



Frequency and levels of mycotoxins in beer from the Mexican market and exposure estimate for deoxynivalenol mycotoxins

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Abstract

The aim of the present study was to evaluate the occurrence of 23 mycotoxins in beer purchased in Mexico and to assess two exposure scenarios in the Mexican population through beer consumption. Multi-mycotoxin analysis of a total of 61 different beers (132 samples) was carried out using UHPLC-MS/MS equipment. Probability density functions were used to describe mycotoxins contamination. The daily intake of mycotoxins was estimated using a semi-probabilistic approach, applying the Monte Carlo method. Deoxynivalenol (DON) and its metabolites (deoxynivalenol-3-glucoside (DON3G) and 3-acetyl-deoxynivalenol (3ADON)) were the mycotoxins found in higher proportions in contaminated samples. None of the other mycotoxins overpassed the limit of quantification (LOQ) of the method. The combined intake of DON and its analogues ranged from 5.24 to 86.59 ng kg⁻¹ bw day⁻¹, which represent from 1.20 to 19.83% of the DON TDI. The results suggest that depending on the individual consumption of beer and depending on the type of beer, the intake of DON via beer could represent a significant percentage of the tolerable daily intake (TDI).

Keywords Mycotoxins · Deoxynivalenol · Beer · Occurrence and estimated daily intake

Introduction

Beer is the most consumed alcoholic beverage worldwide, with an annual per capita consumption greater than 100 L in some European countries (Euromonitor International 2014; Kirin 2016). Mexico, with a production of 10.5 billion litres, is the country with the highest export of beer worldwide. In 2016, Mexico beer exports reached \$2.814 billion, followed by Netherlands (1.905 billion), Belgium (1.438 billion) and Germany (1.307 billion) (INEGI 2017). About 80% of Mexican beer is exported to the USA, the rest being

distributed to more than 184 countries (Kantar Worldpanel Mexico 2015; INEGI 2017).

Cereals used in brewing are mainly barley, wheat and corn (Shetty and Jespersen 2006). These cereals can be subjected to contamination by different mycotoxins. Barley and wheat are mainly contaminated by ochratoxin A (OTA), trichothecenes (deoxynivalenol (DON), nivalenol (NIV), T-2 and HT-2 toxins and zearalenone (ZEN)). Corn is usually infested by *fungi*-producing fumonisins (FBs) and aflatoxins (AFs). All these mycotoxins have been associated with human and animal diseases (Zain 2011). *Alternaria* mycotoxins in cereals have been largely ignored both in Europe and overseas (Müller and Korn 2013). *Alternaria* species produces several mycotoxins, such as alternariol (AOH) and alternariol monomethyl ether (AME). Strong evidence suggests that they are genotoxic (Pfeiffer et al. 2007) and mutagenic (Schrader et al. 2001; Brugger et al. 2006).

The International Agency for Research on Cancer (IARC) classified AFs as a human carcinogen (class 1) and OTA and fumonisin B₁ (FB₁) as possible human carcinogens (class 2B), and DON, ZEN, NIV and T-2/HT-2 toxins were not classifiable as to their carcinogenicity to humans (class 3) (IARC 1993, 2002; FAO/WHO 2006; EFSA 2010b, 2014). The lack of regulation for *Alternaria* toxins worldwide is partially due

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to the limited toxicity data available for them. As a consequence, the European Food Safety Authority (EFSA) used the Threshold of Toxicological Concern (TTC) approach to evaluate the relative level of concern of *Alternaria* toxins for human health. The results demonstrated that dietary exposure to AOH and AME exceeded the TCC value of 2.5 ng/kg body weight per day, indicating the need for additional toxicity data (Arcella et al. 2016; Tralamazza et al. 2018).

