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Occurrence of *Fusarium* mycotoxins and their dietary intake through beer consumption by the European population

Yelko Rodríguez-Carrasco [a], Margherita Fattore [b], Stefania Albrizio [c], Houda Berrada [a,*], Jordi Mañes [a]

[a] Department of Public Health, Faculty of Pharmacy, University of Valencia, Av. Vicent A. Estellés s/n, 46100 Burjassot, Spain
[b] Consorzio Interuniversitario INBB, Viale Medaglie d'Oro, 305, I-00136 Rome, Italy
[c] Dipartimento di Farmacia, Università di Napoli Federico II, via D. Montesano 49, I-80131 Naples, Italy

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ABSTRACT

Since cereals are raw materials for production of beer and beer-based drinks, the occurrence mycotoxins in 154 beer samples was topic of investigation in this study. The analyses were conducted using QuEChERS extraction and gas chromatography–tandem mass spectrometry determination. The analytical method showed recoveries for vast majority of analytes ranged from 70% to 110%, relative standard deviations lower than 15% and limits of detection from 0.05 to 8 μg/L. A significant incidence of HT-2 toxin and deoxynivalenol (DON) were found in 9.1% and 59.7% of total samples, respectively. The exposure of European population to mycotoxins through beer consumption was assessed. No toxicological concern was associated to mycotoxins exposure for average beer consumers. Despite that, for heavy beer drinkers, the contribution of this commodity to the daily intake is not negligible, approaching or even exceeding the safety levels.

1. Introduction

Mycotoxins are toxic secondary metabolites produced naturally by filamentous fungi, such as *Aspergillus*, *Penicillium*, and *Fusarium*. Most mycotoxins are immunosuppressive agents and some are classified as carcinogens, hepatotoxins, nephrotoxins, or neurotoxins. They have been reported in several kinds of food, especially in globally consumed cereals such as wheat, rice, maize and barley (Marroquín-Cardona, Johnson, Phillips, & Hayes, 2014). DON, the most commonly detected *Fusarium* mycotoxin in cereal grains, was related to deleterious health effects like anorexia, weight loss, malnutrition, endocrine dysfunction and immune alterations (Pestka, 2010) but is non-classifiable as carcinogen to humans (IARC, 1993).

As co-occurrence of mycotoxins in cereals for beer production is frequently reported, their levels should be contemplated in exposure estimates studies. Mycotoxin contamination could occur at various stages of the brewing process and would be transmitted from malt into beer as a consequence of their thermal stability and relatively good water solubility (Inoue, Nagatomi, Uyama, & Mochizuki, 2013; Kostelanska et al., 2011). Moreover, during brewing process some mycotoxins can be converted to its metabolites as previously reported Mizutani, Nagatomi, and Mochizuki (2011).

The investigation on a formation/release of DON conjugate during processing of contaminated barley was initiated by studies conducted by Berthiller et al. (2013) who characterize the main DON plant metabolite. However, very scarce literature concerning the toxicokinetics of deoxynivalenol-3-glucoside (D3G) is available. Up to now, D3G seems to be resistant to acidic conditions and thus it is unlikely that D3G can be hydrolyzed into its parent compound in the stomach of mammals (Berthiller et al., 2011).

Important enzymes related to the hydrolysis of plant glucosides such as β-glucosidase, are expressed in human liver and gut. Nonetheless, some naturally occurring glucosides, including D3G, cannot be cleaved by the human cytosolic β-glucosidase. In that sense, only partial hydrolysis was observed upon incubation with several pure cultures of intestinal bacteria as reported Berthiller et al. (2011) and thus, only a fraction of D3G will be bioavailable. In this line, the digestibility and absorption of D3G in *in vitro* models was recently investigated and they reported that no evidence was found for D3G hydrolysis to DON in the digestion model representing the upper part of the gastrointestinal tract. Moreover, it was shown that bioavailability of D3G in humans may be low as compared to DON since Caco-2 cells did not absorb D3G, in contrast to DON (De Nijs et al., 2012).

Currently, there is no regulation for *Fusarium* toxin levels in beer. Maximum limits are set only for raw materials used for production of this commodity (EC No 1881/2006). A provisional maximum tolerable daily intake (PMTDI) of 1 μg/kg bw was established for the sum of DON and its acetylated forms by the Scientific

* Corresponding author. Tel.: +34 963544117; fax: +34 963544954.
E-mail address: Houda.Berrada@uv.es (H. Berrada).

