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EFFECT OF FOOD PRESERVATIVES ON FUNGAL INTERACTION AND AFLATOXIN PRODUCTION

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EFFECT OF FOOD PRESERVATIVES ON FUNGAL INTERACTION AND AFLATOXIN PRODUCTION

BY

SHI-JENQ LEE

A DISSERTATION

Presented to the Faculty of

The graduate College in the University of Nebraska

In partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Major: Food Science and Technology

Under the Supervision of Professor Lloyd B. Bullerman

Lincoln, Nebraska

May, 1986

TITLE

Effect of Food Preservatives on Fungal Interaction and Aflatoxin Production

BY

Lee, Shi-Jeng

APPROVED	DATE
Dr. Lloyd B. Bullerman	3/26/86
Dr. R. Burt Maxcy	3/26/86
Dr. Khem M. Shahani	3/26/86
Dr. James Van Etten	3/26/86

SUPERVISORY COMMITTEE

GRADUATE COLLEGE **UNIVERSITY OF NEBRASKA**

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EFFECTS OF FOOD PRESERVATIVES ON FUNGAL INTERACTION AND AFLATOXIN PRODUCTION

SHI-JENQ LEE, Ph.D.

University of Nebraska, 1986

Advisor: Lloyd B. Bullerman

The effectiveness of potassium sorbate as a preservative for stored corn at 18, 24, and 30% moisture contents was studied. Growth and aflatoxin production by Aspergillus parasiticus decreased with increasing levels of sorbate treatment. The sorbate was more effective on corn with lower moisture contents and in sealed containers. The effect of potassium sorbate on growth and aflatoxin production by aflatoxin producing molds was studied in the presence of selected competing molds in yeast extract sucrose (YES) broth which were separated by a dialysis membrane (MWCO 12,000). Growth of competing molds reduced growth and aflatoxin production by aflatoxin producing molds. The colonizing ability of aflatoxin producing molds in the presence of competing molds was studied on the surface of corn meal agar or corn kernels. Aspergillus flavus was more competitive on damaged corn kernels with 18% moisture than on undamaged kernels with 26% moisture. Aspergillus niger was the competing mold most effective in reducing growth and aflatoxin production by aflatoxin

producing molds in YES broth with or without sorbate treatment. The presence of A. niger and sorbate had an additive effect in reducing aflatoxin production. A. niger was most competitive in YES broth containing 0.25% yeast extract and 20% sucrose. Neither of the aflatoxin producing molds were competitive in glucose ammonium nitrate broth in all compositions studied. Potassium sorbate, calcium propionate, and natamycin were applied to non-sterilized corn kernels containing inoculated spores of A. flavus . All treatments increased the number of corn kernels dominated by A. flavus . Aflatoxin production was reduced by potassium sorbate and calcium propionate at the 0.4% level. When A. flavus and A. niger were inoculated on autoclaved corn, only treatment with potassium sorbate reduced the number of kernels dominated by A. flavus . Aflatoxin production was reduced by 0.2 and 0.4% sorbate, and 0.4% calcium propionate.

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GENERAL INTRODUCTION

Fungal growth on stored cereal grains causes loss of grain quality, and the mycotoxins produced by fungi, are often toxic to humans and animals when consumed. Aflatoxins are a group of bis-furano-isocoumarin derivatives produced by toxigenic strains of Aspergillus flavus and Aspergillus parasiticus . Among the derivatives of aflatoxin, B1 and G1 are likely to be found in cereal grains and in quantities sufficient to be toxic. All cereal grains, with the exception of soybeans, are potential substrates for aflatoxin production. Drying is the most common method used to reduce water activity of cereal grains to levels which prevent fungal growth and toxin production during storage. The increasing cost of energy, however, may make the drying of cereal grains less economically feasible. Alternatives such as controlled atmosphere, irradiation, acid fermentation, and chemical preservatives are used when energy costs are high. Use of chemical preservatives requires less capital input and can be applied quickly if needed. Propionic acid and acetic acid are the two most commonly used preservatives for stored high moisture grains. The corrosive nature of these acids is the major drawback if the surface of the storage bin has not been painted. Sorbic acid or sorbates (sodium or potassium salts of sorbic acid) are effective in retarding

microbial growth in many foods. They are safe to use, non-corrosive and contribute no taste or odor to foods. Sorbic acid and sorbate may have potential to preserve high moisture grains.

Cereal grains are often contaminated with a mixed flora of microorganisms. Microbial competition usually results in less aflatoxin production on such agricultural products contaminated with a mixed microflora. Application of chemical preservatives on cereal grains can reduce microbial competition by retarding the growth of more sensitive microorganisms. Since the chemical is applied on the surface of the cereal grains, the effectiveness is directly related to the evenness of distribution of the chemicals. The more resistant microorganisms will initiate growth and eventually spoil the entire lot when there is less competition. The growth of aflatoxin producing molds under such conditions would be most undesirable. The effect of chemical preservatives on aflatoxin producing molds in the presence of other microorganism is therfore very important for us to understand when evaluating use of chemical preservatives on stored cereal grains.

The general objective of this study was to evaluate potassium sorbate as a preservative for high moisture corn storage within the context of microbial competition. The

specific objectives were: 1). To evaluate the effectiveness of potassium sorbate on growth and aflatoxin production by Aspergillus parasiticus on high moisture corn; 2). To develop proper methods for studying effects of sorbate on mixed fungal populations; 3). To study the effects of growth of Aspergillus niger on growth and aflatoxin production by Aspergillus parasiticus and Aspergillus flavus in different substrates; 4). To evaluate the effects of subinhibitory levels of sorbate and other mold inhibitors on mold development and aflatoxin production on corn contaminated with a mixed microflora.

SECTION I. REVIEW OF LITERATURE

REVIEW of LITERATURE

AFLATOXIN

History.

Discovery of aflatoxin was initiated by an outbreak of"Turkey X disease"in 1960 when more than 100,000 young turkeys died within a few months on poultry farms in the south and east of England (Blount, 1961). It is now known that this toxin also affects many other farm animals. The culture, isolated from a toxic sample of peanuts heavily contaminated with fungi, was later identified as Aspergillus flavus Link ex Fries. A toxic compound produced by this mold was purified by paper chromatography having a Rf value of 0.7 and emitting fluorescence under ultra violet (U.V.) illumination. This toxic compound was later given the name "aflatoxin" in view of its origin (Sargeant et al., 1961).

Chemistry.

Aflatoxin was later shown to be a group of structurally related compounds (Hartley et al., 1963). The structures of some of these toxins and their derivatives are given in Figure 1. Among these, B1, B2, G1, and G2 are naturally synthesized by aflatoxin-producing strains of Aspergillus flavus and Aspergillus parasiticus . All these toxins contain a

Figure 1. The structure of four major aflatoxins and derivatives.