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Mycotoxin analysis of industrial beers from Brazil: The influence of fumonisin B_1 and deoxynivalenol in beer quality

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ABSTRACT

Worldwide, barley is the main source of carbohydrate in the brewing process. However, corn is often used as an adjunct to improve and accelerate the fermentation process. Considering that, these two substrates are susceptible to fungal contamination as well as mycotoxins. The objective of the current study is to determine the incidence of the mycotoxins deoxynivalenol (DON) and fumonisin B_1 (FB_1) in industrial beers.

The method applied for mycotoxin analyses included high performance liquid chromatography. The mean levels for recovery experiments were 89.6% for DON and 93.3% for FB_1. DON was not detected in any of the analyzed samples whereas FB_1 was found in 49% of the 114 samples. The current survey demonstrated levels of FB_1 contamination in industrial beer, possibly due to the addition of contaminated adjuncts. It is necessary to establish maximum levels of mycotoxins in beer in Brazil and other countries in order to reduce health risks.

1. Introduction

Beer is a popular alcoholic beverage in the world context for reasons that transcend social and economic aspects. In Brazil, the beer market continues to grow every year and production reached 13.5 billion liters in 2013. In terms of national average consumption, data has shown a large growth potential of 68.3 L per person in 2014 (CervBrasil, 2014).

Worldwide, the source of fermentable sugars in industrial beer is starch-rich cereals, mainly malted barley. Nevertheless, adjuncts including corn, rice, unmalted barley, wheat starch, oats and sorghum have been also used by the large-scale brewing industry to provide additional sources of fermentable carbohydrates for the yeast (Figueroa, Martínez, & Ríos, 1995; Poreda, Czarnik, Zdaniewicz, Jakubowski, & Antkiewicz, 2014).

The brewing industry has many reasons for the application of adjuncts including better availability on the local market, sensory modification of the beer and, the most important, the lower price of this product in Brazil (Dhellot & Kobawila, 2013; Glatthar, Heinisch, & Senn, 2005). It has been proven that the use of 30% of corn adjunct can give an 8% reduction in total production costs, although this number may vary depending on the local prices of raw materials and other costs of production (Baca, 2001). However, application of adjuncts also has some disadvantages. One of the most relevant is the contamination by fungi and the production of mycotoxins. These will have a negative impact on the final product, mainly because of the health problems associated with mycotoxin contamination (Lancova et al., 2008). Several studies revealed that barley can be contaminated by fungi (Piacentini, Savi, Olivo, & Scussel, 2015), as well as the adjuncts used for brewing (Kawashima, Vieira, & Valente Soares, 2007; Oliveira, Rocha, Sulyok, Krska, & Mallmann, 2016; Queiroz et al., 2012; Van der Westhuizen et al., 2003).

The major mycotoxins found in barley are the trichothecenes type B group, primarily DON and its metabolites (Lancova et al., 2008). Trichothecenes are toxins of the sesquiterpenoid metabolism, produced by some species of *Fusarium* and other fungi in the order Hypocreales (Rocha et al., 2015). DON is a mycotoxin largely produced by the *Fusarium graminearum* species complex (FGSC) and this group is a devastating pathogen causing Fusarium head blight in wheat and barley. DON is considered a potent inhibitor of protein biosynthesis, exhibiting acute adverse effects in animals (Rubella, Goswami, & Kistler, 2004).

Considering that corn is one of the adjuncts used for brewing, detection of fumonisins B_1 and B_2 (FB_1 and FB_2) are expected in

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beer, as these are the most prevalent mycotoxins in this cereal and its processed products (Matumba et al., 2014; Oliveira et al., 2016). Fumonisins is an important mycotoxin group produced mainly by the *Fusarium fujikuroi* species complex (FFSC) (Rocha et al., 2016). *Fusarium verticillioides,* the major fumonisin-producing species within the FFSC, is able to produce levels above 5000 μg/kg as observed by many authors worldwide (Hinojo et al., 2006; Vismer, Marasas, & Schalkwyk, 2004).

FB_1 is known to cause toxicity in animals due to the inhibition of sphingolipid metabolism and cell cycle regulation, resulting in several adverse effects such as leukoencephalomalacia in horses and pulmonary oedema in swine (Desjardins, 2006). It is also associated with oesophageal cancer and neural tube birth defects in humans (Marasas et al., 2004).

Nowadays, there is a lack of regulation for toxin levels in beer, with maximum levels set only for raw materials (Brasil, 2011, 2013; EC No 1881/2006). However, the Scientific Committee for Food (SCF) and FAO/WHO Joint Expert Committee on Food Additives (JECFA) (Bolger et al., 2001) indicated a tolerable daily intake (TDI) of 1 $\mu g/kg^{-1}$ and 2 $\mu g/kg^{-1}$ bw for DON and FB_1, respectively.

For the reasons stated above and considering that the brewing industry in Brazil is continuing to rise, the objective of the study was to determine the influence of the mycotoxins DON and FB_1 on industrial beer quality from Brazil.

2. Materials and methods

2.1. Beer samples

A total of 114 industrial beer cans and bottles (Lager type) consisting of 20 different brands and different batches were randomly chosen to represent the main brewing industries of Brazil. These samples were acquired from markets between September 2015 and January 2016. None of the beers had surpassed their expiry date. Until the sample preparation, they were stored in the dark at room temperature.

