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Occurrence of Deoxynivalenol and Its Major Conjugate, Deoxynivalenol-3-Glucoside, in Beer and Some Brewing Intermediates

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Since deoxynivalenol (DON), the main representative of *Fusarium* toxic secondary metabolites, is a relatively common natural contaminant in barley, its traces can be detected in many commercial beers. Our previous study reporting for the first time the occurrence of relatively high levels of DON-3-glucoside (DON-3-Glc) in malt and beer prepared from relatively "clean" barley (semiscale experimental conditions) induced a follow-up investigation focused on this DON conjugate in commercial beers. The current survey involving in total 176 beers, representing different brands, and collected at various markets, has documented a ubiquitous occurrence of DON-3-Glc in this product. Its levels even exceeded that of free DON in some samples; the highest level found was 37 μg/L. In addition to glucosylated DON, its acetylated forms (ADONs) were also common contaminants in most of the beers. Generally, stronger beers (higher alcohol content) tended to contain higher levels of DON and its conjugates. No distinct relationship between the contamination of malt and beer was observed in samples collected from several breweries. Attention was also paid to comparison of data on malts obtained by LC−MS/MS and ELISA DON-dedicated kits. The latter provided apparently higher levels of DON, the most distinct difference being observed for malts processed at higher temperatures (caramel and roasted malts). The nature of this phenomenon has not yet been explained; in addition to cross-reacting species, other factors, such as the higher content of dark pigment, can also be the cause.

KEYWORDS: Trichothecenes; deoxynivalenol; deoxynivalenol-3-glucoside; LC−MS/MS; ELISA; beer; wort; malt

INTRODUCTION

Fusarium mycotoxins are toxic secondary metabolites produced under field conditions by many species of the *Fusarium* genus in various cereals, including barley (*1, 2*). *Fusarium* head blight (FHB) not only may result in a lower yield of crop but also may reduce technological quality of grains (e.g., gushing of beer prepared from FHB barley is a serious problem) (*2*−*9*). To protect consumers' health, maximum levels have been established for major *Fusarium* toxins, deoxynivalenol (DON), zearalenone, and fumonisins in cereals and some cereal-based products in Commission Regulation (EC) 1881/2006 (*10*). For those products, where no specific Community maximum levels have been set, the need to take into account changes in the concentration of respective contaminants caused by processing is emphasized in Article 2 of this Regulation.

While cleaning the grains during a harvest or their scouring in a first stage of processing may reduce mycotoxins levels, an increase (sometimes very distinct) of DON and other *Fusarium* toxin levels during malting has been reported by several

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Table 1. Beer Samples Analyzed

beer type		no. of samples characterized					
		≤0.5% alcohol by volume	0.5−3.9% alcohol by volume	4.0−4.9% alcohol by volume	5.0−5.9% alcohol by volume	6.0−9.0% alcohol by volume	total no. of samples
light	prepared from barley only	4	1	52	49	6	112
	prepared from barley and adjuncts	4	2	12	22	6	46
	light beers	**8**	**3**	**64**	**71**	**12**	**158**
dark	prepared from barley only	2	0	1	2	1	6
	prepared from barley and adjuncts	2	0	6	3	1	12
	dark beers	**4**	**0**	**7**	**5**	**2**	**18**
	total no. of samples	**12**	**3**	**71**	**76**	**14**	**176**

authors (*2, 3, 8, 9, 11*). Thanks to their thermal stability and relatively good water solubility, *Fusarium* toxins can be transmitted from malt into beer (*2*−*4, 6, 7, 11*). It should be noted that unfortunately it is rather difficult to predict a contamination level of the final product based on analysis of a raw material, since many factors play a role, including the pattern of infection of barley grains and technological conditions employed for production of a particular brand of beer (*8, 9*). In any case, the presence of *Fusarium* and other mycotoxins in this commodity has been investigated in several studies (*11*−*32*). The first one addressing this problem was published as early as in 1974 (*33*). Follow-up research confirmed the occurrence of detectable amounts of DON, the main representative of trichothecene mycotoxins, in almost all commercial beer samples, regardless of the market where they were collected (*11*−*22*). Only a few studies were concerned with other *Fusarium* toxins in this commodity (*11*−*22*). Occasionally, ADONs and NIV were detected, while there is no evidence of transfer of trichothecenes A into the beer until now. It should also be noticed that other mycotoxins such as fumonisins, zearalenone, ochratoxin A, and/or aflatoxins were reported in some studies concerned with beer contamination (*16*−*19, 23*−*32*). Since, generally, high dilution of fungal secondary metabolites occurs during a brewing process, the concentration in beers is typically lower by 1 order of magnitude compared to that contained in raw material taken for its production (in addition to malt, cereal adjuncts such as wheat, maize, or corn grits can be used). However, exposure of consumers to mycotoxins through beer should not be underestimated, particularly in case of high-end drinkers. This widely popular beverage may, under certain conditions, contribute significantly to intake of DON, approaching or even exceeding a tolerable daily intake (TDI) of 1 μg/kg established by the Scientific Committee on Food (SCF) in Commission Regulation 856/2005 (*34*).

