Stability of Aflatoxin B₁ and Ochratoxin A in Brewing

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The stability of aflatoxin B₁ and ochratoxin A in brewing was investigated by adding the purified toxins to the raw materials at 1 and 10 µg/g levels during mashing in a conventional micro-brewing process. The results indicate that both toxins are stable to heat and are insensitive to cooker mash treatment. Both mycotoxins were partially removed in the mashing and brewing processes. About 14 to 18% and 27 to 28% of the added toxins were found in the final beers brewed from starting materials containing 1 and 10 µg, respectively, of either toxin per g. The possible route of transmission of mycotoxins into beer is discussed.

Since the discovery of aflatoxins, a series of potent carcinogens produced by *Asperigillus flavus* and *A. parasiticus*, a number of other fungal metabolites have been demonstrated to be toxic to animals (6, 9). Among these toxins, ochratoxins, a series of nephrotoxins produced by several species of *Aspergillia* and *Penicillia*, have also attracted attention because of their high toxicity and the widespread occurrence to toxin-producing fungi (4). Aflatoxins and ochratoxins have been found in many agricultural commodities (4, 6), including corn (13–15). Because of the unusual stability of these toxins in agricultural commodities, the question of whether they could carry through the food process has attracted considerable attention. Because of the natural occurrence of ochratoxin A (OA), the most potent toxin in the ochratoxin series, in barley and other cereals (10, 12), scientists in the United States and Denmark have studied the possibility of transmitting OA into beer. Fischbach and Rodricks (8) reported that earlier work in the Food and Drug Administration had shown the retention of OA in the beer process, but a survey on OA and ochratoxin B in samples of beer and malted barley from each of the 130 breweries in the United States showed no detection of ochratoxins. These samples were tested by using an analytical method sensitive to 0.01 µg/g (10 µg/kg). Recent experiments by Krogh et al. (11) demonstrated, however, that OA was destroyed during the malting and brewing processing. To determine the stability of aflatoxin B₁ (Afla B₁) and OA in brewing, we added the purified toxins at two stages (to either malt or corn grits) during a micro-brewing process. This paper

presents data on the stability of these two toxins in five major operations of brewing.

MATERIALS AND METHODS

Materials. Afla B₁ and OA were prepared by the methods of Chu (3) and Chu and Butz (5). Malt was prepared from Larker barley (*Hordeum vulgare* L.) that was grown in North Dakota in 1972. Portions (170 g, dry basis) were steeped in water at 16 C until the moisture content was 45%. The barley was allowed to grow at this temperature in the dark. The malting (growth) chamber is a modified drum type that slowly rotates 36 perforated cans containing the portions of barley. Moisture-conditioned air was circulated through the chamber and the samples during the 5-day malting period. The germinated barley was then kilned according to the following program: 9 h at 35 C, 7 h at 44 C, 5 h at 55 C, 5 h at 65 C, and 2 h at 85 C. Rootlets were removed after kilning, and the malt was ground in a Miag mill (Miag Co., Braunschweig, West Germany) prior to mashing. Corn (*Zea mays* L.) grits were obtained from the Krause Milling Co., Milwaukee, Wis. Adsorbosil-5 and Silica Gel G were obtained from Applied Science Laboratories (State College, Pa.) and the Brinkmann Instrument Co. (Westbury, N.Y.), respectively. All other solvents and chemicals were either analytical reagent grade or chemically pure.

Micro-brewing procedures. The brewing procedure was that described by Burkhart et al. (2), in which 90% of the malt is used in the malt mash and 10% in the cooker mash. The essentials of the operation are as follows.

(i) **Cooker mash.** Malt (22 g), corn grits (94 g), and water (489 ml) were mixed and held at 45 C for 10 min. The temperature was increased at 5-min intervals to 53, 61, 69, 77, 85, 93, and 100 C. The mash was boiled for 15 min.

(ii) **Malt mash.** Malt (189 g) and water (718 ml) were mixed at 38 C for 10 min. The mixture was

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allowed to stand at 38 C without stirring for 50 min for optimal hydrolysis of proteins.

(iii) Combined mash. The cooled cooker mash was added, and the temperature was raised to 72 C. The mash was maintained at this temperature for 20 min for optimal carbohydrate hydrolysis. The mash temperature was then raised to 75 C, and the mixture was filtered.