The accumulation of mycotoxins in cereals, or derived foods and feeds, has been sporadically documented in Mexico, reaching concentrations higher than 1000 $\mu\text{g kg}^{-1}$ for ZEN in wheat (Gonzalez-Osnaya and Farres 2011), 200 $\mu\text{g kg}^{-1}$ for AFs in maize and maize products (Martínez-Flores et al. 2003; Castillo-Urueta et al. 2011), 5.8 $\mu\text{g kg}^{-1}$ for OTA (Reyes-Velázquez et al. 2008) and 5600 $\mu\text{g kg}^{-1}$ for FB₁ (Robledo et al. 2001) in maize silage. In Mexico, there is no comprehensive food mycotoxins monitoring program carried out by the governmental agencies (Guzmán-de-Peña and Peña-Cabrales 2005).

AFs are the only mycotoxins legislated in Mexico, as described by the official Mexican norms number NOM-187-SSA1-2002 NOM-247-SSA1-2008 and NOM-243-SSA1-2010. The maximum allowed limit of AFs in cereals for human and animal consumption is 20 $\mu\text{g/kg}$. In the USA, AFs (20 $\mu\text{g/kg}$), DON (1000 $\mu\text{g/kg}$), FBs (2000–4000 $\mu\text{g/kg}$) and patulin (50 $\mu\text{g/kg}$) have been regulated (USDA 2015). European regulations on mycotoxin set maximum levels in foodstuff for 14 compounds (European Commission 2006b; European Commission 2013). Regulation 1881/2006 establishes a limit for fumonisin content in maize-based foods (applicable to beer) intended for human consumption to 1000 $\mu\text{g/kg}$. However, specific regulations for mycotoxins in beer do not exist in any of these countries.

Mycotoxin contamination can occur during cereal growth in the field, during post-harvest storage or during malting (Bertuzzi et al. 2011). Considering mycotoxins thermal stability (AFs, ZEN, and DON) and solubility in water (DON and FBs), they can be partially transferred from cereals to malt and then to beer (Rodríguez-Carrasco et al. 2015). Several authors have studied the occurrence of mycotoxins in industrial and craft beers sold in Argentina (Molto et al. 2000), Brazil (Piacentini et al. 2017), Spain (Torres et al. 1998; Rodríguez-Carrasco et al. 2015; Pascari et al. 2018b), Poland (Kuzdraliński et al. 2013), Belgium (Tangni et al. 2002) and other European countries (Papadopoulou-Bouraoui et al. 2004; Bertuzzi et al. 2011). There are no studies on the occurrence of mycotoxins in beer consumed in Mexico or in the USA; however, some of the surveys mentioned above included Mexican beers in their study detecting: OTA, AOH, DON and ZEN.

To estimate dietary exposure, it is necessary to combine data on food consumption and contamination levels in order to allow conclusions to be drawn about the amount of a

substance being consumed by the population (FAO/WHO 2006). Monte Carlo simulation is a statistical method commonly used in probabilistic approach assessment. Monte Carlo simulation relies on a sequence of random numbers to carry out a simulation. This allows a probability distribution to be obtained and studied, instead of a single value to represent this risk (Landau and Binder 2015).

Among the studies of exposure to mycotoxin through beer intake that have been made so far, none has been conducted exclusively in Mexico. Therefore, the objective of this work was to assess two exposure scenarios to mycotoxins throughout beer consumption, focusing on data for the Mexican population (daily beer consumption, average body weight).

Materials and methods

Chemicals and reagents

The standards of mycotoxins, aflatoxin B₁ (AFB₁), aflatoxin B₂ (AFB₂), aflatoxin G₁ (AFG₁), aflatoxin G₂ (AFG₂), sterigmatocystin (STE), OTA, roquefortine C (ROQ-C), AOH, AME, T-2 toxin (T-2), HT-2 toxin (HT-2), neosolaniol (NEO), diacetoxyscirpenol (DAS), DON, 3-acetyl-deoxynivalenol (3ADON), 15-acetyl-deoxynivalenol (15ADON), deoxynivalenol-3-glucoside (DON3G), NIV, fusarenon-X (F-X), ZEN, fumonisin B₁ (FB₁), fumonisin B₂ (FB₂), and fumonisin B₃(FB₃), were obtained from Sigma-Aldrich (Bornem, Belgium). An internal standard of deepoxy-deoxynivalenol (DOM-1) was obtained from Romer Labs (Getzersdorf, Austria). All mycotoxin solid standards were dissolved in methanol (1 mg/mL) and stored at $-18\text{ }^{\circ}\text{C}$.