2.2. Chemicals and reagents

DON and FB_1 standards were obtained from Sigma Aldrich Chemicals (St. Louis, MO, USA). The DON stock solution was prepared by dissolving 1 mg of DON in 1 ml of ethyl acetate and then dried. The standard curve solutions were prepared from appropriate dilutions of the stock solutions (200 μg/ml) with the mobile phase acetonitrile: water (10:90) in concentrations of 0.1, 0.3, 0.6, 1.25, 2.5, 5, 10 and 20 μg/ml. A stock solution of FB_1 was prepared by dissolving 1 mg of FB_1 in 1 ml of acetonitrile and then dried. The standard curve solutions were prepared from appropriate dilutions of the stock solution (50 μg/ml) with the mobile phase acetonitrile:water: acetic acid (520:480:5) in concentrations equal to 0.025, 0.05, 0.18, 0.37, 0.75, 1.5, 3 and 6 μg/ml.

For HPLC analyses, acetonitrile and methanol were obtained from J.T Baker (Sao Paulo, SP, Brazil) and all were LC grade. Sodium hydroxide was from Biotec (Pinhais, PR, Brazil) and the OPA reagent (0.04 g o-ftaldialdehyde in 1 ml methanol diluted with 5 ml 0.1 M borate buffer and 50 μl of 2-mercaptoethanol) was from Sigma Aldrich Chemicals (St. Louis, MO, USA). High-purity Milli-Q water (18.2 MU/cm) was obtained from the Millipore Synergy system (MA, USA).

2.3. Instruments and apparatus

The following instruments were required for analysis: high performance liquid chromatography (HPLC), Shimadzu (Kyoto, Japan), equipped with an isocratic pump, an SIL-20A autosampler, with an ultraviolet–visible (UV) detector model SPD M20 and a fluorescence detector model RF-10AXL. The chromatographic column used for DON was C18 reversed-phase (Synergi 4 μm particle size, with 250 × 4.60 mm, length and diameter, respectively), model Fusion-RP 80, Phenomenex (Torrance, USA). The chromatographic column used for FB_1 was C18 reversed-phase (particle size 5 μm, with 150 and 4.60 mm), model Luna (Torrance, USA).

2.4. Extraction procedure

To perform DON analysis, the Vicam protocol DON test N° G1005 (2013) with some modifications was used as follows: briefly 84 ml of Milli-Q water was added to 16 ml of a degassed beer sample and mixed for 10 min in the shaker at 150 rpm and then filtered through a filter paper.

For sample cleanup and concentration, an aliquot of 1 ml of the extract was applied to an immunoaffinity column (DON Test WB HPLC – Vicam) at a flow rate of one drop per second. The sample was followed by 2.5 ml of LC grade water to wash the column and the toxin was slowly eluted with 2 ml of 100% LC grade methanol. The eluate was evaporated using a heating block device at 40 °C in a gentle nitrogen stream.

FB_1 analysis was performed according to the Association of Official Analytical Chemists (AOAC) Official Methods of Analysis 995.15 (AOAC, 2005), originally developed for corn and its products, with some modifications. In short, the pH of craft beer samples was brought to a 5.8–6.5 range with 1 N NaOH and the samples were filtered through qualitative filter paper. For sample cleanup and concentration, a 50 ml aliquot of beer was applied to a strong anion exchange SPE column (6 cm^3, 500 mg^{-1}, SAX, Phenomenex, USA), previously conditioned with 10 ml of methanol, followed by 10 ml of methanol:water (3:1). The sample was followed by 10 ml of methanol:water (3:1) and 6 ml of methanol. FB_1 was eluted with 5 ml of methanol:acetic acid (95:5). The elution was dried in a heating block at 60 °C. The dried extract was suspended in 300 μl of acetonitrile:water (1:1) and then was cleaned with a syringe filter (0.45 mm, 13 mm, CA membrane).

2.5. HPLC analysis

The dry residue of DON was resuspended in 500 μl of mobile phase acetonitrile:water (10:90, v/v). The extract (70 μl) was injected into the LC/UV system set at a wavelength of 218 nm and the mobile phase was delivered at a constant flow rate of 1 ml/min.

For FB_1 analysis, 50 μl of the extract was transferred to a reaction vessel and 50 μl of OPA reagent was added. After 60 s of reaction time, 20 μl of the derived sample was injected into an LC-FLD at 335 and 440 nm for excitation and emission, respectively. The mobile phase was acetonitrile:water:acetic acid (520:480:5, v/v) at a flow rate of 1 ml/min.

3. Results

3.1. Validation methods

The samples used for validation were analyzed beforehand to ensure that they did not contain any of the studied compounds. Afterwards, blank samples were selected for spiking and recovery purposes. The spiking concentrations and their recoveries are shown in Table 1 and were carried out in triplicate. The linearity was established by the calibration curve with injections of different standard concentrations aforementioned (0.025–6 μg/ml for FB_1 and 0.1–20 μg/ml for DON). Each standard concentration was carried out in quintuplicate. The coefficient correlations were r2: