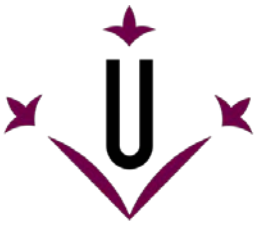

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Survey of mycotoxins in beer and exposure assessment through the consumption of commercially available beer in Lleida, Spain

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Abstract

A multianalyte method, using a MS/MS detector, was applied for a simultaneous determination of 23 mycotoxins in 64 beer products purchased from the supermarket in Lleida, Spain. The samples varied by their origin, brewing technology, alcohol content, etc. The results showed that 20.3% of the tested samples were mycotoxin contaminated overpassing the limit of detection (LOD). None of the alcohol-free samples (17%) were contaminated with mycotoxins. The most frequently occurring toxin was zearalenone (ZEN), being quantified in 65% of the positive samples, with levels ranging from 8.24 to 62.96 µg/L. Regarding the co-occurrence of mycotoxins, three samples were found to contain two or more mycotoxins simultaneously. A deterministic approach was used to evaluate the contribution of beer consumption to daily intake and the proportion of the established tolerable daily intake (TDI) for ZEN and deoxynivalenol (DON) and its metabolite deoxynivalenol-3-glucoside (DON-3-G).

Keywords

Mycotoxins, beer, LC-MS/MS, tolerable daily intake.

1. Introduction

Mycotoxins are natural compounds with a low molecular weight produced by filamentous *fungi* as secondary metabolites with no biochemical significance for fungal development. When exposed to optimal mycotoxin synthesis conditions, they create a toxic substrate which if ingested is able to cause diseases in animals and human beings (Benett & Klich, 2003).

Beer is one of the products that is susceptible to mycotoxin contamination. Spain is the fourth beer producing country in the European Union (Cerveceros de España, 2016). Beer production in the country represents the major economic impact compared to other agrifood sectors (1.4% of GDP) (Cerveceros de España, 2016). As the main ingredient in brewing is barley, *Fusarium, Aspergillus, Penicillium* and *Alternaria* mycotoxins are highly probable to be present, if barley contamination in the field has occurred or good storage practices have not been applied (Medina et al., 2006). The most abundant mycotoxin in beer is found to be deoxynivalenol (DON) (Lancova et al., 2008; Piacentini, Savi, Olivo, & Scussel, 2015). However, other studies did prove the presence of toxins such as zearalenone (ZEN), fumonisins B_1 (FB1), B_2 (FB2) and B_3 (FB3), ochratoxin A (OTA) together with their modified forms (Bauer, Gross, Gottschalk, & Usleber, 2016; Bertuzzi, Rastelli, Mulazzi, Donadini, & Pietri, 2011; Medina, Jiménez, Gimeno-Adelantado, Valle-Algarra, & Mateo, 2005; Rodríguez-Carrasco, Fattore, Albrizio, Berrada, & Mañes, 2015; Rubert, Soler, Marín, James, & Mañes, 2013; Zachariasova et al., 2008). The EU Regulation EC 1881/2006 establishes maximum allowed levels for 13 mycotoxins, however the modified forms are not yet included. The limits for cereal based products (*e.g.* beer) are set as follows: 2 µg/kg for aflatoxin B_1 (AFB1) and 4 µg/kg for total aflatoxins (AFs), 750 µg/kg for DON, 75 µg/kg for ZEN, 400 µg/kg for the sum of FB1 and FB2, and 5 µg/kg for OTA (EC 1881/2006). The majority of modified mycotoxins are less toxic than their parent forms, nevertheless enzymes present in the digestive system may be able to transform the modified forms into parent forms and may have consequences on human health that are difficult to predict (Berthiller et al., 2013).

