FOOD CHEMICAL CONTAMINANTS

A Survey of Ochratoxin A and Aflatoxins in Domestic and Imported Beers in Japan by Immunoaffinity and Liquid Chromatography

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Analyses of ochratoxin A (OTA) and aflatoxins (AFs) in 94 imported beer samples from 31 producing countries and in 22 Japanese beer samples were performed by immunoaffinity column and reversed-phase liquid chromatography (LC) with fluorescence detection. Recoveries of OTA from beer samples spiked at 25 and 250 pg/mL were 86.1 and 88.2%, respectively. Recoveries of AFs were 98.4 and 98.9%, 95.4 and 95.5%, 101.2 and 97.8%, and 98.9 and 96.0%, respectively, from beer samples spiked at 4.1 and 41 pg AF B_1, 4.45 and 44.5 pg AF B_2, 4.7 and 47 pg AF G_1, and 4.65 and 46.5 pg AF G_2/mL. Detection limits were 1.0 pg/mL for OTA, 0.5 pg/mL for AFs B_1 and B_2, and 1.0 pg/mL for AFs G_1 and G_2. OTA was detected in 86 (91.5%) of 94 imported beer samples at a mean level of 10.1 pg/mL and in 21 (95.5%) of 22 Japanese beer samples at a mean level of 12.5 pg/mL. AF B_1 was detected in 11 of 94 imported beer samples at a level of 0.5–83.1 pg/mL and in 2 of 22 Japanese beer samples at 0.5 and 0.8 pg/mL. Except for one beer sample from Peru, the samples contaminated with AFs were also contaminated with OTA. Although OTA was detected in most samples from various countries, AFs were detected in the beer samples from only a limited number of countries where AF contamination might be expected to occur because of their warm climate.

Aflatoxins (AFs) produced by *Aspergillus flavus* and *A. parasiticus,* and ochratoxin A (OTA) produced by *A. alutaceus* and *Penicillium verrucosum* are carcinogenic mycotoxins, and consequently, their levels in various agricultural products have been monitored (1–6).

The possibility of mycotoxins getting into beer from contaminated grains used in brewing was pointed out in the early 1970s (7–11), and mycotoxins in beer recently were reviewed (12). Until the 1990s, contamination of beer with OTA was not reported (8, 12–16), except for one study (17) that showed exceptionally high levels of OTA in beer. Since the introduction of liquid chromatography (LC) with fluorescence detection to determine OTA, low levels of OTA (up to 1.53 ng/mL) in beer have been reported (12, 18–20). Moreover, with the use of immunoaffinity columns in the cleanup steps, detection limits have improved to the parts-per-trillion range. Now, many incidences of OTA contamination of beer have been reported, although levels are very low (up to 0.2 ng/mL; 12, 21–24).

On the other hand, high levels of AFs (up to 262 ng AFs B_1 + G_1/mL) and high incidences (up to 100%) in African beer have been reported (12, 25–27). However, AFs were not detected in several surveys of European beers before 1991 (12–15, 17, 28–30). With the use of immunoaffinity column for analysis of AFs in beer, parts-per-trillion levels of AFs have been found in Mexican and Brazilian beers (31). In Japan, only 2 reports exist concerning OTA and AF B_1 contaminations of a limited number of beer samples (32, 33). This study surveys OTA and AFs in Japanese and imported beers.

METHOD

Safety note: AFs and OTA are carcinogens and should be handled with care (34).

Apparatus

(a) *LC system.*—LC-10AD pump, SIL-10A autoinjector, CTO-10AC column oven, RF-10A spectrofluorometric detector (150 W Xenon lamp), DGU-3A degasser, CBM-10A communications bus module, Class LC-10 chromatography data system (Shimadzu, Kyoto, Japan), stainless steel Capcell Pak C_{18} SG 120, 250 × 4.6 mm column (Shiseido, Tokyo, Japan). Operating conditions: flow rate, 1.0 mL/min; column oven, 40°C.

(b) *Ultrasonic bath.*—Bransonic B-42 (Branson SmithKline Co., Shelton, CT).