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 (MgSO₄) and sodium chloride (NaCl) were purchased from Fischer Scientific (NJ, USA).

Samples

Various types of bottled and canned beers ($n = 61$) were bought from supermarkets and beer stores of Veracruz city (Mexico) between July and October 2017. Every product was purchased by duplicate or triplicate (2 or 3 different lots of each beer) according to their availability at the time of buying (total of 132 samples). Twenty-five different beer-producing companies, originating from eight countries,

Mexico (40), the USA (10), Belgium (4), Germany (3), Spain (1), Netherlands (1), Argentina (1) and Guatemala (1), were chosen for the analysis. To facilitate the interpretation and discussion of results, the samples were grouped as follows: according to their fermentation style—ale (31.1%) and lager (68.9%); their alcohol content—alcohol-free (3.3%), between 4 and 5% vol. (80.3%) and > 5.5% vol. (16.4%); their colour—golden (62.2%), amber (28.0%) and dark coloured (9.8%); and their production method—industrial (73.8%) and craft (26.2%).

Sample pre-treatment

Extraction of beer samples was carried out following a protocol modified from Monbaliu et al. (2009), validated by the Laboratory of Food Analysis from Ghent University, Belgium. Briefly, from each sample, a 100-mL aliquot was taken, degassed, sonicated for 15 min and stored at $-18\text{ }^{\circ}\text{C}$ until analysis. 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 $10\text{ }\mu\text{g L}^{-1}$. The mixture was vigorously shaken for 30 s prior to the addition of premixed 4 g of MgSO_4 and 1 g of NaCl, after which it was shaken again for 60 s and agitated during 30 min at 200 rpm in an orbital rotary shaker (Infors AG CH-4103, Bottmingen, Switzerland). The mixture was then centrifuged at $2336\times g$ during 10 min with a Hettich Universal 320R centrifuge (Tuttlingen, Germany), and 7 mL of supernatant was collected and evaporated to dryness under a low nitrogen stream ($40\text{ }^{\circ}\text{C}$). The dry extract was resuspended in 0.5 mL of methanol:water (95:5, v/v) and filtered (PTFE syringe filter, $0.22\text{ }\mu\text{m}$) before injection in HPLC-MS/MS system.

Mycotoxin 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 $2.1\times 100\text{ mm}$, $1.8\text{ }\mu\text{m}$ column was applied (Milford, MA, USA).

The mobile phase consisted of a gradient with phase A: water:methanol (95:5, v/v) and phase B: methanol:water (95:5, v/v), both buffered with 10 mmol L^{-1} ammonium acetate and acidified with 0.3% of glacial acetic acid.

The phase gradient was adjusted with 5% of solvent B and the rest with solvent A. After 7 min, it was increased linearly at 65% of solvent B, and 4 min later, it was increased to 75% of B. Following that, the proportion dropped to 1% B within 2 min and increased to 99% B the next minute. After that, the proportion of solvent B again decreased to 5% and increased

to 65% B and 75% B in the next 3.5 min and 1 min, respectively. In the following 1.2 min, the proportion of solvent B decreased to 1%, increasing to 5% after 1 min. Then, the solvent B proportion was increased linearly to 65% in 3.5 min to 75% in 1 min and to 99% in the next 1.6 min. In the last 2 min of the chromatogram, solvent proportion was kept at 5% B until the next injection. The flow rate was set at 0.3 mL min^{-1} through the entire analysis process.

The mass spectrometer was operated in positive electrospray ionisation mode (ESI+). The ESI parameters were set up as follows: capillary voltage 30 kV, and nitrogen applied as spray gas; source and dissolution temperatures $150\text{ }^{\circ}\text{C}$ and $200\text{ }^{\circ}\text{C}$, respectively; argon collision gas pressure $9\times 10^{-6}\text{ bar}$; cone gas flow 50 L h^{-1} ; dissolution gas flow 4 mL h^{-1} . 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.