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Committee on Food (SCF, 2002). SCF has also established TDI for other mycotoxins frequently reported in cereals and cereals-based foods. For instance, a TDI for the sum of HT-2 and T-2 toxin of 0.1 μg/kg bw was set (SCF, 2011a) and a temporary tolerable daily intake (t-TDI) of 1.2 μg/kg bw was established for nivalenol (EFSA, 2013). In 2000 the SCF established a t-TDI of 0.2 μg/kg bw for zearalenone. However, in 2011 the SFC concluded that a TDI of 0.25 μg/kg bw can be established based on recent data in the most sensitive animal species (SCF, 2011b).

This widely popular fermented drink may, under certain conditions, contribute significantly to intake of mycotoxins, approaching or even exceeding the safety levels when consuming a regular diet. The latest data available from Food and Agricultural Organization (FAO) reported an annual per-capita consumption of beer exceeding 70 L in 46.4% of European countries, with a maximum of 142.8 L in Ireland (FAO, 2011). Hence, exposure of consumers to mycotoxins through beer should not be underestimated, particularly in case of heavy drinkers (Warth et al., 2012).

Therefore, development of reliable analytical multi-mycotoxin methods becomes necessary. Sample preparation method still remains the bottleneck in the entire protocol because of the wide range of properties of the several mycotoxins and food matrix. Sample preparation techniques such as SPE (Romero-González, Martínez Vidal, Aguilera-Luiz, & Garrido Frenich, 2009), immunoaffinity columns (Bertuzzi, Rastelli, Mulazzi, Donadini, & Pietri, 2011), QuEChERS (Tamura, Uyama, & Mochizuki, 2011) or enzyme-linked immunosorbent assay (Kuzdraliński, Solarska, & Muszyńska, 2013) have been employed in multi-mycotoxin analysis in beer yielding in general satisfactory results. Analytical procedures based on chromatographic principles such as gas chromatography coupled to mass spectrometry (GC–MS) (Scott, Kanhere, & Weber, 1993) and/or liquid chromatography coupled to mass spectrometry (LC–MS), (Malachova, Varga, Schwartz, Krska, & Berthiller, 2012)for developing multi-mycotoxin methods in several foodstuffs have been widely employed. For instance Scott et al. (1993) developed a sensitive method for the determination of five *Fusarium* toxins in beer by capillary gas chromatography–mass spectrometry.

The acquisition of two selected reaction monitoring (SRM) transitions per compound by the triple quadrupole detector (QqQ) and the predetermined selected ions intensity ratio allowed the unequivocal confirmation of positive samples and accomplished the requirements set by the Commission Decision 2002/657/EC (2002/657/EC) as regards criteria and procedures for the validation of analytical methods satisfied by MS/MS. In this sense, high dynamic range and good performance reached in SRM mode make the triple quadrupole one of the most widely employed mass spectrometry analyzer (Rubert, Soler, Marín, James, & Mañes, 2013). The here proposed methodology is presented as a sensitive and robust analytical tool for the simultaneous determination of fourteen mycotoxins by GC–MS/MS. Thus, this work serves as an update of the gas chromatography methods for the determination of mycotoxins in foodstuffs.

Robust and selective methods are highly desirable for the mycotoxin determination. These requirements can be achieved by both GC and LC coupled to mass spectrometry as recently reviewed Pereira, Fernandes, and Cunha (2014). GC offers some benefits over LC methods such as lower instrument cost as well as lower maintenance, even though analysis of mycotoxins by GC requires derivatization. Ion sources used in LC–MS are also related to ion suppression or enhancement with strong consequences on the accuracy, precision and sensitivity of the methods (Ran et al., 2013).

Monitoring studies regarding evaluation of mycotoxins in several foodstuffs should be continuously conducted as recommended EFSA to collect and evaluate occurrence data on mycotoxins in food and feed. The work carried out by Varga, Malachova, Schwartz, Krska, and Berthiller (2013) is one of the largest performed study of the occurrence of DON mainly in Austrian beers. One of the most pressing goals of the here proposed article is to investigate occurrence data of not only deoxynivalenol but also other trichothecenes as well as other *Fusarium* toxins (e.g., zearalenone and its metabolites) in beers. In order to accomplish this, the objectives of this work were to develop an analytical strategy based on a QuEChERS-based extraction and gas chromatography–tandem mass spectrometry for the simultaneous determination of fourteen *Fusarium* toxins and metabolites in a total of 154 beer samples produced mainly in different European countries to estimate the dietary intake of these mycotoxins among the European population.

