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Analytical Methods

A UHPLC method for the simultaneous analysis of biogenic amines, amino acids and ammonium ions in beer

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ABSTRACT

This paper reports a novel UHPLC method for simultaneously quantifying nine biogenic amines, 21 amino acids, and ammonium ions, in beer. Precision values of standard curves slopes were lower than 3.4% and recovery was between 85% and 106%, indicating the absence of matrix effect. Linear calibration curves were obtained for analyte concentrations between two and four orders of magnitude ($R^2 > 0.996$). Repeatability tests returned mean variations of 3.2% and 0.5% for beer and a standard solution, respectively. Sensitivity ranged between 0.03 mg/L and 0.63 mg/L for the biogenic amines, and 0.05 mg/L and 5.19 mg/L for other compounds. Original data on the habitual presence of ethanolamine in beers are presented. The method allows for more samples to be assayed per unit time, it uses less solvent than other techniques and therefore reduces costs and the associated waste. It could be a valuable tool for monitoring the safety and quality of beers.

1. Introduction

Beer is one of the most consumed of all fermented beverages. Its nutritional value, aroma and flavour depend greatly on the proteolytic events that the raw materials (mainly barley and hops) undergo during malting and brewing, resulting in each beer having a particular amino acid composition (Bokulich & Bamforth, 2013; Gorinstein et al., 1999). The amino acid content of a beer also reveals whether wheat adjuncts were present during brewing (Dale, Young, & Brewer, 1989).

Certain amino acids can be decarboxylated by microbial enzymes, producing amino-containing compounds known as biogenic amines (BA) (reviewed in Kalac & Krízek, 2003). For instance, the decarboxylation of histidine renders histamine, while tyrosine, ornithine, lysine and phenylalanine can be decarboxylated to produce tyramine, putrescine, cadaverine and β-phenylethylamine, respectively (Kalac & Krízek, 2003; Linares, Martin, Ladero, Alvarez, & Fernandez, 2011; Lonvaud-Funel, 2001; Spano et al., 2010). High concentrations of BA in food are considered a biological hazard by international regulatory organizations (European Food Safety Authority [EFSA], 2011; Food & Agriculture Organization of the United Nations, 2014; Food and Drug Administration of the United States of America, 2011). Indeed, the ingestion of large quantities of BA can cause toxicological reactions leading to neurological problems, headaches, hypo- or hypertension, nausea, heart palpitations and kidney poisoning etc., with symptoms particularly severe in persons with suboptimal BA-detoxifying amine oxidase capacity (Caston, Eaton, Gheorghui, & Ware, 2002; EFSA Panel on Biological Hazards (BIOHAZ), 2011;

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Ladero, Calles-Enríquez, Fernández, & Alvarez, 2010; Shalaby, 1996). In addition, tyramine and histamine have recently been shown to exert considerable cytotoxic effects *in vitro* (Linares et al., 2016). An extra concern regarding the consumption of alcoholic beverages centres around a potential synergistic effect between ethanol/acetaldehyde and some BA causing the inhibition of detoxifying amino oxidases (Maynard & Schenker, 1962).

The type and concentration of BA in beer are affected largely by the raw materials and brewing techniques employed in the production process, plus the hygiene conditions maintained (Halász, Baráth, & Holzapfel, 1999; Kalac & Krízek, 2003). Different concentrations of histamine, tyramine, putrescine, cadaverine, β-phenylethylamine and tryptamine have been reported in different types of beer (Aflaki, Ghoulipour, Saemian, & Sheibani, 2014; Bunka et al., 2012; Halász et al., 1999; Izquierdo-Pulido, Albalá-Hurtado, Mariné-Font, & Vidal-Carou, 1996; Kalac, Hlavatá, & Krízek, 1997; Slomkowska & Ambroziak, 2002; Zee, Simard, & Desmarais, 1981). However, the presence of ethanolamine and ethylamine, which are known to appear in other alcoholic beverages (including wine; Galgano, Caruso, and Favati (2009)), has been little studied in beer.

The availability of rapid and validated methods for detecting BA in foods is essential if their concentrations are to be minimised (Alvarez & Moreno-Arribas, 2014; EFSA, 2011; Kalac & Krízek, 2003; Spano et al., 2010). Currently, high-performance liquid chromatography (HPLC) is the only reliable technique for monitoring BA concentrations in foods and beverages (EFSA Panel on Biological Hazards (BIOHAZ), 2011). The use of chromatographic columns with particles under 2 μm in diameter (so-called ultra-HPLC [UHPLC]) guarantees shorter elution times, greater sensitivity and improved peak resolution.