The extent of transmission of mycotoxins into the final beer is rather difficult to predict, since it depends on the pattern of grain infection and largely varies with technological conditions employed for production of a particular brand of beer (*8, 9*). This concern might be even more serious when considering other potential sources of dietary DON. Only recently have we reported the presence of relatively large amounts of DON-3-β-D-glucopyranoside (DON-3-Glc) in malt and beer (*11*). While the levels of this "masked" DON in the above-mentioned commodities were comparable to or even exceeded that of its free form, relatively small amounts of DON conjugate (assumed to originate through detoxification processes within phase II of plant metabolism) were found in cereals. For instance, in wheat artificially inoculated with *Fusarium graminearum* and *Fusarium culmorum*, the content of DON (bound in DON-3-Glc) was in the range of 14−29% of that of free DON, and in naturally contaminated grains, the content was 4−29% (*35*).

Until now, no information about the bioavailability of DON-3-Glc has been reported; nevertheless, the potential health risk associated with consumers' dietary exposure should be regarded as an issue of concern since this DON conjugate seems to be ubiquitous in various cereal-based products.

The aim of this study was to obtain more comprehensive information about the occurrence of DON-3-Glc in various beers available on the European market and (to a limited extent) on the North American market. The content of other *Fusarium* toxins was also determined, to assess the overall contamination pattern. Moreover, to obtain more knowledge of the fate of these mycotoxins during the brewing process, samples of malt, sweet wort, and final beer obtained from several breweries were also examined. For the evaluation of alternative approaches applicable for control of DON content in malt, which is the key commodity used for beer production, both DON-dedicated ELISA kits and LC−MS/MS (reference method) were employed for analysis of various brands.

MATERIALS AND METHODS

Standards. Analytical standards, with a purity in the range of 96−99.4%, of *Fusarium* mycotoxins deoxynivalenol (DON), isotope-labeled ($^{13}C_{15}$)DON, deoxynivalenol-3-glucoside (DON-3-Glc), 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol (ADONs), nivalenol (NIV), fusarenon-X (Fus-X), HT-2 toxin, T-2 toxin, and zearalenone (ZON) were purchased from Biopure (Tulln, Austria). ($^{13}C_{15}$)DON was used as an internal standard. Stock standard solutions (1 mg/mL) were prepared in acetonitrile and stored at −20 °C. A composite working standard solution (1000 ng/mL, each analyte) was also prepared in acetonitrile and stored for a maximum of 1 month at 4 °C. For preparation of matrix-matched standards, a solution containing each analyte at 25 ng/mL was prepared in a methanol/water mixture (1:1, v/v).

Chemicals and Reagents. Organic solvents used for LC−MS/MS analysis (HPLC grade methanol and ammonium acetate) as well as other chemicals (Cellite 500 fine and acetonitrile) used for sample preparation were obtained from Sigma-Aldrich (Taufkirchen, Germany). Deionized water was produced with a Milli-Q system (Millipore Corp., Bedford, MA).

Samples. *Beer.* In total, 176 beer samples (bottles and cans; see the overview in **Table 1**) were collected from European and North American markets in 2007. In addition to commercial beers, samples from beer production chain (malt, sweet wort, and beer) were obtained from 14 breweries (see **Table 2**). All the beers were bottom fermented. In production of three of them, adjuncts were employed.

Malts, Sweet Worts, and Beers Collected from the Brewing Process. Fourteen sets of samples representing the production chain malt to sweet wort (the first liquid fraction of beer brewing) and finally to beer obtained from various breweries were examined for the presence of mycotoxins. The characteristics of final products are summarized in **Table 2**. In three of these sample sets (PT 1a−3a and PL 1b−3b), malt was processed in two different ways, to obtain both tap and lager beer.