2. Materials and methods

2.1. Chemical and reagents

Solvents (acetonitrile, hexane and methanol) were purchased from Merck KGaA (Darmstadt, Germany). Anhydrous magnesium sulphate was obtained from Alfa Aesar GmbH & Co. (Karlsruhe, Germany); sodium chloride was purchased from Merck and C18 was purchased from Phenomenex (Torrance, USA).

The derivatization reagent composed of BSA (N,O-bis(trimethylsilyl)acetamide) + TMCS (trimethylchlorosilane) + TMSI (N-trimethylsilyimidazole) (3:2:3) was purchased from Supelco (Bellefonte, PA). Sodium dihydrogen phosphate and disodium hydrogen phosphate, used to prepare phosphate buffer, were acquired from Panreac Quimica S.L.U. (Barcelona, Spain).

2.2. Analytical standards

The standards of the type A and B trichothecenes: deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON), diacetoxyscirpenol (DAS), nivalenol (NIV), fusarenon-X (FUS-X), neosolaniol (NEO), T-2 and HT-2 toxins, zearalenone (ZON) and its derivatives α-zearalenol (α-ZOL), β-zearalenol (ß-ZOL), α-zearalanol (α-ZAL), β-zearalanol (ß-ZAL) and zearalanone (ZAN) toxin were obtained from Sigma–Aldrich (St. Louis, USA). All stock solutions were prepared by dissolving 1 mg of the mycotoxin in 1 mL of pure methanol, obtaining a 1 mg/mL solution and diluted with acetonitrile in order to obtain the appropriate multicompounds working standard solutions. All standards were kept at −20 °C.

2.3. Sampling

A total of one hundred and fifty-four bottled commercial beer samples were randomly purchased from different retail outlets located in Valencia (Spain) from May to July 2013. None of the beers had surpassed their expiration date. Until sample preparation, they were stored in a dark and cold environment at 4 °C. The beer samples were classified based on both country of production and type of beer.

2.3.1. Country of production

The vast majority of beer samples ($n = 60$) were produced in Spain. Fifty-four samples were imported mainly from Europe. The imported beer originated from Germany ($n = 24$), Ireland ($n = 8$), Portugal ($n = 8$), Belgium ($n = 7$), Denmark ($n = 7$), Netherlands ($n = 7$), Czech Republic ($n = 6$), Great Britain ($n = 6$), United States of America ($n = 6$), France ($n = 5$), Mexico ($n = 5$) and Argentina ($n = 5$).

2.3.2. Type of beers

Samples were subdivided into 6 groups: non-alcoholic (n = 17) beers which contained no alcohol or an alcohol content <1% Vol; shandy (n = 14) beers which contained mixtures of different ratios of beer and lemonade; light (n = 16) beers which contained an alcohol content between 1 and 3.5% Vol; lager (n = 58) beers brewed with barley malt and exhibiting a light color; dark (n = 24) any beer exhibiting a darker, brownish color, regardless of the alcohol content and wheat beers (n = 25) brewed with wheat malt alone or in combination with different ratios of barley malt.

2.4. Mycotoxin extraction

Beer samples were processed using the following procedure. First, each bottle of beer sample was gently shaken and approximately 100 mL was degassed by sonication for 15 min. Then, 5 mL of acetonitrile was added to 10 ml of sample and vigorously shaken for 30 s prior the addition of 4 g of anhydrous $MgSO_4$ and 1 g NaCl. The mixture was vortexed for 30 s and sonicated for 3 min prior to be centrifugated for 3 min at 3500 rpm. After the acetonitrile extract was submitted to a dispersive solid phase extraction (d-SPE) into a tube containing 900 mg $MgSO_4$ and 300 mg C18, it was vortexed for 1 min and then centrifuged for 1 min at 3500 rpm. Finally, the supernatant was collected and evaporated to dryness under a gentle nitrogen flow.

The dry extract was treated with 50 μL of BSA + TMCS + TMSI (3:2:3), and the sample was left for 30 min at room temperature. The derivatized sample was diluted to 200 μL with hexane and mixed thoroughly on a vortex for 30 s. The hexane was washed with 1 mL of phosphate buffer (60 mM, pH 7) and transferred to an autosampler vial for the chromatographic analysis.

