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Impact of selected parameters of the fermentation process of wine and wine itself on the biogenic amines content: Evaluation by application of chemometric tools

Justyna Płotka-Wasylka[a,*,1], Vasil Simeonov[b,2], Calum Morrison[c,3], Jacek Namieśnik[a,1]

[a] Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology, Gdańsk, Poland
[b] Faculty of Chemistry and Pharmacy, University of Sofia, Sofia 1126, Bulgaria
[c] Forensic Medicine and Science, School of Medicine, Dentistry and Nursing, College of Medical, Veterinary and Life Sciences, University of Glasgow, United Kingdom

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ABSTRACT

The demand for safer foods has promoted more research into biogenic amines (BAs) over the past few years, however, there are still some questions that remain unanswered. Despite the fact that BAs are present in wine and can cause toxic effect to the body, a shared regulation limiting the amounts of BAs in wine is still lacking. A detailed understanding of their presence in wine is also important for the food trade sector. Therefore, the aim of this work was to determine the level of selected BAs in wine samples origin from Poland. Thereafter, the evaluation of correlation between concentration of BAs and selected parameters including pH, alcohol content and fermentation temperature by application of chemometric analysis was carried out. The BAs were determined by application of previously developed SPME-GC–MS methodology characterized by low detection limits ranged from 0.009 μg/L (tyramine) to 0.155 μg/L (histamine). Data obtained in this study show that none of the wine samples surpassed the toxic levels reported for BAs in the literature (the total BAs content was ranged from 7 to 2174 μg/L), therefore, these wines appear to be safe as regards the risk associated with the intake of potentially toxic BAs. Moreover, several correlations between occurrence, concentration of biogenic amines, important factors of winemaking process as well as physico-chemical parameters of wine were indicated. Even though information on BAs is currently not included in wine composition databases, information on their existence, distribution, concentration and knowledge of existing relationships between BAs and other wine parameters is crucial and may be useful for the food industry, health professionals and consumers.

1. Introduction

The occurrence of biogenic amines (BA) in wine is becoming increasingly important to both consumers and producers due to the potential threat of toxicity to humans and trade implications. Considering the fact that concentration levels of BA can increase (cadaverine, putrescine and tyramine), decrease (spermine and spermidine) or remain constant during the processing and storage of some food products (including wine) their amounts and ratios have been proposed as an index of the hygienic conditions of raw material and/or manufacturing practices [1]. Thus, BA have the potential to be used as indicators of food spoilage as well as authenticity [2].

Biogenic amines are naturally present in wine and it is very difficult, or even impossible, to obtain a wine that does not contain any biogenic amines [3]. The occurrence of BAs in wine may have many different sources: amino acid content at the initial and final phases of alcoholic fermentation, time of wine contact with yeast, but also the type and degree of ripeness of the grapes, the climate and soil of the viticulture area, and the vinification techniques can contribute to the biogenic amines content in wine. Additionally, biogenic amines can be produced during ageing or storage when wine is exposed to the activity of decarboxylase positive microorganisms [4].

The main BA's associated with wine include histamine, putrescine, tyramine and cadaverine, followed by 2-phenylethylamine, tryptamine, agmatine, spermidine, and spermine [5]. Some polyamines such as putrescine may be present in grape skin. They are mainly produced by grape vines in response to stress factors including salt, heat, and water deficiency. Putrescine and cadaverine are also normally associated with

* Corresponding author.
E-mail address: juswasyl@pg.edu.pl (J. Płotka-Wasylka).
[1] Current address: 11/12 Narutowicza Street, 80-233 Gdańsk, Poland.
[2] Current address: 1 James Bourchier Blvd., Sofia 1126, Bulgaria.
[3] Current address: Glasgow G12 8QQ, United Kingdom.

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poor sanitary conditions of grapes. The group of non-volatile BA (including histamine, putrescine, cadaverine, spermine, spermidine, agmatine, tyramine, tryptamine) and 2-phenylethylamine (a volatile amine) are formed mainly by microbial decarboxylation of corresponding amino acids [5]. Generally, lactic acid bacteria (LAB) can produce metabolic energy and/or increase their acid resistance by using catabolic pathways that convert amino acids into amine-containing compounds including BAs.

The main environmental factors which impact on the microbial activities in wine are temperature, concentration of salt and pH. The parameter which significantly correlates with putrescine, cadaverine and tyramine presence in wine is pH. Many studies correlate the formation of BAs with high values of pH in wine. In fact, BAs formulation influences the growth rate of the bacteria species which participate in the micro-biota of wines, and therefore their malolactic activity. A pH under 3.3 may cause a difficult malolactic fermentation, but a high pH can increase the susceptibility of the wine to microbial spoilage [6]. Some authors have established a critical pH level between 3.5 and 3.6, above which it is more difficult to control the microorganism population, with the possibility of problems arising due to the production of BAs [6].

Environmental factors can influence the formation of BA in two ways. First, these factors are responsible for the overall metabolism of the decarboxylating cells and second the activity of decarboxylases depends on the same parameters. In fact, the optimal values of environmental parameters for these two aspects can be different, thus the final amount of biogenic amines is the result of this double influence [7, 8].