(c) *Immunoaffinity columns.*—AflaTest P columns and OchraTest columns (Vicam, Watertown, MA), fitted with 30 mL Sep-Pak reservoirs (Waters, Milford, MA).

(d) *Vacuum manifold.*—16 ports (Waters).

(e) *Glass microfiber filter paper.*—Whatman GF/B, 6 cm (Whatman International Ltd., Maidstone, UK).

Received December 1, 1998. Accepted by AP February 8, 1999.

(f) *Mixer.*—Vortex.

(g) *Screw-cap vials.*—1.5 mL autosampler vials with Teflon liners.

(h) *Syringes.*—Continuously adjustable pipets with disposable tips, capacities: 10, 200, and 1000 μL, and 5 and 10 mL.

(i) *Aluminum block heater.*—Dry Thermo Unit DTU-2C (Taitec Co., Saitama, Japan).

Reagents

(a) *Solvents.*—LC grade water, acetonitrile, and methanol (Katayama Chemical, Osaka, Japan) and reagent grade *n*-hexane.

(b) *General chemicals.*—LC grade trifluoroacetic acid (Wako Pure Chemical Industries, Ltd., Osaka, Japan), reagent grade cetyltrimethyl ammonium bromide (CTA; Tokyo Kasei, Tokyo, Japan), KH_2PO_4, and K_2HPO_4 (Katayama Chemical).

(c) *0.2M phosphate buffer, pH 8.5.*—Prepare from 0.2M K_2HPO_4 in water by titration to pH 8.5 with 0.2M KH_2PO_4 in water.

(d) *0.2M phosphate buffer, pH 5.0.*—Prepare from 0.2M KH_2PO_4 in water by titration to pH 5.0 with 0.2M K_2HPO_4 in water.

(e) *Acetonitrile solutions.*—(*1*) *15% (v/v) acetonitrile in water.*—Dilute 15 mL acetonitrile to 100 mL with water. (*2*) *30% (v/v) acetonitrile in water.*—Dilute 30 mL acetonitrile to 100 mL with water.

(f) *Aflatoxin mix standards.*—Makor Chemicals, Jerusalem, Israel. (*1*) *Stock solution.*—Prepare from crystalline standards in benzene–acetonitrile (98 + 2, v/v) as described in AOAC Methods **971.22A** and **B** (34) to contain 0.82 μg AF B_1, 0.89 μg AF B_2, 0.94 μg AF G_1, and 0.93 μg AF G_2/mL. (*2*) *Working standard dilutions.*—Prepare 10-fold and 100-fold dilutions of stock solution in benzene–acetonitrile. Use 10 μL of these solutions to prepare spiking solutions. Transfer 10 μL of the 10-fold working dilution to a vial. Evaporate to dryness under a gentle stream of nitrogen and use as analytical standard, which should be prepared daily.

(g) *OTA standards.*—Makor Chemicals. (*1*) *Stock solution.*—Prepare from crystalline standard in benzene–acetic acid (99 + 1, v/v) as described in AOAC Method **973.37C** (34) to contain 1.0 μg/mL. (*2*) *Working dilutions.*—Prepare 10-fold dilution of stock solution in benzene–acetic acid. Use 50 and 5 μL of this solution to prepare spiking solution. Transfer 5 μL of working dilution to a vial and evaporate to dryness under a gentle stream of nitrogen. Dissolve residue in 1.0 mL 30% acetonitrile by using an ultrasonic bath for 1 min. Mix for 30 s on a Vortex mixer and use as analytical standard, which should be prepared daily.

(h) *LC mobile phase.*—Acetonitrile–methanol–0.2M phosphate buffer, pH 5.0–water (23 + 4 + 3 + 70, v/v/v/v) for AF analysis; acetonitrile–0.2M phosphate buffer, pH 8.5–water (60 + 3 + 37, v/v/v) containing 3 mM CTA for OTA analysis.