Considering the existing studies on the carryover of mycotoxins from barley to beer (Inoue, T., Nagatomi, Y., Uyama, A. & Mochizuki, N., ~~et al.~~, 2013; Kostelanska et al., 2011; Lancova et al., 2008) and the few mycotoxin survey studies in Spain, the aim of this work was to study the occurrence of mycotoxins in 64 different beer products, varying by their origin and brewing technology, purchased in the area of Lleida, Spain. In the present research it was aimed to use an analytical LC-MS/MS method for the simultaneous determination of diacetoxyscirpenol (DAS), DON, deoxynivalenol-3-glucoside (DON-3-G), 3- and 15-acetyl-deoxynivalenol (3ADON and 15ADON), fusarenon-X (F-X), the three main fumonisins

(FB1, FB2, FB3), neosolaniol (NEO), nivalenol (NIV), T-2 and HT-2 toxins, zearalenone (ZEN), four aflatoxins (AFB1, AFB2, AFG1, AFG2), sterigmatocystin (STE), ochratoxin A (OTA), roquefortine-C (ROQ-C), alternariol (AOH) and alternariol-methyl-ether (AME). Also, an assessment of population exposure to mycotoxins through beer consumption was performed.

2. Materials and Methods

2.1 Chemicals and reagents

The standards of DAS, DON, DON-3-Glc, 3ADON, 15ADON, F-X, FB1, FB2, FB3, NEO, NIV, T-2, HT-2, ZEN, AFB1, AFB2, AFG1, AFG2, STE, OTA, ROQ-C, AOH and AME were obtained from Sigma Aldrich (Bornem, Belgium). Internal standard deepoxy-deoxynivalenol (DOM-1) was obtained from Romer Lab (Getzersdorf, Austria). All mycotoxin solid standards were dissolved in methanol (1 mg/mL) and stored at -18 °C.

Water was obtained from a Milli-Q® SP Reagent water system from Millipore Corp. (Brussels, Belgium). Disinfectol® (denaturated ethanol with 5% ether) was supplied by Chem-Lab (Zedelgem, Belgium). Methanol (LCMS grade) was purchased from BioSolve (Valkenswaard, the Netherlands), while acetonitrile (Analar Normapur), was obtained from VWR International (Zaventem, Belgium). Acetic acid (glacial, 100%) was supplied by Merck (Darmstadt, Germany). Magnesium sulphate and sodium chloride were purchased from Fischer Scientific (New Jersey, USA).

2.2 Samples

Various bottled and canned beers (*n=64*) were bought from supermarkets of the area of Lleida between May and July 2017. Every product was purchased in a duplicate or triplicate (2 or 3 different lots of each beer) according to their availability at the time of buying (*n=165 samples*). Fourteen different brands originating from nine countries, namely Spain (*5*), Germany (*2*), France (*1*), Belgium (1), Netherland (*1*), Scotland (*1*), Czech Republic (*1*), Argentina (*1*) and Mexico (*1*) were chosen for the analysis according to their availability. The samples were bought considering the differences in consumer preferences, *i.e.* with respect to their fermentation style, ale (9.4%) and lager (90.6%); their alcohol content, alcohol free (17.2%), between 4 and 5 % vol. (60.9%) and >5.5% vol. (21.9%); their colour, yellow (75%), amber (15.6%) and dark coloured (9.4%). Because of the high number of analysed samples, Table 1 regroups only the description of the samples that were found to be contaminated with mycotoxins.

2.3 Sample preparation

Beer samples purification was carried out following a protocol validated by the Laboratory of Food Analysis from Ghent University, Belgium. Briefly, from each bottle (or

can) 100 mL of sample was fractioned and degassed by sonication during 15 min (Branson 2800, Newtown, USA). Then, 18 mL of extraction solvent composed by acetonitrile: water: acetic acid (59:40:1, v/v/v) was added to 2 mL of degassed beer sample containing the internal standard (DOM-1) at a concentration of 10 µg/L. The mixture was vigorously shaken for 30 s prior to the addition of premixed 4 g of MgSO4 and 1 g of NaCl. Afterwards, it was again intensively shaken for 60 s and agitated during 30 min at 200 rpm (Infors AG CH-4103, Bottmingen, Switzerland). The mixture was then centrifuged at 4500 rpm during 10 min with Hettich Universal 320R centrifuge (Tuttlingen, Germany) and 7 mL of supernatant were collected and evaporated to dryness under a low nitrogen stream (40 °C). The dry extract was reconstituted with 200 µL of methanol:water (95:5, v/v) and membrane filtered (0.45 µm).

