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Fate of Mycotoxins during Beer Brewing and Fermentation

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Mycotoxins are frequent contaminants of grains, and breweries need, therefore, to pay close attention to the risk of contamination in beer made from such grains as barley and corn. The fate of 14 types of mycotoxin (aflatoxins, fumonisins, ochratoxin A, patulin, trichothecenes, and zearalenone) during beer brewing was investigated in this study. Malt artificially spiked with each mycotoxin was put through the mashing, filtration, boiling and fermentation processes involved in brewing. After brewing, the levels of aflatoxins, ochratoxin A, patulin, and zearalenone were found to have decreased to less than 20% of their initial concentration. They had been adsorbed mainly to the spent grain and removed from the unhopped wort. Additionally, as zearalenone was known, patulin was metabolized to the less toxic compound during the fermentation process. The risk of carry-over to beer was therefore reduced for half of the mycotoxins studied. However, attention still needs to be paid to the risk of trichothecene contamination.

Key words: mycotoxin; beer; brewing; fermentation; high-pressure liquid chromatography tandem mass spectrometry (LC-MS/MS)

Approximately 300 or more chemical compounds are currently recognized as mycotoxins which are toxic secondary metabolites produced by fungi. Spores from these fungi can be found floating in the air and, under favorable conditions of temperature, moisture and oxygen concentration, can grow and produce mycotoxins. Moreover, mycotoxins are generally thermostable and can remain present in crops even after all signs of the fungus itself have been removed. Fungi and mycotoxins are therefore a potential problem for farmers and food manufacturers that can adversely affect production.

In particular, there are more than 10 kinds of mycotoxin that need to be carefully controlled due to their toxicity and widespread occurrence. The most virulent of these is the aflatoxin group, represented by aflatoxin B_1 (AFB_1) which has one of the highest levels of carcinogenicity of any natural toxin and is classified as group 1 (carcinogenic to humans) by the International Agency for Research on Cancer (IARC).[1] Other significant mycotoxins include trichothecenes (deoxynivalenol [DON], nivalenol [NIV], T-2 toxin [T-2] and HT-2 toxin [HT-2]), ochratoxin A (OTA), fumonisins (FMB_1, FMB_2 and FMB_3) and zearalenone (ZON) in cereals. These toxic chemical compounds can be produced in such raw materials as barley, corn and rice used in beer manufacture. Many studies have been conducted on mycotoxin contamination of commercial beer and its raw materials. Trichothecenes, OTA, and ZON have been frequently detected in barley,[2–5] which is the main raw material of beer, and AFB_1 and FMB_1 contamination has also been reported.[5] PAT has been recognized as a potential contaminant of malt made from barley.[6] Corn and rice have also been found contaminated with AFs, DON, OTA, FMs, and ZON.[7–10] It has in fact been reported that such mycotoxins as trichothecenes, OTA, and FMs were transferred to commercial beer.[11–16]

Studies have also been carried out on the fate of such specific mycotoxins as AFB_1, DON, HT-2, OTA, FMB_1, and ZON during beer brewing.[17–23] Despite the fact that many mycotoxins can contaminate the beer or raw materials used in beer manufacture, not all have yet been comprehensively studied.

This study is focused on 14 mycotoxins (Fig. 1) that are potent toxicants and are occasionally found in the raw materials used in beer manufacture. In a laboratory-scale investigation, beer was brewed from malt artificially spiked with the 14 selected mycotoxins (Fig. 2). By comparing their residual ratios in the intermediates produced at key steps throughout the various brewing processes, we aimed to predict which mycotoxins need to be controlled with special care.

