# 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,*], Vasil Simeonov [b], Calum Morrison[c], Jacek Namieśnik[a]

[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*

[a] Current Address: 11/12 Narutowicza Street, 80-233 Gdańsk, Poland
[b] Current Address: 1 James Bourchier Blvd., Sofia 1126, Bulgaria
[c] Current Address: Glasgow G12 8QQ, United Kingdom
*Corresponding author
E-mail: juswasyl@pg.edu.pl; plotkajustyna@gmail.com

## 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.

## Key words

Wine; biogenic amines; food analysis; correlations; SPME; cluster analysis

## 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 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 analysed, 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 choroformate 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 $^{o}$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.

**Table 1.** Fragments, relative intensities and retention times (Rt) of analytes characteristic for procedure of determination of BAs in wine samples based by application of gas chromatography-mass spectrometry technique

| **Analytes** | **Rt [min]** | **m/z SIM ions (intensity)** |
|---|---|---|
| 2-phenylethylamine | 9.992 | 130 (99.8), 104 (79.7), 91 (76.1), 221 (31), 148 (18.5) |
| 1,7-Diaminoheptane (I.S.) | 11.137 | 130 (99.8), 112 (42.3), 157 (38.7), 155 (31.2), 140 (27.0), 182 (26.3) |
| Putrescine | 11.981 | 170 (99.8), 130 (63.7), 288 (12) |
| Tryptamine | 13.109 | 130 (99.8), 143 (59.0), 260 (19.4), 187 (4.1) |
| Tyramine | 13.234 | 120 (99.8), 107 (27.5), 176 (4.9), 237 (2.0), 337 (1.6) |
| Cadaverine | 13.491 | 130 (80), 84 (81), 129 (72), 302 (12) |
| Histamine | 14.138 | 194 (99.9), 238 (16.9), 138 (25.6) |

### *2.5. Quality assurance*

The linearity of the method was determined by preparing 6 aqueous solutions containing all analytes at different concentrations ranging from 10 to 1000 μg/L. The correlation coefficient observed was ranged between 0.991-0.996. The precision of the analytical method calculated with the ratio between the area peaks of the sample spiked with a known concentration of BA and with the spiked water solution between 6 measurements in the lowest and in the highest concentration were obtained. To determine the recovery of procedure, the comparison of peak area obtained for unspiked wine samples and for spiked samples of wine. The intra-day precision was determined by analysing in the same day six replicates of wine samples spiked at 6 levels (10, 100, 250, 500, 750, 1000); each replicate was submitted to the overall developed method. The limits of detection (LOD) and limits of quantification (LOQ) were

175 calculated from spiked samples (*n*=4), as the minimum detectable amount of the target
176 compound with a signal to noise ratio of 3 and 10, respectively. Information on selected
177 validation parameters and recovery are presented in Table 2.

**Table 2.** Information on limits of quantification (LOQ [µg/L]) and limits of detection (LOD [µg/L]), average recoveries (%), and intra-day repeatability (% RSD) obtained with the application of SPME-GC-MS method in spiked wine samples, (*n* = 4).

| Analyte | Coefficient | LOD (µg/L) | LOQ (µg/L) | Concentration levels | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 10 µg/L | | 100 µg/L | | 250 µg/L | | 500 µg/L | | 750 µg/L | | 1000 µg/L | |
| | | | | Rec (%) | RSD% | Rec (%) | RSD% | Rec (%) | RSD% | Rec (%) | RSD% | Rec (%) | RSD% | Rec (%) | RSD% |
| **2-PE** | 0.993 | 0.031 | 0.102 | 85 | 3 | 93 | 7.3 | 81 | 1 | 97 | 2 | 98 | 3 | 102 | 2.7 |
| **PUT** | 0.993 | 0.025 | 0.081 | 77 | 7 | 71 | 7.4 | 79 | 10 | 96 | 8 | 98 | 11 | 103 | 0.5 |
| **CAD** | 0.994 | 0.125 | 0.414 | 70 | 1 | 67 | 8.0 | 77 | 5 | 75 | 3 | 73 | 2 | 77 | 6.3 |
| **TRYP** | 0.993 | 0.065 | 0.215 | 65 | 11 | 41 | 4.4 | 60 | 3 | 69 | 3 | 70 | 5 | 69 | 10.4 |
| **TYR** | 0.996 | 0.009 | 0.028 | 84 | 3 | 74 | 1.4 | 76 | 5 | 79 | 3 | 76 | 3 | 79 | 4.2 |
| **HIS** | 0.991 | 0.155 | 0.512 | 86 | 9 | 91 | 8.8 | 82 | 9 | 98 | 2 | 92 | 6 | 87 | 1.1 |