2.4 LC-MS/MS analysis

A Waters Acquity UHPLC system coupled to a Quattro XEVO TQ mass spectrometer (Waters, Milford, MA, USA) was used to analyse the samples. Data acquisition and processing were performed with MassLynx™ version 4.1 and QuanLynx® version 4.1 software (Waters, Manchester, UK). A Waters Acquity UPLC ® HSS T3 2.1 x 100 mm, 1.8 µm column was applied (Milford, Massachusetts, US).

The mobile phase consisted of water:methanol (95:5, v/v (A)) and methanol:water (95:5, v/v (B)), both buffered with 10 ~~mM~~ mmol/L ammonium acetate and adjusted with 0.3% of glacial acetic acid.

The flow rate was set at 0.3 mL/min. Initially, the mobile phase gradient was set at 5% of the solvent B. Then, it was changed linearly to 65% B in 7 min and to 75% B in the next 4 min. Following that, the proportion dropped to 1% B within 2 min and increased to 99% B in the next minute. Afterwards, the proportion of the solvent B came back to 5 % within 0.1 min, increased to 65 % B and 75% B in the next 3.5 min and 1 min, respectively. The next 1.2 min was characterized by a drop to 1% of solvent B and its increase to 5 % in the following minute. Then, the solvent B proportion was linearly increased to 65% in 3.5 min, to 75% in 1 min and to 99% in the next 1.6 min. The last 2 min of the chromatogram, solvents proportion was kept at 5% B until the next injection.

The mass spectrometer was operated in the positive electrospray ionisation mode (ESI+). The capillary voltage was 30 kV, and nitrogen was applied as spray gas. Source and dissolution temperatures were set at 150 °C and 200 °C, respectively. The argon collision gas pressure was 9×10^{-6} bar, the cone gas flow 50 L/h and the dissolution gas flow 4 mke/h. Two selected reaction monitoring (SRM) transitions with a specific dwell time were chosen for each analyte, in order to increase the sensitivity and the selectivity of the mass spectrometric conditions.

2.5 LC-MS/MS method validation

The LC-MS/MS method was successfully validated based on European Commission Decision 401/2006 laying down the rules for the analytical methods to be used in the testing of official samples. Matrix-matched calibration plots were constructed for the determination of the analytes. DOM-1 was used as internal standard in the multi-mycotoxin analysis. Evaluating the linearity, the homogeneity of variance was checked before fitting the linear model. The linearity was interpreted graphically using a scatter plot. The precision was calculated in terms of the relative standard deviation (RSD) and the bias of the method (uncertainty related to the reference standard, the accuracy of the bias and the root mean square (RMS_{bias})), represented by measurement uncertainty (MU). The MU evaluation was performed according to European Union Decision 2002/657/EC, which corresponds to a confidence interval of 95%. Limit of detection (LOD) was calculated as three times the standard error of the intercept, divided by the slope of the standard curve; the limit of quantification (LOQ) was similar, differing by six times the standard error. The calculated LOD and LOQ were verified by the signal-to-noise ratio (s/n), which should be more than 3 and 10, respectively according to the IUPAC guidelines (Curie, 1995)~~(IUPAC, 1995)~~. The results of the performance characteristics of the LC-MS/MS method were in good agreement with the criteria mentioned in European Commission Decision 401/2006. Table 2 describes the above described parameters.