LC-MS/MS method validation

The LC-MS/MS method for the simultaneous detection of 23 mycotoxins was successfully validated in-house based on European Commission 401/ 2006a. Validation data for each selected compound are presented in Table 1. Matrix-matched calibration plots were constructed for the determination of the analytes. Linearity and the homogeneity of variance were checked for each mycotoxin studied. The linearity was interpreted graphically using a scatter plot. The precision was represented in terms of relative standard deviation (RSD) and the bias of the method represented by measurement uncertainty (MU). The MU evaluation was performed according to European Regulation (European Commission 2002/657), which corresponded to a confidence interval of 95%. Limit of detection (LOD) and limit of quantification (LOQ) were calculated as three and six times the standard error of the intercept divided by the slope of the calibration curve, respectively. 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 (IUPAC, prepared by Currie 1995). The results of the performance characteristics of the LC-MS/MS method were in good agreement with the criteria mentioned in European Commission 401/ 2006a.

The resulted detection and quantification limits are higher compared to the ones obtained in similar studies (Bertuzzi et al. 2011; Rodríguez-Carrasco et al. 2015; Bauer et al. 2016; Piacentini et al. 2017); however, none of them performed a simultaneous multi-analysis study of 23 mycotoxins with conversion rates close to 100%.

Table 1 Validation parameters for the LC-MS/MS method for mycotoxins analysis in beer

Analyte	LOD ($\mu\text{g L}^{-1}$)	LOQ ($\mu\text{g L}^{-1}$)	Recover range (%)	Lowest level recovery was tested ($\mu\text{g L}^{-1}$)	Recover range at lowest level (%)	RSD r % ($n = 10$)	CC α ($\mu\text{g/L}$)	CC β ($\mu\text{g/L}$)	Measurement uncertainty ($2\times$)
AFB ₁	3.22	6.43	107.86	10.00	103.74	3.86	1.77	2.39	3.48
AFB ₂	2.29	4.57	106.00	10.00	104.47	3.38	0.97	2.35	5.71
AFG ₁	2.10	4.20	105.83	10.00	107.02	3.16	1.10	1.46	2.68
AFG ₂	1.16	2.23	103.11	10.00	103.65	1.60	0.69	1.42	2.48
STE	5.27	10.54	104.41	25.00	107.60	3.65	2.70	3.51	0.46
OTA	4.04	8.08	122.08	25.00	107.50	9.71	2.25	2.46	15.46
ROQ-C	0.67	1.34	105.90	2.50	104.47	1.24	0.35	0.42	2.41
AOH	7.78	15.57	104.37	50.00	104.57	1.14	3.71	6.18	2.45
AME	24.73	49.47	109.06	100.00	111.12	2.62	12.23	22.60	7.96
T-2	8.23	16.46	105.50	50.00	105.20	1.19	5.03	7.45	3.83
HT-2	6.39	12.79	102.61	50.00	104.82	2.0	3.47	4.25	1.35
NEO	9.58	19.16	104.11	50.00	103.86	1.65	4.57	8.68	4.73
DAS	0.52	1.03	104.76	5.00	104.47	1.68	0.29	0.55	3.74
DON	51.76	103.53	107.21	200.00	108.85	0.84	27.16	32.44	4.58
DON3G	22.36	44.71	101.32	20.00	102.59	1.08	12.21	12.28	0.16
3ADON	4.97	9.95	103.59	25.00	102.57	3.08	2.82	3.34	4.95
15ADON	2.65	5.29	106.18	12.50	103.97	4.08	1.52	1.84	1.88
NIV	31.75	63.50	107.71	100.00	103.49	3.22	18.26	23.07	4.51
F-X	20.68	41.35	104.59	100.00	106.78	1.53	11.25	11.47	0.32
ZEN	14.12	28.23	103.11	50.00	108.24	1.60	7.42	9.24	3.88
FB ₁	42.77	85.54	106.96	200.00	108.23	3.76	19.87	59.66	7.70
FB ₂	172.91	345.82	123.51	200.00	124.23	10.42	102.48	159.31	31.40
FB ₃	23.20	46.40	105.90	125.00	105.26	2.06	11.76	25.02	6.20

CC α decision limit, CC β detection capability

Treatment of left-censored data

Analytical methods are defined by LOD and LOQ; to express quantitatively the result below these limits, several techniques can be used. EFSA published a scientific report evaluating the accuracy of methods currently used and providing recommendations for more advanced alternative statistical approaches. WHO has proposed recommendations for replacing the non-detected samples by LOD/2 or 0 and LOD according to the percentage of non-detects in the samples; similar guidelines have been provided in the case of non-quantified values (EFSA 2010a).

