Food Chemistry 274 (2019) 110–117

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

A simple dispersive solid phase extraction clean-up/concentration method for selective and sensitive quantification of biogenic amines in wines using benzoyl chloride derivatisation

Juliana Milheiro[a,1], Leonor C. Ferreira[a,1], Luís Filipe-Ribeiro[a], Fernanda Cosme[b], Fernando M. Nunes[a,*]

[a] Chemistry Research Centre – Vila Real (CQ-VR), Food and Wine Chemistry Lab, Chemistry Department, University of Trás-os-Montes and Alto Douro, School of Life Sciences and Environment, 5000-801 Vila Real, Portugal

[b] Chemistry Research Centre – Vila Real (CQ-VR), Food and Wine Chemistry Lab, Biology and Environment Department, University of Trás-os-Montes and Alto Douro, School of Life Sciences and Environment, 5000-801 Vila Real, Portugal

ARTICLE INFO

Keywords:
Wine
Biogenic amines
Dispersive solid phase extraction
Histamine
Benzoyl chloride
Derivatisation

ABSTRACT

A simple, quick, cheap and green dispersive solid phase extraction (dSPE) method followed by benzoyl chloride pre-column derivatisation for HPLC-UV determination of twelve biogenic amines (BAs) in wines is proposed for the first time. The dSPE using a strong cation exchange resin increased the selectivity and sensitivity of the analysis by elimination of interfering compounds and a five-fold enrichment of BAs. The method presented an adequate precision and linearity with detection limits ranging from 0.133 to 0.509 mg/L. Recoveries ranging from 72 to 99% prove the accuracy of the method for determining BAs in red, white and Tawny Port wine samples yielding chromatograms clean from interferents. The method was applied successfully to the analysis of 31 young red wines from different Portuguese wine regions. The dSPE method although has a potential of broader application to other food matrixes, other derivatisation procedures than benzoyl chloride and other detectors.

1. Introduction

Biogenic amines (BAs) are organic bases found in several foods like fish, chocolate, beer, wine and cheese (Onal, 2007). They can have aliphatic structure (putrescine, cadaverine, spermine and spermidine), aromatic (tyramine and phenylethylamine) and heterocyclic structure (histamine and tryptamine) (Kelly, Blaise, & Larroque, 2010; Onal, 2007; Smit, du Toit, & du Toit, 2008). In low concentration, BAs contribute to the normal physiological functions like regulation of body temperature, stomach pH or brain activity (Smit et al., 2008). Normal intakes of BAs are metabolised in the intestinal tract by the activities of monoamine oxidase (MAO; EC 1.4.3.4) and diamine oxidase (DAO; EC 1.4.3.6). However, the consumption of foods containing high amounts of BAs several toxicological effects may occur such as headaches, renal intoxication, nausea, hypotension, and hypertension and in severe situations intracerebral haemorrhage or even death (Hlabangana, Hernández-Cassou, & Hlabangana, 2006; Manetta et al., 2016). The toxicity depends on the effectivity of detoxification which varies in individuals. Alcohol, acetaldehyde and anti-depressive drugs can interfere with the amino oxidase enzymes activities (Brink, Damink, Joosten, & Huis in t'Veld, 1990). Also histamine metabolism can be inhibit by tyramine, putrescine and cadaverine by competing for binding sites in the gastrointestinal tract or by saturating the activity of mono- or diamine oxidases (Kanny et al., 2001). In wine, BAs can have three possible origins: they can be already present in the grape must, can be formed during alcoholic fermentation by yeasts or during malolactic fermentation by action of lactic acid bacteria that can decarboxylate amino acids present in the wine (Anli & Bayram, 2008). Their presence and levels are determined by the availability of free amino acids, presence of decarboxylase-positive microorganisms and

Abbreviations: AQC, 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate; BAs, biogenic amines; Bnz-Cl, benzoyl chloride; dSPE, dispersive solid phase extraction; Dabs-Cl, dabsyl chloride; Dns-Cl, dansyl chloride; HorRat, Horwitz ratio; LOD, detection limit; LOQ, quantification limit; NQS, 1,2-naphthoquinone-4-sulphonate; OPA, *o*-phthalaldehyde; $PRSD_{IR}$, predicted relative standard deviation of repeatability; $PRSD_R$, predicted relative standard deviation of intermediate repeatability; QC_{mean}, quality coefficient

* Corresponding author.
E-mail address: fnunes@utad.pt (F.M. Nunes).