(i) *Sample collection.*—Beer samples were purchased in Nagoya City, Japan, in 1998. The 116 samples of different brands were composed of 22 Japanese beer samples; 17 beer samples imported from the United States; 15 from Belgium; 7 from Germany; 6 from Holland; 5 from the United Kingdom; 4 from Mexico; 3 each from Canada, Denmark, Italy, and Thailand; 2 each from Brazil, Australia, Austria, France, Portugal, Indonesia, and Singapore; and 1 each from Jamaica, Trinidad, Bolivia, Peru, New Zealand, Tahiti, Kenya, Spain, China, India, Israel, the Philippines, Taiwan, and Vietnam. They were stored at 4°C until opened for analysis. For method recovery studies, 10 μL AF mix working dilutions or 5 or 50 μL OTA working dilution was transferred to a 100 mL Erlenmeyer flask and evaporated to dryness under a gentle stream of nitrogen. Beer sample (20 mL) was added, and the mixture was dissolved by immersion in an ultrasonic bath for 1 min and mixed well.

Cleanup with Immunoaffinity Column

Filter beer through glass microfiber filter, and degas for 30 min by using an ultrasonic bath. Insert needle into top cap of affinity column. Remove top cap and cut off tip. Connect reservoir to cap and attach column. Remove end cap and set at vacuum manifold. Immediately pipet 20 mL beer into reservoir and allow to drain by gravity. Wash column with 5 mL water. Remove column from manifold. Place 10 mL glass test tube under column and then elute toxin with 2 mL methanol by gravity. Place test tube in an aluminum block heater set at 40°C, and evaporate eluate to dryness under a gentle stream of nitrogen. For OTA, use the OchraTest column, dissolve resi-

Table 1. Recovery of ochratoxin A (OTA) and aflatoxins (AFs) B_1, B_2, G_1, and G_2 from spiked beer samples

Spiking level	No. of replicates	Mean recovery, % (RSD, %)				
		OTA	AF B_1	AF B_2	AF G_1	AF G_2
High[a]	5	88.2 (2.4)	—	—	—	—
Low[b]	5	86.1 (2.4)	—	—	—	—
High[c]	5	—	98.9 (0.8)	95.5 (1.5)	97.8 (1.1)	96.0 (2.4)
Low[d]	5	—	98.4 (2.5)	95.4 (2.2)	101.2 (2.3)	98.9 (3.7)

[a] 250 pg OTA/mL.

[b] 25 pg OTA/mL.

[c] 41.0 pg AF B_1/mL, 44.5 pg AF B_2/mL, 47.0 pg AF G_1/mL, and 46.5 pg AF G_2/mL.

[d] 4.10 pg AF B_1/mL, 4.45 pg AF B_2/mL, 4.70 pg AF G_1/mL, and 4.65 pg AF G_2/mL.