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2.6 Risk assessment and mycotoxin daily intake

A deterministic approach was used in order to evaluate the probable daily intake of mycotoxins throughout beer consumption based on the obtained mycotoxin levels and beer consumption data available, considering an average body weight of 70 kg (Juan~~-~~C., Berrada, H., Manes, J. & Oueslaty, S., ~~et al.~~, 2017). Taking into account that more than 80% of the samples were found to be below the detection limit (left-censored data), the recommendations of EFSA applying the substitution method (best case scenario – the <LOD values were considered equal to zero, worst case scenario – the <LOD values were equalled to LOD) were followed (European Food Safety Authority, 2010). Afterwards, the following equation was used to calculate the PDI (1):

 (1)

PDI: probable daily intake for each mycotoxin (ng/kg bw/day);

C_m: mean of mycotoxins in the analysed samples (ng/L);

K: average beer consumption (L/day);

bw: body weight (kg).

3. Results and discussions

3.1 Mycotoxin contamination

From 64 analysed beers, thirteen (20.3%) were found to be contaminated with mycotoxins (table 3). However, none of the contaminated samples overpassed the maximum allowed limits. From the thirteen positive samples, only in one sample (01) the three batches were mycotoxin contaminated and in four samples (03, 05, 06 and 10) 2/3 batches were contaminated. None of the eleven analysed alcohol free samples (17%) contained mycotoxins, which is in accordance with previously published researches (Kostelanska et al., 2009; Varga, E., Malachova, A., Schwartz, H., Krska, R. & Berthiller, F. ~~et al.~~, 2013). However, the lack of knowledge concerning the raw materials does not let us explain the different incidence of mycotoxin in alcohol free and the beer containing alcohol.

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According to previously reported studies, the unmalted adjuncts (e.g. maize) are susceptible to be contaminated with mycotoxins and to transfer them to the beer (Torres M.R., Sanchis, V. & Ramos, A.J. ~~et al.~~, 1998). Nevertheless, no significant correlation between mycotoxin contamination and the presence of maize adjuncts was found in this particular case.

DON, DON-3G, ZEN, FB1 and HT-2 toxin were found in the mycotoxin positive samples with and average concentration in the positive samples of 31.28, 13.19, 15.06, 32.78 and 23.72 µg/L, respectively (Table 3). The most frequently encountered mycotoxin resulted to be ZEN (in 65% of positive samples) which concentration ranging between 8.24 and 62.96 µg/L (legal limit for ZEN in beer is 75 µg/L) (Regulation EC 1881/2006). However, none of other monitored toxins were found in the tested beer samples (AOH, AME, NIV, AFs, STE, 3ADON, 15ADON, F-X, DAS, ROQ-C, NEO, OTA, T-2 toxin, FB2 and FB3).

In one of the samples (021) the co-occurrence of three mycotoxins was identified, namely DON, DON-3G and FB1 with concentrations of 20.97, 13.05 and 32.78 µg/L, respectively. In two samples the co-occurrence of DON and DON-3-G was observed which can be explained by a possible conversion from one form to another during the stages of brewing (Kostelanska et al., 2011). However, in this case the ratio DON-3-G/DON <1 (0.53), which is in opposition with mentioned study. Another study performed by Inoue et al. (2013) on the fate of mycotoxins during brewing showed a reduction of DON levels up to 50% compared to the initial contamination but DON-3-G was not an object of the study, thus DON reduction was attributed only to its possible adsorption on spent grains. Also, two samples contained only DON and other two only DON-3-Glc, which prove, in line with other published researches, that there is not a unique correlation of transformation from one form to another during brewing processes, but more complexed origins of these two toxins are

modulating their concentration in the final product (nature of contamination of raw materials, enzymatic activity etc.) (Habler & Rychlik, 2016; Kostelanska et al., 2011; Scott, 1996; Wolf-Hall, 2007).

One of the samples (09) was found to contain HT-2 toxin in a concentration of 23.72 µg/L. HT-2 toxin's main source is wheat and the HT-2 toxin contaminated sample is a wheat beer, which explains that a possible contamination at the level of raw materials occurred (Schothorst & Van Egmond, 2004).

The fact that none of the samples overpassed the legal limits suggests that good reception and storage practices are applied, yet that at the level of reception, the rejection of the contaminated raw materials is an important preventive measure that companies are implementing (Medina et al., 2006).

