APPLIED MICROBIOLOGY, Nov. 1969, p. 714-717

Vol. 18, No. 5
Printed in U.S.A.

Aflatoxin Production in Meats

I. Stored Meats[1]

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Received for publication 11 August 1969

Aflatoxins were produced on fresh beef (in which bacterial spoilage was delayed with antibiotics), ham, and bacon inoculated with toxinogenic fungi and stored at 15, 20 and 30 C. Meats stored at 10 C were spoiled by bacteria and yeast before detectable levels of aflatoxins were produced. High levels of aflatoxins were formed in meats stored at 20 C; one sample supported the production of 630 μg of aflatoxins per g of meat, the major portion (580 μg) of which was aflatoxin G_1. Meats stored below 30 C developed higher levels of aflatoxin G_1 than B_1, but at 30 C *Aspergillus flavus* produced equal amounts of B_1 and G_1, whereas *A. parasiticus* continued to produce more G_1 than B_1.

Aflatoxins are metabolites of *Aspergillus flavus* Link and *A. parasiticus* Speare (1, 5, 10). The compounds are both toxic and carcinogenic to a wide range of animals (16). A recent review by Ciegler and Lillehoj (4), complete with bibliography, deals extensively with chemical properties, production conditions, and biological effects of the aflatoxins.

A number of investigators have studied the formation of aflatoxins on human food. Lie and Marth (8, 9) observed aflatoxin production on Cheddar cheese and casein, which had been inoculated with *A. flavus* and *A. parasiticus*. Frank (7) studied the production and diffusion of aflatoxins in apple juice, rye and wheat breads, and Tilsit cheese by using a strain of *A. flavus* isolated from food. Wildman et al. (15) inoculated a large number of sterilized and nonsterilized solid foods and fruit juices with *A. flavus* and obtained aflatoxins. Sterilized beef infusion and beef pieces supported yields of 15 and 11 μg of aflatoxin per g of meat, respectively, but no aflatoxins were produced on raw beef because of bacterial overgrowth. Frank (6) obtained aflatoxins from *A. flavus* grown on a large number of foods including smoked bacon, condensed and powdered milk, and egg noodles.

Molds capable of producing aflatoxins are occasional contaminants of foods. Van Walbeek et al. (14) found that 16 of 128 fungi isolated from 74 food samples produced toxins when cultured on complex media and on shredded wheat. Molds often grow on meats, especially cured meats during storage or aging. Some of these molds have toxinogenic potential. In a recent study in our laboratory (2), a strain of *A. flavus* isolated from cured meat was found capable of producing aflatoxins. The present study was undertaken to determine the quantities of aflatoxins produced on fresh and cured meats during storage at various temperatures.

MATERIALS AND METHODS

The effects of type of meat, strain of mold, storage temperature, length of storage, and number of mold spores present were investigated. The meats studied were beef, smoked ham, and smoked bacon. The use of beef was intended to simulate beef undergoing aging. To keep bacterial contamination at a minimum, the beef was obtained as one large piece (beef round, ca. 4.5 kg) and ground with the coarse cutting blade of a sterilized grinder. To further prevent bacterial overgrowth, the following antibiotics were thoroughly mixed with the beef: chlorotetracycline, 15 μg/g of meat; polymyxin B, 20 units/g of meat; penicillin G, 0.5 units/g of meat. The meat-antibiotics mixture was stored overnight at 2 to 3 C to allow the antibiotics to become evenly distributed. The meat was then dispensed in 100-g portions into sterile pint Mason jars (470 ml) closed with sterilized Mason lid assemblies modified to contain two sheets of Whatman no. 1 filter paper (diameter 7.0 cm) in place of the regular flat portion of the lid.

The ham used in this study was freshly produced, boneless, smoked ham. The ham was cut into small pieces (ca. 1- to 2-cm cubes) and dispensed in 100-g portions into sterile pint Mason jars, and the jars were closed as previously described. Care was taken

[1] Journal paper no. J-6336 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, project no. 1264.

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to use good sanitation procedures and to avoid gross contamination of the meat during this procedure.

The bacon used in this study was thick-sliced bacon which was cut across the slice to make slices of about 6-cm lengths. A sufficient number of slices was used to make a sample size of 100 g. A procedure similar to that described for hams was used in placing the bacon samples in sterile pint Mason jars.

The toxic mold strains used were *A. parasiticus* Speare CMI 15957 (Commonwealth Mycological Institute Culture Collection, Surrey, England), *A. flavus* Link NRRL 2999 (Northern Regional Research Laboratory, U.S. Department of Agriculture Culture Collection, Peoria, Ill.), and *A. flavus* NRRL A-16100 (2).

Times and temperatures of storage were 28 and 56 days at 10 C, 14 and 28 days at 15 C, 7 and 14 days at 20 C, and 7 days at 30 C.

