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Analysis of biogenic amines in wines by salting-out assisted liquid–liquid extraction and high-performance liquid chromatography with fluorimetric detection

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ABSTRACT

Biogenic amines are nitrogenous organic compounds of low molecular weight that are either formed or metabolized in cells of living organisms and can be found in several food products, being produced mainly by amino acid decarboxylation. When ingested in high concentrations they can induce several health problems in humans. In alcoholic beverages, and especially in wine, they are formed during the vinification process as a result of the action of microorganisms.

In this work it is proposed a new methodology for the determination of biogenic amines in wines, which includes a sample preparation approach based on salting-out assisted liquid–liquid extraction, the use of dansyl chloride for the derivatization and chromatographic separation by high-performance liquid chromatography with fluorimetric detection. The salting-out effect is used to promote phase separation between water and a water-miscible organic solvent, while improving the extraction of organic or inorganic species. Several extraction parameters were optimized, such as the dansyl chloride concentration, pH and the effects caused by the order in which the extraction and derivatization were performed. Extraction of amines, and consequent detection, depends on the presence of dansyl chloride in solution prior to extraction. The results showed the possibility to simultaneously perform the extraction and the derivatization, making sample preparation easier and less time-consuming. The methodology was successfully applied to the determination of biogenic amines in five wines (white, red and *rosé*). This method has the potential to be a good alternative to existing methods since it is cheaper, easier and simplifies the sample preparation step.

1. Introduction

Biogenic amines (BA) are a very important group of organic compounds that are commonly found in a wide variety of foods and beverages such as cheese, wine, beer, fishery products and meat [1–6]. They are generally formed by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones [7,8]. BA are commonly divided in three groups, according to their chemical structure: aliphatic (methylamine (MA), dimethylamine (DMA), ethylamine (EA), putrescine (PUT), cadaverine (CAD), isopentylamine (ISO), spermidine (SPD), spermine); aromatic (tyramine, phenylethylamine (PHE)) and heterocyclic (histamine (HIS), tryptamine). BA are food quality markers associated to the degree of degradation and fermentation in foods. These compounds can either be beneficial and harmful to human health, as in low concentrations they can be easily tolerated by the human body and actually help regulate several physiological functions; on the other hand at high concentrations they can induce neurological disorders, headaches, hypo- or hypertension, nausea, cardiac palpitations, renal intoxication among others [7,9,10].

In wine, BA are formed throughout the vinification process due to the action of various microorganisms during alcoholic and malolactic fermentations. The malolactic fermentation, which is catalyzed by the lactic acid bacteria (LAB), is considered one of the most important steps during winemaking and it is also when most BA are produced. This production is dependent on the presence of LAB, the availability of the precursor amino acids, the duration of the initial fermentation phase, the levels of sulfur dioxide, the pH and the period of contact between must and grape skin [11,12]. For those reasons, red wines have generally higher contents of BA than white wines. As for beer, the formation and presence of BA is related to the action of several yeast strains, LAB and the quality of the precursor raw materials [13]. The presence of BA in alcoholic beverages has received continuous attention since it was found that the interaction between ethanol and BA might be synergistic, as ethanol can directly or indirectly inhibit amine oxidases (monoamine oxidase) [14,15].

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The simultaneous and rapid determination of BA in wines requires inexpensive, reliable and simple methodologies. This is not an easy task, due to their low levels in complex matrices such as wine. In the literature we found several methodologies used for the determination of biogenic amines that are based on capillary electrophoresis (CE), gas chromatography (GC) and most commonly high-performance liquid chromatography (HPLC), in combination with several detectors [16–18]. For HPLC analysis using spectrophotometric detectors the determination of BA needs a derivatization because most BA lack of a chromophore. This derivatization, that occurs via amino groups with different tagging reagents, helps to improve selectivity and sensitivity of the methodology. The most common derivatizing reagents used are *o*-phthalaldehyde (OPA) [19,20], dansyl chloride (DNS-Cl) [21–23], phenyl isothiocyanate (PITC) [3], 4-chloro-3,5-dinitrobenzotrifluoride (CNBF) [24] and 1,2-naphthoquinone-4-sulfonate (NQS) [25]. DNS-Cl is probably the most used because derivatives are more stable than those obtained with other reagents and can react with primary and secondary amines [21] (Fig. 1).

Regardless of the derivatization reagent used, to obtain optimal analysis conditions, a clean-up or concentration step is required for the determination of biogenic amines in complex matrices, such as wine and beer. Many analytical methods reported in the literature, such as liquid–liquid extraction (LLE), include these steps, but usually involve the use of large quantities of hazardous solvents [26,27] while being a time-consuming step. To overcome this, other techniques like solid-phase extraction (SPE) [28], solid-phase microextraction (SPME) [29] or dispersive liquid–liquid microextraction (DLLME) [30] have been used.

