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Biogenic amines in liqueurs: Influence of processing and composition

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ABSTRACT

With the aim to evaluate the presence of biogenic amines (BA) in liqueurs, a previously developed dispersive liquid–liquid microextraction (DLLME) gas chromatography-mass spectrometry (GC–MS) method was validated for a reliable and sensitive analysis of 18 BA from different chemical classes (aliphatic, heterocyclic and aromatic). The proposed method is based on an advanced procedure that allows simultaneous extraction and derivatization of all the amines in study through a simple and fast way. Very good figures for linearity (correlation coefficient >0.999), intra- and inter-day repeatability (maximum coefficient of variation of 10%) and recovery (79–108%) were obtained for all BA under study. Detection and quantification limits were lower than 4 μg/L and 10 μg/L, respectively. The validated method was used to screen the presence of BA in liqueurs from different origin and processing mode in a total of 27 samples, including 11 fruit liqueurs, 8 herbs liqueurs, 5 coffee liqueurs, 2 honey liqueurs, and 1 milk liqueur. The volatile amines methylamine, ethylamine, and dimethylamine, and the non-volatile amines morpholine, cadaverine, and histamine, were the BA more often detected. Although found in only 15 out of 27 samples, putrescine showed the higher mean content. Overall, coffee, honey and fruits liqueurs had significantly higher levels of BA ($p < 0.05$) than those found in milk and herb liqueurs. The variability observed between samples was influenced by the type of components as well as by the different modes of production (homemade or industrial). Indeed, homemade sample had significantly higher amounts of BA ($p < 0.05$) than industrial samples.

1. Introduction

Liqueurs are highly flavoured sweetened spirit beverages (15–30% alcohol; >10% sugar) typically consumed in small quantities. Already known in ancient Roman times, liqueurs were developed in the Middle Ages through experiments with distillation of herbs, fruits, and flowers, aiming to achieve recipes with medical properties (Buglass, 2012). Basically, a liqueur consists in a distilled spirit based on ethyl alcohol (e.g. cognac, rum, or whiskey) or a neutral distillate of agricultural origin flavored with fruit, cream, herbs, spices, flowers or nuts and bottled with added sugar, honey, or high-fructose corn syrup (Reg. 110/2008; Murphy, 2013). The secret of success of this drink is to develop formulations that combine harmoniously the aroma and flavor of vegetal products with alcohol and sugar, providing a product of great acceptance by the consumers. The high levels of alcohol and sugar avoid the use of chemical preservatives, which is an important advantage over other types of beverages.

Liqueurs are produced mainly by three basic means: i) heat or infusion method, which could be done in the presence of the alcohol base or merely in hot water being the distilled spirit subsequently added, often preferred when herbs, peels or roots are used, as heat can add to extract flavouring substances. ii) cold or maceration method, usually done in large oak casks in the presence of distilled alcohol, best when fruits and flowers are used, because heat could destroy some of the most favorable aromatic substances; iii) distillation, in which alcohol and flavouring agents are blended before being distilled (Murphy, 2013). Besides sugar, a few numbers of food additives are allowed to be added to alcoholic macerates: sweeteners such as stevioside or thaumatin or stabilizers such as calcium or trisodium citrate (Buglass, 2012). The quality of the final product depends crucially on the raw materials used (Gutiérrez et al., 1995).

Although liqueurs are not strictly fermented beverages, the alcohol used in their production is always obtained by fermentation and further distillation of crop products, so liqueurs may contain secondary products of fermentation, such as BA mainly

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originating from amino acids by the action of microorganisms. Moreover, some of the vegetal material used in the maceration process may have poor quality, and again BA may be leached to the final product at worrying levels.

BA are nitrogenous organic bases of low molecular weight of aliphatic (putrescine, cadaverine, spermine and spermidine), aromatic (tyramine, phenylethylamine) or heterocyclic (histamine, pyrrolidine) structure that are synthesized during cellular metabolic processes such as decarboxylation of amino acids and transamination of aldehydes and ketones (Smit et al., 2008; Mayer and Fiechter, 2013).

BA presence in food is a critical point on both safety and quality given their involvement in toxic incidents. Indeed, consumption of foods containing high levels of BA can cause rash, headache, nausea, hypo- or hypertension, and cardiac palpitations, or even more serious damages such as intracerebral hemorrhage and anaphylactic shock, especially if alcohols or monoamine oxidase inhibitors are simultaneously ingested (Maynard and Schenker, 1996; Medina et al., 1999; Sarkadi, 2009). In addition, the presence of BA in foods, especially histamine, is a typical consequence of the use of poor quality raw materials, microbial contamination and inappropriate conditions during storage and food processing. There are some evidences that as the hygienic quality of the product decreases, BA content increases (EFSA, 2011). Despite toxic and safety food evidence, no official limits have been set for BAs in liqueurs or other alcoholic beverages as wine or beers in the European Union (EU).