Table 2. Beers Obtained from Characterized Brewing Processes

final product	sample code	% alcohol by volume	addition of adjuncts	malt description
pale tap	PT 1a	4.5	yes	Pilsner
	PT 2a	3.9	no	mixture of two Pilsners
	PT 3a	4.0	yes	Pilsner
	PT 4	4.1	no	Pilsner
	PT 5	4.0	no	Pilsner
	PT 6	4.5	no	mixture of one Pilsner and two caramels
	PT 7	4.1	no	mixture of three Pilsners
pale lager	PL 1b[a]	5.2	no	Pilsner
	PL 2b[a]	4.8	yes	mixture of two Pilsners
	PL 3b[a]	5.0	yes	Pilsner
	PL 8	5.0	no	mixture of one caramel and three Pilsners
	PL 9	5.0	no	mixture of two caramels and one Pilsner
	PL 10	5.0	yes	Pilsner
	PL 11	5.1	yes	Pilsner

[a] Beer samples were derived from the same malts and sweet worts as PT 1a, PT 2a, and PT 3a, respectively.

Comparison of Two Analytical Approaches for Quantification of DON in Malt. Ten malts prepared by several types of malting technologies were examined for DON content by both an immunoassay (DON-dedicated ELISA kits) and LC−MS/MS. While the maximum temperature in production of Pilsner (pale) malts PM1−PM4 was only 85 °C during the kilning stage, temperatures of ∼120 °C were used when caramel malts CM1−CM4 were prepared. A special kilning process inducing crystallization and caramelization of carbohydrates contained in malt at temperatures of up to 120−180 °C was used for production of caramel malts. Temperatures as high as 220 °C were used for production of roasted malts RM1 and RM2.

Analysis of *Fusarium* Toxins. LC−MS/MS employed for analysis of seven *Fusarium* toxins and DON-3-Glc in beers, sweet worts, and malts was described in detail in our previous paper (*11*). Briefly, 12.5 g of representative malt samples was extracted with 50 mL of an acetonitrile/water mixture (84:16, v/v) for 60 min. When liquids were analyzed, sweet wort and beer, 84 mL of acetonitrile and 3.2 g of Cellite were added to 16 mL of a degassed sample. The slurry was then shaken for 60 min. Aliquots of crude extracts (4 mL for malts and 5 mL for liquid samples) were evaporated to dryness under vacuum, and the residue was then transferred into 1 mL of a methanol/water mixture (1:1, v/v). For chromatographic separations, an HP 1100 LC system (Agilent Technologies) with a 150 mm × 3 mm (inside diameter), 4 μm, Synergi Hydro RP reverse phase column (Phenomenex, Torrance, CA) was used. The mobile phase consisted of 10 mM ammonium acetate in water (mobile phase A) and methanol (mobile phase B); gradient elution started at 20% B, than was linearly changed to 70% B over 8 min, and held at 70% B until 15 min, and the postrun was 6 min with 20% B. The flow rate of 0.5 mL/min and column temperatures of 40 °C were used. The identification and quantification of target analytes were carried out by tandem mass spectrometry (MS/MS) employing an ion trap analyzer (Finnigan LCQ Deca) with atmospheric-pressure chemical ionization (APCI) source operated in both positive and negative modes. Capillary and vaporizer temperatures were 150 and 325 °C, respectively. Nitrogen sheath and auxiliary gas flow rates were 1.2 and 3 L/min, respectively, and the discharge needle voltage was 6 kV. Helium was used as a collision gas for collision-induced dissociation (CID). Ions were scanned in selected reaction monitoring (SRM) mode. The precursor (P), quantification (Q), and identification (I) ions (trichothecenes B and zearalenone ions in forms $[M + CH_3COO]^-$, trichothecenes A ions in forms $[M + NH_4]^+$) of target mycotoxins were as follows: DON 355 (P), 295 (Q), 265 (I); ($^{13}C_{15}$)DON 370 (P), 310 (Q), 279 (I); DON-3-Glc 517 (P), 457 (Q), 427 (I); ADONs 397 (P), 337 (Q), 307 (I); NIV 371 (P), 311 (Q), 281 (I); Fus-X 413 (P), 353 (Q), 263 (I); HT-2 442 (P), 273 (Q), 299 (I); T-2 488 (P), 305 (Q), 245 (I); ZON 317 (P), 273 (Q), 299 (I).

The method used in this study was revalidated for all matrices involved; the performance characteristics are summarized in **Table 3**. To enable better comparison of results, concentrations of DON-3-Glc and ADONs (DON conjugates) are expressed as DON concentration in these two compounds throughout the paper.