2.5. GC–MS/MS analysis

The final extract (1 μL) was injected in splitless mode at 250 °C in programmable temperature vaporization (PTV) using an Agilent 7890A GC system coupled with an Agilent 7000A triple quadrupole mass spectrometer with inert electron-impact ion source and an Agilent 7693 autosampler (Agilent Technologies, Palo Alto, CA). The mass spectrometer was operated in electron impact ionization (EI, 70 eV). The source and transfer line temperatures were 230 °C and 280 °C, respectively. The collision gas for MS/MS experiments was nitrogen, and the helium was used as carrier gas at fixed pressure of 20.3 psi, both at 99.999% purity supplied by Carburos Metálicos S.L. (Barcelona, Spain). Data was acquired and processed using the Agilent MassHunter version B.04.00 software. Analytes were separated on a HP-5MS 30 m × 0.25 mm × 0.25 μm capillary column. The oven temperature program was initially 80 °C, and the temperature was increased to 245 °C at 60 °C/min. After a 3 min hold time, the temperature was increased to 260 °C progressively at 3 °C/min and finally to 270 °C at 10 °C/min and then held for 10 min. The analysis was performed with a solvent delay of 3 min in order to prevent instrument damage.

The criteria established in Document No. SANCO 12495/2011 (SANCO, 2011) was achieved for quantitation purpose. For each analyte, two SRM transitions for each compound and compliance of the SRM ratio, defined as the relative ion intensities between the area of both the quantitation (Q) and confirmation transition (q), were required. The most intense SRM transition was selected for quantitation purposes. The specific MS/MS parameters for each mycotoxin are shown in Table 1.

2.6. Method validation

A prior analysis of the samples was performed in order to ensure they did not contain any of the studied compounds. Then, blank samples were selected for spiking, calibration curves and recovery purposes. Calibration functions of both neat solvent standards and spiked samples were established by plotting peak areas versus analyte concentrations in the measured solutions and performing linear regression. Linear range was tested from 0.1 to 500 μg/L by spiking at eight concentration levels. Spiking at each level was carried out in triplicate. In order to reveal the presence of matrix effects, matrix-matched calibration prepared by spiking extracts of blank samples with mycotoxins at similar concentrations than the calibration built in neat solvent without any matrix were compared. The slopes of the resulting linear calibration functions were compared and the signal suppression/enhancement (SSE) due to matrix effects was determined according to Eq. (1):

$$\text{SSE}\ (\%) = \text{Slope matrix} - \text{matched calibration}/\text{Slope standard in solvent} \times 100 \quad (1)$$

A SSE of 100% indicates that no matrix effect occurred in the concentration range investigated. A SSE above 100% revealed signal enhancement, while a SSE below 100% signal suppression.

For recovery studies, the samples obtained from the local market were spiked with the standard solution at the appropriate levels. Following this method, 10 mL sample were fortified with the working standard solution. The samples were then allowed to stand overnight until analysis. The final spiking concentration levels in the samples for recovery studies were 50, 100, and 200 μg/L. Precision studies were determined in fortified beer samples at the

Table 1
GC–MS/MS parameters for the analytes under study.

Analyte	Retention time (min)	Quantitation transition			Confirmation transition			SRM ratio (%)
		Q1	Q3	CE, eV (Dt, ms)	Q1	Q3	CE, eV (Dt, ms)	
DON	8.6	392	259	10 (25)	407	197	10 (25)	41.6
3-ADON	9.68	392	287	5 (35)	467	147	10 (25)	47.5
FUS-X	9.73	450	260	10 (35)	450	245	20 (35)	11.9
DAS	9.85	350	229	15 (35)	378	124	10 (25)	56.9
NIV	10.15	289	73	15 (35)	379	73	15 (35)	29.6
NEO	11.68	252	195	10 (25)	252	167	15 (35)	40.6
HT-2	14.73	347	157	10 (25)	347	185	10 (25)	86.7
T-2	14.8	350	259	10 (25)	350	229	15 (35)	81.9
ZAN	15.15	307	235	15 (25)	449	335	10 (25)	59.9
α-ZAL	15.45	433	309	20 (35)	433	295	20 (35)	26.1
ß-ZAL	15.68	433	295	15 (35)	307	73	10 (35)	82.2
ZON	15.95	462	151	10 (25)	462	333	10 (25)	76.9
α-ZOL	16.45	305	73	15 (25)	305	289	15 (20)	12.7
ß-ZOL	16.82	536	333	10 (35)	536	446	15 (20)	66.1

Q1: precursor ion; Q3: product ion; CE: collision energy; Dt: dwell time.

same spiking levels above mentioned and calculated as percentage of relative standard deviation (RSD,%). Both recovery and precision studies were conducted in triplicate in the same day (intra-day precision) and in four different days (inter-day precision). Limits of detection (LODs) and quantitation (LOQs) were calculated as the concentrations for which signal-to-noise ratios were 3 and 10, respectively.