CAD, Cadaverine; HIS, Histamine; 2-PE, 2-phenylethylaminePUT, Putrescine; TRYP, Tryptamine; TYR, Tyramine

Rec, Recovery average

### *2.6. Chemometric analysis*

Cluster analysis (hierarchical and non-hierarchical clustering) is one of the most applied chemometric methods for multivariate data interpretation [11]. It is thoroughly described as an unsupervised pattern recognition approach (hierarchical clustering) or supervised method (non-hierarchical clustering) which makes it possible to reveal groups of similarity (clusters) within a large and generally diffuse data set. The cluster formation could be achieved with respect to the objects of interest (described by various parameters, features, variables) or with respect to the variables identifying the objects. In order to perform the hierarchical clustering procedure several steps are necessary – data standardization (in order to eliminate the role of variables dimension on the clustering), determination of the distances between the objects by some similarity measure equation (usually Euclidean distances), and linkage of the similar (close) objects in clusters (very often the Ward's method is preferred). The graphical output of the analysis is a tree-like diagram called dendrogram. Usually, statistical significance of the clusters has to be determined in order to better identify significant clusters. In the nonhierarchical clustering approach the members of the pre-defined clusters are automatically given as well as the average values of the variables for each cluster. In addition, principal components analysis (PCA) was also performed. PCA is a typical display method allowing reduction of the number of the input variables by introducing new coordinates of the system in consideration called latent factors or principal components. They are linear combinations of the old variables used in the such a way that the first principal component explains the biggest part of the total variance, the second – lesser part, the third – less that the second etc. The optimal number of the newly introduced latent factors is often determined by empirical rules, e.g. the introduction of new coordinates stops when a certain amount of total variance (e.g. 60 or 70 % of the total) is already explained. Very often cluster analysis and principal components analysis are parallel applied for verification of the results obtained. Missing data are replaced by the value LOD/2. The software package used was STATISTICA 8.0

## 3. Results and discussion

This work was intended to determine the biogenic amines in wine samples origin from Poland, made from different varieties of grape as well as to assess the possible correlation between the content of biogenic amines as well as parameters of fermentation process and wine itself by application of chemometric tools. All the parameters taking into consideration for this study are presented in Table 3. It can be seen that the wine considered in this study are different in terms of grape used for its production, fermentation temperature applied during fermentation process and container type used. Moreover, the alcohol content as well as pH were measured to characterize wine samples.

Table 3. Information on characteristic parameters of wine samples (color, alcohol level, pH, fermentation temperature, filtration during winemaking performed, container type employed during malolactic fermentation, year of production, biogenic amines content, total BA, content calculated as mean (*n*=4)). **For chemometric analysis concentrations of appropriate BA determined as <LOD are replaced by the value LOD/2.**

| Sample | Color | Year | Grape variety | Yeast | Fermentation temperature | Container type | Filtration (yes/no) | Alcohol [%] | pH | HIS (µg/L) | 2-PE (µg/L) | PUT (µg/L) | CAD (µg/L) | TRYP (µg/L) | TYR (µg/L) | Total BA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1W | W | 2016 | Hibernal | UCLM325 | 20 | OB | YES | 13.6 | 3.05 | 757±21 | 36.34±0.45 | <LOD (<0.025) | 68.19±0.97 | <LOD (<0.065) | 110.1±1.5 | 972 |
| 2W | W | 2016 | Solaris | UCLM325 | 22 | SS | YES | 12.9 | 3.09 | 416±13 | <LOD (<0.031) | 52.12±0.78 | <LOD (<0.125) | 134.0±1.3 | 23.09±0.16 | 625 |
| 1R | R | 2016 | Frontenac | MurvinB | 17 | OB | YES | 12.9 | 3.59 | 598±11 | 31.31±0.21 | 1148±29 | 397±10 | <LOD (<0.065) | <LOD (<0.009) | 2174 |
| 2R | R | 2016 | Regent, Rondo | Wild&Pur | 20 | OB | YES | 12.1 | 3.68 | 855±23 | 9.11±0.19 | 435±12 | 12.09±0.78 | <LOD (<0.065) | <LOD (<0.009) | 1311 |
| 3W | W | 2016 | Seyval Blanc | Lalvin71B | 18 | OB | YES | 9.5 | 3.04 | 194.1±5.4 | <LOD (<0.031) | 696±17 | 55.26±0.39 | 19.07±0.23 | 10.21±0.10 | 975 |
| 4W | W | 2016 | Seywal Blanc | Lalvin71B | 18 | SS | YES | 10.1 | 2.98 | 29.01±0.30 | <LOD (<0.031) | <LOD (<0.025) | <LOD (<0.125) | 20.00±0.12 | <LOD (<0.009) | 49 |
| 3R | R | 2016 | Rondo | Lalvin71B | 17 | SS | NO | 13.5 | 3.94 | 228.0±4.9 | <LOD (<0.031) | 312±10 | <LOD (<0.125) | 1.034±0.014 | <LOD (<0.009) | 541 |
| 4R | R | 2016 | Regent | Lalvin71B | 17 | OB | NO | 13.5 | 4.02 | 111.3±1.7 | <LOD (<0.031) | 198.2±6.8 | <LOD (<0.125) | <LOD (<0.065) | <LOD (<0.009) | 310 |
| 5W | W | 2016 | Bianca | CKS102 | 12 | SS | NO | 12 | 3.25 | 172.1±3.2 | <LOD (<0.031) | 260±10 | <LOD (<0.125) | 10.11±0.10 | <LOD (<0.009) | 442 |
| 6W | W | 2016 | Solaris | CKS102 | 12 | OB | NO | 17 | 3.43 | 128.0±2.0 | <LOD (<0.031) | 759±21 | 12.00±0.12 | 30.15±0.17 | <LOD (<0.009) | 929 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1Re | Re | 2016 | Regent, Rondo | Lalvin71b | 16.5 | OB | NO | 11 | 3.43 | 64.01±0.69 | <LOD (<0.031) | 859±19 | 48.11±0.19 | <LOD (<0.065) | <LOD (<0.009) | 971 |
| 7R | R | 2014 | Rondo | Lalvin71b | 17 | SS | YES | 12 | 3.62 | 257.1±2.1 | <LOD (<0.031) | 84.3±1.3 | <LOD (<0.125) | <LOD (<0.065) | <LOD (<0.009) | 342 |
| 8R | R | 2015 | Regent | Lalvin71b | 17 | SS | YES | 12 | 3.65 | 169.0±1.6 | 9.09±0.16 | 131.5±3.6 | <LOD (<0.125) | <LOD (<0.065 | <LOD (<0.009) | 310 |
| 2Re | Re | 2014 | Rondo Rose | Lalvin71b | 17 | OB | YES | 11.5 | 3.56 | 317.0±4.4 | <LOD (<0.031) | 457±10 | <LOD (<0.125) | <LOD (<0.065) | <LOD (<0.009) | 774 |
| 10W | W | 2015 | Bianca | Lalvin71b | 17 | SS | YES | 12.5 | 3.62 | 101.6±1.2 | 13.10±0.23 | 147.3±8.1 | <LOD (<0.125) | 2.001±0.012 | <LOD (<0.009) | 264 |