Quantification of *Fusarium* Toxins by the LC−MS/MS Method. Due to high variability of matrix effects, matrix-matched standards

Table 3. Performance Characteristics of the LC−MS/MS Method

			Fusarium toxins							
sample	parameter	unit	DON	DON-3-Glc	ADONs	NIV	Fus-X	HT-2	T-2	ZON
malts	LOQ	μg/kg	1	1	2.5	10	1	3	1	3
	recovery	%	88	85	82	74	86	87	86	73
	RSD	%	4.2	4.5	7.6	9.2	3.9	6.1	6.0	5.8
sweet wort and beers	LOQ	μg/kg	2.5	2.5	5	10	2.5	5	2.5	5
	recovery	%	84	81	78	74	82	84	73	72
	RSD	%	6.2	5.5	8.8	9.9	4.9	8.7	7.5	6.7

Table 4. Contamination of Light and Dark Beers Grouped According to Alcohol Content

	DON (μg/L)			DON-3-Glc (μg/L)			ADONs (μg/L)		
% alcohol by volume (*n*)	mean value	concentration range	RSD (%)	mean value	concentration range	RSD (%)	mean value	concentration range	RSD (%)
				Light Beers					
<0.5 (8)	1.6	1.0−3.7	119	1.7	1.4−3.1	79	3.9	1.0−10.7	151
0.5−3.9 (3)	3.6	4.0−6.2	80	3.8	4.8−6.1	77	1.7	1.0−4.0	121
4.0−4.9 (64)	4.5	1.8−23.0	111	4.5	1.2−37.0	131	5.0	3.0−25.0	102
5.0−5.9 (71)	6.1	3.0−35.9	109	5.5	1.2−27.0	126	4.0	3.5−24.0	117
6.0−9.0 (12)	9.2	1.0−28.1	125	5.8	2.0−14.6	90	5.8	4.0−12.7	78
				Dark Beers					
<0.5 (4)	1.3	1.0−2.7	79	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
0.5−3.9 (0)[a]									
4.0−4.9 (7)	5.3	4.7−15.8	106	4.9	4.1−12.0	97	3.5	6.0−16.2	169
5.0−5.9 (5)	3.8	1.3−16.0	182	7.3	9.0−26.0	152	9.5	1.0−24.0	130
6.0−9.0 (2)	11.2	10.0−12.4	15	7.8	1.5−15.1	132	13.7	13.0−14.3	6

[a] None of the samples belonged to this alcohol group.

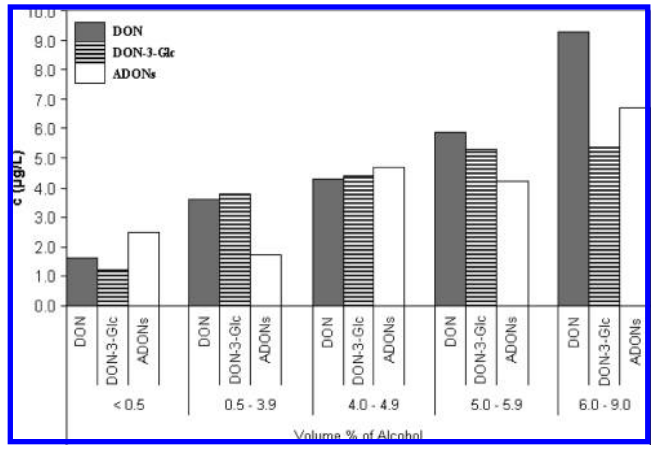

Figure 1. DON and its conjugates in light and dark beers.