Materials and Methods

Materials. The mycotoxin standard solutions, aflatoxin B_1 (AFB_1; $3\,\mu g\,mL^{-1}$ in benzene/acetonitrile, a 98/2 solution), aflatoxin B_2 (AFB_2; $0.5\,\mu g\,mL^{-1}$ in benzene/acetonitrile, a 98/2 solution), aflatoxin G_1 (AFG_1; $3\,\mu g\,mL^{-1}$ in benzene/acetonitrile, a 98/2 solution), aflatoxin G_2 (AFG_2; $0.5\,\mu g\,mL^{-1}$ in benzene/acetonitrile, a 98/2 solution), patulin (PAT; $100\,\mu g\,mL^{-1}$ in a chloroform solution) and ochratoxin A (OTA; $10\,\mu g\,mL^{-1}$ in an acetonitrile solution), were purchased from Supelco (Bellefonte, PA, USA). Nivalenol (NIV; $100\,\mu g\,mL^{-1}$ in acetonitrile) was purchased from Sigma Aldrich Corp. (St. Louis, MO, USA). Deoxynivalenol (DON; $100\,\mu g\,mL^{-1}$ in acetonitrile), T-2 toxin (T-2; $100\,\mu g\,mL^{-1}$ in acetonitrile) and HT-2 toxin (HT-2; $100\,\mu g\,mL^{-1}$ in acetonitrile) were purchased from Wako

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Abbreviations: AFB_1, aflatoxin B_1; AFB_2, aflatoxin B_2; AFG_1, aflatoxin G_1; AFG_2, aflatoxin G_2; DON, deoxynivalenol; NIV, nivalenol; T-2, T-2 toxin; HT-2, HT-2 toxin; OTA, ochratoxin A; FMB_1, fumonisin B_1; FMB_2, fumonisin B_2; FMB_3, fumonisin B_3; OTA, ochratoxin A; ZON, zearalenone; β-ZOL, β-zearalenol; LC-MS/MS, high-pressure liquid chromatograpy tandem mass spectrometry; IARC, International Agency for Reserch on Cancer; ESI, electrospray ionization; MRM, multiple-reaction monitoring; RSD, residual standard deviation; LOQ, limit of quantification; SD, standard deviation