| 11W | W | 2015 | Hibernal | Lalvin71b | 17 | OB | YES | 12.5 | 3.1 | 802±24 | 192±10 | 242±6.7 | 85.09±0.99 | 12.02±0.15 | 54.24±0.24 | 1387 |
| 12W | W | 2012 | Hibernal | Lalvin71b | 17 | SS | YES | 17 | 3.31 | 258.5±3.6 | 48.28±0.66 | 68.09±0.98 | 138.1±1.7 | 38.21±0.32 | 432±10 | 983 |
| 13 W | W | 2016 | Hibernal | Lalvin71b | 17 | SS | NO | 23 | 3.82 | <LOD (<0.155) | <LOD (<0.031) | <LOD (<0.025) | <LOD (<0.125) | 7.002±0.032 | <LOD (<0.009) | 7 |
| 14W | W | 2015 | Hibernal | CK S102 | 12 | SS | NO | 13 | 3.88 | 415.0±3.5 | <LOD (<0.031) | 232±13 | <LOD (<0.125) | 4.100±0.020 | <LOD (<0.009) | 651 |
| 15W | W | 2015 | Jutrzenka | Lalvin71B | 17 | OB | YES | 10 | 2.99 | 100.0±1.4 | <LOD (<0.031) | 669±19 | 25.09±0.16 | 2.068±0.009 | <LOD (<0.009) | 796 |
| 16W | W | 2014 | Jutrzenka | Enartis Ferm Aroma White | 17 | SS | NO | 11 | 3.16 | <LOD (<0.155) | <LOD (<0.031) | 115.1±2.3 | <LOD (<0.125) | <LOD (<0.065) | <LOD (<0.009) | 115 |
| 17W | W | 2015 | Aurora, Bianca | Fermivin PDM, Bio L1 | 17 | SS | YES | 10 | 3.01 | 43.06±0.69 | 8.17±0.20 | 259±2.7 | <LOD (<0.125) | 1.110±0.009 | <LOD (<0.009) | 311 |
| 18W | W | 2016 | Aurora, Bianca | Oenoferm Inter Dry F3 | 16 | SS | YES | 12 | 3.19 | 159.0±1.4 | 9.09±0.18 | 239±3.1 | <LOD (<0.125) | 1.151±0.010 | <LOD (<0.009) | 408 |
| 19W | W | 2014 | La Crescent | ENOVI | 17 | OB | NO | 11.5 | 3.4 | 122.9±1.0 | 3.023±0.065 | 76.1±1.0 | 55.45±0.39 | <LOD | <LOD | 258 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | (<0.065) | (<0.009) | |
| 20W | W | 2015 | La Crescent, St. Pepin | Fermivin | 17 | SS | NO | 12 | 3.23 | 118.1±1.0 | 10.01±0.10 | 328±2.6 | 188.1±4.5 | 3.01±0.09 | <LOD (<0.009) | 647 |
| 23W | W | 2015 | Seywal Blanc | Enartis Ferm Aroma White | 17 | SS | YES | 13 | 2.94 | 130.1±1.8 | 27.41±0.25 | 164±1.4 | 37.31±0.21 | <LOD (<0.065) | <LOD (<0.009) | 359 |
| 24W | W | 2016 | La Crescent, St. Pepin | Oenoferm Color F3 | 17 | SS | YES | 16 | 3.37 | 131.2±1.8 | 14.09±0.20 | 167±1.5 | 83.0±1.1 | <LOD (<0.065) | <LOD (<0.009) | 395 |
| 10R | R | 2015 | Frontenac | Enartis Ferm Red Fruit | 17 | OB | NO | 13 | 3.37 | 1639±48 | 24.31±0.22 | 482±13 | 96.01±0.91 | 3.04±0.10 | <LOD (<0.009) | 2244 |
| 11R | R | 2016 | Frontenac | Oenoferm Color F3 | 17 | OB | NO | 13 | 3.62 | 873±24 | <LOD (<0.031) | 334±11 | 168.1±4.6 | 1.020±0.008 | <LOD (<0.009) | 1376 |
| 12R | R | 2015 | Regent | Aromatic Wine complex yeast est. 2005 Spititferm | 17 | OB | NO | 12 | 3.5 | 26.09±0.29 | 21.17±0.20 | 289.9±8.5 | <LOD (<0.125) | 3.21±0.11 | <LOD (<0.009) | 340 |
| 14R | R | 2016 | Heridian | Oenoferm Color F3 | 17 | SS | NO | 11 | 3.62 | 211.1±2.1 | 9.08±0.19 | 249.1±8.1 | 32.11±0.16 | <LOD (<0.065) | <LOD (<0.009) | 501 |