The simultaneous analysis of amino acids and BA is difficult given their different structures and the absence of a specific chromophore; a pre- or post-column derivatization step is therefore usually required. Among the derivatizing reagents used for the simultaneous analysis of these compounds in food matrices diethyl ethoxymethylenemalonate (DEEMM) has many advantages: its coupling with primary and secondary amino compounds, the good stability of the aminoenone derivatives produced, simplicity of use, and the absence of post-reaction by-products (Alaiz, Navarro, Girón, & Vioque, 1992; Gómez-Alonso, Hermosín-Gutiérrez, & García-Romero, 2007; Redruello et al., 2013; Wang, Ye, Zhu, Wu, & Duan, 2014). Further, it is the official derivatizing agent of the *Organisation International du Vin* (OIV, 2014).

A DEEMM derivatization procedure coupled to UHPLC-photodiode array detection (DAD) has been reported reliable for the simultaneous analysis of amino acids and BA in cheeses and red wines (Redruello et al., 2013; Wang et al., 2014). However, no reports exist on the use of this technique for analysing beers. The present work reports the development and validation of a UHPLC-based method for the simultaneous analysis of the aminoenone derivatives of the BA, amino acids and ammonium ions present in beer.

2. Materials and methods

2.1. Reagents and beer samples

HPLC-grade acetonitrile and sodium hydroxide were purchased from VWR (Barcelona, Spain), methanol and hydrochloric acid from Merck (Darmstadt, Germany), boric acid from USB (Cleveland, OH, USA), and ammonium acetate, sodium azide, DEEMM, L-2-aminoadipic acid (internal standard), amino acids, BA and ammonium chloride from Sigma-Aldrich (Madrid, Spain). All solutions were made with Milli-Q water. Eleven beers (lager or ale) made by different European brewers and produced using different techniques, were chosen for analysis. Four of them (two lager and two ale) were used during the matrix effect analysis (see Section 2.4.1 for samples description). All samples were purchased in retail stores in Spain.

2.2. Derivatization reaction

Containers of beer were left open for 2 h at room temperature to remove their carbon dioxide content. Samples of the decarbonated beers were then centrifuged at 8000 *g* for 5 min to eliminate any particulate matter. DEEMM derivatization reactions were performed as described in Redruello et al. (2013), using 100 μl samples (or of standard solution when constructing calibration curves). When necessary, samples were diluted with 0.1 N HCl. After derivatization, samples were filtered through 0.22 μm polytetrafluoroethylene (PTFE) membranes (VWR) into conical vials (VWR) prior to injection into the UHPLC system.

2.3. Equipment and chromatographic conditions

The chromatographic column and the UHPLC equipment used were those described in Redruello et al. (2013). The mobile phase consisted of 25 mM acetate buffer plus 0.02% sodium azide (eluent A; pH 6.7), methanol 100% (eluent B) and acetonitrile 100% (eluent C). Samples (1 μl) were applied to the column and eluted at a flow rate of 0.45 mL/min according to the ternary gradient shown in Table 1. The column was then returned to the initial conditions within 1 min, and allowed to equilibrate for 4.5 min before the next injection. Data were acquired and analysed using Empower 2 software (Waters). The target compounds were identified by their retention times compared to standards, and quantified using the internal standard method.

2.4. Method validation

2.4.1. Evaluation of matrix effect

The precision value (RSD, relative standard deviation) of standard curves slopes in five different lots of a biofluid was used to evaluate the existence of matrix effect (Matuszewski, 2006). This value should not exceed 4% for the method to be considered free from matrix effect (Matuszewski, 2006). Additionally, the RSD of the peak areas measured at all the spiked concentrations in the five lots of biofluids should not exceed 10–15% (Matuszewski, 2006). We used as five different biofluids four beer samples of different matrix complexity (an alcohol-free French lager, an artisan spanish lager, and two abbey-style dark belgian ale beers) and a 0.1 N HCl solution acting as solvent matrix. Mixtures of analytes at five different concentrations (15, 30, 60, 120 and 240 μM) were added (spiked) to each of the five matrices. The calibration curves obtained for each analyte in the five matrices were then calculated and their slopes compared. The RSD of the standard curves slopes as well as the RSD of the peak areas measured at all spiked concentration were then calculated.

Recovery of each analyte was calculated from the calibration data obtained as described above as [(area measured in the spiked sample) − (area measured in the non-spiked sample)/(area measured in the solvent 0.1 N HCl solution] × 100. Total recovery for each analyte was the average of the individual recoveries calculated from all the spiked concentrations and all the matrices.

2.4.2. Linearity, repeatability and sensitivity

Linearity, repeatability and sensitivity were validated according to Taverniers, De Loose, and Van Bockstaele (2004). Linearity was tested by regression analysis from three independent calibration curves that contained all the analytes (a mixture of amino acids,