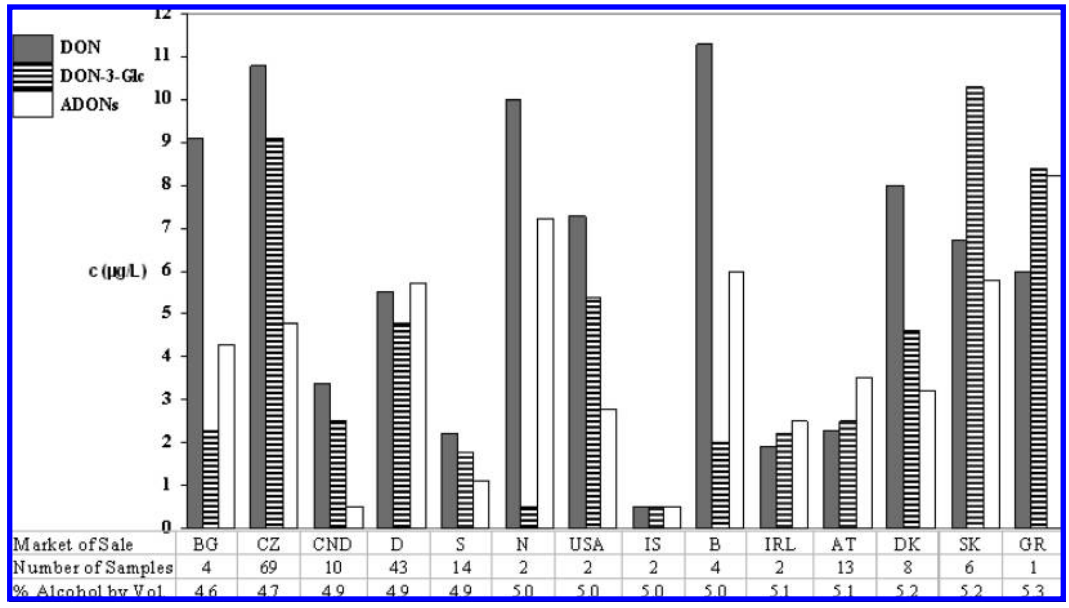

Figure 2. *Fusarium* mycotoxins in commercial beer samples (BG, Bulgaria; CZ, Czech Republic; CND, Canada; D, Germany; S, Sweden; N, The Netherlands; IS, Iceland; B, Belgium; IRL, Ireland; AT, Austria; DK, Denmark; SK, Slovak Republic; GR, Greece).

(concentration of each analyte, 25 ng/mL) were prepared for each sample by dissolving the residue that was left after the evaporation of a crude extract (sample preparation procedure described above) in 1 mL of standards in a methanol/water mixture (1:1, v/v). All of these standards also contained 25 ng/mL internal standard ($^{13}C_{15}$)DON.

Determination of *Fusarium* Toxins by an Immunoassay. Four types of commercial direct competitive ELISA kits were used for screening of 10 malts (PM, CM, and RM; see above) for DON content: (i) Ridascreen DON (R-Biopharm), (ii) AgraQuant DON Assay 0.25/5.0 Test Kit (Romer Laboratories), (iii) Veratox 5/5 DON (Neogen Corp.), and (iv) Deoxynivalenol EIA (Euro-Diagnostica). Sample processing (extraction, dilution, etc.) was carried out according to the particular producer's recommendation.

RESULTS AND DISCUSSION

Commercial Beers. The release of large amounts of DON-3-Glc during malting and brewing processing experiments has been reported in our recently published pilot study (*11*). To find out whether this masked DON is commonly present in commercially available beers, and, if so, in what quantities, an extensive market survey was conducted in 2007. Altogether, 176 samples representing a wide range of beer brands were collected for analysis of *Fusarium* toxins and DON-3-Glc. Interestingly, almost 74% of the samples contained DON-3-Glc at levels exceeding the detection limit of the LC−MS/MS method (1 µg/L), which was greater than the incidence of DON positive samples (64%) found in the beer set examined. Also, other DON conjugates, ADONs (3- and 15-isomers) which could not be resolved by using LC−MS/MS method, neither chromatographically nor spectrally, were present in almost 50% of beers; 86% of samples contained at least one of the investigated DON metabolites. On the other hand, none of other monitored *Fusarium* toxins (NIV, Fus-X, HT-2, T-2, and ZON) was detected.

As in other similar studies (*15, 20*), the data generated in our experiments did not allow any generalization of the relationship between contamination levels of raw material and those in final product. Nevertheless, it is well-known that both the quality of barley/malt and conditions within particular malting/brewing stages may significantly influence the extent of transfer of *Fusarium* toxins across the beer making chain. Since no detailed information was available about the technology employed for production of beers involved in this survey, the only parameter according to which the samples could be reliably

Table 5. Levels of DON and Its Conjugates Determined in Brewing Process Samples and Transfer of Mycotoxins from Malt to Beer