CAD, Cadaverine; HIS, Histamine; OB, oak barrel; 2-PE, 2-phenylethylamine; PUT, Putrescine; SS, stainless steel; TRYP, Tryptamine; TYR, Tyramine

### *3.1. Occurrence of biogenic amines in wine samples*

The information on BA content (µg/L) in wine samples calculated as a mean (n=4) is given in Table 4. The compounds of interest were effectively separated (Fig 1b). The biogenic amines were determined in all samples, however, the type of BA as well as the quantity depends on the sample analyzed. The BA that were present in most of analyzed samples are: histamine and putrescine. The relative concentrations of BAs (µg/L) followed the order: histamine > putrescine > cadaverine> 2-PE > tryptamine = tyramine. Tyramine only occurred in 5 samples.

Amongst the aromatic and heterocyclic biogenic amines, which exhibit negative effect after ingestion of high doses, histamine, 2-phenylethylamine, tyramine and tryptamine were found in the analyzed wines, histamine is described as the most toxic for human. This BA is the causative agent of physiological distresses experienced by some individuals following wine ingestion [12]. The symptoms commonly reported include intense headache, heart palpitation, low blood pressure, facial flushing, edema, rashes, thirst, nausea, swelling, diarrhea, and vomiting. Histamine was present in 28 samples, with levels ranging from 26.09±0.29 to 1639±48 µg/L. Compounds including 2-phenylethylamine, tyramine and tryptamine are associated with increasing blood pressure, and can cause migraines. Tryptamine was determined in 18 samples: 4 red wines and 14 white wines, with levels ranging from 1.020±0.008 to 3.21±0.11 µg/L and from 1.110±0.009 to 134.0 ±1.3 µg/L, respectively. Thus, it can be concluded, that tryptamine occurrence is mainly associated with white variety grapes.

2-phenylethylamine was found in 10 white (from 3.023±0.065 to 192±10 µg/L) and 6 red (from 9.08±0.19 to 31.31±0.21 µg/L) Polish wines. Tyramine was determined in 5 white wines (from 10.21±0.10 to 432±10 µg/L). It is worth noting that the toxic effects of this group of BA are potentiated in the presence of alcohol, acetaldehyde and other amines. Taking into consideration the toxic dose of BA in alcoholic beverages which varies between 8 and 20 mg/L for histamine, between 25 and 40 mg/L for tyramine, and 3 mg/L for phenethylamine [4], none of the examined sample exceeds toxic doses of these compounds.

Two other compounds considered in this study (putrescine and cadaverine) are associated with sanitary conditions. These compounds were also found in the analyzed samples, however, the level was different depending on the compounds. Putrescine was determined in 28 samples, with levels ranging from 52.12±0.78 to 1148±29 µg/L, while cadaverine was found only in 16 samples, with levels ranging from 12.00±0.12 to 188.1±4.5 µg/L.

The total amount of BA determined in wine samples varied widely among types of wines included in this study, with higher total levels for red wine numbered as sample R10 (2244 µg/L), followed by sample R1 (2174) and R11 (1376 µg/L), compared to white wines where the higher total levels are noted for samples W11 and W12 (1387 µg/L and 983 µg/L, respectively). The total level of biogenic amines in rose wines is from 774 µg/L to 971 µg/L. Putrescine and histamine were the amines that mainly contributed the most to total levels.

### *3.2. Correlations between content of BA and selected parameters of wine samples*

From an initial assessment of the obtained results it can be concluded that there is a correlation between wine age, variety of grape used for production, container type, and the content of particular BA in wine. Higher total amounts of BA are generally found in the younger wines (sample no 1R, 2R, 10R, 11R, 11W; 2015-2016 year) what was surprising. It is also noticeable that the content of BA is correlated with type of container employed during malolactic fermentation. And so, the higher concentration level of biogenic amines was mainly determined for wines kept in oak barrel (sample 1R, 2R, 10 R, 11R, 1W, 1Re) and the

average concentration of total BA was cc. 1000 µg/L. The wines kept in stainless steel were characterized by lower of total concentration of BA (cc. 200-500 µg/L).
Considering the total BA concentration in the analysed wines and variety of grape used for production it can be concluded that the highest total concentration of BA in red wines was noted for samples produced from the same variety of grape, namely Frontenac, while in the case of white wines, the highest total concentration of BA was assigned to wines originating from Hibernal variety of grape. Other correlations are not visible at first look, therefore, the chemometric analysis was performed.