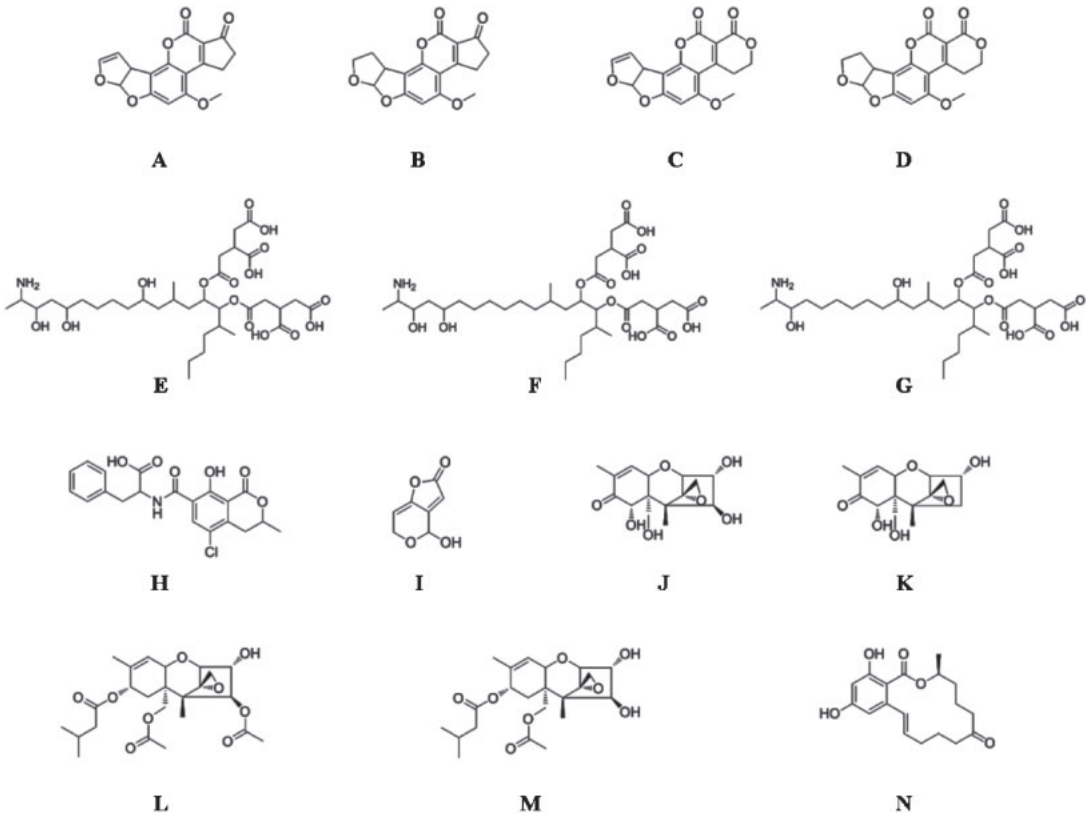

Fig. 1. Structures of the Mycotoxins.
A) AFB_1, B) AFB_2, C) AFG_1, D) AFG_2, E) FMB_1, F) FMB_2, G) FMB_3, H) OTA, I) PAT, J) NIV, K) DON, L) T-2, M) HT-2, N) ZON.

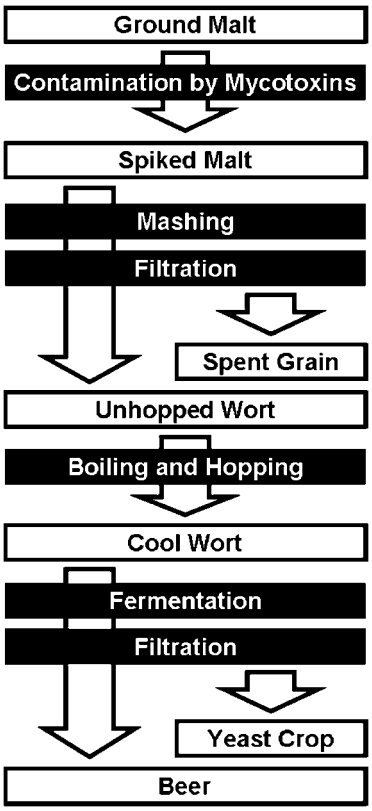

Fig. 2. Scheme for the Principal Stages of the Laboratory-Scale Brewing Process.

The analytes were sampled from the unhopped wort, spent grain, cool wort, yeast crop, and beer. Refer to "Laboratory-scale beer brewing using malt spiked with mycotoxins" in the Materials and Methods section for details of the lab-scale brewing method.

Pure Chemical Industries (Osaka, Japan). Zearalenone (ZON; $100\,\mu g\,mL^{-1}$ in acetonitrile), fumonisin B_1 (FMB_1; $50\,\mu g\,mL^{-1}$ in acetonitrile/water, a 1/1 solution), fumonisin B_2 (FMB_2; $50\,\mu g\,mL^{-1}$ in acetonitrile/water, a 1/1 solution) and fumonisin B_3 (FMB_3; $50\,\mu g\,mL^{-1}$ in acetonitrile/water, a 1/1 solution) were purchased from Biopure Corp. (Cambridge, MA, USA).

Acetonitrile (LC/MS grade), methanol (LC/MS grade) and ammonium acetate (JIS-guaranteed reagent), purchased from Kanto Chemical Co. (Tokyo, Japan), and acetic acid (LC/MS grade), obtained from Wako Pure Chemical Industries (Osaka, Japan), were used as solvents.

Samples were prepared by using an Oasis HLB column (6 cc, 200 mg) provided by Waters (Milford, MA). Analytical samples were passed through a PTFE filter with a mesh size of 0.2 µm that was purchased from Advantec Toyo Kaisha (Tokyo, Japan).

Apparatus. The samples were analyzed by using liquid chromatography-tandem mass spectrometry (LC-MS/MS). An Acquity UPLC system (Waters) equipped with an Acquity BEH C18 column (1.7 µm, 50×2.1 mm) was used for liquid chromatography. A Xevo TQ MS system (Waters) was used for highly sensitive tandem mass spectrometry and was interfaced with the LC unit.

Analytical method. Five microliters of each sample was injected into the column with the temperature controlled at 40 °C. The flow rate for gradient elution was $0.5\,mL\,min^{-1}$. The mobile phase eluents were 2% acetic acid and 0.1 mM ammonium acetate in methanol (A), and water (B).