3.2 Exposure assessment

Results allowed the evaluation of the probable daily intake for ZEN and the sum of DON and DON-3-G as they were the most frequently and significantly occurring mycotoxins in the tested samples (table 3). The exposure was assessed using the available national beer consumption data for 2016 provided by two sources: Spanish Brewers Association (Cerveceros de España, 2016) and Spanish Ministry of Agriculture, Fishing, Alimentation and Environment (MAPAMA). The databases showed a slightly different annual per capita consumption, namely 46.4 L/person (Cerveceros de España, 2016) and 40.67 L/person (MAPAMA, 2016). In the light of the knowledge that in alcohol free beers mycotoxins contamination has not occurred (Kostelanska et al., 2009; Varga et al., 2013) and, considering an average proportion of alcohol free beer in the diet of Spanish consumer of 14% (Cerveceros de España, 2016) and 13.3 % (MAPAMA, 2016), the annual consumption levels were considered as 39.9 L/person (corresponding to 109 mL/day) and 35.27 L/person (corresponding to 97 mL/day), respectively. The established tolerable daily intake (TDI) for ZEN is 0.25 µg/kg body weight (EFSA, 2014). In 2010, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) extended the group of DON and DON-3-G by including 3 and 15- ADON as a factor increasing consumers exposure risk to these toxins and established a TDI of 1 µg/kg body weight for the sum of the four toxins (JECFA/FAO, 2011).

The left-censored data approach was used to treat the obtained dataset. Two exposure scenarios, the lower bound (LB) or the best-case scenario and the upper bound (UB) or the worst-case scenario, were defined (table 4). Regarding the LB scenario, the obtained PDI is less than 1% from the established TDI for both sum of DONs and ZEN (0.15 and 0.65 %, respectively). In the case of UB scenario, the PDI for the sum of DONs and for ZEN represent an average of 5.81 and 4.75 % from the recommended TDI for these toxins.

In a study performed by Juan et al. (2017) on consumers' exposure to mycotoxins through the consumption of barley derived products in Tunisia, beer represented the highest contribution to the TDI compared to other analysed products. Nevertheless, studies evaluating the exposure of the European population to mycotoxins, specially dedicated to DON and its metabolites, found out that beer is not a significant source of exposure, unless it is consumed in high amounts (e.g. more than 0.5 L/day) (Pietri, A., Bertuzzi, T., Agosti, B. & Donadini, G, ~~et al.~~, 2010; Varga et al., 2013).

Only several toxicological studies are published investigating the combined toxic effect on health of two or more simultaneously present or ingested mycotoxins (Speijers & Speijers, 2004). This, considering the findings of the present work (several samples were found contaminated with more than one mycotoxin), proves the need for establishing more combined TDI for the mycotoxins that have additive or synergic effects as the effect of multiple mixtures of mycotoxins must be better understood. Also, considering that beer is only a part of daily diet, studies on the interaction of mycotoxins and other contaminants (e.g. heavy metals) are needed, yet that DON is already known to be decreasing micro-nutrients absorption at intestinal level (Hunder et al., 1991).

4. Conclusions

From the 64 tested beer products, 20.3% were found to contain mycotoxins over the limit of detection (LOD). Ordered by their prevalence in the tested beer samples, the found mycotoxins were ZEN, DON, DON-3-G, FB1 and HT-2. Three samples were characterized by a co-occurrence of two or more mycotoxins. In none of the cases the contamination exceeded the legally established maximum limits for mycotoxins. Regarding the mycotoxin exposure risk assessment, it was found that, according to the available national data on beer consumption, the consumers are not at risk (<1% from the TDI for LB scenario and about 5% from the TDI for the UB scenario). However, the situation might change in the case of heavy drinkers (>0.5 L/day).