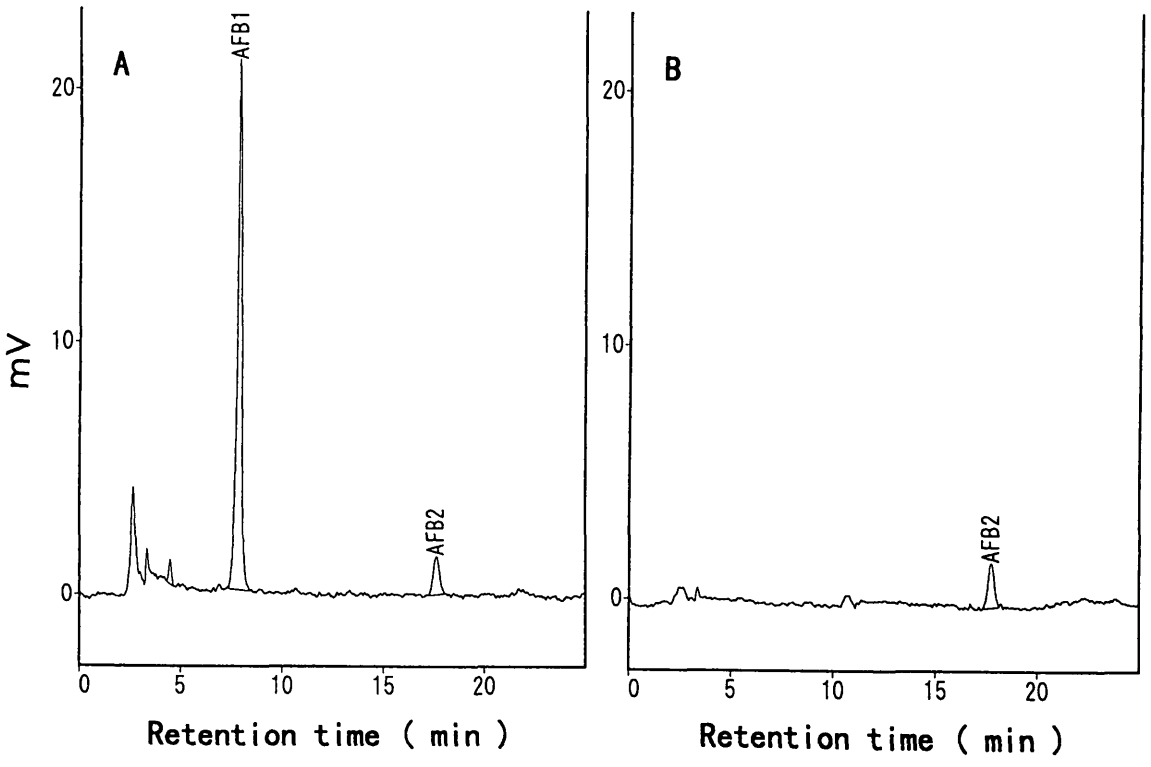

Figure 1. Chromatograms of aflatoxins B_1 (as B_{2a}) and B_2 in beer sample from the Philippines: (A) with trifluoroacetic acid treatment, 10.3 pg AF B_1/mL, 1.2 pg AF B_2/mL and (B) with no trifluoroacetic acid treatment.

due in 1 mL 30% acetonitrile in an ultrasonic bath for 1 min, and mix for 30 s on a Vortex mixer. Transfer solution to autosampler vial. For AFs, use the AflaTest P column, add 200 µL *n*-hexane and 50 µL trifluoroacetic acid to residue, cap, and incubate 5 min in an aluminum block heater set at 40°C. Evaporate mixture to dryness under a gentle stream of nitrogen. Dissolve residue in 1 mL 15% acetonitrile in an ultrasonic bath for 1 min, and mix for 30 s on a Vortex mixer. Transfer solution to autosampler vial. Repeat with AF standard.

Confirmation

If sample is positive for AF B_1, confirm presence of AF B_1 by the method of Scott and Lawrence (31). Dissolve dried extract from affinity column in 1 mL 15% acetonitrile without derivatization of trifluoroacetic acid, and observe absence of AF B_{2a} peak.

If sample is positive for OTA, methylate OTA by the method reported by Terada et al. (35) with some modifica-

