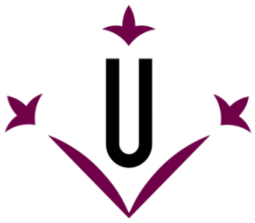

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Frequency and levels of mycotoxins in beer from the Mexican market and exposure estimate for deoxynivalenol mycotoxins

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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 litres 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 dollars, 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 United States, 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), OTA and fumonisin B_1 (FB_1) as a possible human carcinogen (Class 2B), 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 to the limited toxicity data available for them. As a consequence, the 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 $\mu g\ kg^{-1}$ for ZEN in wheat (Gonzalez-Osnaya and Farres 2011), 200 $\mu g\ kg^{-1}$ for AFs in maize and maize products (Martínez-Flores et al. 2003; Castillo-Urueta et al.

2011), 5.8 μg kg^{-1} for OTA (Reyes-Velázquez et al. 2008) and 5600 μg kg^{-1} for FB_1 (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-Cabriales 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 μg/kg. In the United States, AFs (20 μg/kg), DON (1000 μg/kg), FBs (2000-4000 μg/kg), and patulin (50 μg/kg) have been regulated (USDA 2015). European regulations on mycotoxin set maximum levels in foodstuff for 14 compounds (European Commission 1881/2006; European Commission 2013/165/EU). Regulation 1881/2006 establishes a limit for fumonisin content in maize-based foods (applicable to beer) intended for human consumption to 1000 μg/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 United States, 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 B_1 (AFB_1); aflatoxin B_2 (AFB_2); aflatoxin G_1 (AFG_1); aflatoxin G_2 (AFG_2), sterigmatocystin (STE); OTA; roquefortin 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 B_1 (FB1); fumonisin B_2 (FB2) and fumonisin B_3(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 °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 ($MgSO_4$) and sodium chloride (NaCl) were purchased from Fischer Scientific (New Jersey, 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), Unites States (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 color – golden (62.2 %), amber (28.0 %) and dark colored (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 minutes and stored at -18 º 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 µg 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 shaked 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 x g during 10 min with a 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 resuspended in 0.5 mL of methanol:water (95:5, *v/v*) and filtered (PTFE syringe filter, 0.22 µm) 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 × 100 mm, 1.8 µm column was applied (Milford, Massachusetts, US).

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 minutes; it was increased linearly at 65 % of solvent B, and 4 minutes 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 %, 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 minute. 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. The last 2 min of the chromatogram, solvents 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 ionization 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 °C and 200 °C, respectively; argon collision gas pressure 9×10^{-6} bar; cone gas flow 50 L h^{-1}; dissolution gas flow 4 mke 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 401/2006. 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 401/2006.

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

Analyte	LOD (µg L^{-1})	LOQ (µg L^{-1})	Recover Range (%)	Lowest level recovery was tested (µg L^{-1})	Recover Range at lowest level (%)	RSD r % (n = 10)	CCα (µg/L)	CCβ (µg/L)	Measurement uncertainty (2x)
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

CCα = decision limit.
CCβ = detection capability

169 The resulted detection and quantification limits are higher compared to the ones obtained in similar studies
170 (Bertuzzi et al. 2011; Rodriguez-Carrasco et al. 2015; Bauer et al. 2016; Piacentini et al. 2017), however none
171 of them performed a simultaneous multi-analysis study of 23 mycotoxins with conversion rates close to 100
172 %.

173 **Treatment of left-censored data**

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

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

184 **Theoretical distribution of mycotoxin beer contamination**

185 Using the Risk 7.5 (Palisade, Inc.) risk software, a comparison of different probability distribution functions
186 was carried out. Considering the asymmetry of the histogram of mycotoxin contamination in beer, the data
187 were adjusted to an exponential function. Probability density functions and descriptive statistics (the mean,
188 median, standard deviation and the 95th percentile) of mycotoxin concentration in beer were also determined
189 and analysed. The Monte Carlo method was applied with the iterations number (10,000) recommended by
190 international agencies (US-EPA 1997).

191 **Data used for body weight population and beer consumption**

192 The high variability of alcohol consumption within the population makes it one of the most difficult food
193 items for exposure assessment studies. According to the FAO/WHO (2014), in Mexico alcohol consumption
194 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 equation (1):

$$EDI = \frac{Mc * Bc}{bw} \ldots (1)$$

Where:

$EDI =$ Probability Density Function of Estimated Daily Intake (ng mycotoxin kg^{-1} bw d^{-1})

$Mc =$ Probability Function Density of mycotoxin concentration in beer (ng L^{-1})

$Bc =$ Beer consumption (L d^{-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 are summarised in Table 2.