In this study, taking into account that more than 60% but less than 80% of the samples were found to be below the detection limit (with <25 results quantified), EFSA's recommendations were applied: lower bound (LB) or best-case scenario, where the < LOD values were considered equal to zero and upper bound (UB) or worst-case scenario, where the < LOD values were equalled to LOD (EFSA 2010a).

Theoretical distribution of mycotoxin beer contamination

Using the Risk 7.5 (Palisade, Inc.) risk software, a comparison of different probability distribution functions was carried out. Considering the asymmetry of the histogram of mycotoxin contamination in beer, the data were adjusted to an exponential function. Probability density functions and descriptive statistics (the mean, median, standard deviation and the 95th

percentile) of mycotoxin concentration in beer were also determined and analysed. The Monte Carlo method was applied with the iteration number (10,000) recommended by international agencies (US-EPA 1997).

Data used for body weight population and beer consumption

The high variability of alcohol consumption within the population makes it one of the most difficult food items for exposure assessment studies. According to the FAO/WHO (2014), in Mexico, alcohol consumption is six times higher in men (12.4 L of pure alcohol per year) than in women (2.6 L of pure alcohol per year) and 76% of the alcohol consumed comes from the intake of beer. Because there are no available studies describing the behaviour of beer consumption in groups of population, such as age, gender, region or socioeconomic level, the national average volume of 60 L of beer per year, equivalent to 164.38 mL/day, established by the Mexican Ministry of Economy (Secretaría de Economía 2015), will be applied in the present publication. To estimate the levels of intake in high drinkers, the beer consumption average of Czech Republic (143.3 L per year), the country with the highest consumption of beer in the world, was used.

The benchmark body weight used was that established by CANAIVE (2012) for an average Mexican (71.7 kg) (Cámara Nacional de la Industria del Vestido, (CANAIVE 2012).

Estimation of mycotoxins daily intake and exposure risk

Daily intake was then calculated under a semi-probabilistic approach by Eq. (1):

$$EDI = \frac{Mc \times Bc}{bw} \quad (1)$$

where:

EDI= Probability density function of estimated daily intake (ng mycotoxin kg⁻¹ bw day⁻¹)

Mc= Probability function density of mycotoxin concentration in beer (ng L⁻¹)

Bc= Beer consumption (L day⁻¹)

bw= Body weight (kg)

In the case of mycotoxins that are not classified as genotoxic or carcinogenic, the exposure estimates were compared with the guidance values of tolerable daily intake (TDI). TDI used in the present study is summarised in Table 2.

Results and discussion

Occurrence of mycotoxin in beer

Mycotoxins were detected in 16 of the 61 analysed samples (26.2% positive samples); however, none overpassed the limits of quantification of the methodology used. Only one beer presented contamination in the two analysed replicates (different production batches).

The samples were purchased in supermarkets and beer stores in Veracruz city, so there is no information available on the traceability of the raw material or of the process; however, all the mycotoxins detected are produced by *Fusarium* fungi, which are characterised by invading cereals in the field (Gimeno and Martins 2003). Thus, the contamination probably originates in the field, with minimal possibility of contamination during storage or processing. From the analysed

samples, nine presented contamination with DON, two with 3ADON, six with DON3G and three with FB₁.

Similar results were reported by Pascari et al. (2018b) in beer purchased in Lleida, Spain, with 20.3% of samples contaminated by DON, DON3G, ZEN, HT2 and FB₁. Kuzdraliński et al. (2013) and Rodríguez-Carrasco et al. (2015) reported contamination by DON in 100% of beers analysed; however, all samples showed contamination less than 48 µg L⁻¹. This concentration is lower than the LOQ of our methodology, so decreasing the LOQ of our methodology, the proportion of positive samples would probably increase to a large extent.