(iv) Wort. The entire filtrate (wort) was boiled with 1.5 g of hops for 90 min. Twenty minutes before the boiling was completed an additional 2.0 g of hops was added. The boiled wort was filtered and cooled to 12 C.

Fermentation and storage. The wort was pitched with 5 g of moist cake yeast (1 g, dry basis) (*Saccharomyces cerevisiae*) and fermented in a covered, but not sealed, flask at 12 C for 8 days. A 64-mg amount of commercial chill-proofing preparation (crude papain) was added, and the beer was transferred to a storage flask and maintained at 0 C under 12 lb/in^2 pressure of carbon dioxide for 14 days. The beer was then filtered and bottled under 12 lb/in^2 carbon dioxide pressure. For pasteurization the bottled beer was kept at 60 C for 20 min and then cooled to room temperature in 15 min. The beer was stored thereafter at 12 C.

Spiking of the purified toxins. For Afla B$_1$, appropriate amounts of toxin in 5 ml of chloroform were added to the malt or corn grits at the beginning of the cooker and malt mashing stages at 0, 1, and 10 μg/g levels (weight basis). The chloroform was removed by evacuation. For OA, the toxin was dissolved in 1 to 2 ml of 1% NaHCO$_3$ and added to cooker and malt mash directly before processing. Duplicate experiments were made for each level of toxins added. The amounts of toxins spiked in each step were: (i) cooker mashes: 0, 116, and 1,160 μg of toxins; (ii) malt mashes: 0, 189, and 1,890 μg of toxins; (iii) combined mashes: 0, 189, and 1,890 μg toxins; (iv) worts: 0, 189, and 1,890 μg of aflatoxin (made from cooker mashes without toxin and malt mashes with each toxin level), or 0, 305, and 3,050 μg of ochratoxin (made from cooker mashes with each level of toxin and malt mashes with the corresponding level of toxin); and (v) beers: 0, 189, and 1,890 μg of toxins contributed by the malt mash.

Extraction of brewing preparations and toxin analyses. Duplicate samples were analyzed at five stages during the brewing process: (i) the completed cooker mash; (ii) the malt mash at the end of the protein hydrolysis stage; (iii) the combined mash at the end of the carbohydrate hydrolysis stage; (iv) the wort after addition of hops and boiling; and (v) the bottled beer after pasteurization. The following procedures were used for toxin analysis.

(i) Afla B$_1$. For Afla B$_1$, official methods of analysis were followed (1). Samples of 100 g or 100 ml were extracted with chloroform according to Sect. 26.018 (a) (ref. 1). After evaporation of the chloroform from the extracts, the samples were redissolved in 1 to 2 ml of chloroform and chromatographed as described in Sect. 26.019 (a) (ref. 1). The chloroform elution fraction was evaporated to dryness, redissolved quantitatively in 1 to 5 ml of chloroform, and then

chromatographed on a glass plate (20 by 20 cm) with a 250-μm layer of Adsorbosin-5; methanol-chloroform (3:97) was the developing solvent. Quantitations were made by a fluorodensitometric method (5), except that a photo volt densitometer model III Turner fluorometer TLC scanner (G. K. Turner Associates, Palo Alto, Calif.) with primary and secondary filters of Turner no. 110-811 and 110-816 was used.

(ii) OA. For OA determination, 100 g each of treated brewing product and a control was blended with 100 ml of 2% NaHCO$_3$ solution for 5 min and then centrifuged at 3,000 × *g* for 10 min. The supernatants were transferred to separatory funnels. The residues were blended twice with 150 ml of 1% NaHCO$_3$ for 5 min and centrifuged again as described above. All of the supernatants of each batch were then pooled and acidified with concentrated HCl to pH 2. Likewise, 100 ml of beer was directly acidified to pH 2.0 with HCl. The acidified solutions were extracted twice with equal volumes of chloroform in separatory funnels. The chloroform extract was then evaporated to dryness and dissolved quantitatively in 8 ml of chloroform for thin-layer chromatography analysis on Adsorbosil-5 as described before; the solvent system was benzene-acetic acid (9:1; 7). Quantitation was done on a Turner fluorometer as described for Afla B$_1$.