The 14 target mycotoxins were assigned to two groups, and linear gradient profiles were set up on the liquid chromatograph for each group. The first profile was set for PAT, AFB_1, AFB_2, AFG_1, AFG_2, DON, NIV, HT-2, T-2 and ZON, while the second was for OTA, FMB_1, FMB_2 and FMB_3. The first group was analyzed under the following conditions: 0 min (5% A, 95% B), 4.5 min (80% A, 20% B), 4.51 min (5% A, 95% B), and 6 min (5% A, 95% B). In order to avoid matrix effects, the following conditions were used for yeast: 0 min (5% A, 95% B), 10 min (80% A, 20% B), 10.5 min (5% A, 95% B), and 11 min (5% A, 95% B). The second group was analyzed under the

Table 1. Precursor to the Fragment Transitions, Optimum Cone Energy, and Optimum Collision Energy of Each Mycotoxin

Name		Mode	Precursor	Fragment	Cone	Collision
DON	MS1	ESI(+)	297.1	249.0	28	12
	MS2	ESI(+)	297.1	230.9	28	13
AFB_1	MS1	ESI(+)	313.1	240.9	40	35
	MS2	ESI(+)	313.1	268.7	40	32
AFB_2	MS1	ESI(+)	315.1	286.9	55	25
	MS2	ESI(+)	315.1	258.9	55	35
AFG_1	MS1	ESI(+)	329.1	242.8	55	25
	MS2	ESI(+)	329.1	310.9	55	20
AFG_2	MS1	ESI(+)	331.1	313.0	48	24
	MS2	ESI(+)	331.1	244.9	57	35
HT-2	MS1	ESI(+)	442.2	214.9	23	13
	MS2	ESI(+)	442.2	263.0	23	13
T-2	MS1	ESI(+)	484.2	305.0	27	13
	MS2	ESI(+)	484.2	214.9	27	20
PAT	MS1	ESI(−)	152.9	108.8	16	8
	MS2	ESI(−)	152.9	78.6	16	24
ZON	MS1	ESI(−)	317.2	174.8	47	24
	MS2	ESI(−)	317.2	273.0	47	20
NIV	MS1	ESI(−)	371.1	280.9	25	17
	MS2	ESI(−)	371.1	311.0	25	10
OTA	MS1	ESI(+)	404.1	238.7	23	25
	MS2	ESI(+)	404.1	357.9	23	15
$FMB_{2,3}$	MS1	ESI(+)	706.3	336.3	57	37
	MS2	ESI(+)	706.3	318.3	57	40
FMB_1	MS1	ESI(+)	722.3	334.3	57	45
	MS2	ESI(+)	722.3	352.3	57	40

Refer to "Apparatus" in the Materials and Methods section for details of the equipment conditions.

following conditions: 0 min (55% A, 45% B), 2 min (80% A, 20% B), 2.01 min (55% A, 45% B), and 3 min (55% A, 45% B).

Each target substance was treated in the positive or negative electrospray ionization (ESI) mode. The MS/MS properties were set as follows: ion source voltage, 3 kV; ion source temperature, 150 °C; and desolvation temperature, 500 °C. Data for quantification and confirmation were acquired in the multiple-reaction monitoring (MRM) mode. The precursor to fragment transitions and the optimum cone and collision energies for each compound are shown in Table 1.

Laboratory-scale beer brewing using malt spiked with mycotoxins. Ground malt (110 g) was artificially spiked with 14 different mycotoxin standard solutions at a concentration of 100 ng mL^{-1} (except for PAT [250 ng mL^{-1}] and AFB_1, AFB_2, AFG_1 and AFG_2 [20 ng mL^{-1}]). The mycotoxin-spiked ground malt was added to hot water (50 °C, 200 mL), and the first mashing was conducted at 55 °C for 1 h. The mash obtained was added to mashed corn starch made from corn starch (50 g) and hot water (50 °C, 400 mL), diluted with hot water (50 °C), and made up to a total of 800 mL. The second mashing was conducted at 65 °C for 1 h and 76 °C for 5 min. An unhopped wort (680 mL) was obtained after the mash had been filtered, and spent grain (200 g) was obtained as a byproduct. The wort was boiled with pelletted hops (approximately 0.5 g) in an oil bath at 140 °C for 1 h. It was then cooled and filtered from the trub (7.9 g) to obtain a cool wort (570 mL). This cool wort (200 mL) was added to bottom-fermenting yeast (*Saccaromyces pastorianus*, 1.0 g) and mixed by a magnetic stirrer at 10 °C for 7 d. Further fermentation was carried out under static conditions at 10 °C for 7 d and at 4 °C for 4 d. The beer was finally recovered by filtering out the yeast (Fig. 2).