5. Acknowledgement

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Table 1

Table 1: Description of the contaminated beers purchased in Lleida, Spain

Sample*	Country of origin	Alc. content, % vol.	Colour	Malt type	Fermentation style	Unmalted adjuncts
01	Spain	7.5	Ambar with orange reflections	Barley	Lager	None
02	France	5.0	Golden yellow	Barley	Lager	None
03	Spain	7.2	Golden yellow	Barley	Lager	Maize, rice
04	Spain	4.8	Cloudy golden	Barley and Wheat	Lager	None
05	Spain	7.5	Golden	Barley	Lager	Maize
06	Spain	6.8	Ambar	Barley	Lager	Maize
07	Belgium	6.0	Blond	Barley and wheat	Lager	None
08	Spain	5.4	Golden bright	Barley	Lager	Rice
09	Spain	5.2	Blond	Barley and wheat	Lager	None
10	Germany	5.0	Blond	Barley and wheat	Lager	None
11	Spain	4.8	Golden	Barley	Lager	Maize
12	Czech Republic	4.4	Pale to golden yellow	Barley	Lager	None
13	Spain	4.0	Bright yellow	Barley	Ale	None

Table 2

Table 2: MS/MS parameters for the analysis of the target analytes by MRM ESI (+) positive mode ionization

Mycotoxin[h]	Precursor ion (m/z)	Product ions (m/z)	CE[a] (eV)	CV[b] (v)	Retention time (min)	LOD[c] (µg/L)	LOQ[d] (µg/L)	CCα[e], µg/L	CCβ[f], µg/L	MU[g] (2x), %
NIV	313.1	175.0/177.0	21/16	35	3.02	31.75	63.50	18.26	23.07	4.51
DON-3-G	476.1	249.0/297.0	18/12	15	3.75	22.36	44.71	12.21	12.28	0.16
DON	297.0	249.0/231.0	15/10	26	4.43	51.76	103.53	27.16	32.44	4.58
3-ADON	356.1	203.1/339.2	16/15	25	5.53	4.97	9.95	2.82	3.34	4.95
15-ADON	356.1	339.2/137.4	25/8	18	5.47	2.65	5.29	1.52	1.84	1.88
F-X	355.0	137.1/247.1	21/12	16	5.37	20.68	41.35	11.25	11.47	0.32
ZEN	319.2	187.2/203.0	19/20	27	11.54	14.12	28.23	7.42	9.24	3.88
STE	325.0	281/310.0	24/30	40	11.25	5.27	10.54	2.70	3.51	0.46
DAS	384.1	247.1/307.1	12/9	35	7.59	0.52	1.03	0.29	0.55	3.74
AOH	258.9	185.1/213.1	30/26	40	9.56	7.78	15.57	3.71	6.18	2.45
AME	272.9	199.3/258.2	30/26	57	12.11	24.73	49.47	12.23	22.60	7.96
AFB1	313.0	241.1/270.1/	32/35	65	8.21	3.22	6.43	1.77	2.39	3.48
AFB2	315.0	259.0/286.9	28/40	25	7.95	2.29	4.57	0.97	2.35	5.71
AFG1	329.0	243.0/311.0	24/20	50	7.20	2.10	4.20	1.10	1.46	2.68
AFG2	331.0	285.0/313.0	28/24	40	6.84	1.16	2.33	0.69	1.42	2.48
NEO	400.0	215.0/305.0	12/9	30	5.34	9.58	19.16	4.57	8.68	4.73
OTA	403.9	239.0/358.0	22/12	40	11.36	4.04	8.08	2.25	2.46	15.46
HT2	442.3	215.7/263.2	12/12	40	9.13	6.39	12.79	3.47	4.25	1.35
T2	484.3	215.9/305.2	18/12	40	9.74	8.23	16.46	5.03	7.45	3.83
FB1	706.2	354.3/530.2	30/28	70	10.51	42.77	85.54	19.87	59.66	7.70
FB2	706.1	336.2/354.2	36/30	70	11.15	172.91	345.82	102.48	159.31	31.40
FB3	722.1	334.2/352.1	36/32	40	9.65	23.20	46.40	11.76	25.02	6.20