The screening of BA has been carried out by several authors in a variety of foods, including cheese (Pinho et al., 2004; Gosetti et al., 2007), fish (Al Bulushi et al., 2009) and meat (Ruiz-Capillas and Jiménez-Colemenero, 2004) as well as in beverages such as wines (Fernandes and Ferreira, 2000; Fernandes et al., 2001; Cunha et al., 2011) and beers (Almeida et al., 2012) homemade wines (Płotka-Wasylka et al., 2016). However, very few data have been published in beverage spirits such as liqueurs, whiskey, vodka, and brandy. The aim of this work was to assess the presence of BA in industrial and homemade liqueurs, and evaluate the influence of the different raw materials and modes of production in BA content. A total of twenty-seven liqueur samples commercialized in Portugal, including twelve from industrial production and fifteen homemade liqueurs, from 5 different types (fruits, herbs, coffee, honey and milk) were analysed. To achieve this purpose a previously developed method, based on DLLME with simultaneous derivatization using isobutyl chloroformate, followed by GC–MS analysis, was validated for this kind of matrix. As far as we known, there is no data concerning the BA content in liqueurs samples neither any application of DLLME in this kind of matrix, therefore, this study is intended as an enhancement of the knowledge about liqueur composition and analysis.

2. Experimental

2.1. Reagents and materials

BA standards (purity >98%) were obtained, mostly as hydrochloride salts, from Sigma (St Louis, MO, USA), Aldrich (Milwaukee, WI, USA), and Fluka (Buchs, Switzerland). Deuterated internal standards (IS), ethyl[$^2H_5$]amine·HCl, α,α,β·β-[$^2H_4$]histamine·2HCl, methyl[$^2H_3$]amine·HCl, 1,4-butane[$^2H_8$]diamine·2HCl (or [$^2H_8$]putrescine·2HCl), and 2,2,3,3,4,4,5,5[$^2H_8$]pyrrolidine were supplied by CDN isotopes (Québec, Canada) through Regie (Montlugon, France) all with purity >98%. The other IS, amphetamine and hydroxyamphetamine sulfate were from Sigma. Stock standard solutions (2.0 mg/mL) of each compound were prepared in 0.1 M HCl. Working standard solutions were prepared by dilution with 0.1 M HCl.

The derivatizing reagent isobutyl chloroformate (IBCF) was supplied by Sigma. DLLME extractive and dispersive solvents, toluene, acetonitrile (MeCN) and methanol (MeOH), respectively, were HPLC high purity grade from Fluka. HCl 0.1 M and NaOH 10 M were also obtained from Fluka. Other chemicals were of analytical grade. The solution of alkaline methanol was prepared by dissolving KOH (Prolabo – Leuven, Belgium) in methanol (Sigma-Aldrich), until saturation. Ultra-pure water used in the preparation of working solutions was obtained through a Milli-Q System (USA).

Ultrahigh purity He (helium) for GC–MS and N_2 (nitrogen) for solvent evaporation were obtained from Gasin (Maia, Portugal).

2.2. Sampling

A total of 27 samples comprising 11 fruit liqueurs, 8 herb liqueurs, 5 coffee liqueurs, 2 honey liqueurs, and 1 milk liqueur were obtained in local markets (12 samples) or acquired in farmer producers (homemade, 15 samples) (see supplement Table S1). All the samples were stored at room temperature (20 °C) and protected from light.

2.3. Optimized sample preparation

The whole sample preparation procedure is presented in Fig. 1. Briefly, five millilitres of sample were placed into a 25 mL vial spiked with all ISs (50 μL of an HCl 0.1 M solution containing methyl[$^2H_3$]amine, ethyl[$^2H_5$]amine, [$^2H_8$]putrescine, [$^2H_8$]

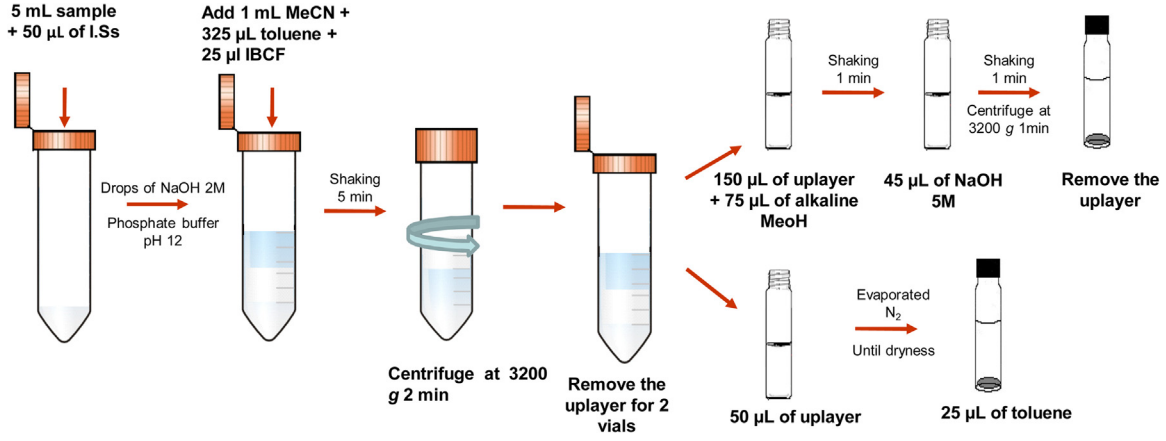

Fig. 1. Procedure of sample preparion by DLLME with simulatneous derivatization using IBCF.