Student's *t*-test statistical analysis was performed for data evaluation; p values < 0.05 were considered significant.

3. Results and discussion

3.1. Analytical features of the proposed method

The here proposed procedure for the determination of fourteen mycotoxins in beer was validated in lager beer, the most numerous group of the collected samples (58 out of 154) as a representative of the whole beer samples.

The good performance of the method was confirmed by the validation data reported in the Table 2. The analyses of blank samples showed that no interfering signals were presented at the retention time of the investigated analytes, assessing method specificity. Regression equations were obtained using eight standard concentrations on the abscissa and the area of the chromatogram peaks as vertical coordinates. Linear range was tested at eight concentration levels in triplicate from 0.1 to 500 μg/L. Relative standard deviations among the triplicate were below 5% at all calibration curve points. The determination coefficients (R^2) of all analytes were >0.995. Matrix effect was observed (from 52% to 79%), and thus matrix-matched calibration curves were used for quantification purposes.

Accuracy and precision data were provided by recovery tests conducted in triplicate using the fortified blank samples at three fortification levels (50, 100 and 200 μg/L) within laboratory reproducibility conditions. Considering all the concentration levels, mean recoveries (n = 3) obtained with matrix-matched calibration curves were in the range of 68–109% (Table 2) with RSD values between 4% and 12%. Global inter-day precision was estimated as RSD of 12 determinations and was between 6% and 15%. The sensitivity of the method was expressed in terms LODs and LOQs, which ranged between 0.05–8 μg/L and 0.1–16 μg/L, respectively (Table 2). Results showed the suitability of the developed method for the determination of trace amounts of the selected mycotoxins in beer samples.

3.2. Exposure data: occurrence of mycotoxins in beer samples

The above described analytical method was applied to one hundred and fifty-four beer samples collected from 13 countries yielded data of exposure assessment to consumers. Table 3 displays the concentration of the occurrence of mycotoxins quantified in this study. An important incidence of DON (59.7%) was

Table 2
Validation parameters of the GC–MS/MS method.

Mycotoxin	LOD (μg/L)	LOQ (μg/L)	SSE (%)	Recovery (%)			Intra-day precision (RSD%; n = 3)			Inter-day precision (RSD%; n = 3)		
				50 μg/L	100 μg/L	200 μg/L	50 μg/L	100 μg/L	200 μg/L	50 μg/L	100 μg/L	200 μg/L
DON	0.05	0.10	61	76	82	79	9	7	8	13	10	9
3-ADON	2	4	63	82	71	76	6	10	9	10	7	13
FUS-X	8	16	53	98	95	81	8	5	10	8	12	14
DAS	4	8	66	77	83	85	6	9	5	12	9	7
NIV	0.5	1	79	78	75	84	12	8	6	15	11	14
NEO	2	4	73	82	84	78	8	5	10	10	6	12
HT-2	2	4	52	98	101	108	9	7	7	8	14	15
T-2	4	8	59	109	97	109	7	5	8	10	6	9
ZAN	8	16	58	68	69	72	9	10	12	12	13	15
α-ZAL	4	8	71	70	74	72	6	9	10	9	12	12
ß-ZAL	4	8	76	73	68	70	8	7	11	10	9	13
ZON	8	16	70	75	73	69	5	6	9	13	15	8
α-ZOL	2	4	78	83	80	75	6	4	5	8	9	7
ß-ZOL	4	8	62	72	75	71	8	7	9	10	8	13

SSE: signal suppression/enhancement calculated based on Eq. (1).

Table 3
Occurrence of mycotoxins in the analyzed beer samples based on country of production.