RESULTS AND DISCUSSION

The validity of the present data rests primarily on the method of extraction and analysis; thus, control experiments, in which test mycotoxins were added to the brewing products after each process, with subsequent analysis of toxin content, were carried out. The results showed that the toxin recovery ranged from 78 to 91% for each step, with an overall average of 85%. Since the recovery yield falls into the range of mycotoxin analysis in other food (4), the analytical methods used in the present study might also be used for routine analysis of these mycotoxins in beer or related products. The presence of alcohol was removed by evaporation under vacuum, and the remaining material was analyzed for toxins. The results showed that both the treated (alcohol removed) and the untreated samples gave similar recoveries (86.6 versus 89.3%). The toxins were then directly extracted from the beer or acidified beer (for OA) with organic solvents before analyzing.

The levels of Afla B$_1$ and OA in brewing at five stages are given in Table 1. In general, both mycotoxins were partially lost during the brewing. The patterns for the loss of both mycotoxins were similar; both were relatively stable in the cooker mash step but were more sensitive to later treatments, especially the protein hydrolysis, wort boiling, and final fermentation. Since both mycotoxins are relatively stable to heat (4, 9, 16), it is not surprising that more than 90% of

TABLE 1. *Stability of toxins in brewing*

Treatment	Toxin added (µg)		Toxin recovered (µg)		% Recovery	
	Aflatoxin	Ochratoxin	Aflatoxin	Ochratoxin	Aflatoxin	Ochratoxin
Complete cooker mash	1,160	1,160	938	946	81 (95)[a]	82 (96)[a]
	116	116	74	90	63 (74)	77 (91)
Malt mash after protein hydrolysis	1,890	1,890	1,392	1,415	74 (87)	75 (88)
	189	189	118	122	62 (73)	65 (76)
Combined mash after carbohydrate hydrolysis	1,890	1,890	1,225	1,355	65 (76)	72 (85)
	189	189	98	109	52 (61)	58 (68)
Boiled wort	1,890	3,050	800	1,644	42 (49)	46 (54)
	189	305	65	106	34 (40)	41 (48)
Completed beer	1,890	1,890	440	446	23 (27)	24 (28)
	189	189	28	24	15 (18)	12 (14)

[a] Values in parentheses indicate a correction of 15% due to loss in the analysis.

the added toxins were in the sample after cooker mash treatment. Removal of toxin from the product was very significant in the malt mash (with a loss of 12% for a 10 µg/g sample and 24 to 27% for a 1 µg/g sample), in boiled wort (20 to 30% loss of material added to the malt mash), and in the final fermentation (another 20 to 30% loss from the boiled wort step) steps. Since it has been observed that OA can be hydrolyzed by acid and some proteases to ochratoxin α and phenylalanine (4) and since ochratoxin α has been detected in the wort when OA-contaminated barley was used in the brewing (10), removal of OA in the brewing process may primarily be due to such hydrolysis. The mechanism of Afla B$_1$ removal, however, is not known. The significant loss of both mycotoxins between the combined mash and boiled wort steps may be due to the nonspecific interaction or adsorption of both mycotoxins by the solid particles removed by the filtration process.

Both mycotoxins were partially removed in brewing, but considerable amounts were transferred into the beer, especially if the contamination level was high. Approximately 25% of the added toxins were found in the final product when 10 µg of the toxin per g in the cereal was tested. At the lower testing level (1 µg/g), recovery was about 15%. Our results on OA differ from those reported by Krogh et al. (11). They found significant loss in mashing (70% lost before boiled wort), and less than 10% of OA was recovered when barley, containing 1 to 5 µg of toxin per g, was used in the brewing. It is not known whether these contradictions are due to a difference in their brewing processes, in which lactic acid and bacterial enzymes were used, or

to the method of extraction and analysis for which recovery yield in the control sample was not reported by the investigators.

Inasmuch as mycotoxins in contaminated materials are not completely removed from the beer in brewing and might be converted to some unknown products which might also be toxic, the best protection would be to prevent the use of contaminated raw materials in brewing. In order that barley germinate and grow (malt) satisfactorily, and therefore meet the requirements of the malting industry, it must be stored under conditions which would not promote the growth of storage molds. Although Krogh et al. (11) demonstrated that when barley was heavily contaminated with OA it thus failed to germinate and would be rejected for malting, it is not known whether or not aflatoxin would also affect the malting process. Moreover, the material used as adjunct (the constituent other than malt in the cooker mash) functions primarily as a source of starch and other carbohydrates which are largely hydrolyzed to maltose during the "conversion" or carbohydrate hydrolyzing stage of mashing. Possibly these adjuncts (corn syrups, ground rice, and ground barley are commonly used), if they are already contaminated in the field or if they are stored under adverse conditions, could contribute mycotoxins to the mash.

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