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

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 characterized 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 FB_1.

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 FB_1. Kuzdraliński et al. (2013) and Rodríguez-Carrasco et al. (2015) reported contamination by DON in 100 % of beers analyzed; 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 200; 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 analyze α-zearalenone (α-ZEL) and β-zearalenone (β-ZEL) to discard contamination by ZEN metabolites (Karlovsky et al. 2016).

FB_1 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 (Rodriguez 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 analyzed, 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 (AFB_1, OTA, ZEN, FBs, DON, T-2, and HT2) in craft beer than in industrial beer from 1,000 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.

The Mexican-brand or Mexican-made beers presented contamination in 27.5 %. Although with a non-representative sample size (3 positive samples from a total of 7 analyzed), 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 µg 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 adsorption 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 recommendation of EFSA (2014) to 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).

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 (µg L^{-1})	Lower bound (LB)	Upper bound (UB)
Mean	5.24	86.59
Median	3.64	85.02
Standard Deviation	5.24	4.79
95^{th} Percentile	15.71	96.05
99^{th} Percentile	24.12	103.77

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 Bryla et al. (2018) (9.0 μg L^{-1}), Kuzdraliński et al. (2013) (20.66 μg L^{-1}) and Rodriguez 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 1 a) 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 Figure 1 b) and Table 4.

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})
95^{th} Percentile	36.00	220.23	(ng kg^{-1} bw day^{-1})
99^{th} 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 50^{th} percentile	1.20	19.83	%
TDI 50^{th} percentile for a high consumer	2.87	47.36	%

The EDI average was 12.03 ng kg^{-1} bw day^{-1} (LB) and 198.31 ng kg^{-1} bw day^{-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 d^{-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 that than obtained by Bauer et al. (2016) (5-10 %) and Rodríguez-Carrasco 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 d^{-1}) and cookies (5.7 ng kg^{-1} bw d^{-1}) in the population of Brazil (Savi et al. 2016) and pasta (22 ng kg^{-1} bw d^{-1}) in Spain. It is lower than corn flour (1600 ng kg^{-1} bw d^{-1}) and greater than of oat flakes (0.07 ng kg^{-1} bw d^{-1}) in China (Ji et al. 2018).