Conidia used for inocula were produced by growing the individual molds at 25 to 30 C for 7 to 10 days on thin layers of potato dextrose agar. The conidia were harvested and washed by using a sterile 0.05% Tween 80 (Mann Research Laboratories, Inc., New York, N.Y.) solution and then suspended in 100 ml of sterile Tween 80 solution. The spores were quantitated by using a Petroff-Hauser bacterial counting chamber, and portions of each spore suspension were diluted to obtain levels of 10^3 and 10^6 conidia per ml. The meat samples were inoculated with 1 ml of inoculum to obtain spore levels of 10^2 and 10^6 conidia per 100 g of meat for each type of meat at each storage temperature and time.

The molds used on meats were also inoculated into 50-g portions of sterile rice, at spore levels of 10^2 and 10^6 spores per 50 g of rice, by using methods for growth, extraction, and analysis of aflatoxins similar to those of Sorenson et al. (13). These cultures were incubated at each of the temperatures and for the longest times used with the meats and were used as controls.

After the proper storage time had elapsed, samples were placed in a freezer at −18 C until they could be analyzed. Mold growth was estimated visually. Since the growth of species of *Aspergillus* was readily distinguishable by characteristic color and appearance, distinction was made between the growth of the aspergilli and that of other fungi.

The aflatoxins were extracted from both the meats and rice with chloroform and then quantitated by visual comparison with aflatoxin standards on thin-layer chromatography plates by the method of Bullerman et al. (3).

RESULTS AND DISCUSSION

No aflatoxins were detected in any of the meat samples stored at 10 C. Yet, *A. flavus* NRRL 2999 produced 6 μg/g each of aflatoxins B_1 and G_1 when grown in pure culture on rice at 10 C for 56 days. Other workers (12, 13) have observed trace amounts of aflatoxin production at 10 to 11 C in pure cultures of *A. flavus* grown on rice. With the long incubation periods required for the growth of *A. flavus* and *A. parasiticus* at 10 C, bacteria and yeasts naturally present on the meats tended to overgrow the toxic molds. These data indicate that meats stored at 10 C or less would become spoiled by the growth of bacteria and yeasts before they would become toxic as the result of growth and toxin production by *A. flavus* or *A. parasiticus*.

The highest levels of total aflatoxins detected (41 μg/g) at 15 C were in beef inoculated with 10^6 spores of *A. parasiticus* and stored for 28 days (Fig. 1). Low levels of aflatoxin (0.12 to 0.36 μg/kg) were produced by all three organisms on ham and bacon stored for 14 days and inoculated with either 10^2 or 10^6 conidia/100 g of meat. No aflatoxins were detected in beef inoc-

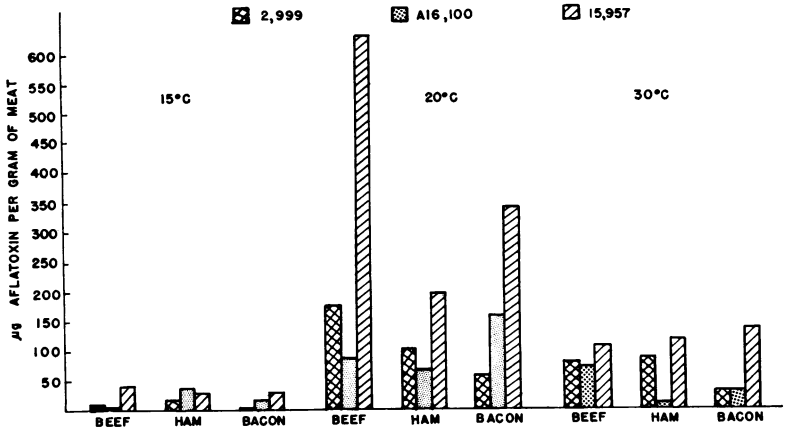

FIG. 1. *Amounts of aflatoxins (μg/g) observed on meats inoculated with 10^6 conidia of A. flavus NRRL 2999, A. flavus NRRL A-16100, or A. parasiticus CMI 15957 and stored at 15 C (28 days), 20 C (14 days), and 30 C (7 days). The aflatoxin levels represent an average of four samples.*

ulated with 10^2 spores of *A. flavus* and incubated for 14 days, whereas, under these same conditions *A. parasiticus* produced 0.05 μg of both aflatoxins B_1 and G_1 per g, even though there was no visible growth of the organism. When beef samples were inoculated with 10^2 spores and stored for 28 days at 15 C, neither *A. flavus* nor *A. parasiticus* produced detectable amounts of aflatoxin.

The high levels of aflatoxin produced by *A. parasiticus* consisted primarily of aflatoxin G_1. The *A. flavus* strains also produced more aflatoxin G_1 than B_1 at 15 C. Also, the accumulation of G_1 over B_1 was greater on beef than on either ham or bacon.

Although the highest level of aflatoxin production at 15 C was by *A. parasiticus* CMI 15957 on beef, the two *A. flavus* strains produced more toxin on ham than on beef (Fig. 1). *Aspergillus flavus* NRRL 2999 produced its lowest level of aflatoxin on bacon, whereas *A. flavus* NRRL A-16100 produced its lowest level of aflatoxin on beef.

