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Fate of Ochratoxin A and Citrinin During Malting and Brewing Experiments

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The fate of ochratoxin A and citrinin during malting and brewing processes was studied by the use of naturally contaminated lots of barley, as well as by the addition of crystalline toxins to the mash. Complete degradation was observed for ochratoxin A from moderately contaminated barley lots and for citrinin added to mash. The use of highly contaminated barley resulted in transmission of ochratoxin A into the beer, but only 2 to 7% of the initial content was detected, corresponding to levels of 6 to 20 μg of ochratoxin A per liter of beer. Barley lots with this high ochratoxin contamination (1,000 to 5,000 μg/kg) will be easily detected and, therefore, because of pronounced deterioration, should be rejected during inspection upon admittance to the breweries.

Ochratoxin A and citrinin are mycotoxins with nephrotoxic properties, and they have been found as causal determinants in a naturally occurring kidney disease, porcine nephropathy (6). Kidney lesions have been induced experimentally in many animal species, and it is reasonable to expect the toxins to act on human kidneys as well. Ochratoxin A as a natural contaminant was first reported from the U.S.A. (12), where a sample of maize was found to be contaminated with approximately 150 μg of ochratoxin A per kg. In inspection of maize from different regions of the U.S.A., ochratoxin A has repeatedly been found at levels of from 83 to 166 μg/kg (13, 14). This mycotoxin has also been detected in American barley samples (S. Nesheim, Annu. Meet. Ass. Offic. Anal. Chem., 85th, Washington, D.C., Abstr., 1971) at levels of 12 to 38 μg/kg. Ochratoxin A was found in concentrations of 30 to 27,000 μg/kg in 18 out of 29 samples of Canadian grain stored under damp conditions (11). The cereal samples consisted mainly of wheat but also included oats, barley, and rye. Contamination of Canadian wheat with ochratoxin A at levels of 20 to 100 μg/kg was previously reported (10). Thirteen of the above mentioned samples of heating grain were simultaneously contaminated with citrinin at levels of 70 to 80,000 μg/kg. The citrinin-contaminated samples included wheat, oats, barley, and rye. In Denmark, ochratoxin A-contaminated samples of barley and oats have frequently been observed at levels of 28 to 27,500 μg/kg (6). Some of these samples were simultaneously contaminated with citrinin (160 to 2,000 μg/kg). In Sweden, barley and oats have been found to be contaminated with ochratoxin A at levels of 16 to 410 μg/kg (5).

Most of the cereal samples reported here have been collected from lots which were intended to be used as animal feed. The breweries perform careful control of barley and other cereals before acceptance for malting and brewing purposes, because moldy cereals will spoil the flavor and other qualities of the beer, e.g., by causing gushing (3). In spite of the control, contaminated lots of cereals may pass, because organoleptical changes are minor and germination tests and microbiological control cannot prevent the passing of low-contaminated lots, which can only be detected by chemical analysis for mycotoxins.

The fate of ochratoxin A and citrinin during the malting and brewing processes is therefore of considerable interest from a food hygiene and food safety point of view.

(Some of the results presented here were included in a report at the 14th Congress of the European Brewery Convention, Salzburg, May 1973.)

MATERIALS AND METHODS

Barley. Lots naturally contaminated with ochratoxin A and/or citrinin were collected from farms, where the lots had caused kidney disease in pigs (6). Malting barley, of normal high quality, was obtained from the Carlsberg Breweries and used as uncontaminated barley (control).

Crystalline mycotoxins. Crystalline ochratoxin A was obtained from Macor Chemicals Ltd., Jerusalem, Israel. Crystalline citrinin was obtained from *Aspergillus niveus* NRRL 1955 (strain kindly supplied by C. W. Hesseltine, Northern Regional Research Laboratory, USDA, Peoria, Ill.), according to the procedure of Rodig et al. (9).

Malting and brewing. (i) Micromalting. Portions (70 g) of barley were steeped in 250-ml plastic beakers with 12 holes (2-mm diameter) in the bottom. Alternating periods of 8 h with water and 16 h without water were applied at 12 C. Steeping was finished when a moisture content of 43 to 44% was obtained, and germination was carried out in the same beakers at 12 C. Once a day the barley was taken out for inspection, and the kernels were loosened from each other. Total steeping and germination time was 9 days. The samples were kilned in nylon bags for 8 h at 45 C and 16 h at 75 C.