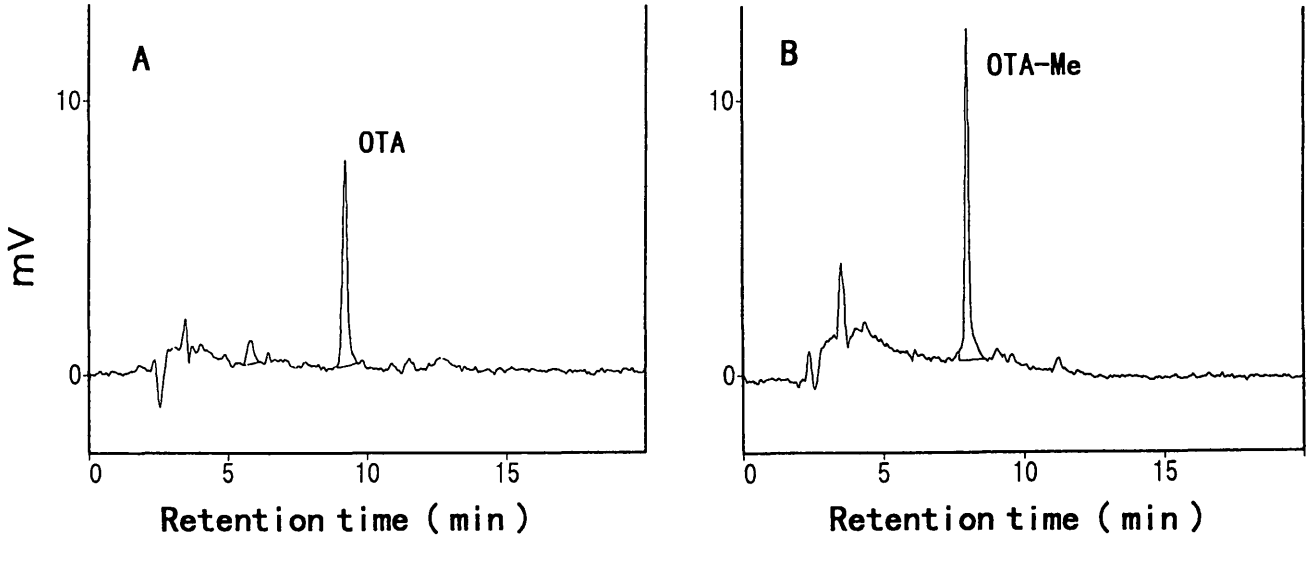

Figure 2. Chromatograms of (A) ochratoxin A in Japanese beer sample, 23.8 pg ochratoxin A/mL and (B) methyl ester of ochratoxin A in Japanese beer sample.

Table 2. Ochratoxin A (OTA) and aflatoxins (AFs) in imported and Japanese beer samples

	OTA			AFs	
Region or country	Incidence	Mean,[a] pg/mL	Range, pg/mL	Incidence	Concentration, pg/mL
North America	20/20	9.6	2.0–31.1	1/20	1.0
Canada	3/3	6.3	3.3–9.5	0/3	ND[b]
United States	17/17	10.1	2.0–31.1	1/17	1.0
Central America	6/6	10.2	2.3–21.7	2/6	AF B_1 0.5, 1.8
Jamaica	1/1	21.7	21.7	0/1	ND
Mexico	4/4	8.5	2.3–10.8	2/4	AF B_1 0.5, 1.8
Trinidad	1/1	5.2	5.2	0/1	ND
South America	2/4	3.4	3.7–9.9	3/4	AF B_1 1.4–12.1
Bolivia	1/1	9.9	9.9	1/1	AF B_1 8.7, AF B_2 1.3
Brazil	1/2	1.9	3.7	1/2	AF B_1 1.4
Peru	0/1	ND	—[c]	1/1	AF B_1 12.1, AF B_2 2.0
Oceania	2/4	1.4	2.2–3.4	0/4	ND
Australia	0/2	ND	—	0/2	ND
New Zealand	1/1	2.3	2.3	0/1	ND
Tahiti	1/1	3.4	3.4	0/1	ND
Africa: Kenya	1/1	2.0	2.0	1/1	AF B_1 1.9
Europe	43/46	11.4	1.7–66.2	1/46	AF B_1 3.3
Austria	2/2	4.8	3.4–6.2	0/2	ND
Belgium	12/15	11.2	2.3–66.2	0/15	ND
Denmark	3/3	7.6	2.3–17.8	0/3	ND
France	2/2	11.9	9.5–14.2	0/2	ND
Germany	7/7	16.8	6.6–36.4	0/7	ND
Holland	6/6	11.0	3.0–25.2	0/6	ND
Italy	3/3	7.9	4.8–9.9	0/3	ND
Portugal	2/2	6.5	6.0–6.9	0/2	ND
Spain	1/1	26.1	26.1	1/1	AF B_1 3.3, AF B_2 1.3
United Kingdom	5/5	11.0	1.7–34.6	0/5	ND
Asia	12/13	11.8	1.2–49.2	3/13	AF B_1 1.3–83.1
China	1/1	49.2	49.2	0/1	ND
India	1/1	1.2	1.2	1/1	AF B_1 83.1, AF B_2 8.6
Indonesia	2/2	1.9	1.8–1.9	0/2	ND
Israel	1/1	36.2	36.2	1/1	AF B_1 1.3
Philippines	1/1	1.9	1.9	1/1	AF B_1 10.3, AF B_2 1.2
Singapore	1/2	7.8	15.5	0/2	ND
Taiwan	1/1	2.9	2.9	0/1	ND
Thailand	3/3	13.1	3.0–27.5	0/3	ND
Vietnam	1/1	4.0	4.0	0/1	ND
Overall	86/94 (91.5%)	10.1	1.2–66.2	11/94	AF B_1 0.5–83.1
Japan	21/22 (95.5%)	12.5	2.2–44.8	2/22	AF B_1 0.5, 0.8