Fermentation of beer from the cool wort spiked with mycotoxins. In order to observe the fate of the mycotoxins during the fermentation step, the cool wort produced from non-contaminated ground malt was artificially spiked with mycotoxins before fermentation. Fourteen different mycotoxin standard solutions were added to the cool wort (200 mL) at a concentration of 50 ng mL^{-1}, except for PAT (125 ng mL^{-1}) and AFB_1, AFB_2, AFG_1 and AFG_2 (10 ng mL^{-1}). Yeast (1 g) was then added to the contaminated cool wort, and fermentation was carried out at 10 °C for the first 7 d, using a magnetic stirrer. Further fermentation was carried out under static conditions at 10 °C for the next 7 d and then at 4 °C for the last 4 d. Beer was obtained after filtering out the yeast. The progress of the fermentation process was monitored daily by measuring the brix level (°Bx) which denotes the sugar content in an aqueous solution.

Sample preparation for unhopped wort, cool wort and beer. Samples of unhopped wort, cool wort and beer were taken during the beer brewing process and pretreated in an Oasis HLB solid-phase column. Three replicates of each sample from the brewing process were prepared. Each sample (1 mL) was added to 5% methanol/water (3 mL) and sonicated for 5 min. This solution was loaded into the HLB column conditioned with 50% methanol (5 mL) and 5% methanol (5 mL), washed three times with 5% methanol (4 mL), and then eluted with methanol (5 mL). The eluate was dried in a nitrogen atmosphere at 40 °C, and the dried residue was dissolved in an 85/15 solution (1 mL) of 10 mM ammonium acetate in water/acetonitrile. The analytical solution was passed through a PTFE filter with a 0.2 µm mesh and then transferred to a 2-mL glass vial.

Sample preparation for the spent grain and yeast crop. The mycotoxins in the spent grain or yeast crop needed to be extracted with an organic solvent before being cleaned up with a solid-phase column. The spent grain (1 g) or yeast crop (1 g) was centrifuged (3000 rpm for 5 min), and 80% methanol (3 mL) was added. This suspension was shaken vigorously for 10 min and then centrifuged (3000 rpm for 5 min). The resulting supernatant was dried in a nitrogen atmosphere and dissolved with 5% methanol (3 mL).

After the mycotoxins had been extracted and dissolved, the same sample preparation procedure was followed as that described in the previous section for unhopped wort, cool wort and beer.

Method validation. The experimental method was statistically validated by comparing the relative standard deviation of the repeatability (RSD), recovery, and linearity (r). The repeatability and recovery were calculated from six experimental replicates per sample. Each non-contaminated sample was spiked with mycotoxins at 50 ng mL^{-1} (except for PAT [125 ng mL^{-1}]; AFB_1, AFB_2, AFG_1 and AFG_2 [10 ng mL^{-1}]) before and after pretreatment. The linearity of the standard addition calibration curves was estimated from the eight data points of 0, 2, 5, 10, 20, 50, 70, 100, and 200 ng mL^{-1}.

Analysis. The residual level of each mycotoxin in the sample was quantified by using the standard addition calibration curve over a range of 2–200 ng mL^{-1}. The residual ratio was calculated from the concentration level of each mycotoxin in the sample, with the initial concentration for the ground malt set at 100%. Each key intermediate produced from the non-spiked malt was analyzed to confirm whether or not it was free from natural mycotoxin contamination.