Country	Deoxynivalenol				HT-2 toxin			
	Incidence	Positive samples (%)	Mean (μg/L)	Range (μg/L)	Incidence	Positive samples (%)	Mean (μg/L)	Range (μg/L)
AR	1/5	20	28.2	28.2	n.d.	–	–	–
BE	7/7	100	30.5	27.1–40.3	1/7	14	31.3	31.3
CZ	2/6	33.3	35.1	29.3–42.5	n.d.	–	–	–
DE	20/24	83	32.3	24.5–43.1	9/24	37.5	33.6	29.3–38.2
DK	n.d.	–	–	–	n.d.	–	–	–
ES	45/60	75	27.0	25.2–47.7	2/60	3.3	27.0	25.1–30.4
FR	5/5	100	30.6	27.3–42.5	1/5	20	29.5	29.5
GB	1/6	16.7	26.7	26.7	1/6	16.7	24.2	24.2
IE	n.d.	–	–	–	n.d.	–	–	–
MX	5/5	100	26.2	25.1–27.3	n.d.	–	–	–
NL	1/7	14.3	28.9	28.9	n.d.	–	–	–
PT	3/8	37.5	31.4	30.3–32.8	n.d.	–	–	–
US	2/6	33.3	26.3	25.0–26.4	n.d.	–	–	–
Total samples (n = 114)	92/154	59.7	28.9[a] (*17.2*)	24.5–47.7[a]	14/154	9.1	30.9[a] (*2.8*)	24.2–38.2[a]

[a] Data calculated based on positive samples only. Italic number: data calculated based on all samples.

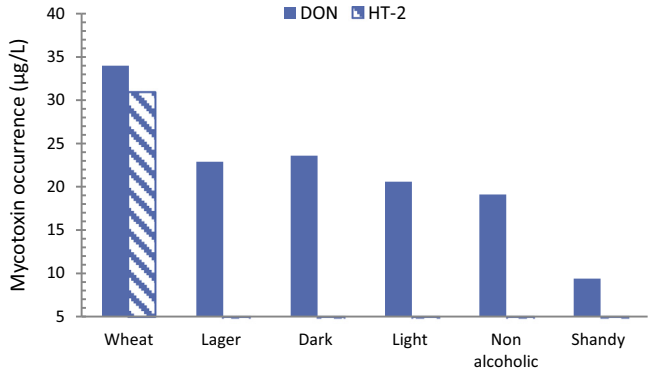

Fig. 1. Mean levels of quantified mycotoxins in samples classified by type of beer.

found in the total samples assayed. Moreover, HT-2 co-occurred in 9.1% of total samples. The overall average of all 154 beer samples was 17.2 µg/L for DON and 2.8 µg/L for HT-2. Taking into consideration only the contaminated beers above the LOQ, the average contents reached 28.9 µg/L for DON (range: 24.5–47.7 µg/L) and 30.9 µg/L for HT-2 (range: 24.2–38.2 µg/L). The results of this survey were in agreement with previous European studies: Papadopoulou-Bouraoui, Vrabcheva, Valzacchi, Stroka, and Anklam (2004) detected DON in 87% of analyzed beer samples (n = 313) at level of 4–56.7 µg/L. Kostelanska et al. (2009) quantified DON in 64% of samples (n = 176) in a concentration range of 1–35.9 µg/L whereas Bertuzzi et al. (2011) detected DON in 66% of samples (n = 106) at level of <0.5–18.6 µg/L. Recently, Kuzdraliński et al. (2013) quantified DON in 100% assayed beer samples (n = 91) at mean level of 20.66 µg/L and Varga et al. (2013) reported an average contamination of DON at 13.6 µg/L in the 77% of positive samples (n = 374). So far, a limited number of studies have reported HT-2 contamination in beer. The values here reported are also in good agreement with previous findings. For instance, Romero-González et al. (2009) detected HT-2 toxin in 26.7% of the analyzed beer samples (n = 15) but at levels of 1 µg/L. A low incidence of HT-2 toxin (6.1%) was detected by Rubert et al. (2013) in 49 beer samples but at contents similar to those here found (concentration range of 15.1–20 µg/L).

No significant statistical differences for a confidence interval of 95% in mycotoxin occurrence were observed between samples from different countries as previously described by Bertuzzi et al. (2011) and Kuzdraliński et al. (2013). Nonetheless, different mycotoxin occurrence was detected taking into consideration the different types of beers regardless of the country of production (Fig. 1). Wheat-based beers showed the highest mycotoxin incidence for both DON (76%) and HT-2 toxin (56%). In fact, the 14 out of 154 samples (9.1% of total beers) contaminated by HT-2 belong to this beer category (range total samples: <LOQ to 38.2 µg/L). In addition, wheat-based beers also showed the highest mean level of DON at 34.0 µg/L (range total samples: <LOQ to 47.7 µg/L). A similar trend was observed by Varga et al. (2013) who reported DON contamination in 78.3% of the 46 analyzed wheat beers at average content of 18.4 µg/L and maximum level of 49.6 µg/L. The high mycotoxin occurrence in this type of beers could be justified taken into account that the growth of *Fusarium graminearum* and *Fusarium culmorum*, the major plant pathogens that are capable of producing trichothecenes in infected grains, is slightly more predominant in wheat than in barley and hence a greater mycotoxin contamination in wheat-based products is expected (Krstanović, Klapec, Velić, & Milaković, 2005).

