Journal of Chromatography A, 1217 (2010) 7385–7392

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Rapid automated high performance liquid chromatography method for simultaneous determination of amino acids and biogenic amines in wine, fruit and honey

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ARTICLE INFO

Article history:
Received 31 May 2010
Received in revised form 30 August 2010
Accepted 14 September 2010
Available online 22 September 2010

Keywords:
Amino acid
Biogenic amine
HPLC
o-Phthaldialdehyde
N-acetyl-L-cysteine
Fluorescence
Grape juice
Wine
Honey
Physalis

ABSTRACT

This paper reports a new, simple, rapid and economical method for routine determination of 24 amino acids and biogenic amines in grapes and wine. No sample clean-up is required and total run time including column re-equilibration is less than 40 min. Following automated in-loop automated pre-column derivatisation with an *o*-phthaldialdehyde, N-acetyl-L-cysteine reagent, compounds were separated on a 3 mm × 25 cm C_{18} column using a binary mobile phase. The method was validated in the range 0.25–10 mg/l; repeatability was less than 3% RSD and the intermediate precision ranged from 2 to 7% RSD. The method was shown to be linear by the 'lack of fit' test and the accuracy was between 97 and 101%. The LLOQ varied between 10 μg/l for aspartic and glutamic acids, ethanolamine and GABA, and 100 μg/l for tyrosine, phenylalanine, putrescine and cadaverine. The method was applied to grapes, white wine, red wine, honey and three species of *physalis* fruit. Grapes and *physalis* fruit were crushed, sieved, centrifuged and diluted 1/20 and 1/100, respectively, for analysis; wines and honeys were simply diluted 10-fold. It was shown using this method that the amino acid content of grapes was strongly correlated with berry volume, moderately correlated with sugar concentration and inversely correlated with total acidity.

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1. Introduction

Amino acids contribute to the nutritional value of several fresh foods and their importance in grape juice is that primary amino acids are a significant source of yeast nutrition. Microbial and enzymatic conversion of amino acids produces key aroma and flavour compounds in foods such as cheese, wine, honey and other fermented foodstuffs [1], and decarboxylation of amino acids by bacteria leads to the formation of physiologically active biogenic amines.

There is growing interest in the analysis of individual amino acids in grape juice due their pivotal role as precursors to aromas released during fermentation or ageing. For example, isoamyl, isobutyl and phenylethyl alcohols are derived from respectively leucine, isoleucine and valine [2]. The same authors also showed that threonine, phenylalanine and aspartic acid are the amino acids which most influence the fermentation process. A relationship was demonstrated between the amino acid profile in grape juice and the concentration of some important volatile compounds in wine [3]. It has also been shown [4] that the amino acids remaining in wine after fermentation have an influence on aromas during the maturing process.

It has recently been shown that adding ammonium salts to grape juice (to increase its fermentability) can reduce by up 30% the production of aromatic thiols such as 4-methyl-4-mercaptopentan-2-one (4MMP) from their precursors through the phenomenon of NCR—nitrogen catabolic repression [5]. For this reason, new research is beginning to focus on adapting vineyard practice (for example the judicious application of nitrogen fertilisation, irrigation or fertigation) in order to increase the concentration of amino acids in the grapes at harvest.

The demand by consumers for better and healthier foods has led to renewed interest in biogenic amines, given their importance for human health and food safety. The aliphatic polyamines, putrescine, cadaverine, spermine and spermidine, are pharmacologically active and reportedly toxic [6,7]. Putrescine and cadaverine play an important role in food poisoning as they can enhance the toxicity of histamine [8]. They play an essential part in tissue growth, and because of this, it has been suggested that they may be involved in the development of tumours [9,10]. Furthermore, putrescine and cadaverine can react with nitrite to form

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0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.09.047

heterocyclic nitrosamines which are carcinogenic [11]. Thus, the determination of biogenic amines in foods is of interest not only due to their possible toxicity, but also due to their role as potential indicators to determine the quality of freshness or spoilage of food products.

Current analytical methods for the determination of biogenic amines in foods have recently been reviewed [12]. High performance liquid chromatography is by far the most widely used technique for the determination of amino acids and biogenic amines in a diversity of matrices. More recently, liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) has been shown to be a very specific and sensitive technique for the determination of underivatized amino acids [1,13], but reported applications of these methods to food measurements are limited as LC–MS/MS instrumentation is expensive, requires a higher level of technical skill than GC–MS and is not available in many research laboratories.

In recent years, the original HPLC technique of ion-exchange chromatography followed post-column derivatisation with ninhydrin on a dedicated amino acid analyser has been largely supplanted by pre-column derivatisation, due to the flexibility of the technique and relative simplicity of the apparatus without the requirement for a dedicated instrument. A variety of derivatising reagents are available for pre-column derivatisation followed by HPLC with fluorescence detection, each with their advantages and drawbacks. For example, the advantage of fluorenylmethyl chloroformate (FMOC-Cl) [14], the use of which has been recently reviewed [15], is that it reacts with both primary and secondary amines, but it is troublesome in that requires quenching and even then produces a large reagent peak in the chromatogram. Dabsyl chloride also reacts with both primary and secondary amines, produces stable derivatives and allows for sensitive detection, however, the methods described for its application in the analysis of biogenic amines are rather complex, involving heating at 70 °C, intermediate mixing and cooling in an ice bath [16,17]. A relatively recent fluorescent derivatising reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) was first reported in 1993 [18] is available commercially from the Waters Corporation. It provides selective fluorescence detection of stable derivatives with no significant interference from the only major fluorescent reagent by-product, 6-aminoquinoline. This technique is growing in popularity, though it does suffer the drawback of requiring heating, and a reaction time of 15 min is essential for optimum results.