On the other hand, if the environmental factors significantly impact on the rate and accumulation of biogenic amines in wine (and fermented foods) their modulation is limited by the conditions which allow fermentation and ripening processes and by health trends, as in the case of the reduction of NaCl content [7].

Special attention should be paid to some oenological practices frequently used to enhance wine complexity and increase the precursor amino acids concentration, such as the ageing of wines with lees or longer maceration times. Bacteria and yeasts lees can indirectly play an important role on the BA production, since they affect the amino acid composition during the alcoholic fermentation or during autolysis. Moreover, they can be a source of decarboxylase enzymes that could be involved in amines production [9]. In addition, the container type employed during malolactic fermentation (stainless steel or oak barrel) seems to affect the biogenic amine content of wines, suggesting that the components of wood, mainly phenolic compounds, may influence the production of BAs by LAB.

The influence of processing parameters such as grape composition and the treatment of wine has been analyzed, and there is general agreement on the importance of these factors in reducing the presence of BA in fermented beverages including wine. Knowledge of the metabolic pathways involved in BA production, but also the factors affecting BA accumulation in food may be useful in suggesting possible means of reducing BA contents. Finally, although biogenic amines occur in many different foods as well as beverages and their concentrations vary widely between and within food types, a shared regulation limiting the amounts of these compounds in foods and beverages is still lacking (except for histamine in fish and fish products) [9]. In fact, knowledge regarding their occurrence in foods and beverages is also very important for the food trade sector because recommended upper levels of content of biogenic amines vary between countries [10].

Therefore, the aim of this work was to determine the level of biogenic amines in wine samples origin from Poland. Moreover, the possible correlation between concentration of biogenic amines and selected parameters such as pH, alcohol content as well as fermentation temperature are evaluated by application of chemometric analysis.

Based on the results of literature studies, it can be argued that this work is the first attempt to find correlations between such a wide range of parameters that may contribute to the occurrence of given biogenic amines in wine samples at lower or higher concentration levels. Even though information on BA is currently not included in wine composition databases, information on their existence, distribution and concentration in wine is crucial and may be useful for the food industry, health professionals and consumers.

2. Materials and methods

2.1. Reagents and materials

All reference materials of biogenic amines: 1.7 diaminoheptene (internal standard, IS), cadaverine hydrochloride, histamine dihydrochloride, putrescine dihydrochloride, tryptamine hydrochloride, tyramine hydrochloride and 2-phenylethylamine hydrochloride were purchased from Sigma–Aldrich (St. Louis, MO, USA). Isobutyl chloroformate used as derivatization agent was obtained from Sigma-Aldrich. The ultrapure water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Stock solutions of amines and IS (both at 1 mg/mL) were prepared in the ultrapure water and stored + 4 °C. Working solutions were prepared daily by appropriately diluting stock solutions with water.

All SPME elements (SPME Fiber-Polyacrylate with 85 μm, SPME holder, manual holder and SPME manifold) were supplied by Supelco. After every injection, a -carry over- injection was applied until the interferences and ghost peaks disappeared completely, and low baseline noise was reached.

2.2. Samples

A total of 31 samples prepared from different grape varieties were obtained from Polish vineyards in different region of Poland. All the samples were stored at room temperature (21 °C) and protected from light. The original bottle of samples was opened in the analysis time.

2.3. Biogenic amines determination by application of solid phase microextraction

Each sample was diluted with the deionized water (1:2). 5 mL of pH 12 sample solution was immersed in screw top vials with phenolic cap and PTFE/silicon septa. Next, the 50 μL of isobutyl chloroformate was added to the solution together with sodium chloride (15% NaCl), and then the solution was stirred with a magnetic stirrer for 2 min. Thereafter, the extraction took place with immersing the SPME fiber into the solution for 40 min. All reactions were carried out at room temperature. After extraction, the fiber was carefully removed and inserted directly into the GC–MS system. Desorption time was 10 min. The schematic representation of this procedure is presented in Fig. 1a.

2.4. Equipment used

The GC 7890A (Agilent Technologies) system equipped with an electronically controlled split/splitless injection port was interfaced to a mass selective detector (5975C, Agilent Technologies) with electron impact ionization chamber. Chromatographic separation was achieved using a ZB-5MS capillary column (30 m × 0.25 mm I.D., 0.25 μm) obtained from Zebron Phenomenex. The injector temperature (splitless mode) and the interface were set at 250 °C. Sample injection volume was 2 μL. The oven temperature program was as follows: 100 °C min held for 1.2 min, increased to 160 °C at 10 °C/min, and finally ramped to 280 °C at 25 °C/min, and held for 12 min (total run time 25 min). Helium was used as the carrier gas at 1.0 mL/min. Spectra were obtained at 70 eV. For improved selectivity and sensitivity, the analysis was performed in Selected Ion Monitoring mode (SIM). The ionic fragments of BA together with the relative ion intensities are given in Table 1. The presence of fragments, relative ion intensities and retention times were considered as the valid identification criteria.