[1] Both authors contributed equally to this work.

https://doi.org/10.1016/j.foodchem.2018.08.116
Received 22 April 2018; Accepted 24 August 2018
Available online 25 August 2018

conditions that allow bacterial growth, decarboxylase synthesis and activity (Anli & Bayram, 2008; Beneduce et al., 2010). The amount of biogenic amines present in wines will vary with the grape variety, soil composition and fertilisation, winemaking process, microbial populations and wine oenological treatments (Anli & Bayram, 2008). The total concentration of BAs in red wines has been reported in a range of few milligrams per litre to about 50 mg/L (Beneduce et al., 2010). Red wines are often reported as containing higher amounts of BAs than white wines due to their lower acidity (higher pH) and occurrence of malolactic fermentation that is much less common in white wines (Anli & Bayram, 2008). Besides the potential toxic effects, the presence of BAs in wines can have negative implications in the wine sensory characteristics; nevertheless this topic is still controversial (Lehtonen, 1996; Wankte et al., 2008). The quantification of BAs in food matrixes by liquid chromatography (LC), including wine, can be highly problematic as there are present simultaneously various BAs that have to be separated for individual analysis and they are present in low amounts. Moreover most of the BAs do not present good spectroscopic properties for being detected by the common LC UV and florescence detectors and therefore a derivatisation step is required. The derivatisation also increases BAs chromatographic performance by increasing their hydrophobicity as underivatised BAs are small polar molecules with low retention on LC reversed phase columns. Nevertheless, BAs derivatisation in wine occurs in a matrix containing a diversity of interfering substances that can consume the derivatisation reagent like amino acids, phenols and alcohols or change the derivatisation conditions like tartaric, malic and lactic acids that may lead to a significant drop in the reaction pH in comparison to pure standards that may hinder the quantitative formation of BAs derivatives (Hernández-Cassou & Saurina, 2011). Therefore highly selective and sensitive methods are needed for their accurate quantification, being more demanding when working with low volume of the samples where the absolute quantities of BAs in the total sample are low. Mass spectrometry detection has been used for biogenic amine analysis with high sensitivity and selectivity (Malec et al., 2017), nevertheless MS detectors heavily suffer from signal suppression and enhancement effects caused by the presence of undesired components that co-elute in the chromatographic separation and alter the ionisation process (Gosetti, Mazzuco, Zamperi, & Gennaro, 2010) corrected by using ^{13}C-labbeled internal standards for each compound. Besides the high cost of MS detectors when compared to UV-detectors the need of ^{13}C labelled internal standards increases further the cost of the analysis. Derivatisation with dansyl chloride (Dns-Cl) (Konakovsky et al., 2011; Manetta et al., 2016; Soufleros, Bouloumpasi, Zotou & Loukou, 2007; Zotou, Loukou, Soufleros & Stratis, 2003), dabsyl chloride (Dabs-Cl) (Hernández-Cassou & Saurina, 2011), benzoyl chloride (Bnz-Cl) (Özdestan & Üren, 2009), *o*-phthalaldehyde (OPA) (Busto, Miracle, Guasch & Borrull, 1997; Kelly et al., 2010), 1,2-naphthoquinone-4-sulphonate (NQS) (Hlabangana et al., 2006) or 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) (Hernández-Orte, Peña-Gallego, Ibarz, Cacho & Ferreira, 2006) have been used. The two most common derivatisation procedures used in wine BAs analysis employ the Dns-Cl and OPA. Although OPA reacts quickly with BAs, it only reacts with primary amines and gives unstable derivatives (Alberto, Arena, & Manca de Nadra, 2002). On the other hand Dns-Cl is light sensitive and has limited stability (Karovicová & Kohajdová, 2005). Benzoyl chloride offers some important advantages as BAs derivatisation reagent including short elution time, stability, yielding BAs derivatives not sensitive to light, being relatively inexpensive and easily accessible, reacting with both primary and secondary amines forming stable derivatives (Hornero-Méndez & Garrido-Fernández, 1997; Karovicová & Kohajdová, 2005). Redmond and Tseng (1979) were the first to use Bnz-Cl for derivatisation of