[a] Mean values were calculated by assuming that samples containing levels less than detection limit contained OTA at 0 pg/mL.
[b] ND = not detectable (less than detection limit).
[c] — = not applicable.

tions. Add 2 mL methanol (dried with anhydrous Na_2SO_4) and 0.1 mL H_2SO_4 to dried extract from affinity column. Cap and heat for 20 min at 100°C. Add 2 mL water to reaction mixture, and extract with 1 mL $CHCl_3$ 3 times. Wash $CHCl_3$ layer with 1 mL water. Evaporate $CHCl_3$ layer to dryness under a gentle stream of nitrogen. Dissolve residue in 1 mL 30% acetonitrile. Confirm disappearance of OTA peak and appearance of OTA methyl ester peak.

LC Determination

Using an autosampler, inject 100 µL sample extract (equivalent to 2 mL beer) or appropriate standard. For detection, set wavelengths for AF B_1 or OTA by using the scanning mode of the LC system as follows: for AF B_1, excitation, 355 nm; emission, 430 nm; for OTA, excitation, 383 nm; emission 432 nm. Compare peak heights of AFs or OTA in sample extract and each standard for the determination. Typical retention times are 6.4, 7.9, 13.1, 17.7, 7.9, and 9.2 min for AF G_{2a} (corresponding to AF G_1), AF B_{2a} (corresponding to AF B_1), AF G_2, AF B_2, OTA methyl ester, and OTA, respectively.

Results and Discussion

Scott and Lawrence (31) recommended the use of silanized vials to evaporate the methanol eluate from the immunoaffinity column. We checked the recoveries of toxins from silanized and nonsilanized vials and found no differences in recoveries of toxins when the residue was dissolved in an ultrasonic bath. The results of a recovery study with beer spiked with 2 levels of AFs or OTA, using a blank beer that contained no detectable AFs and OTA, are summarized in Table 1. Recoveries of AFs or OTA spiked at high (41.0 pg AF B_1, 44.5 pg AF B_2, 47.0 pg AF G_1, 46.5 pg AF G_2, and 250 pg OTA/mL) and low (4.10 pg AF B_1, 4.45 pg AF B_2, 4.70 pg AF G_1, 4.65 pg AF G_2, and 25 pg OTA/mL) levels were more than 95% for AFs and 86% for OTA. Relative standard deviations (RSDs) ranged from 0.8 to 3.7%.

For OTA analysis, we used ion-pair chromatography in the alkaline mobile phase. The fluorescence intensity of OTA increases under alkaline condition (35, 36). Therefore, the detection limit of OTA in beer (signal-to-noise ratio of 3:1) improved to 1.0 pg/mL beer, which is an order of magnitude lower than that of another method (23). Also for AF analysis, by setting the maximum excitation and emission wavelengths of AF B_1 under the used mobile phase through the scanning mode of the LC system, the detection limit of AF B_1 in beer was sufficiently low for a survey of AFs in beer. Detection limits were 0.5 pg AF B_1 and AF B_2/mL beer and 1.0 pg AF G_1 and AF G_2/mL beer. These limits are comparable with those reported by Scott and Lawrence (31), even though the injection volume of beer sample used in the current study (equivalent to 2 mL beer) was less than that of the other method (equivalent to 7 mL beer).

The immunoaffinity columns removed matrix interferences, as shown by the liquid chromatograms (Figures 1 and 2). The identity of AF B_1 was confirmed by the disappearance of the AF B_{2a} peak without trifluoroacetic acid derivatization (Figure 1B). In the case of OTA, a positive confirmation of identity was provided by the disappearance of the OTA peak at a typical retention time of 9.2 min and appearance of the OTA methyl ester peak at a typical retention time of 7.9 min when methylation was done (Figure 2B). Although the OTA methyl ester peak appeared after the OTA peak under an acidic mobile phase (23), the OTA methyl ester peak appeared before the OTA peak under an ion-pair chromatography alkaline mobile phase (35).

Results of OTA analysis of the beer samples from various countries are shown in Table 2. OTA was detected in imported beer samples at levels ranging from 1.2 to 66.2 pg/mL at a high incidence (91.5%) and in Japanese beer at levels ranging from 2.2 to 44.8 pg/mL at a high incidence (95.5%). Recently, Thellman and Weber (24) detected OTA in 3 malt samples at levels ranging from 0.1 to 0.92 µg/kg. In our results, 100% malt (no adjuncts) beer samples from Germany and other countries also contained OTA at levels ranging from 6.9 to 36.4 pg/mL. These results indicate that OTA is likely to enter the final beer from malt. The mean OTA concentration in Japanese beer samples was similar to that in North American or European beer samples. In Japan, most malts for beer are imported from North America and Europe, and so it is not unlikely that the concentrations of OTA in Japanese beer would be similar to those in North American and European beers. However, one Japanese beer sample made from only domestic barley contained OTA at 5.9 pg/mL. This result suggests that Japanese barley also may be contaminated with OTA or OTA-producing fungi.

AFs were detected in beer samples from countries where AF contamination might be expected to occur because of their warm climate. AF B_1 was detected in 11 imported beer samples from the United States, Mexico, Bolivia, Brazil, Peru, Kenya, Spain, India, Israel, and the Philippines at levels ranging from 0.5 to 83.1 pg/mL. The samples from Bolivia, Peru, India, and the Philippines also contained AF B_2. The levels of AF B_1 in beer samples are comparable with those reported by Scott and Lawrence (31). Two Japanese beer samples also contained AF B_1 at very low levels (0.5 and 0.8 pg/mL). Except for one beer sample from Peru, samples contaminated with AFs were also contaminated with OTA. In conclusion, because of the worldwide occurrence of OTA in beer, surveys of beer for OTA and other mycotoxins should be continued.

References

(1) Scott, P.M., van Walbeek, W., Kennedy, B., & Anyeti, D. (1972) *J. Agric. Food Chem.* **20**, 1103–1109

(2) Scott, P. M., & Kennedy, B.P.C. (1973) *J. Assoc. Off. Anal. Chem.* **56**, 1452–1456

(3) Beljaars, P.R., Schumans, J.C.H.M., & Koken, P.T. (1975) *J. Assoc. Off. Anal. Chem.* **58**, 263–271

(4) Shotwell, O.L., Goulden, M.L., & Hesseltine, C.W. (1976) *J. Assoc. Off. Anal. Chem.* **59**, 122–124

(5) Jelinek, C.F., Pohland, A.E., & Wood, G.E. (1989) *J. Assoc. Off. Anal. Chem.* **72**, 223–230

(6) Tabata, S., Kamimura, H., Ibe, A., Hashimoto, H., Iida, M., Tamura, Y., & Nishima, T. (1993) *J. AOAC Int.* **76**, 32–35

(7) Gjertsen, P., Myken, F., Krogh, P., & Hald, B. (1973) in *Proceedings of the 14th Congress of the European Brewery Convention*, Salzburg, Austria, Elsevier, Amsterdam, The Netherlands, pp 373–380