The most frequent contaminants were DON and its metabolites, detected in 87.5% of the positive samples. In two samples, co-occurrence of DON and 3ADON was detected, which could have been due to their release from barley matrix during mashing and subsequent transfer to wort and beer because of their relatively high solubility in water (Samar et al. 2001; Kostelanska et al. 2011). Similarly, the presence of DON3G in five samples can be attributed to DON conversion during malting due to grain defence mechanisms against the presence of the contaminant, as reported by Lancova et al. (2008). ZEN was not detected in any of the samples. It would have been advisable to analyse α-zearalenone (α-ZEL) and β-zearalenone (β-ZEL) to discard contamination by ZEN metabolites (Karlovsky et al. 2016).

FB₁ contamination was found in three analysed beers; this could be a consequence of the use of corn as an unmalted adjunct—corn grits are commonly used in order to achieve a greater degree of lightness in colour, clarity, calories and flavour (Bertuzzi et al. 2011). Corn has been proven susceptible to infestation by FB-producing *Fusarium*, which would explain the abovementioned finding (Robledo et al. 2001; Mendoza et al. 2017).

There are limited surveys that classify samples for data analysis (Rodríguez-Carrasco et al. 2015; Peters et al. 2017; Pascari et al. 2018a). In our study, beers with an alcohol content greater than 5.5% had mycotoxin contamination in 60% of the samples analysed, similar to the results reported by Pascari et al. (2018b). A possible explanation would be the necessity to use more grain in high-density malt wort to reach these alcohol levels, which could contribute to greater mycotoxin contamination. Light and non-alcoholic beers did not show contamination above LOD.

Craft beer presented a higher percentage of mycotoxin contamination (56.3%) than industrial beers (15.55%). In the same way, Peters et al. (2017) detected more mycotoxins (AFB₁, OTA, ZEN, FBs, DON, T-2, and HT2) in craft beer than in industrial beer from 1000 beers analysed. It is recommended that small craft breweries consider the implementation of rapid analysis techniques for mycotoxins in cereals to control purchased malts and adjuncts as well as their final products.

Table 2 Compilation of tolerable daily intake (TDI) values for mycotoxins issued by the European Union

Mycotoxins	Tolerable daily intake (ng kg ⁻¹ bw day ⁻¹)	Reference
OTA	17	EFSA 2010b
T-2	60	EFSA 2011a
HT-2	100	EFSA 2011a
DON	1000	SCF 2000
NIV	700	SCF 2002
ZEN	250	EFSA 2011b
FBs	2000	SCF 2003

Table 3 Fitted exponential probability density function (PDF) parameters for the content of DON mycotoxins (DON + DON3G + 3ADON + 15ADON) in beer marketed in Veracruz (Mexico)

PDF parameters ($\mu\text{g L}^{-1}$)	Lower bound (LB)	Upper bound (UB)
Mean	5.24	86.59
Median	3.64	85.02
Standard deviation	5.24	4.79
95th percentile	15.71	96.05
99th percentile	24.12	103.77

The Mexican-brand or Mexican-made beers presented contamination in 27.5%. Although with a non-representative sample size (three positive samples from a total of seven analysed), the results agree with that reported by Bauer et al. (2016), who found a high frequency of mycotoxin contamination (75% for DON) although in low concentrations (2.2–

20 $\mu\text{g L}^{-1}$) in European beers. Regarding the colour classification, similar contamination was found, dark beers presented 33%, amber 26% and golden 23%. Finally, as for the fermentation style, ale beers had a higher percentage of contamination (42%) than lager (29%), which could be probably explained by different adsorptions of the toxins to the yeast cell during fermentation (Lancova et al. 2008); nonetheless, more investigation is needed to confirm this statement.