[a] CE=collision energy; [b] CV=cone voltage; [c] LOD= limit of detection; [d] LOQ= limit of quantification; [e] CCα=decision limit; [f] CCβ=detection capability; [g] MU=measurement uncertainty, [h] NIV = nivalenol, DON-3-G = deoxynivalenol-3-glucoside, DON = deoxynivalenol, 3ADON = 3- -acetyl-deoxynivalenol, 15ADON = 15-acetyl-deoxynivalenol, F-X = fusarenon-X, ZEN = zearalenone, STE = sterigmatocystin, DAS = diacetoxyscirpenol, AOH = alternariol, AME = alternariol-methyl-ether, AFB1, AFB2, AFG1, AFG2 = four aflatoxins B_1, B_2, G_1, G_2, NEO=neosolaniol, OTA = ochratoxin A, HT-2 and T-2 toxins, FB1, FB2, FB3 = fumonisins B_1, B_2, B_3.

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Table 3

Table 3: Mycotoxin levels (µg/L) in contaminated beer samples purchased from Lleida, Spain

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Sample ID		DON	DON-3-G	ZEN	HT-2	FB1
01						
	011	<LOD	<LOD	**8.77**	<LOD	<LOD
	012	<LOD	<LOD	**8.95**	<LOD	<LOD
	013	<LOD	<LOD	**8.24**	<LOD	<LOD
02						
	021	**20.97**	**13.05**	<LOD	<LOD	**32.78**
03						
	032	<LOD	<LOD	**9.98**	<LOD	<LOD
	033	**46.74**	<LOD	<LOD	<LOD	<LOD
04						
	041	<LOD	<LOD	**10.98**	<LOD	<LOD
05						
	051	<LOD	<LOD	**11.66**	<LOD	<LOD
	052	<LOD	<LOD	**10.72**	<LOD	<LOD
06						
	061	<LOD	<LOD	**8.69**	<LOD	<LOD
	062	<LOD	<LOD	**8.53**	<LOD	<LOD
07						
	072	<LOD	**13.76**	<LOD	<LOD	<LOD
08						
	083	<LOD	<LOD	**10.33**	<LOD	<LOD
09						
	091	<LOD	<LOD	<LOD	**23.72**	<LOD
10						
	101	**26.82**	<LOD	<LOD	<LOD	<LOD
	102	**26.13**	**11.94**	<LOD	<LOD	<LOD
11						
	112	<LOD	**14.00**	<LOD	<LOD	<LOD
12						
	121	<LOD	<LOD	**62.96**	<LOD	<LOD
13						
	131	<LOD	<LOD	**20.97**	<LOD	<LOD

DON = deoxynivalenol, DON-3-G = deoxynivalenol-3-glucoside, ZEN = zearalenone, HT-2 toxin, FB1 = fumonisin B_1.

Table 4

Table 4: Results of the probable daily intake (PDI) assessment for the tested mycotoxins expressed as LB and UB scenarios (ng/kg bw/day).

Mycotoxin[f]	TDI[c], (ng/kg bw/day)	PDI, ng/kg bw/day			
		LB[d]	**%TDI**	**UB**[e]	**%TDI**
ZEN	250	[a]1.71 [b]1.52	[a]0.68 [b]0.61	[a]12.56 [b]11.18	[a]5.02 [b]4.47
Sum of DON, DON-3-G, 3ADON, 15ADON	1000	[a]1.64 [b]1.46	[a]0.16 [b]0.15	[a]61.45 [b]54.69	[a]6.14 [b]5.47

[a] Spanish Brewers Association; [b] MAPAMA; [c] TDI=Tolerable Daily Intake; [d] LB=Lower bound; [e] UB=Upper Bound, [f] ZEN = zearalenone, DON = deoxynivalenol, DON-3-G = deoxynivalenol-3-glucoside, 3ADON = 3-acetyl-deoxynivalenol, 15ADON = 15-acetyl-deoxynivalenol.