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Occurrence of biogenic amines in beers from Chilean market

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ABSTRACT

The presence of tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine was determined for the first time in beers commercialized in Chilean market. Dansylated amines were separated on a Zorbax-XDB C_{18} column using a binary mobile phase composed of acetonitrile and 20 mmol L^{-1} ammonium formate, pH 5.5. Chromatographic conditions were optimized using an experimental design, and validation was carried out following ICH recommendations. Calibration data fitted a linear regression model with $R^2 > 0.996$. Repeatability ($n = 6$) and intermediate precision ($n = 3$) in matrix showed RSD values lower than 5.00% and 5.21%, respectively. Recoveries at three different concentrations ranged from 75.50 to 96.48%. The proposed method was applied to determine the biogenic amines content in 101 beer samples produced by macro- ($n = 65$) and microbreweries ($n = 36$) using low ($n = 63$) and high fermentations ($n = 38$). Biogenic amines content in beer samples ranged from 0.53 to 85.04 mg L^{-1}, from which beers produced by microbreweries (19.13 ± 16.92 mg L^{-1}) showed a significant higher biogenic amines content ($P = 0.0021$) than beers produced by macrobreweries (9.65 ± 4.50 mg L^{-1}). Putrescine was the principal biogenic amine found in all kind of beers regardless origin, and the type of fermentation and brewery. Only 2 samples presented relevant levels of histamine and tyramine, but both below the limits reported by the European Food Safety Authority (EFSA). Thus, it can be concluded that beers commercialized in Chile are not a serious toxicological risk (e.g. hypertensive crisis) regarding the type and content of biogenic amines.

1. Introduction

Beer is the fifth most consumed beverage in the world (Sohrabvandi, Mortazavian, & Rezaei, 2011); being China the largest beer producer with over 500 millions of hectoliters produced during 2014, followed by USA with 225 million of hectoliters. In Latin America, Brazil and Mexico were ranked in the third and fifth position of the largest beer producers in 2014. Chile currently ranks in the 37th position of the world beer producers with 6.2 million of hectoliters (Barth-Haas Group, 2015). The principal Chilean brewery is *Compañia de Cervecerias Unidas (CCU)* located in the 23th position of the largest brewing groups in the world with 10.9 millions of hectoliters and 0.6% of world beer production (Barth-Haas Group, 2015). Regarding consumption, Czech Republic is the country with the highest consumption with 144 L per capita, followed by Germany and Austria, both with 108 L per capita. In Latin America the principal consumers are Venezuela, Brazil and Mexico with 75, 62 and 60 L per capita, respectively (Barth-Haas Group & Hansmaennel, 2013). In Chile, beer consumption has grown more than 60% in the last 10 years achieving 43 L per capita. Thus, beer has become the most consumed alcoholic beverage representing 64% of sales followed by wines with 28% and liquors with 6%. These figures demonstrate the importance of brewing industry for Chile. Therefore, it seems imperative to avoid or control the presence of deleterious compounds that could negatively affect the beers quality. Among these compounds biogenic amines (BA) have become a worldwide concern, where several efforts have been made to reduce its content in fermented food products (European Community, 2011). BA are well-known organic nitrogenous compounds, naturally synthetized in animals, plants and microorganisms. These amines are generally formed by decarboxylation of free amino acids or by amination or transamination of aldehydes and ketones. BA at low concentrations are relevant for several physiological functions, e.g. body temperature regulation and gastric acid secretion (Coton et al., 2010). Nevertheless, the intake of foods containing large amounts of BA may cause important adverse

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effects such as headache, hypotension or hypertension, cardiac palpitations, hot flushes and respiratory discomfort (Anli & Bayram, 2009). BA have been detected in numerous kinds of foods and beverages, e.g. cheese, fish, vegetable, meat, wine and beer (Bedia Erim, 2013; Önal, Tekkeli, & Önal, 2013; Spano et al., 2010). Its presence is generally related with food deterioration and contamination as well as with sanitary-type deficiencies (EFSA., 2011). In beers, the type and content of BA are explained by the contribution of two different sources. BA produced by microbial contamination, mainly bacterial, e.g. histamine, tyramine and cadaverine (Almeida, Fernandes, & Cunha, 2012) and BA present in raw materials (malted barleys, yeasts and hop) represented by spermidine, phenylethylamine, and spermine. Due to its source the latter are denominated "natural" (Kalač & Křížek, 2003). Putrescine has a dual origin because it can be contributed by raw materials as well as produced by bacterial contamination (Loret, Deloyer, & Dandrifosse, 2005). BA content in beer is also related with the type and conditions of fermentation (Gloria & Izquierdo-Pulido, 1999), where spontaneous fermentations showed the highest BA content (Loret et al., 2005). From a toxicological point of view, beers could also increase BA adverse effects due to the concomitant intake of ethanol, which reduce/inhibit the activity of monoamine oxidase and diamine oxidase, enzymes responsible of BA metabolism. Other compounds such as trimethylamine and acetaldehyde as well as the BA putrescine, cadaverine, spermine and spermidine could increase the histamine toxicity (Cardona-Gálvez & González-Domínguez, 2005). There are a small number of studies that evaluated the toxicological risk of BA presence in beers. Hypertensive crises have been reported after beer consumption in patients treated with drugs that inhibit monoamine oxidase enzyme (Tailor, Shulman, Walker, Moss, & Gardner, 1994). This adverse effect was observed with both draft and alcohol-free beers and it was ascribed to tyramine. Beers with tyramine content higher than 10 mg L^{-1} could be considered unsafe for this kind of patients; however, these levels were not dangerous for healthy consumers (Tailor et al., 1994). The determination of BA in beers is relevant for three main reasons, first, due to the toxicological risk that they could represent; second, because their possible usefulness as microbial spoilage indicator; and third, to evaluate the need of carrying out corrective actions that can reduce or prevent its presence, e.g. improving/modifying best practices guides (European Community, 2011). There has been considerable research about BA, several articles have reported its occurrence in beers from different countries, e.g. Greece (Loukou & Zotou, 2003b), Belgium (Loret et al., 2005), Spain (Cortacero-Ramirez, Arraez-Roman, Segura-Carretero, & Fernandez-Gutierrez, 2007), Turkey (Anli, Vural, Demiray, & Mert, 2006), Czech Republic (Bunka et al., 2012), Portugal (Fernandes, Judas, Oliveira, Ferreira, & Ferreira, 2001), Bulgaria (Lozanov, Petrov, & Mitev, 2004), Poland (Slomkowska & Ambroziak, 2002), China (Tang et al., 2009) and Korea (Choi, Lee, Shukla, & Kim, 2012). In South America only Brazil (Gloria & Izquierdo-Pulido, 1999) and Venezuela (Camacho et al., 2007) have reported the BA content in beers. To the best of our knowledge, the present work reports for the first time the occurrence of BA in beers commercialized in Chilean market.