Estimation of the DON intake via beer consumption in various scenarios

Due to the limited number of positive samples contaminated with FB_1 and other mycotoxins, only an assessment of the intake of DON through beer consumption was performed, considering the recommendations of EFSA (2013, 2014) to

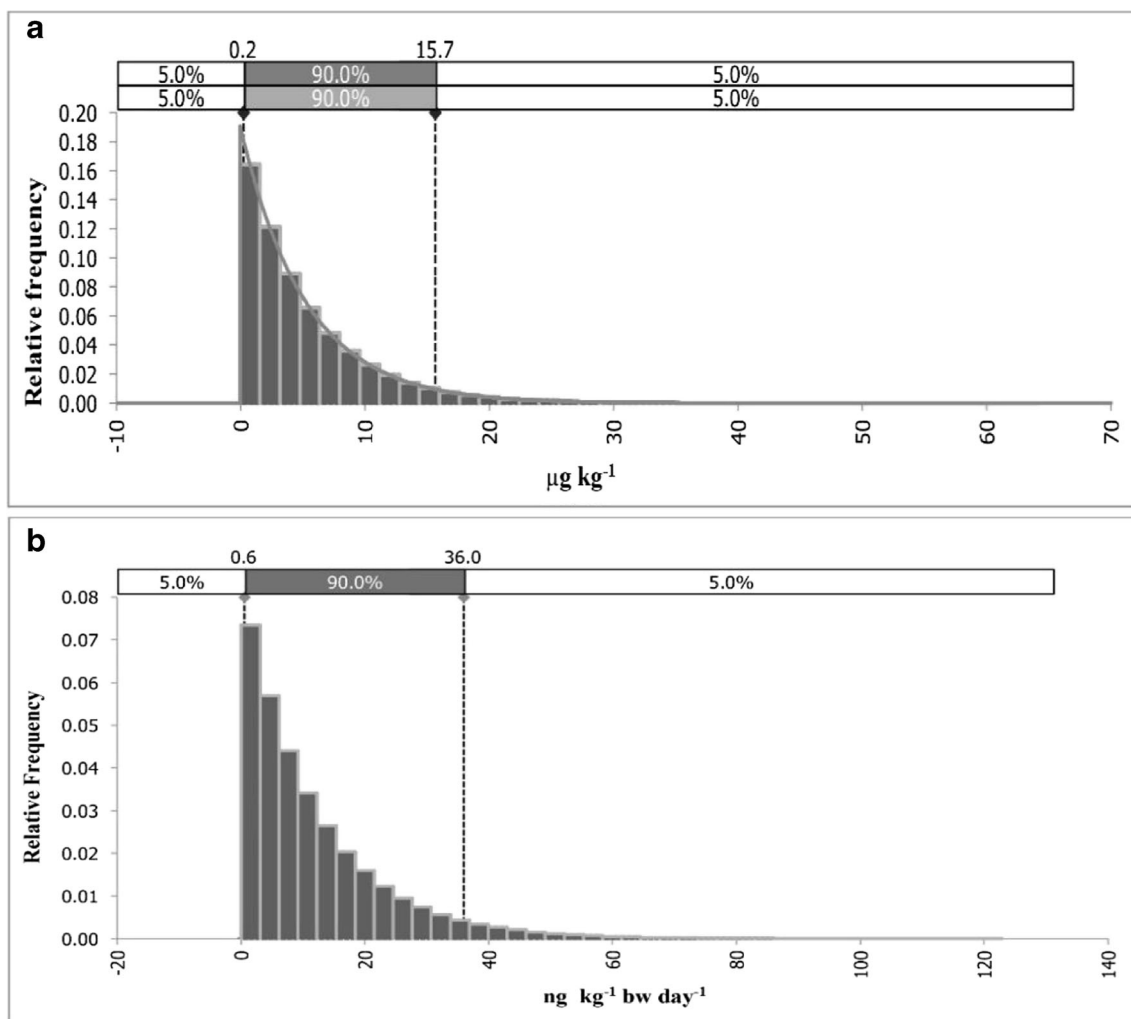


Fig. 1 **a** Probability density function fitted exponential distribution (solid line) for DON contamination in beer marketed in Mexico (lower bound values), obtained by the Monte Carlo method, showing contamination in the 5th and 95th percentiles (broken line). **b** Probability density function

for estimated daily intake of DON (Lower bound values) though beer, obtained by Monte Carlo method, showing exposure in the 5th and 95th percentiles (broken line)

