 486-3540

Project: Almond Diseases Mycotoxins and Consulting Work

<u>Objectives</u>: To develop a sampling plan for accurate estimation of the aflatoxin content of manufactured cut almonds (diced, sliced).

<u>Progress</u>: Most aflatoxins in almonds appear in the manufacturing stock and reject materials because processing removes them from whole almonds. Thus a sampling plan is needed to accurately estimate the amount of aflatoxin in manufactured nutmeats. Adequate analytical techniques exist.

The sampling plan is a multi-step plan where samples are drawn and analyzed sequentially. If in the first sample aflatoxin content is too high the lot is rejected; if low enough the lot is accepted. If the analysis shows an intermediate aflatoxin content, sampling and analysis are repeated until a decision is reached to either accept or reject the lot. This type of sampling plan results in accurate, fast, and less expensive sampling to get reliable answers to the lot quality.

<u>Plans</u>: (1) To refine and publish methodology for sampling based on the analysis of the products we had last year; (2) to continue work to develop instrumental techniques for detecting contamination in individual nuts. The approach will be to grow <u>A. flavus</u> and <u>A. parasiticus</u> molds on almonds to obtain nuts contaminated at high level. These will be examined using a spectrofluorometer on both blanched and unblanched nuts. The object will be to obtain an instrumental method to "see" aflatoxin fluorescence where a visual method fails.

Almond Industry Participation

\$1,000



UNITED STATES DEPARTMENT OF AGRICULTURE SCIENCE AND EDUCATION ADMINISTRATION

AGRICULTURAL RESEARCH WESTERN REGION WESTERN REGIONAL RESEARCH CENTER 800 BUCHANAN STREET BERKELEY, CALIFORNIA 94710

Identifying Molds on Food Products

Isolating, identifying and counting molds from foods, such as almonds, is often complicated by overgrowth of some colonies in the petri plate by rapidly growing spreading molds. These molds such as <u>Rhizopus</u> and <u>Botrytis</u> species produce woolly or cottony colonies that fill the plate and obscure the majority of the colonies that are slower growing and which form low colonies with only a small amount of aerial growth. These smaller colonies such as <u>Alternaria</u>, <u>Aspergillus</u>, <u>Cladosporium</u> and <u>Penicillium</u> species are frequently the molds of interest since they cause spoilage and can produce mycotoxins such as aflatoxin, citrinin, etc.

We have developed a medium that was designed to inhibit the rapidly growing molds yet still let them form small colonies and at the same time allow development of the remaining mold flora. This medium consists of a nutrient basal medium at a pH that allows optimum growth plus added inhibitors. Chlortetracycline is added to inhibit bacterial growth. Rose bengal and dichloran inhibit the proliferation of the spreading molds while influencing the remaining molds only slightly when added at the appropriate concentrations.

Sterilized agar is poured into petri plates and allowed to solidify. Then the inoculum is added to the surface (0.1 ml) and spread evenly. After 4-5 days at 25°C (77°F) the plates can be examined and individual colonies selected for isolation.

The medium formulation is:

Glucose Peptone Magnesium	sulfate heptahydrate	MgS04·7H20	10.0 g 5.0 g 0.5 g
Potassium Agar	phosphate, monobasic	^{KH} 2 ^{P0} 4	1.0 g 15.0 g
Distilled	water		11

Final pH 5.6

Rose bengal (certified) is made up in water 0.5 g/20 m water and 1 ml of this stock solution is added per liter of medium (25 ppm rose bengal final concentration).

Dichloran (2,6-dichloro-4-nitroaniline) is made up in ethyl alcohol 0.02 g/10 ml and 1 ml added per liter for a final concentration of 2 ppm. (Refrigerate to prevent alcohol loss from stock solution). The medium is sterilized 15 minutes at 15 PSIG and a chlortetracycline added to the medium cooled to 50°C. Chlortetracycline stock solution (keep in refrigerator) is prepared by dissolving 0.1 g/100 ml water and filter sterilizing. This sterile solution is added 1 ml/100 ml of medium for a final concentration of 10 ppm.

Sources of the specialized reagents are:

Peptone - Difco, Detroit, or BBL Microbiology Systems, Cockeysville, MD

Rose Bengal - Eastman Organic Chemicals, Rochester, NY

Tetracycline, hydrochloride - Calbiochem-Behring, La Jolla, CA

Dichloran - Aldrich Chemical Co., Milwaukee, WI

Reference:

King, A. Douglas, Jr., Ailsa D. Hocking, and John I. Pitt. Dichloran-Rose Bengal Medium for Enumeration and Isolation of Molds from Foods. Appl. & Environ. Microbiol. 37:959-964, 1979.

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A. Douglas King, Jr. Glenn Fuller

Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.