### *3.3. Chemometric analysis*

In the present study an input data matrix consisting of 31 object explained by 9 variables (wines origin from Poland as objects and chemical compounds as descriptors) was interpreted by the use of hierarchical and non-hierarchical cluster analysis. Thereafter, PCA was carried out. The major goal of the study was to reveal patterns of similarity between the different wines and specific indicators (discriminating) responsible for speciation of the wines.
The input data were subject to normalization (z-transform). The hierarchical clustering was performed by the use of Euclidean distances as similarity measure (squared Euclidean distances) and Ward's method of linkage and K-mean mode was applied for non-hierarchical clustering.

#### *3.3.1. Hierarchical clustering results*

In Fig 2a the hierarchical dendrogram for linkage of 9 variables is shown.
Four clusters are formed as follows:

K1 (PUT CAD)
K2 (2-PE HIS)
K3 (TRYP TYR FT)
K4 (Alcohol pH)

This clustering is on level of cluster significance $1/3D_{max}$. For the significance level of $2/3D_{max}$ K1 and K2 are linked into one bigger cluster (PUT, CAD, 2-PE, HIS) and K3 and K4 remain as independent structures.
This way of clustering indicates similarity between organic chemical compounds, responsible for a toxic effect when consumed in high dose but also at good concentration level for "organic" flavor of the wines (one of the latent factors for taste – PUT, CAD, 2-PE, HIS). The second factor is related to alcoholic content and acidity of the wine (Alc, pH) and a third latent factor linked to fermentation temperature and the relatively low concentrations of TRYP and TYR, being function of the fermentation temperature, but also color of wine.
In Fig 2b the hierarchical dendrogram for clustering of 31 wine products is shown.
It can be assumed that 5 major clusters and one specific outlier are found. It could be stated that the red and white wines are, in general, clustered separately in two smaller clusters (1W, 2R, 11R,10R, 1R is the cluster with dominantly red wines) and (5W, 6W, 13W, 14 W is the group only of white wines). The other two clusters are bigger but, again, one of them consists of dominantly white wine types (3W, 15W, 1Re, 4 W, 16W, 17W, 18W, 23W, 20W) and the other is rather of mixed nature ( 3R, 4R, 7R, 8R, 10W, 14R, 19W, 2Re). Two of the wine (2W 12W) form an outlying cluster which differs significantly from the other four.

#### *3.3.2. Non-hierarchical clustering results*

Keeping in mind the results from the hierarchical clustering we have tried to achieve a more detailed classification expertise by applying non-hierarchical clustering mode (K-mean) with a priori selected number of clusters to be considered.

For variables non-hierarchical clustering, four numbers of clusters were aimed. The results confirm, in general, the outcome of the hierarchical approach.

K1 (HIS PUT CAD)
K2 (2-PE TYR)
K3 (Alc pH)
K4 (TRYP FT)

Two of the clusters are related to the content of the organic compounds determined and the other two – with the specific wine characteristics like acidity, alcoholic content and fermentation temperature.

The non-hierarchical clustering of the wine samples reveals six patterns of the classification as follows:

K1 (4W 5W 7R 2Re 8R 10W 16W 17W 18W 19W 20W 23W 24W 12R 14R)
K2 (3R 6W 13W 14W 4R)
K3 (1W 11W 12W)
K4 (3W 1Re 15W)
K5 (2W)
K6 (1R 2R 10R 11R)

Again, one reveals two small specific clusters for red and white wines (K6 and K3), two slightly mixed small clusters (K2 and K4), one outlier (2W) and a big mixed cluster (K1).

In order to interpret the results and select specific discriminating factors both for the groups of variables or for wine samples mean values of features for each cluster were compared. In Fig 3a the mean values of the four identified clusters of parameters (variables) are presented.

