Persistence of Aflatoxin During the Fermentation of Soy Sauce

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Aflatoxin was produced by Aspergillus parasiticus NRRL 2999 but not by A. oryzae during fermentation of soy sauce. Little aflatoxin was degraded within 6 weeks unless Lactobacillus delbrueckii also was present.

Sudden outbreaks of “turkey X disease,” later considered to be aflatoxicosis, have been attributed to the toxic metabolites of Aspergillus flavus Link ex Fries (1). Strains of Aspergillus, e.g. A. oryzae, are popularly used in Asia in the manufacture of soy sauce as well as other varieties of fermented foods (4). Although a number of strains of A. oryzae have been shown not to produce aflatoxin (5), it is difficult to distinguish A. oryzae from A. flavus or A. parasiticus contaminants in substrates. Because both are widely distributed, either could be involved in an impure fermentation. Another microorganism, Lactobacillus delbrueckii, is commonly used with A. oryzae for producing soy sauce (4). Therefore, the research reported here was undertaken to determine whether an aflatoxin producing A. parasiticus strain could grow with A. oryzae and with L. delbrueckii in soybean koji, whether aflatoxin would be present in the final soy sauce, or whether aflatoxin appeared at some point during preparation, but then was degraded at a later stage in the process.

A. parasiticus NRRL 2999, A. oryzae NRRL 1988, and L. delbrueckii NRRL B-445 were obtained from the Northern Utilization Research and Development Div., U.S.D.A., Peoria, Ill.; aflatoxin standards were obtained from the Southern Utilization Research and Development Div., U.S.D.A., New Orleans, La. The procedure for soy sauce production cited by Hesseltine and Wang (6) was adopted, but was modified as indicated in Fig. 1.

Fernbach flasks (2.8 liters) were used instead of conventional open “koji” boxes to prevent undesirable contamination, and a clean room was utilized as an incubation chamber. The substrates were inoculated with 8 ml of a culture of A. oryzae NRRL 1988 (1 \times 10^6 spores/ml) and a 5-ml suspension of L. delbrueckii (1 \times 10^6 organisms/ml) into the flasks. Then they were stored at room temperature. To study aflatoxin production during the fermentation of soy sauce, a 2 \times 10^6 spores/ml suspension of A. parasiticus NRRL 2999 was added to the substrate immediately after inoculation with A. oryzae and L. delbrueckii. For the aflatoxin B1 degradation study, an aqueous preparation of aflatoxin B1 (5 \mu g/kg of substrate) and 0.5 M lactic acid (100 ml/kg of substrate) were introduced immediately after inoculation with A. oryzae and L. delbrueckii.

Extraction followed the Ass. of Official Analytical Chemists method suggested by Epplle (3). Soy sauce substrate (50 g) containing wheat, soybeans, and liquid (50 ml) was shaken vigorously with chloroform (50 ml) and decanted. The procedure was twice repeated and

\begin{align*}
400 \text{ G OF SOYBEANS} & \quad 100 \text{ G OF WHEAT} \\
\text{SOAKED IN WATER} & \quad \text{ROASTED FOR} \\
\text{FOR 10 H} & \quad \text{5 MIN} \\
\text{DRAINED BEANS} & \quad \text{LIGHTLY CRUSHED} \\
\text{MIXED IN 2.8-LITER FERNBACH FLASK} & \\
1.1 \text{ LITER OF WATER ADDED} & \\
\text{AUTOCLAVED AT} & 121 \text{ C (15 LB/IN}^2) \text{ FOR} \\
15 \text{ MIN} & \\
\text{INOCULATED} & \\
\text{INCUBATED FOR 7 DAYS AT} & 25 \text{ C} \\
18 \text{ TO} 20% \text{ SALT BRINE ADDED} & \\
\text{AGED FOR 6 WEEKS} & \\
\text{Fig. 1. Production of soy sauce.}
\end{align*}

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Fig. 2. Persistence of aflatoxin B₁ during the production of soy sauce.
the three extracts were combined in a 500-ml Erlenmeyer flask. Aflatoxin determinations were then made using Silica gel-GHR-thin-layer chromatographic (TLC) plates developed in chloroform-acetone (97:3) for 45 min. Each sample was done in triplicate.

**Production of aflatoxin.** Data in Fig. 2 show the amount of aflatoxin in each sample during fermentation and throughout the processing of the koi into soy sauce. The sample containing only *A. oryzae* and lactic acid bacteria showed no production of aflatoxin during the entire fermentation period (sample I). Aflatoxin (8,500 µg/kg) production by *A. parasiticus* NRRL 2999 alone peaked after 1 week (sample II). Aflatoxin (7,000 µg/kg) was produced in the sample inoculated with *A. parasiticus* NRRL 2999 and *L. delbrueckii* (sample III). Lower quantities of toxin (2,100 µg/kg) were detected in sample IV after 1 week, i.e., koi inoculated with *A. parasiticus*, *A. oryzae*, and lactic acid bacteria.

**Persistence of aflatoxin.** Observations made with TLC plates indicated that aflatoxin degradation starts immediately after bringing in samples II, III, and IV. The TLC chromatograms of all three samples were nearly identical. No aflatoxin was detected in soy sauce containing only *A. oryzae* and *L. delbrueckii* (sample I). Sample II showed only 8% degradation of the aflatoxin in 6 weeks, whereas sample III showed 45% conversion. During this same time interval, there was 55% degradation of the toxin in sample IV. Acid production by *L. delbrueckii* may have catalyzed the conversion of aflatoxin B₁ to B₂a. Direct addition of aflatoxin B₁ and lactic acid (sample V) to the fermentation medium resulted in conversion of aflatoxin B₁ to derivatives comparable in *Rₐ* value to aflatoxin B₂a, a comparatively nontoxic form (2). Lindenfelser and Ciegler (7) found the acid-catalyzed conversion of aflatoxin B₁ to B₂a if a sufficient amount of lactic acid was supplied. The mechanism of the reactions among participating organisms is not clear, but the degradation products from samples III, IV, and V show *Rₐ* values lower than those of aflatoxin B₁ and B₂ on TLC.

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