Table 4 Probability density functions (PDF) parameters for estimated daily intake of DON mycotoxins (DON + DON3G + 3ADON + 15ADON) through beer consumption in Veracruz (México)

PDF parameters	Lower bound (LB)	Upper bound (UB)	Units
Mean	12.03	198.31	(ng kg ⁻¹ bw day ⁻¹)
Median	8.33	194.93	(ng kg ⁻¹ bw day ⁻¹)
Standard deviation	12.04	10.98	(ng kg ⁻¹ bw day ⁻¹)
95th percentile	36.00	220.23	(ng kg ⁻¹ bw day ⁻¹)
99th percentile	55.35	237.91	(ng kg ⁻¹ bw day ⁻¹)
DON TDI	1000	1000	(ng kg ⁻¹ bw day ⁻¹)
Mean for a high consumer	28.69	473.64	(ng kg ⁻¹ bw day ⁻¹)
TDI 50th percentile	1.20	19.83	%
TDI 50th percentile for a high consumer	2.87	47.36	%

use the sum of DON and its modified forms (DON3G, 3ADON, and 15ADON) for calculation.

Table 3 shows the statistical parameters of the probability density function for mycotoxin contamination in beer for the two risk scenarios (LB and UB). It can be seen that even in the 99th percentile, the values are below the DON TDI of 1000 ng kg⁻¹ bw day⁻¹ (SCF 2000). Similar concentrations were presented by Bryła et al. (2018) (9.0 µg L⁻¹), Kuzdraliński et al. (2013) (20.66 µg L⁻¹) and Rodríguez-Carrasco et al. (2015) (28.9 µg L⁻¹) in beer from different countries. The data on contamination by DON and its metabolites were adjusted to an exponential function. Figure 1a presents the probability density function of DON contamination in LB scenario.

Probability density function and probability density function parameters of the EDI calculated by the Monte Carlo method are shown in Fig. 1b and Table 4. The EDI average was 12.03 ng kg⁻¹ bw day⁻¹ (LB) and 198.31 ng kg⁻¹ (UB) or 28.69 ng kg⁻¹ bw day⁻¹ (LB) and 473.64 ng kg⁻¹ bw day⁻¹ (UB) in the high consumption scenario. Those are lower than the recommendation of the JEFCA (2010) of 1000 ng kg⁻¹ bw day⁻¹. The percentage of TDI of DON mycotoxins that beer provides as a result of LB consumption is similar that reported by Pascari et al. (2018a) in Spain (1.6%) and lower than that obtained by Bauer et al. (2016) (5–10%) and Rodríguez-Carrasco et al. 2015 (10%) in beer consumers from Germany and Ireland respectively. Regarding other products, TDI in the LB scenario that beer provides for exposure to DON is similar to bread (5.3 ng kg⁻¹ bw day⁻¹) and cookies (5.7 ng kg⁻¹ bw day⁻¹) in the population of Brazil (Savi et al. 2016) and pasta (22 ng kg⁻¹ bw day⁻¹) in Spain. It is lower than corn flour (1600 ng kg⁻¹ bw day⁻¹) and greater than of oat flakes (0.07 ng kg⁻¹ bw day⁻¹) in China (Ji et al. 2018).

This is the first study with a large number of mycotoxins analysed in beer commercialised in Mexico, the country with the largest world export of beer. Mycotoxins were present in a greater proportion in craft beers than in commercial beers. DON and its modified forms (DON3G, 3ADON) were the

most frequently occurring mycotoxins compared to other analyzed compounds. Although the contamination data obtained in the present study were not above the legal limits, DON intake through beer consumption should not be ignored (contribution to exposure from 1.20 to 19.83% of TDI). An even greater contribution may take place for the population consuming a daily amount of beer above the national average, such as the Mexican male population (according to WHO reports, men consume six times more alcohol than women).

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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