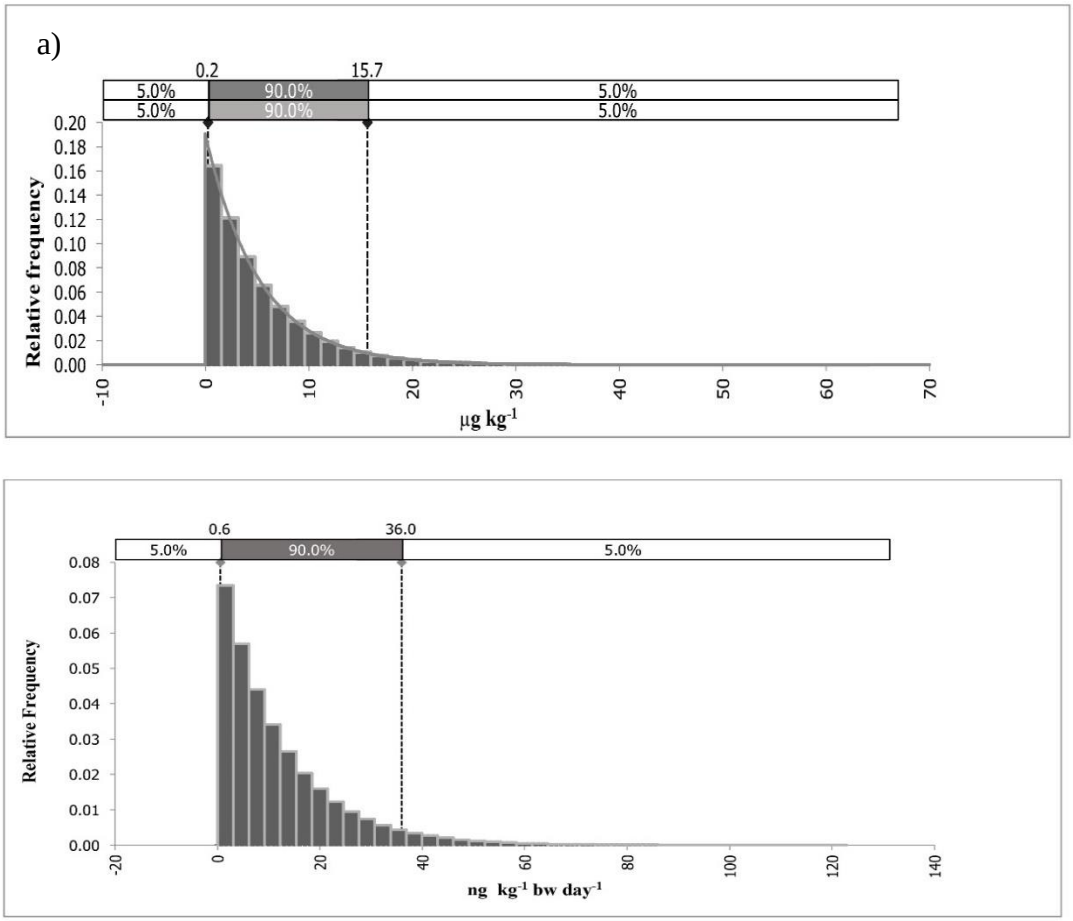

Fig 1. a) Probability density function fitted exponential distribution (solid line) for DON contamination in beer marketed in Mexico (Lower bound values), obtained by the Monte Carlo method, showing contamination in the 5th and 95th percentiles (broken line); b) Probability density function for estimated daily intake of DON (Lower bound values) though beer, obtained by Monte Carlo method, showing exposure in the 5th and 95th percentiles (broken line).

313 This is the first study with a large number of mycotoxins analysed in beer commercialised in Mexico, the
314 country with the largest world export of beer. Mycotoxins were present in a greater proportion in craft beers
315 than in commercial beers. DON and its modified forms (DON3G, 3ADON) were the most frequently
316 occurring mycotoxins compared to other analized compounds. Although the contamination data obtained in
317 the present study were not above the legal limits, DON intake through beer consumption should not be
318 ignored (contribution to exposure from 1.20 to 19.83 % of TDI). An even greater contribution may take place
319 for the population consuming a daily amount of beer above the national average, such as the Mexican male
320 population (according to WHO reports, men consume six times more alcohol than women).

321

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326

327 **CONFLICTS OF INTEREST**

328 The authors declare to have no conflicts of interest to disclose, have full control of all the primary data and
329 grant the journal access to review their data if requested.

330

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MATERIAL SUPLEMENTARY

Beer classification and mycotoxin contamination

Sample	Country of origin	Mycotoxin detected	Process (Craft/Industrial)	Fermentation style	Colour	Contamination replicate	Alcohol content	Type of Malt	Unmalted Adjuncts
1	Belgium	DON	Industrial	Ale	Golden	No	> 5.5 %	Barley	None
2	Belgium	DON	Industrial	Ale	Amber	No	> 5.5 %	Barley	None
3	Belgium	DON	Industrial	Ale	Dark	No	> 5.5 %	Barley	None
4	Mexico	DON	Industrial	Lager	Amber	Yes	Between 4 and 5 %	Barley	Coffee and chocolate
5	USA	DON	Industrial	Lager	Golden	No	Between 4 and 5 %	Barley	Maize
6	Mexico	DON, FB1	Craft	Lager	Golden	No	Between 4 and 5 %	Barley	Maize
7	Mexico	DON, 3ADON	Craft	Ale	Amber	No	> 5.5 %	Barley	None
8	Mexico	DON, 3ADON	Craft	Ale	Dark	No	> 5.5 %	Barley	None
9	Mexico	DON3G	Craft	Ale	Amber	No	> 5.5 %	Barley	None
10	Mexico	DON3G	Craft	Lager	Amber	No	Between 4 and 5 %	Barley	Wheat
11	Mexico	DON3G	Craft	Lager	Amber	No	Between 4 and 5 %	Barley	None
12	Mexico	DON3G	Craft	Ale	Amber	No	Between 4 and 5 %	Barley	None
13	Mexico	DON3G	Craft	Ale	Amber	No	Between 4 and 5 %	Barley	None
14	Mexico	DON3G	Industrial	Lager	Amber	No	Between 4 and 5 %	Barley	None
15	Mexico	FB1	Craft	Lager	Golden	No	Between 4 and 5 %	Barley	Maize
16	Guatemala	FB1	Industrial	Lager	Amber	No	Between 4 and 5 %	Barley	Maize