Results and Discussion

Method validation

The analysis methods used to detect the presence of each mycotoxin in the cool wort, beer, spent grain and yeast crop were validated. The ideal criteria for validation are defined as follows: RSD of less than 20%, recovery between 70% and 120%, and correlation coefficient for linearity of more than 0.99.[24] The results for the cool wort, beer, spent grain and yeast crop are shown in Table 2. All of the mycotoxins in the cool wort and beer gave good results. The method for mycotoxins from the spent grain and yeast crop achieved ideal repeatability and linearity, but only DON, T-2 and HT-2 for the spent grain, and FMB_1, OTA, DON and ZON for the yeast crop were recovered well. A standard addition calibration curve was hence adopted in this study to estimate the residual concentrations. The lowest level for the limit of quantification (LOQ) for all mycotoxins except DON and NIV was 2 ng mL^{-1}, this being at the lowest limit of the analytical curve for the cool wort, beer and spent grain. Since DON and NIV (with a higher polarity) were affected by matrix effects, their LOQ was set at 10 ng mL^{-1}.

Fate of the mycotoxins during beer brewing

The residual ratios of all mycotoxins present were calculated at each step of the brewing process for beer made from the ground mycotoxin-spiked malt. Analytical samples were taken from i) unhopped wort, ii) spent grain, iii) cool wort, and iv) beer. Table 3 shows the residual ratios of the mycotoxins, calculated from their concentration levels at each step. The concentrations of half of the mycotoxins tested (OTA, AFB_2, FMB_2, AFG_1, AFB_1, ZON and PAT) decreased to less than 20% of their initial level. This result shows good agreement with previous studies on DON carried out by Lancova *et al.*[19] and those carried out on AFB_1 and FMB_1 by Pietri *et al.*[21] The largest decrease in mycotoxin levels was observed during mashing, followed by smaller changes in all subsequent steps. On the other hand, the NIV, DON and HT-2 levels in beer remained at more than 50% of their initial concentrations. This indicates a higher risk of residual mycotoxins due to the presence of trichothecenes in beer, even after brewing (in contrast to the results obtained for OTA, AFB_2, FMB_2, AFG_1, AFB_1, ZON and PAT).

Residual ratios for the spent grain

The residual levels of mycotoxins in the spent grain were also analyzed with the results shown in Table 3.

Table 2. Results of Validation Tests (%RSD, Recovery Rate and Linearity) for Each Mycotoxin Found in the Cool Wort, Beer, Spent Grain and Yeast Crop

Mycotoxin	Cool wort			Beer			Spent grain			Yeast crop		
	RSD (%)	linearity	Recovery (%)	RSD (%)	linearity	Recovery (%)	RSD (%)	linearity	Recovery (%)	RSD (%)	linearity	Recovery (%)
AFB_1	5.5	1.00	87	1.7	1.00	88	4.3	1.00	40	4.3	1.00	43
AFB_2	4.8	1.00	95	0.3	1.00	91	3.8	1.00	47	9.8	1.00	52
AFG_1	14.4	1.00	76	3.0	1.00	85	6.3	1.00	37	6.6	1.00	40
AFG_2	11.0	1.00	82	2.4	1.00	84	4.7	1.00	37	8.0	1.00	43
FMB_1	10.5	1.00	118	10.6	1.00	88	11.6	1.00	49	6.9	1.00	71
FMB_2	11.6	1.00	110	7.9	1.00	86	15.7	1.00	45	4.7	1.00	67
FMB_3	13.6	1.00	90	8.7	1.00	90	15.6	1.00	50	3.1	1.00	64
OTA	4.5	1.00	94	0.6	1.00	91	12.0	1.00	66	7.3	1.00	96
PAT	2.9	1.00	71	2.9	1.00	88	3.0	1.00	56	—	—	—
NIV	11.0	1.00	101	7.9	1.00	79	3.8	1.00	75	14.8	0.99	53
DON	3.2	1.00	95	1.9	1.00	86	3.9	1.00	63	8.9	1.00	94
T-2	10.2	1.00	83	1.5	1.00	95	6.0	0.99	71	17.8	0.99	33
HT-2	3.6	1.00	93	1.3	1.00	96	4.3	1.00	76	8.6	1.00	30
ZON	19.8	1.00	100	9.7	1.00	91	11.7	1.00	60	14.0	1.00	83

Refer to "Method validation" in the Materials and Methods section for details of the validation process.