The growth of the toxic strains at 15 C was greatest on ham inoculated with 10^6 conidia per 100 g of meat and stored for 28 days. Bacon supported the least mold growth, with the growth confined to the lean portion. Members of the penicillia were the most prevalent competing organisms on ham and bacon. A *Rhizopus* species grew profusely on the beef and covered much of the meat surface. Colonies of *Aspergillus* species could be seen growing, intermixed with the black mold. Samples stored for 28 days at 15 C supported greater mold growth than samples stored for 14 days. This was also generally true for aflatoxin production, with highest levels occurring with longer storage time. The toxic molds were better able to compete with the other organisms with an inoculum of 10^6 condidia per 100 g of meat than with an inoculum of 10^2 conidia per 100 g of meat, and toxin levels were higher with the larger inoculum.

All meats stored at 20 C contained aflatoxins. *A. parasiticus* CMI 15957 produced more aflatoxins than did the *A. flavus* strains on every type of meat studied at this temperature. Maximal amounts of aflatoxins produced by *A. parasiticus* were 630 μg/g of beef, 340 μg/g of bacon, and 190 μg/g of ham, when samples were inoculated with 10^6 conidia/100 g and stored for 14 days. *A. parasiticus* CMI 15957 and *A. flavus* NRRL A-16100 produced more aflatoxins on beef and bacon than on ham; *A. flavus* NRRL 2999 produced more aflatoxins on beef than on ham and bacon (Fig. 1).

At 20 C, *A. parasiticus* CMI 15957 produced more aflatoxin G_1 than B_1 in every instance. In most of the samples, the two *A. flavus* strains also produced more G_1 than B_1. Higher levels of aflatoxins were produced in 14 days than in 7 days of storage; however, there did not appear to be any substantial increase in growth at the longer incubation time. The larger inoculum usually resulted in more growth and higher levels of aflatoxins on all the meats than the smaller inoculum.

At 30 C, abundant to profuse growth of the toxic mold strains occurred in most of the meat samples. The growth was equal to or greater than that observed at 20 C, but toxin production was lower than that found at 20 C (Fig. 1). At 30 C, *A. parasiticus* produced consistently higher amounts of aflatoxins than either of the *A. flavus* strains on all the meats. *A. parasiticus* produced 135 μg/g on bacon; this was the largest amount of aflatoxin produced on any of the substrates at 30 C, but bacon supported the least amount of toxic mold growth of the three meats studied. *A. flavus* NRRL 2999 produced more aflatoxins on ham than on any other substrate, and *A. flavus* NRRL A-16100 produced the most aflatoxin on beef. Strain A-16100 grew less and produced less aflatoxins overall than either of the other two strains (Fig. 1).

The ratio of the aflatoxin B_1 to G_1 was quite different at 30 C than at the lower temperatures. With the *A. flavus* strains, the relative amounts of the two toxins were approximately equal, although, with strain A-16100, G_1 tended to predominate slightly on beef and bacon. In some instances, with strain 2999, B_1 slightly predominated; in others G_1 predominated. With *A. parasiticus*, G_1 predominated on all meats studied; however, the degree of predominance of G_1 was less at this temperature than at lower temperatures. These data clearly show the effects of temperature on the ratio of B_1 to G_1; although less total toxin was detected at 30 C, meat samples stored at this temperature may be more toxic than meat samples stored at lower temperatures because of a higher proportion of aflatoxin B_1 in these meats.

The 10^6 inoculum level resulted in more mold growth and greater production of aflatoxin than did the 10^2 inoculum level. When the 10^6 inoculum level was used, no fungal growth other than the *Aspergillus* species could be detected at 30 C, but, at the 10^2-inoculum level, presence of other fungi was observed although *Aspergillus* species still predominated. Of the four temperatures studied, the optimal temperature for aflatoxin production by all three organisms growing on meats was 20 C. However, growth of all three organisms was better at 30 C than at 20 C. Other workers have found optimal temperatures for aflatoxin production in the range of 20 to 30 C, depending on cultural conditions (11, 12, 13).

The data presented in this study suggest that

aflatoxins could develop on meats in storage when the storage temperature was not kept at 10 C or below. That aflatoxins were produced at 15 C on meats with mixed cultures is significant since this approaches refrigeration temperatures. Since cured meats such as ham and bacon are less susceptable to bacterial spoilage than fresh meats, they are more apt to be mishandled in terms of poor control of storage temperatures. If the temperature of a display case or storage chamber containing cured meats was allowed to rise slightly, or if cured meats were stored in the home in a cool but unrefrigerated place, growth of *A. flavus* or *A. parasiticus* and subsequent aflatoxin production could result.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service Fellowship 5-F1-GM29, 037 from the National Institute of General Medical Sciences and by Public Health Service research grant UI-698 from the Division of Urban and Industrial Health.

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