		malt (μg/kg)			sweet wort (μg/L)			beer (μg/L)			% of DON and its conjugates transferred to beers[a]		
final product	sample code	DON	DON-3-Glc	ADONs	DON	DON-3-Glc	ADONs	DON	DON-3-Glc	ADONs	DON	DON-3-Glc	ADONs
pale tap	PT 1a	17.8	14.2	15.6	2.5	<LOQ	5.6	3.1	<LOQ	5.7	113	*b*	237
	PT 2a	24	57	<LOQ	11.4	10.4	<LOQ	19.8	18.4	<LOQ	536	210	*b*
	PT 3a	17.9	41.4	<LOQ	6.7	5.6	<LOQ	4.7	3.5	<LOQ	171	55	*b*
	PT 4	11.6	9.3	10.1	<LOQ	2.9	<LOQ	<LOQ	<LOQ	<LOQ	*b*	*b*	*b*
	PT 5	22.2	42.7	<LOQ	9.8	6.6	<LOQ	<LOQ	2.1	<LOQ	*b*	*b*	*b*
	PT 6	50.6	85.9	8,4	16.1	11.7	7.8	13.8	9.7	8.3	177	73	642
	PT 7	33.2	60.8	12.1	11.5	12.2	8.3	10.5	13.5	10.2	205	144	547
pale lager	PL 1b	17.8	14.2	15.6	2.5	2.2	5.6	<LOQ	2.3	<LOQ	*b*	*b*	*b*
	PL 2b	24	57	<LOQ	11.4	10.4	<LOQ	18.8	16.3	<LOQ	406	148	*b*
	PL 3b	17.9	41.4	<LOQ	6.7	5.6	<LOQ	5.4	3.7	<LOQ	156	46	*b*
	PL 8	34.4	50.7	7.8	22.2	42.7	<LOQ	11.1	7.8	9.9	167	80	657
	PL 9	33.2	65.8	13.4	13.7	12.5	6.1	3.8	2.7	7.7	59	21	297
	PL 10	10.3	8.2	12.8	3.7	2.4	6.8	<LOQ	<LOQ	7.6	*b*	*b*	307
	PL 11	11.6	9.3	14.5	3.4	1.7	<LOQ	<LOQ	<LOQ	8.2	*b*	*b*	293

[a] Total amount of DON in processed malt batch is 100%. [b] Analytes were not detected in beer.

Table 6. Levels of DON and Its Metabolites in Malts As Determined by LC−MS/MS and ELISA Methods

		LC−MS/MS (μg/kg)				ELISA (μg/kg)	
		DON	DON-3-Glc	ADONs	∑DONs	Ridascreen	AgraQuant
Pilsner	PM 1	120.1	164.6	51.2	335.9	458.0	365.7
	PM 2	56.3	93.7	11.7	161.7	172.8	563.7
	PM 3	42.1	3.6	92.3	138.0	145.2	485.8
	PM 4	46.6	17.0	74.8	138.4	121.8	419.1
caramel	CM 1	<LOD	20.2	<LOD	20.2	282.6	731.4
	CM 2	4.3	3.8	2.5	9.7	55.2	349.7
	CM 3	<LOD	<LOD	<LOD	<LOD	147.7	775.4
	CM 4	<LOD	<LOD	17.3	17.3	218.6	918.5
roasted	RM 1	<LOD	<LOD	<LOD	<LOD	221.3	2147.5
	RM 2	<LOD	<LOD	<LOD	<LOD	315.1	2469.8

grouped was their alcohol content declared at the label. An overview of the levels of target mycotoxins in both light and dark beers is given in **Table 4**; the contamination pattern for the whole sample set is illustrated in **Figure 1**.

The data clearly document the exponential increase in mean DON concentrations with alcohol content, probably due to use of larger amounts of wort extract for production of these ("stronger") beer brands. A similar trend in the correlation between alcohol and DON levels was reported in a European screening survey (*20*). Interestingly, the mean concentration of this mycotoxin determined by ELISA in 313 commercial beers was 2 times higher (13.5 μg/L) than that in our study (6.3 μg/L) in which the LC−MS/MS method was employed for accurate DON analysis. Although this large difference may be due to interannual variability of barley (and, consequently, malt) contamination, it is also conceivable that DON metabolites (contained in examined beers in large amounts) cross reacting in most ELISA test kits (*22*) could be responsible for overestimation of DON content in the earlier study. As documented in **Table 4** and **Figure 1**, the levels of DON-3-Glc and ADONs were almost the same as the level of free DON and also were highest in high-alcohol beers. The low content of DON and its metabolites in nonalcoholic beers was probably related to their limited release from malt and other cereal adjuncts under the technological conditions that restrict alcohol production during the brewing process (such as control of mashing schedule, stopped fermentation, and use of special yeasts).

Figure 2 shows a comparison of a typical contamination pattern as determined in a set of retail beers collected in 2007 in 15 countries. It should be noted that some differences existed within the most common beers with alcohol content in the range 4−6 vol. % (v/v); nevertheless, the correlation between DON and this parameter (alcohol content) was not so distinct as found for aggregated data shown in **Table 4**. The relative molar ratios of DON and its conjugates varied among the beers from different markets, and it is worth noting that DON-3-Glc was even the dominant *Fusarium* metabolite in some beers. With regard to ADONs, no general trend could be observed for them in this context. In any case, differences in production technologies as well as the quality of raw materials used for beer making should always be considered when interpreting beer contamination data.