Since it was first described as a fluorigenic reagent by Roth in 1971 [19] OPA (*o*-phthaldialdehyde) has become arguably the most widely used derivatising agent in the chromatographic determination of primary amino acids and biogenic amines. The reaction takes place almost instantaneously at room temperature at alkaline pH in the presence of a thiol-containing reducing agent, the most commonly used of which is as 2-mercaptoethanol (MCE) [20]. However, the isoindole derivatives produced by OPA–MCE are unstable, and more bulky thiols such as N-acetyl-L-cysteine (NAC) or 3-mercaptopropionic acid (MPA), provide more stable derivatives [21]. The characteristics and stability of OPA–NAC-amine derivatives have been intensively investigated [21–26].

There are several publications on the simultaneous analysis of amino acids and biogenic amines, using OPA either alone [13,21–26] or combined with FMOC [27–30]. These methods provide varying degrees of sensitivity, selectivity and ease of execution, however, in general they involve long analysis times (>60 min) with flow-rates as high as 1.8 ml/min [28] resulting in high solvent consumption. Therefore, given growing awareness of environmental issues, the rising costs of organic solvents (especially acetonitrile) and of their disposal, the objective of this study was to develop and validate a rapid and economical method for routine analysis of several amino acids and biogenic amines common to wines, grapes and other food matrices without compromising on selectivity or sensitivity.

2. Materials and methods

2.1. Reagents

All chemicals and reagents were of analytical or HPLC grade or equivalent. Methanol, acetonitrile, acetic acid, sodium acetate, potassium chloride, boric acid, hydrochloric acid 0.1 M and sodium hydroxide 1 M were obtained from Carlo Erba (Carlo Erba Réactifs, BP 615, Val de Reuil, France). The 16 amino acids as their hydrochloride salts and the seven biogenic amines in addition to *o*-phthaldialdehyde (OPA) and N-acetyl-L-cysteine (NAC) were purchased from Sigma (Sigma–Aldrich Chimie, Lyon, France). Doubly distilled water was used to prepare solutions and for washing all consumable materials.

2.2. Analyte solutions

Stock solutions (approximately 4 g/l accurately weighed) of the analytes were made with 0.1 M HCl, except for tyrosine, which was prepared in 0.1 M NaOH. These solutions stored at −20 °C and were stable for several months. A stock mixture containing approximately 40 mg/l of the analytes was prepared in freshly distilled water on a weekly basis, however, it was stable at −20 °C for several weeks. Calibration standards of 0.25–0.5–1–2–5–10 mg/l were prepared on a daily basis by serial dilution of the stock mixture in freshly distilled water.

2.3. Samples

The method was applied to the determination of amino acids and biogenic amines in grape juice, wine, honey and physalis fruit. Frozen grapes were thawed at room temperature, crushed in a mortar and pestle, sieved to remove solid matter and then centrifuged at 7000 × *g*. Physalis fruit was homogenised in a domestic blender and then filtered to remove solid matter. The grape juice supernatant was diluted 20-fold with freshly distilled water and filtered using a 0.45 μm membrane. The physalis fruit filtrate was diluted 100-fold and also filtered. Wine and honey were diluted 1 in 10 (v/v) and (w/v), respectively with distilled water and filtered using a 0.45 μm membrane. Prepared samples were placed in the autosampler for in-loop derivatisation.

2.4. Derivatisation reagent

Fifty mg of *o*-phthaldialdehyde (OPA) were dissolved in 10 ml methanol and 400 mg N-acetyl-L-cysteine (NAC) were dissolved in 50 ml of a 0.2 M borate buffer adjusted to pH 9.5 with sodium hydroxide. The derivatisation reagent consisting of 2 ml NAC solution and 0.5 ml OPA solution was prepared on a daily basis and allowed to stabilise at room temperature for 90 min before use. The OPA solution was stable for >10 days but was prepared weekly and the NAC solution was prepared every 14 days.

2.5. Instrumentation and operating conditions

A Hewlett-Packard (Agilent Technologies Massy, France) 1100 series HPLC instrument was used, consisting of a model G1322A degasser, a G1312A binary pump, a model G1313A autosampler and a G1321A fluorescence detector set at excitation and emission wavelengths of 330 nm and 440 nm, respectively. Separations were carried out on a 250 mm × 3 mm Equisil® column (CIL, Bordeaux, France), protected by a 1 mm C18 SecurityGuard® cartridge supplied by Phnomenx (France). Mobile phase A consisted of 95%

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