Table 3. Residual Ratios of Mycotoxins (%) in i) Unhopped Wort, ii) Cool Wort, iii) Beer, and iv) Spent Grain

	NIV	DON	HT-2	T-2	FMB_1	FMB_2	FMB_3	OTA	AFB_1	AFB_2	AFG_1	AFG_2	ZON	PAT
i) unhopped wort (%)	77.0	64.1	54.5	33.9	47.2	19.3	34.7	22.5	15.3	30.6	21.8	19.5	<12.4	<12.4
	(5.6)	(1.9)	(1.4)	(3.2)	(1.7)	(1.2)	(2.4)	(0.6)	(0.9)	(1.4)	(1.5)	(2.9)		
ii) cool wort (%)	79.3	68.4	43.5	27.9	32.4	17.0	26.8	17.0	10.7	17.0	16.9	26.4	<10.4	<10.4
	(15.0)	(6.3)	(3.3)	(0.5)	(2.7)	(1.1)	(3.6)	(1.1)	(0.1)	(1.0)	(0.3)	(0.7)		
iii) beer (%)	71.2	61.2	51.6	35.3	46.1	10.6	25.3	15.5	5.4	12.1	9.0	27.7	<10.4	<10.4
	(3.6)	(4.8)	(2.9)	(2.6)	(3.5)	(0.4)	(1.3)	(1.3)	(0.3)	(0.7)	(0.6)	(0.7)		
iv) spent grain (%)	<9.1	14.4	16.4	20.7	16.4	38.2	20.6	39.6	52.2	63.2	41.7	32.3	48.0	<3.6
		(2.4)	(2.2)	(3.6)	(2.2)	(2.2)	(0.4)	(0.4)	(3.3)	(3.6)	(0.2)	(0.8)	(0.3)	

The first figure is for the residual ratio (%) in each step, whereas the figure in parentheses is for the standard deviation (SD) of three replicates. Refer to "Laboratory-scale beer brewing from malt spiked with mycotoxins" in Materials and Methods section for details of the conditions used in the brewing process.

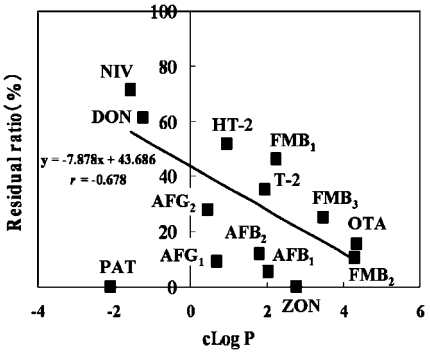

Fig. 3. Correlation Chart Illustrating the Relationship between the clogPow Value and the Residual Ratio in Beer.

Each residual ratio (refer to the analysis section in the Materials and Methods section for further details) was plotted *vs.* clogPow ($r = -0.68$).

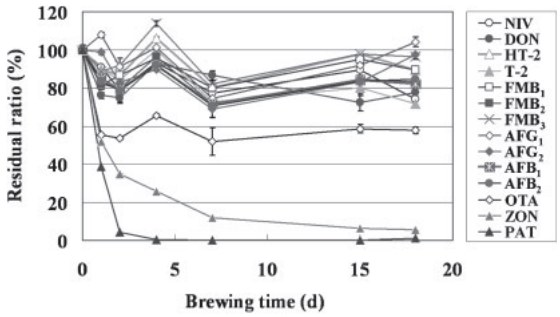

Fig. 4. Residual Ratios during Fermentation.

Each residual ratio was plotted with a standard deviation (SD) bar for three replicates analyzed. Refer to "Fermentation of beer from cool wort spiked with mycotoxins" in the Materials and Methods section for details of the fermentation conditions.

Seven out of the 14 mycotoxins examined (AFB_2, AFB_1, AFG_2, AFG_1, OTA, FMB_2 and ZON) remained at 30% to 60% of their initial concentrations. Their residual ratios were 15–31% in the unhopped wort and around 10% in the beer. However, the trichothecene mycotoxins (NIV, DON, HT-2 and T-2), FMB_1 and FMB_3, which showed lower levels in the spent grain, displayed higher residual ratios in the beer itself. To some extent, the mycotoxins could be decomposed during mashing and boiling because not all of the spiked malt was found in the wort and spent grain. However, decomposed compounds were not studied in this research. Adsorption to the spent grains was thus identified as a major determinant in the removal of mycotoxins from the beer. The hydrophobicity of each mycotoxin was presumably a key factor determining the extent of such adsorption. Figure 3 shows the residual ratio of each mycotoxin present in the beer in this study plotted against its calculated logPow (clogPow) value. The clogPow values were calculated by using Advanced Chemistry Development (ACD/Labs) V11.02 software.