Brewing Technology. Until now, the transfer of DON-3-Glc from malt to beer has been documented only in our previous pilot study carried out in a small scale, in the experimental brewery house. In the current follow-up study, we monitored the concentration changes of DON and its conjugates under real-life conditions. For this purpose, 14 sets of samples, each consisting of malt, sweet wort, and beer, were examined by LC−MS/MS for the presence of *Fusarium* toxins; the results are summarized in **Table 5**. As shown here, all of the tested light malts contained detectable concentrations of free DON as well as its conjugates (glucosylated and acetylated DON). The average concentrations of DON, DON-3-Glc, and ADONs were 23.3, 39.9, and 8.9 μg/kg, respectively. Also, the worts were contaminated with DON and DON-3-Glc at levels slightly exceeding method detection limits; the mean values were established as 8.8 μg/L for DON and 9.2 μg/L for DON-3-Glc. ADONs occurred in 64% of wort samples.

The availability of data documenting contamination of brewing intermediates is important for the elucidation and control of the fate of toxins during malt processing. Nevertheless, for the risk assessment process, the extent of contamination of the final beers has to be known. The results obtained in this part of study are listed in **Table 5**.

Since no detailed information on brewing technology was available, the estimation of the transfer of *Fusarium* toxins from malt to beer was made on the assumption that typically 15.4 kg of light malt is used for production of 100 L of tap beer while 19.3 kg of this raw material is needed to obtain the same amount of lager. The data calculated in this way are listed in **Table 5**.

An extremely large variability in the DON levels and the levels of its conjugates during the brewing is illustrated here.

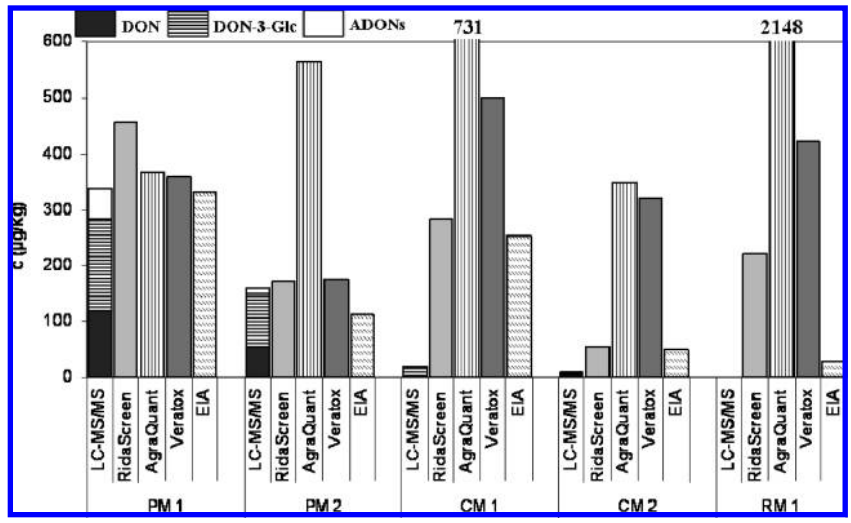

Figure 3. Concentrations of DON and its major conjugates as determined in malt samples by the LC−MS/MS method and four different ELISA kits (Ridascreen, AgraQuant, Veratox, and Deoxynivalenol EIA) (PM, Pilsner malts; CM, caramel malts; RM, roasted malts).

Interestingly, the current data sets obtained on samples from a real-life industrial processes show rather different trends compared to those reported in our previous pilot study conducted in an experimental brewery house (*11*). Large increases in the levels of DON and ADONs (up to 530 and 650%, respectively, when calculated on the basis of malt aliquot contained in beer) occurred in most cases, while the increase in the level of DON-3-Glc was a maximum of 210%. With regard to the addition of adjuncts (e.g., maltose, caramel, saccharose, syrups, artificial sweetener, and maize or wheat malt), we were not able to find any distinct impact on contamination of beer prepared with their addition.

ELISA and LC−MS/MS in the Analysis of Malts. In the final part of this study, we focused on the assessment of analytical approaches applicable for control of malt contamination. At this point, it is necessary to refer to the results of our recent study (*11*) documenting the potential of some barleys with relatively low DON levels, and negligible concentrations of DON-3-Glc and ADONs, to release large amounts of these DON conjugates during the malting process. In other words, the apparently reasonable practice of examination of grains for *Fusarium* toxins prior to processing does not necessarily enable a reliable prediction of their levels in malt (and later on in beer since the content of mycotoxins may further increase during the brewing process). Therefore, only systematic control of malt prior to its use for technological processing may prevent production of beers with high levels of these mycotoxins, which may pose a health risks for consumers.