Concerning the lager beers, 42 out of 58 samples showed DON contamination at average content of 22.9 µg/L (range total samples: <LOQ – 42.0 µg/L). Similar results in terms of occurrence were obtained for dark beer samples (range total samples: <LOQ – 32.8 µg/L; mean content: 23.6 µg/L). Fig. 2 shows a SRM chromatogram of a beer artificially contaminated by DON at 100 µg/L as well as SRM chromatograms of a wheat-based and barley-based beer naturally contaminated by DON at 47.7 µg/L and 24.5 µg/L, respectively.

The lowest averages of DON were found in light, non-alcoholic and shandy beers (20.6, 19.1 and 9.4 µg/L, respectively). These results could be probably related to the differences in the technological process involved, such as earlier stopped fermentation or the use of specific yeast, as highlighted in previous studies (Kostelanska et al., 2009; Papadopoulou-Bouraoui et al., 2004). Therefore, a longer fermentation process could possibly have contributed to the highest level of DON transfer from malt to beer being in agreement with the results here reported for wheat, pale and dark beers. In case of shandy, apart from the above mentioned it has also to be considered the dilution of this type of beer with different ratios of lemonade.

Left-censored results (i.e., data below the analytical limits) were processed according to the dietary exposure assessment of chemicals in food recommendations (substitution method) that

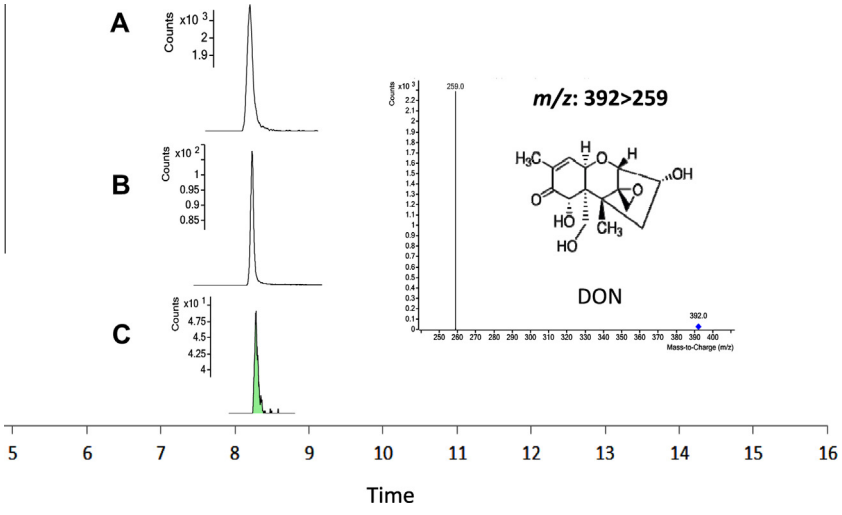

Fig. 2. SRM chromatogram of: beer artificially contaminated by DON at 100 µg/L (A), wheat-based beer naturally contaminated by DON at 47.7 µg/L (B), and barley-based beer naturally contaminated by DON at 24.5 µg/L (C).

are the most commonly used and then evaluating how the exposure estimates change (IPCS, 2009, chap. 6). Two exposure scenarios were then defined: the lower bound scenario (LB) and the upper bound scenario (UB). The LB was obtained by assigning a zero value to those samples in which the analyte was non-detected or non-quantified and using these values to estimate dietary exposure. An UB dietary exposure was estimated by assigning the LOD to all samples with non-detected results and the LOQ to all samples with less than the LOQ but more than the LOD. It is widely considered that the LB scenario generally underestimates contamination and exposure levels and that the UB scenario overestimates them (EFSA, 2010). Hence, considering the LB and UB values of DON and HT-2 obtained from this survey, the latest mean beer consumption data in the European countries reported by FAO (FAO, 2011) and the default body weight for adults (70 kg) recommended by the Scientific Committee (EFSA, 2012), the daily average exposure was calculated.