The clogPow value indicates the octanol-water partition coefficient. When these values were plotted, all results except those for PAT and ZON (degraded) produced a good correlation between the residual ratio and clogPow ($r = -0.68$). The mycotoxins with a higher clogPow value tended to be found at lower levels in the beer. Although this study was made with malt artificially spiked with mycotoxins, this result was able to predict the residue of mycotoxins naturally contaminated. PAT did not follow this trend because its disappearance was not primarily due to adsorption, but to hydrolysis or pyrolysis. The reported decrease of PAT in apple juice after pasteurization[25,26] suggests a possibility of PAT was decomposed during mashing.

Fate of mycotoxins during fermentation

An investigation of the fate of mycotoxins during brewing showed that some had been decomposed or removed from the wort (Table 3). In particular, ZON and PAT completely had disappeared in the cool wort. Such other mycotoxins as AFs, FMs, OTA, HT-2, and T-2 remained in quite small amounts in the cool wort, these amounts being too small to assign to the effect of fermentation. Although the fate of ZON during fermentation had been previously studied in our laboratory,[23] the other 13 mycotoxins had not been examined. It was therefore necessary to conduct a detailed investigation of the fermentation step by using the cool wort artificially spiked with these particular mycotoxins. Their fate during 18 d of fermentation is shown in Fig. 4. ZON and PAT exhibited enzymatic degradation and showed a substantial decline. ZON is known to be metabolized to β-zearalenol (β-ZOL), which had weaker estrogenic activity than that of ZON, during beer fermentation.[23,27,28] PAT also decomposed quite rapidly, being reduced to less than 10% of its initial concentration within 2 d and completely decomposed within 4 d. PAT is known to be converted to ascladiol by *Saccharomyces cervisea*, a top-fermenting yeast, during the fermentation of apple juice to produce cider.[29] The acute toxicity of ascladiol is approximately one quarter that of PAT.[30] The OTA level dropped to less than 60% of its initial concentration during fermentation. This was mainly caused by adsorption to the yeast (Table 4). OTA decomposition to phenylalanine and α-OTA or other metabolites has been potentially suggested,[31] although those degradation compounds could not be detected in this study.

Table 4. Residual Ratios of Mycotoxins Found in Yeast Crop

Mycotoxin	Adsorption ratio (%)
NIV	0.42 (0.00)
DON	0.28 (0.04)
HT-2	0.28 (0.00)
T-2	0.33 (0.00)
FMB_1	1.15 (0.06)
FMB_2	10.86 (0.29)
FMB_3	3.87 (0.12)
OTA	15.85 (0.25)
AFG_2	1.47 (0.02)
AFG_1	3.10 (0.01)
AFB_2	2.62 (0.00)
AFB_1	5.11 (0.68)
ZON	0.44 (11.84)
PAT	—

The first figure is for the adsorption ratio (%), whereas the figure in parentheses is for the standard deviation (SD) of three replicates. Refer to "Fermentation of beer from cool wort spiked with mycotoxins" in Materials and Methods section for details of the fermentation conditions.

In summary, the fate of 14 mycotoxins during a laboratory-scale brewing process was clarified and explained. It was found that the residual concentrations of OTA, AFB_2, FMB_2, AFG_1, AFB_1, ZON and PAT had

decreased to less than 20%. This reduction was mainly due to adsorption to the spent grain. They therefore disappeared naturally in the course of the brewing process and only pose a low health risk. As in a previous study, ZON was metabolized to a less toxic compound during the fermentation process. Moreover, PAT was metabolized to less toxic compounds in the fermentation stage. The beer contamination risk posed by these two mycotoxins can therefore be considered to be quite low. In contrast, the mycotoxins belonging to the trichothecene group, such as DON, NIV and HT-2, have to be paid close attention to, especially when producing and storing the raw ingredients for beer making. The data obtained in this study will help to improve risk management and reduce the potential for mycotoxin contamination during beer manufacture.

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