2. Materials and methods

2.1. Regents and chemicals

Putrescine dihydrochloride (≥98%, Put), 2-phenylethylamine hydrochloride (≥98%, Phe), cadaverine dihydrochloride (≥99%, Cad), tryptamine hydrochloride (≥99%, Try), histamine dihydrochloride (≥99%, His), tyramine hydrochloride (≥98%, Tyr), spermidine trihydrochloride (≥98%, Spd), spermine tetrahydrochloride (Spm), 1,7 diaminoheptane (98%, IS, Dha), cross-linked polyvinylpolypyrrolidone (PVPP) and dansyl chloride (≥99%) were obtained from Sigma (St. Louis, MO, USA). Sodium carbonate anhydrous (>99.5%), sodium hydroxide (≥99%), sodium hydrogen carbonate (>99%), acetone (HPLC grade), acetonitrile (HPLC grade) and ammonia (25% v/v) were purchased from Merck (Darmstadt, Germany). 1 N hydrochloric acid (HCl) solution was obtained from Fischer Scientific (Fair Lawn, NJ, USA). Ultra-pure water (18.2 MΩ cm) was produced using a Simplicity system from Millipore (Bedford, MA, USA). Filter paper N°4 was obtained from Whatman (Clifton, NJ, USA) and Millex polyvinylidene difluoride (PVDF) 13 mm syringe filters (0.45 μm) were purchased from Millipore.

2.2. Standard and derivatization solutions

BA stock solutions were individually prepared in 0.1 N hydrochloric acid for a given concentration of 1 mg mL^{-1}. Pooled standard solutions containing all BA were prepared by aliquot dilution from stock solutions. All BA solutions stored refrigerated at 4 °C were stable at least for 20 days. 0.4 g L^{-1} of 1,7-diaminoheptane (internal standard) stock solution was also prepared in 0.1 N HCl. Sodium carbonate-bicarbonate buffer, pH 10.00, was prepared weekly. 10 mg mL^{-1} dansyl chloride solution (Dns-Cl) was prepared in acetone just prior to use.

2.3. Samples

A total of 101 beer samples were analyzed in the period 2013–2015. 99 samples were purchased directly from Chilean market (supermarket and specialized stores) and two samples were elaborated in our lab-scale microbrewery (25 L per batch). Considering its origin, samples were classified as Chilean produced ($n = 77$) and imported beers ($n = 24$), representing 50 brands. According to the kind of brewery, 36 samples were produced by microbreweries (<3000 L per week) and 65 by large-scale or macrobreweries. Regarding fermentation type, 44 Chilean produced and 19 imported beers were lager (low fermentation) and 33 Chilean produced and 5 imported beers were ale (high fermentation). All samples were codified, stored refrigerated at 4 °C and protected from light. Two independent samples of each beer sample were analyzed in duplicate ($n = 4$).

2.4. Sample preparation and derivatization

50 mL of beer sample were degassed in ultrasonic bath for 30 min at 20 °C. 50 μL (20 μg) of internal standard stock solution were added to 10 mL of degassed beer aliquot before the addition of 500 mg of PVPP. The mixture was shaken in a Boeco OS-20 orbital shaker for 15 min at 180 rpm and then filtered through filter paper N°4. Derivatization procedure was carried out following the method reported by Henríquez-Aedo et al. (Henríquez-Aedo, Vega, Prieto-Rodríguez, & Aranda, 2012) for BA determination in wines. Briefly, into a 1.5 mL micro-tube, 100 μL of filtrate (or standard) were dansylated adding 400 μL of carbonate-bicarbonate buffer, pH 10.0, 300 μL of acetone and 200 μL of Dns-Cl solution. The mixture was vortex-mixed during 30 s and then incubated for 60 min at 47 °C, afterward the remaining dansyl chloride was removed (consumed) by addition of 100 μL ammonia (25% v/v). After 30 min reaction (protected from light), the sample was filtered through a 13 mm PVDF syringe filter (0.45 μm) and injected into HPLC system.

2.5. Chromatography

BA analysis was performed using a Shimadzu (Kyoto, Japan)