(ii) Experimental brewing. Ten kilograms of malt was sprayed with 400 g of water and ground 10 min later. The mashing was an infusion system starting with 35 liters of water at 39 C. After 40 min, the temperature was raised to 51 C for 60 min, and 15 g of lactic acid was added. Eight minutes later, the mash was heated 1 C per min to 65 C, and after 40 min of saccharification the temperature was again raised in 1-C increments per min to 75 C and then held for 30 min. The mash was transferred to the lauter tun for filtration and sparging with 44 liters of water.

The wort was boiled with hops for 90 min, cooled, and aerated. The wort was distributed into two vessels, each containing 25 liters of wort, and was fermented with 90 g of centrifuged yeast in each vessel at 10 C for about 7 days. After racking, the beer was stored at 5 C for 1 week and at 0 C for 4 weeks, after which it was filtered, carbonated, bottled, and pasteurized.

(iii) Experimental brewing with barley and enzymes. Five kilograms of barley was steeped for 30 min at 50 C. The steep water was used later for mashing. After air-drying, the barley was ground and mashed in 20 liters of water at 50 C with addition of 5 g of bacterial amylase (BAN). The temperature was raised to 80 C in 60 min, and, after 30 min at this temperature, the barley-mash was pumped to the malt-mash prepared from 5 kg of ground malt and 17 liters of water at 20 C. To the mixed mash at 50 C was added 5 g of BAN and 5 g of bacterial proteinase (BPN). BAN (EC 3.2.1.1) and BPN (EC 3.4.4.16) were both obtained from *Bacillus subtilis*. The enzyme preparations were obtained from Novo, Copenhagen.

After 30 min at 50 C, the temperature was raised in 1-C increments per min to 63 C, which was held for 45 min. Mashing was finished at 75 C, and the brewing was continued as described above.

Germination of the barley was determined (2), and the following analyses were carried out on the wort. Extract concentration (% Plato) (1), attenuation (degree of fermentation) (1), nitrogen (7), viscosity by use of Höppler viscosimeter, and color were measured in a spectrophotometer at 465 nm in a 1-cm cell.

Mycotoxin analysis. (i) Solid samples. Samples (50 g) of solid material (barley, malt, spent grains) were ground and water was added and, after acidification, they were extracted with chloroform. Quantitation of citrinin (4) and ochratoxin (8) was carried out by the use of densitometer techniques.

(ii) Liquid samples. Samples (350 ml) of liquid material (wort, beer) were acidified and extracted with chloroform. Quantitation was made as above.

During the investigations, the following experiments were carried out: (I) Micromalting of two barley lots (MT 480 and MT 481) contaminated with ochratoxin A, 830 and 420 μg/kg, respectively, was carried out. (II) Experiment II consisted of experimental brewing of two lots of barley (MT 106 and MT 100) heavily contaminated with ochratoxin A (2,060 and 27,500 μg/kg, respectively). The two lots had a germination capacity below 2% and could therefore not be malted. They were then brewed with the use of added bacterial enzymes in the following way. Brew no. 1318 included 5 kg of ochratoxin A-contaminated barley (MT 106) and 5 kg of malt; brew no. 1321 included 1.8 kg of ochratoxin A-contaminated barley (MT 100) and 8.2 kg of malt. (III) We carried out experimental brewing with addition of crystalline ochratoxin A to the mash (brew no. 1322). Ochratoxin A (10 mg) was dissolved in 15 ml of 0.05 N $NaHCO_3$ and mixed with 10 kg of normal malt, after which brewing was carried out. (IV) Experimental brewing with addition of crystalline citrinin to the mash (brew no. 1320) was performed. Citrinin (10 mg) was dissolved in 15 ml of ethanol and mixed with 10 kg of normal malt, after which brewing was carried out.

RESULTS

Ochratoxin A. Moderately contaminated barley samples were used during experiment I, and, although the germination percentage of lot MT 480 was a little below the normal specification, a reasonable malting experiment could be performed. The results of ordinary malt analyses were normal, although there was a moldy smell during the malting, and no ochratoxin was detected in the malt (Table 1).

Two heavily contaminated lots of barley were used for experiment II (experimental brewing). The lots had a moldy smell, very pronounced in MT 100, and because of a very low germination percentage, malting was out of the question, and brewing could be carried out only with

TABLE 1. *Malting of barley naturally contaminated with ochratoxin A*

Sample no.	Germination (%)	Concentration of ochratoxin A (μg/kg)	
		Before malting	After malting
MT 480	87	830	ND[a]
MT 481	97	420	ND

[a] ND, not detectable.

TABLE 2. *Brewing of barley naturally contaminated or with addition of crystalline ochratoxin A*

Sample no.	Brew no.	Analysis for ochratoxin A						
		Amt in mash (μg)	Wort (61 liters)[b]		Beer (61 liters)[b]		Spent grains (13 kg)[a]	
			μg/liter	% of initial amt	μg/liter	% of initial amt	μg/kg	% of initial amt
MT 106	1318	10,300	33	19	11	7	80	10
MT 100	1321	49,500	94	11	20	2	210	6
	1322[c]	10,000	25	16	6	4	86	10

[a] Twelve kilograms of spent grains was used for the experiment with brew no. 1322.
[b] Sixty-six liters of wort and beer was used for the experiment with brew no. 1322.
[c] Brewing with addition of ochratoxin A to the mash.

TABLE 3. *Wort analysis*

Brew no.	Barley sample no.	Extract (% Plato)	mg of N/ 100 ml	Viscosity (cP)	Color ($100 \times E_{465}$)	Attenuation (%)
1316	Normal	10.7	92.2	1.67	19.6	68.7
1318	MT 106	10.7	101.3	1.65	31.7	64.7
1321	MT 100	10.5	118.9	1.55	70.0	63.2

addition of bacterial enzymes. As indicated in Table 2, there was a strong degradation of ochratoxin A during the brewing process, with the final beer containing 2 to 7% of the initial amount of ochratoxin A, resulting in a concentration of 11 to 20 μg/liter. Spent grains contained a proportional higher level, with a concentration of 80 to 210 μg/kg. The normal wort analyses (Table 3) showed an increase of soluble nitrogen and more pronounced color. The smell of the wort and the flavor of the beer was abnormal, especially in brew no. 1321.

To show whether barley-malt enzymes had a different effect on the degradation than did bacterial enzymes, experiment III was conducted, with the addition to normal malt of crystalline ochratoxin A. A similar degradation, as in the previous experiment, was observed (Table 2) with 4% of the initial amount found in the beer, equal to 6 μg/liter. On the thin-layer chromatography plates, fluorescent spots different from ochratoxin A and ochratoxin α were present in the wort but absent in the final beer.

Citrinin. Malting and brewing experiments were intended to be done with the use of a naturally contaminated lot of barley. However, the germination percentage was zero, and no malting was possible. Instead, crystalline citrinin (10 mg) was added to normal malt (experiment IV), and no citrinin could be detected in the wort and spent grains.

DISCUSSION

The malting process completely degrades ochratoxin A present in moderately contaminated barley lots. When heavily contaminated lots are used for mashing, a pronounced reduction of ochratoxin A takes place during the process, indicated by the presence in the wort of only 11 to 19% of the initial toxin content in the barley. The subsequent fermentation process degrades ochratoxin further, so that only 2 to 7% of the initial content is present in the beer, corresponding to 6 to 20 μg/liter. However, the barley used to produce this level in the beer was so heavily contaminated and deteriorated that similar lots would not be used by breweries. Citrinin degraded at an even faster rate than ochratoxin during the mashing process and was not present in detectable amounts in the wort.

Only the use of highly contaminated lots of barley will result in production of ochratoxin-contaminated beer. As these lots will be rejected during inspection upon admittance to the breweries, the problem of transmission of these mycotoxins from cereals to the beer seems very unlikely. The limitation of the present investigations is the use of TLC techniques only for toxic metabolite detection; no biological test systems for detection of possible toxic, nonfluorescent break-down products of ochratoxin A and citrinin were employed.

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