Fig 3a illustrates that 2-PE and TYR are almost constant (concentration in most of the cases close to detection limit) for all wine samples (cluster 2). Only 11W and 12 W indicate specificity to 2-PE and TYR with increased levels of phenylethylamine and tyramine. High levels of HIS, PUT and CAD (cluster 1 members) are typical for several red wines (1 R and 10 R). Several other red wines (3R, 4R) are sensitive to cluster 3 members – higher alcoholic content and acidity, the same holds true for the white wine type 13W. Wine sample 2 W differs from the rest of samples by enhanced fermentation temperature (member of cluster 4) and appears as specific outlier.

In Fig 3b the mean values for each identified cluster of wine samples are presented.

Cluster one being a mixed cluster of red, white and rose wines is characterized by almost equal means for all parameters and could be classified as a "baseline" wine pattern. No specific minima or maxima are observed. Cluster 2 is characterized by increased pH value (lower acidity) and is also of mixed origin – both white and red wine samples. Cluster 3 (only few white wine samples) differs from the rest of samples by higher levels of 2-phenylethylamine. The forth identified cluster (only three wine samples) is specific by increased putrescine level. The outlier 2W reveals a wine pattern with high fermentation temperature and maximal tryptamine level. Finally, cluster 6 (only red wines) shows specificity with respect to histamine and cadaverine content.

The results from PCA (Fig 4) confirms entirely the conclusions made from the cluster analysis – the grouping of the variables is the same as the linkage by hierarchical and non-hierarchical clustering. Three latent factors were identified. They explain over 60 % of the total variance of the system.

This chemometric expertise of the wine quality could be summarized as presented in Table 4.

Table 4. Summary of the chemometric expertise of the wine.

| Descriptors of wine quality | Wine patterns | Note |
|---|---|---|
| ***"Background"*** descriptor levels | 4W 5W 7R 2Re 8R 10W 16W 17W 18W 19W 20W 23W 24W 12R 14R | The alcohol content mainly ranged from 10 to 12 % |
| ***Low acidity*** level | 3R 6W 13W 14W 4R | No filtration was performed during production of wine.<br>The level of alcohol was mainly 13 or 13.5 %. |
| ***2-Phenylethylamine*** increase | 1W 11W 12W | Wine produced from HEBERNAL variety of grape.<br>Filtration was performed for all of wine.<br>Oak barrel employed for malolactic fermentation. |
| Putrescine increase | 3W 1Re 15W | Produced by using Lalvin 71B wine yeast.<br>The temperature of fermentation was ranged from 16.5 to 18 $^{o}$C. |
| High ***fermentation temperature*** and high ***tryptamine*** level | 2 W (outlier) | High temperature of fermentation. |
| High ***histamine*** and ***cadaverine*** levels | 1R 2R 10R 11R | Wine produced from FRONTENAC variety of grape.<br>Oak barrel employed for malolactic fermentation. |

## 4. Summary

The type of wine can be chosen depending on taste, aroma and beneficial health expectations. In this paper, the Polish regional wines were analysed in terms of selected biogenic amines as well as primary physico-chemical parameters (pH, alcohol level) to access not only the presence of selected BA but also to evaluate the correlation between the selected factors which can impact on the presence and content of biogenic amines.

Data obtained in this study show that none of the wine samples surpassed the toxic levels reported for BAs in the literature, therefore, these types of Polish wines seem to be safe as regards the risk associated with the intake of potentially toxic BA. Moreover, the obtained results allow determination of certain dependencies between the content of biogenic amines and selected factors of winemaking as well as physico-chemical parameters. The correlation between the age of wine, variety of grape used for production, container type, and the content of particular BA in wine was visible. Higher total amounts of BA are generally found in the younger wines what was surprising. Furthermore, the container type employed for malolactic fermentation also impact on total BA content. The higher concentration level of biogenic amines was mainly determined for wines kept in oak barrel than in those kept in stainless steel. These results were confirmed by chemometric analysis, which presented additional correlation, for instance that the filtration performed during winemaking process impact on the BA content in wine as well as on pH of wine. Moreover, the high temperature performed during fermentation process ($\geq$22$^{o}$C) affect high tryptamine level.