BAs, and the derivatisation procedure was further improved concerning the time, temperature, phase mixture and solvents used by Asotra, Mladenov and Burke (1987), Hwang, Chang, Shiua and Chai, (1997), Hornero-Méndez and Garrido-Fernández (1997), Křížek and Pelikánová (1998) and Özdestan and Üren (2009). Nevertheless when applied to wines the procedure described by Özdestan and Üren (2009) using Bzn-Cl results in a number of intense peaks appearing on the chromatogram, a major drawback of using Bnz-Cl as derivatisation reagent for wine BAs determination and UV detection. Recently Malec et al. (2017) develop a LC-MS method for determination of 13 biogenic amines in wines after Bnz-Cl derivatisation with increased selectivity and sensitivity by using dynamic multiple reaction monitoring and ^{13}C-labbeled internal standards for each compound (by using ^{13}C labelled Bnz-Cl). For avoiding the interferences which can cause further problems with detection using less selective detectors, not an exclusive feature of the use of Bnz-Cl, and in order to increase the sensitivity and selectivity of the analysis in food matrixes, cleaning procedures have been used that include, liquid-liquid extraction (Lehtonen, 1986) and solid phase extraction (SPE) (Busto et al., 1997; Soufleros et al., 2007; Vazquez-Lasa, Iniguez-Crespo, Gonzalez-Larraina & Gonzalez-Guerrero, 1998), nevertheless these procedures increase the cost, time and complexity of the analysis. On the other hand the absence of clean-up procedures in wine and other matrixes increases the amount of substances injected into the column and in the case of MS detectors, reaching the MS source, decreasing column lifetime and increasing source clean-up frequencies. Dispersive solid phase extraction (dSPE) simplifies SPE clean-up, reducing the extraction/clean-up time, avoiding the conditioning step of SPE, flow rate optimisation toavoidchannelling, offering a more immediate and effective contact between phases. Also dSPE is suitable for the direct analysis of samples containing microparticles or microorganisms (a common situation in wines) which may block the cartridges and lead to extraction failures on conventional SPE, allowing more samples to be analysed simultaneously, is quite rapid, and requires low solvent consumption (Fontana, Camargo, Martinez & Altamirano, 2011). Therefore the aim of this work was to develop a simple dSPE clean-up/concentration procedure in order to remove the huge amounts of high concentrated interfering compounds present in wine matrixes, increasing the method selectivity and sensitivity, in order to use the advantageous Bnz-Cl derivatisation procedure for determination of BAs in wines using the most common UV–Vis detector. When combined with liquid-liquid extraction of the Bnz-Cl derivatisation mixture also allows to remove the interfering amino acids. Despite the large number of methods for BA analysis reported in the literature, some studies do not present validation results of the methods developed (Soufleros et al, 2007). The performance characteristics of the proposed method were validated for twelve biogenic amines ethylamine, propylamine, butylamine, putrescine, cadaverine, tryptamine, β-phenylethylamine, amylamine, spermidine, hexylamine, spermine and histamine in three wine matrixes, red, white and Tawny Port wine. As a practical application of the proposed method, the BAs content in thirty-one young red wines from different Portuguese wine regions was performed. The dSPE protocol developed in this work has great potential to be used for biogenic amines analysis in wines with any detector, fluorescence and MS, and for other derivatisation procedures as efficiently recovers biogenic amines and reduces/eliminates interfering compounds present in the sample for derivatisation. Furthermore no problem is anticipated in its use for other fermented beverages like beer and solid foods after efficient extraction of the biogenic amines from the solid matrix. To the best of our knowledge this is the first time that dSPE has been applied for the analysis of biogenic amines.

2. Experimental

2.1. Reagents and chemicals

High performance liquid chromatography (HPLC) grade methanol, methanol, Dowex® 50W X8, diethyl ether, hydrochloric acid, ethylamine, propylamine, butylamine, putrescine, cadaverine, tryptamine, β-phenylethylamine, amylamine, spermidine, hexylamine, spermine,