In this work we propose a simple and quick methodology for the determination of nine BA found in wines, using a straightforward sample preparation procedure based on a salting-out assisted liquid–liquid extraction (SALLE) and derivatization with DNS-Cl for the analysis by high-performance liquid chromatography with fluorimetric detection (HPLC-FLD). This methodology uses the salting-out effect as the basis for a homogeneous liquid–liquid extraction, and besides promoting phase separation the addition of salt improves the extraction of molecular species to the organic phase. This methodology, that already proved to be valuable in the determination of α-dicarbonyl compounds [31], is a simple, quick and reliable way to determine BA in wine samples.

2. Materials and methods

2.1. Chemicals and samples

All the reagents used in this work, except when mentioned otherwise, were of analytical grade and were used without further purification. Ultrapure water with a resistivity not lower than 18.2 MΩ cm (Direct-Q® 3UV water purification system) was used for all chemical analyses and glassware washing. HPLC grade acetonitrile was from Fisher (USA). All chromatographic eluents were filtered through a Nylon filter of 0.45 μm pore size (Whatman, USA) prior to use. DNS-Cl and all BA were from Sigma-Aldrich (Steinheim, Germany).

Individual stock standard solutions (1 g L^{-1}) of each biogenic amine were prepared by diluting or dissolving the appropriate amount of the commercial reagent in high-purity water and stored at 4 °C. The working solutions were daily prepared by dilution of the stock solutions (1 g L^{-1}). Phosphate buffer 0.2 mol L^{-1} (pH 12) was prepared by dissolving the appropriate amount of disodium hydrogen phosphate in water, and the pH was adjusted with sodium hydroxide (4 mol L^{-1}); acetate buffer 0.01 mol L^{-1} (pH 4.0) was prepared with sodium acetate and acetic acid. These reagents were from Merck.

The DNS-Cl solution (3.5 mg mL^{-1}) was prepared by dissolving 80 mg of the reagent in 25 mL of acetonitrile; it was stored at 4 °C until use.

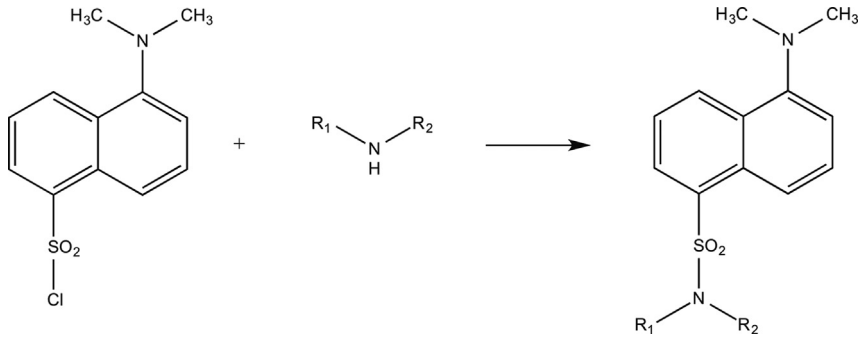

Fig. 1. Representation of the reaction between biogenic amines and dansyl chloride.

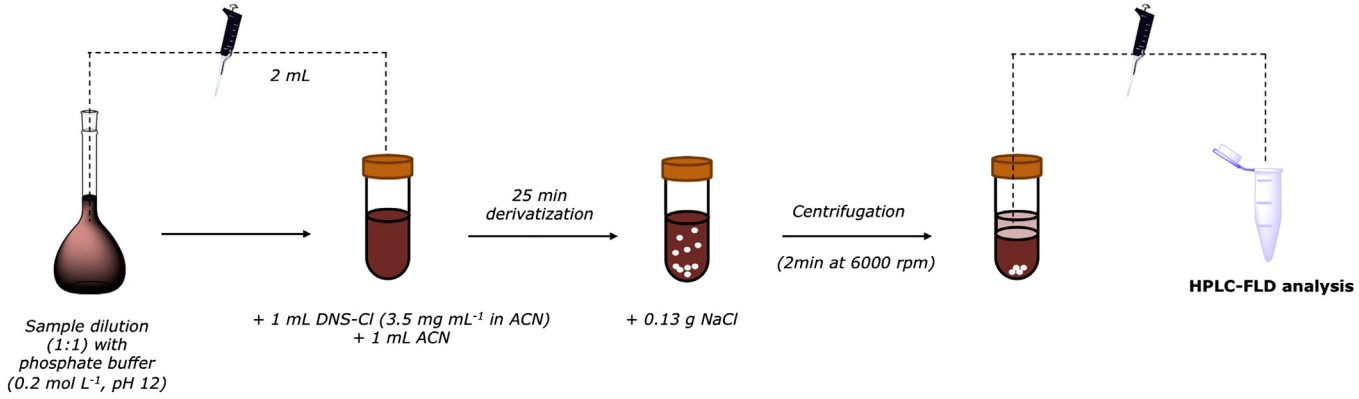

Fig. 2. Scheme of the experimental procedure proposed for the extraction of BA.