Even though information on biogenic amines is currently not included in wine composition databases, information on their existence, distribution, concentration and

knowledge of existing relationships between biogenic amines and other wine parameters is crucial and may be useful for the food industry, health professionals and consumers. Therefore, the obtained data in this study not only characterized wine samples origin from Poland, but also give some important information about parameters that can impact on occurrence of biogenic amines. The detailed information can be useful for the producers of wine not only on an industrial scale but also for personal use.

**Acknowledgements**

The research is funded by Faculty of Chemistry, Gdańsk University of Technology within the mini-grant project (Decision no 4914/E-359/M/2017).

The authors would like to thank Zodiak Vineyard, PrzyTalerzyku Vineyard, Kozielec Vineyard, Pod Orzechem Vienyard, Stok Vineyard, Spotkaniówka Vineyard, and Dwór Kombornia Vineyard for the samples of wine.

***Conflict of Interest:*** Justyna Płotka-Wasylka has received research grants from the Polish Ministry of Science and Higher Education and she declares no conflict of interest. Vasil Simeonov declares that he has no conflict of interest. Calum Morrison declares that he has no conflict of interest. Jacek Namieśnik declares that he has no conflict of interest.

**References**

[1] J. Stadnik, Z.J. Dolatowski, Biogenic amines in meat and fermented meat products, ACTA Scientiarum Polonorum Technologia Alimentaria, 9 (2010) 251- 263.

[2] J.L. Mietz, E. Karmas, Chemical quality index of canned tuna as determined by highpressure liquid chromatography, J. Food Sci. 42 (1997) 155-158.

[3] I.M. Ferreira, O. Pinho, Biogenic amines in Portuguese traditional foods and wines, J Food Prot. 69 (2006) 2293-2303.

[4] K.K. Ganić, L. Gracin, D. Komes, N. Ćurko, T. Lovrić, Changes of the content of biogenic amines during winemaking of Sauvignon wines. Food Sci. Technol. 1 (2009) 21-27.

[5] A.Y. Smit, W.J. du Toit, M. du Toit, Biogenic amines in wine: Understanding the headache. S. Afr. J. Enol. Vitic. 29 (2008) 109-127.

[6] G.E. Bousbouras, R.E. Kunkee, Effect of pH in malolactic fermentation in wine. Am. J. Enol. Vitic. 22 (1971) 121-126.

[7] M. Coton, A. Romano, G. Spano, K. Ziegler, C. Vetrana, D. Desmarais, A. Lonvaud-Funel, P. Lucas, E. Coton, Occurrence of biogenic amine-forming lactic acid bacteria in wine and cider. Food Microbiol. 27 (2010) 1078-1085.

[8] F. Gardini, Y. Özogul, G. Suzzi, G. Tabanelli, F. Özogul, Technological Factors Affecting Biogenic Amine Content in Foods: A Review. Fron. Microbiol. 7 (2016) 1218-1236.

[9] P.V. Pramateftaki, M. Metafa, S. Kallithraka, P. Lanaridis, Evolution of malolactic bacteria and biogenic amines during spontaneous malolactic fermentations in a Greek winery. Lett Appl Microbiol. 43 (2006) 155-160.

[10] G. Spano, P. Russo, A. Lonvaud-Funel, P. Lucas, H. Alexandre, C. Grandvalet, E. Coton, M. Coton, L. Barnavon, B. Bach, F. Rattray, A. Bunte, C. Magni, V. Ladero, M. Alvarez, M. Fernández, P. Lopez, P.F. de Palencia, A. Corbi, H. Trip, J.S. Lolkema, Biogenic amines in fermented foods. Eur. J. Clin. Nutr. 64 (2010) S95-S100.

[11] D.L. Massart, L. Kaufman, The Interpretation of Analytical Chemical Data By the Use of Cluster Analysis. Elsevier, Amsterdam, 1983.

[12] S.C. Souza, K.H. Theodoro, É.R. Souza, S.D. Motta, M.B.A. Glória, Bioactive amines in Brazilian wines: types, levels and correlation with physico-chemical parameters. Braz. Arch. Biol. Technol. 48 (2005) 53-62.