Basically, two alternative strategies are conceivable for laboratory screening of malt for DON. To obtain accurate results for the whole spectrum of target analytes in respective samples, LC−MS/MS currently represents the most common method of choice. However, expensive instrumentation and highly qualified personnel are required for analyses performed by this technique. Therefore, fast and simple bioassays employing commercial ELISA DON kits are preferred by many control laboratories. The latter approach, however, may provide rather distorted data because of cross reactivity of some antibodies to DON metabolites that may be contained in fairly large amounts in malts (see **Table 5**). All of the kits were used according to producers' recommendation within the study. It also should be noted that other factors such as sample preparation, dilution, the wavelength for absorbance measurement, and malt color intensity may influence, to different extents, the final results obtained by a particular ELISA kit. **Table 6** summarizes the results obtained by examination of 10 malt samples by LC−MS/MS and two commercial ELISA kits, which were shown in our previous study (*22*) to be largely different in terms of cross reactivity to DON-3-Glc and ADONs. A trend similar to that observed earlier for beers was found now for malts: the apparent DON content determined by AgraQuant was, without any exception, higher compared to that determined by Ridascreen. The difference between the results generated by these two DON kits increased in the following order: Pilsner malt < caramel malt < roasted malt, while for pale Pilsner malts, the sum of DON + DON-3-Glc + ADONs determined by LC−MS/MS was in relatively good agreement with Ridascreen results. Very low levels, or even no mycotoxins, were determined above the limit of detection by the instrumental method in caramel and roasted malts for production of which temperatures exceeding 200 °C were used. To document the performance of other common ELISA DON kits available at EU market, five samples of malts were also analyzed by Veratox and Deoxynivalenol EIA (**Figure 3**). Similar to our earlier results obtained for beers (*22*), Veratox was another kit prone to overestimation. On the other hand, EIA showed the lowest results which, although rather overestimated, were declining in line with the drop in DON content determined by LC−MS/MS. In any case, the discrepancies between the DON levels determined by the instrumental method and those determined by an ELISA need to be explained. Our follow-up research will focus on identification of cross-reactive compounds. Theoretically, these can be either matrix components that originated from natural precursors at higher temperatures or, alternatively, "unknown" DON-related compounds released or formed during heat processing.

Traces of *Fusarium* toxins, mainly type B trichothecenes, can be detected in almost all world beers. It is worth noting that the contamination pattern of this popular alcoholic beverage often largely differs from that commonly found in barley, the raw material used for its production. This study, conducted on a large set of beers, confirms the ubiquitous occurrence of relatively high levels of DON-3-Glc, release of which from barley grains and further from malt during

malting and brewing was reported for the first time in our previous paper. In addition to DON-3-Glc, an increase in the levels of ADONs across the beer production chain also occurs. While their mean concentrations in barley were around 38% of free DON content, the levels of these conjugates in beers were found to be comparable or even higher than the level of the major *Fusarium* toxin. The data generated in this study also document that beers with higher alcohol content tend to be more contaminated, presumably due to a higher content of malt extract equivalent. However, prediction of mycotoxin levels on the basis of malt analysis is hardly possible, since besides technological conditions, the nature of the mycotoxin pool may also play a role in their release. As far as the choice of analytical method for control of mycotoxins in malt is considered, the cross reactivity of some antibodies employed in DON-dedicated kits should be taken into account. Future research should explain large differences between the results obtained by some immunoassays and LC−MS/MS observed mainly in caramel and roasted malts. Formation of cross-reactive species at elevated temperatures used for production of these commodities could cause overestimation of DON concentrations.

ABBREVIATIONS USED

ADONs, sum of 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol; APCI, atmospheric-pressure chemical ionization; CID, collision-induced dissociation; CM, caramel malt; DON, deoxynivalenol; DON-3-Glc, deoxynivalenol-3-β-D-glucopyranoside (DON-3-glucoside); EC, European Commission; ELISA, enzyme-linked immunosorbent assay; FHB, *Fusarium* head blight; Fus-X, Fusarenon-X; HPLC, high-performance liquid chromatography; HT-2, HT-2 toxin; I, identification ion; LC−MS/MS, liquid chromatography coupled to tandem mass spectrometry; NIV, nivalenol; P, precursor ion; PM, Pilsner malt; Q, quantification ion; RM, roasted malt; SCF, Scientific Committee on Food; T-2, T-2 toxin; TDI, tolerable daily intake; ZON, zearalenone.

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