Table 4 shows the exposure estimates for all European countries based on the here reported mycotoxin occurrence. Generally, DON mean contribution to the tolerable intake in European population from beer is 5% (LB and UB) and HT-2 mean contribution ranged from 7% (LB) to 12% (UB). Consequently, the values obtained in this study demonstrate that the intake of DON though average beer consumption is not a matter of concern from a toxicological point of view, in agreement with previous studies (Bertuzzi et al., 2011; Harcz et al., 2007; Varga et al., 2013). Despite that, the intake of HT-2 from beer consumption in some countries should be taken into account. In fact, a 10% of the HT-2 TDI was surpassed by 21.4% and 53.6% of European countries based on LB and UB, respectively. Irish population was the most exposed due to the highest mean beer consumption (0.39 L/day) amounting 10% of DON PMTDI and 15–25% of HT-2 TDI. Similar exposure estimates were here obtained for Czech and Austrian population (Table 4). Moreover, for high drinkers, beer could suppose an important source of exposure to mycotoxins. For instance, considering a daily consumption of two pints (1 L) and the average contents here reported, a significant exposure to mycotoxins in both scenarios were calculated (25% of the DON PMTDI and from 37% (LB) to 64% (UB) of the HT-2 TDI). Assuming even a worst case: consumption of 1L of the most contaminated beer here analyzed (42 μg/L of DON and 33 μg/L of HT-2), a DON contribution of 60% of PMTDI was calculated whereas HT-2 could exceed the TDI by up to 5-fold.

Note that beer is not the only foodstuff contributing to mycotoxin exposure. Cereals and cereal-based foods can also contain significant amounts of mycotoxins (Rodríguez-Carrasco, Moltó, Berrada, & Mañes, 2014) and should be taken into consideration in exposure assessment studies as recommended by SCOOP, Directive 93/5/EEC.

Table 4
Daily average exposure (expressed as % tolerable daily intake) of Europeans through beer consumption.

Country	Consumption[a] (kg/year)	% DON PMTDI		% HT-2 TDI	
		LB[b]	UB[c]	LB[b]	UB[c]
Austria	107	7	7	11	19
Belgium	88.7	6	6	9	15
Bulgaria	8.2	1	1	1	1
Croatia	83.9	6	6	9	15
Cyprus	2.5	0	0	0	0
Czech Republic	136.6	9	9	14	24
Denmark	66.7	5	5	7	12
Estonia	20.1	1	1	2	4
Finland	85.7	6	6	9	15
France	28.4	2	2	3	5
Germany	97.9	7	7	10	17
Greece	34.7	2	2	4	6
Hungary	69.1	5	5	7	12
Ireland	142.8	10	10	15	25
Italy	28.2	2	2	3	5
Latvia	1.5	0	0	0	0
Lithuania	13.5	1	1	1	2
Luxembourg	97.1	7	7	10	17
Malta	36.6	2	2	4	6
Netherlands	3	0	0	0	1
Poland	95.6	6	6	10	17
Portugal	50	3	3	5	9
Romania	1.4	0	0	0	0
Slovakia	73.4	5	5	8	13
Slovenia	81.2	5	5	8	14
Spain	75.3	5	5	8	13
Sweden	54.5	4	4	6	9
United Kingdom	79.1	5	5	8	14
Eastern Europe	74	5	5	8	13
Northern Europe	79.2	5	5	8	14
Southern Europe	48.3	3	3	5	8
Western Europe	69.8	5	5	7	12
European Union	70.1	5	5	7	12

[a] Consumption data in the European countries reported by FAO.
[b] LB: lower bound.
[c] UB: upper bound.

4. Conclusions

A suitable method based on a QuEChERS extraction using GC–MS/MS was developed and successfully validated for the detection and quantitation of mycotoxins in beer. The here proposed methodology was able to determine simultaneously fourteen mycotoxins in beer proving a sensitive and robust technique. The occurrence of the studied analytes was evaluated in 154 beer samples from different countries of production. Two mycotoxins were found in a relatively high number of samples (59.7% for DON and 9.1% for HT-2). The overall average contents of 17.2 μg/L for DON and 2.8 μg/L for HT-2 contribute on average of 5% of the DON PMTDI and from 7% (LB) to 12% (UB) of HT-2 TDI for Europeans. Conclusively, a moderate consumption of beer do not raise any toxicological concern as regards exposure to mycotoxins. Nonetheless, for heavy drinkers, beer consumption could imply an important source of mycotoxins.

Conflict of interest

The authors declare that there are no conflicts of interest.

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