(8) Fischbach, H., & Rodricks, J.V. (1973) *J. Assoc. Off. Anal. Chem.* **56**, 767–770

(9) Cook, P., & Collis, C.H. (1972) *Lancet* **7758**, 1014

(10) Peers, F.G., & Linsell, C.A. (1973) *Br. J. Cancer* **27**, 473–484

(11) Mannio, M., & Enari, T.-M. (1973) *Brauwissenschaft* **26**, 134–137

(12) Scott, P.M. (1996) *J. AOAC Int.* **79**, 875–882

(13) Tressl, R., Hommel, E., & Helak, B. (1989) *Monatsschr. Brauwiss.* **42**, 331–335

(14) Vecchio, A., Finoli, C., Peccedi, M., & Cerutti, G. (1985) *Tecnol. Aliment. (Milano)* **8**, 84–87

(15) Cerutti, G., Vecchio, A., Finoli, C., & Trezzi, A. (1987) *Monatsschr. Brauwiss.* **40**, 455–457

(16) Majerus, P., & Woller, R. (1983) *Monatsschr. Brauwiss.* **36**, 335–336

(17) Payen, J., Girard, T., Gaillardin, M., & Lafont, P. (1983) *Microbiol. Aliment. Nutr.* **1**, 143–146

(18) El-Dessouki, S. (1992) *Dtsche. Lebensm. Rundsch.* **88**, 354–355

(19) Majerus, P., Cutka, I., Dreyer, A., El-Dessouki, S., Eyrich, W., Reush, H., Schurer, B., & Waiblinger, H.U. (1993) *Dtsche. Lebensm. Rundsch.* **89**, 112–114

(20) Jiao, Y., Blaas, W., Rühl, C., & Weber, R. (1994) *Dtsche. Lebensm. Rundsch.* **90**, 318–321

(21) Jenkins, R. (1994) *Brew. Dist. Int.* November, pp 18–19

(22) Zimmerli, B., & Dick, R. (1995) *J. Chromatogr. B* **666**, 85–99

(23) Scott, P.M., & Kanhere, S.R. (1995) *Food Addit. Contam.* **12**, 591–598

(24) Thellmann, A., & Weber, W. (1997) *Dtsche. Lebensm. Rundsch.* **93**, 1–3

(25) Alozie, T.C., Rotimi, C.N., & Oyibo, B.B. (1980) *Mycopathologia* **70**, 125–128

(26) Okoye, Z.S.C., & Ekpenyong, K.I. (1984) *Trans. Royal Soc. Trop. Med. Hyg.* **78**, 417–418

(27) Obasi, O.E., Ogbadu, G.H., & Ukoha, A.I. (1987) *Trans. Royal Soc. Trop. Med. Hyg.* **81**, 879

(28) Galea, V., & Bara, A. (1976) *Igiena* **25**, 123–128

(29) Woller, R., & Majerus, P. (1982) *Brauwissenschaft* **35**, 88–90

(30) Fukal, L., Prosek, J., & Rakosova, A. (1990) *Monatsschr. Brauwiss.* **43**, 212–215

(31) Scott, P.M., & Lawrence, G.A. (1997) *J. AOAC Int.* **80**, 1229–1234

(32) Ono, H., Seo, T., Cho, E., Nakagawa, T., Tomita, M., Tsuboi, S., & Iwamura, N. (1982) *ICMR Ann.* **2**, 127–134

(33) Ueno, Y. (1998) *Mycotoxins* **47**, 25–32

(34) *Official Methods of Analysis* (1995) 16th Ed., AOAC INTERNATIONAL, Arlington, VA, Chapter 49

(35) Terada, H., Tsubouchi, H., Yamamoto, K., Hisada, K., & Sakabe, Y. (1986) *J. Assoc. Off. Anal. Chem.* **69**, 960–964

(36) Nakajima, M., Terada, H., Hisada, K., Tsubouchi, H., Yamamoto, K., Uda, T., Itoh, Y., Kawamura, O., & Ueno, Y. (1990) *Food Agric. Immunol.* **2**, 189–195