Article

A Survey of Biogenic Amines Profile in Opened Wine Bottles

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Abstract: 1) Background: A survey of biogenic amines profile in opened wine bottles has been established. Opened bottles of red and white wine were submitted to different temperature as well as different kind of stopper (screw cap, cork stopper) and use of vacuum devices. A total of six wine made from different variety of grapes were obtained from Polish vineyard places in different region of Poland; 2) Results: DLLME-GC-MS procedure for biogenic amines determination was validated and applied for wine samples analysis. The total content of BAs in white wines ranged from 442 µg/L to 929 µg/L, while in red wines ranged from 669 µg/L to 2244 µg/L the set of just opened wine samples. The most abundant biogenic amines in the six analysed wines were histamine and putrescine; 3) Conclusion: Considering the commercial availability of the analysed wines, there was no relationship between the presence of biogenic amines in a given wine and their availability on the market. However, it was observed that the different storage conditions employed in this experiment affect not only the biogenic amines profile, but also the pH. The results were confirmed by chemometric analysis.

Keywords: Biogenic amines; chemometric analysis; DLLME, GC-MS; storage conditions; stopper type

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1. Introduction

Biogenic amines (BAs), a compounds which are naturally synthesized in microorganisms, animals and plants, are generally considered as a food hazard. And although there is not a threshold for these biomolecules in the European legislation (except for histamine in fish and its products), many scientist are focused on them. This is mainly due to the fact that BAs can influence the important physiological processes in the organism, but the amount of necessary for physiological body functions are limited, thus, excess concentrations many often taken via food ingestion are reported to cause toxicological effects to the organisms [1]. Moreover, among the beneficial contribution of BAs, some are reported as important to the flavor and taste of food.

Biogenic amines are mainly produced by microbial decarboxylation of some amino acids, but also, volatile amines can be formed by amination and transamination of aldehydes and ketones [2]. Because BAs are stable compounds, and if they are formed it is difficult to eliminate them. The most popular health effect of BAs is known as food poisoning implicated with different type of food products, mainly fish but also meat, cheese, alcoholic beverages, etc. The most important biogenic amines occurring in foods and beverages are histamine, β-phenylethylamine, cadaverine, putrescine, tyramine, serotonine, tryptamine, spermine, and spermidine. Although, all of the mentioned BAs are of high importance when present in food, histamine is the main causative biogenic amine to induce food poisoning covering the majority of reported food poisoning cases [3]. Moreover, some of the other biogenic amines have been claimed to potentiate histamine food poisoning. In addition, BAs produced due to decomposition of food including cadaverine and putrescine, or during processing (e.g. tyramine) are reported to have the potential to cause illness, even the absence of histamine [3].

Due to the fact that alcohol is an inhibitor of monomine oxidase, the monitoring and control of BAs in fermented beverages including wine is considerably important for the health of consumers. In fact, the BAs content in wine could also impact on commercial import and export difficulties. Three possible origins of BAs in wine are reported [4]. BAs can be present in the must, can be produced by yeast during malolactic fermentation, or originate from the action of bacteria involved in malolactic fermentation. Besides, other factors may play an important role in the final concentration of BAs in wine. Thus, nitrogenous fertilization, geographic location of grape and its variety, climatic conditions during growth, or the level of maturation may cause changes in the amino acids profile in grapes [5]. In addition, the concentration of amino acids may be changed by different prefermentative treatments such as clarification, crushing or duration of maceration process [5]. On the other hand, it is also reported that conditions for the BAs formation are mostly related the factors affecting to the growth of microorganisms that have decarboxylating activity and to initialize the decarboxylating reaction of enzymes [3]. To these factors pH, temperature, oxygen content, salt and sugar contents can be also included. For example, it has been reported that decarboxylase activity of amino acids is stronger in an acidic environment [3]. Previously, the optimum pH for decarboxylating activity was suggested in a range of 2.5-6.5, but nowadays, it is limited to 3.5-5.5. It is also often reported that the quantitative production of biogenic amines is time/temperature dependent. Thus, the amine production rate usually increase with the increasing of temperature up to certain level while the production is minimum at low temperatures due to the inhibition of microbial growth and the reduction of enzyme activity [3]. It is reported, that optimum temperature for the BAs formation by mesophilic bacteria range between 20 °C to 37 °C, whereas the BAs production decrease above 40 °C and below 5 °C [6]. Due to the fact that BAs in wine origin from many sources, this alcoholic beverage has specifically been studied throughout its different stages of elaboration and storage. Therefore, the concentration of biogenic amines has been determined at different stages of wine production, starting from in grapes [7] and musts [8-10] throughout the alcoholic and malolactic fermentation [10-13], aging in barrels or tanks [14,15] and in a closed bottles [16-18]. However, reports focusing on the changes in BAs concentration in an opened wine bottle are scarce.

It is a popular problem that wine consumers many often drink wine several days after opening the wine bottle and sometimes they keep it at room temperature. Moreover, in the restaurant sector wine is also usually be kept in opened bottles. It seems important important to monitor the level of BAs in opened bottles with time and kept at different conditions. Therefore, this work is focused on evaluation of the profile of selected biogenic amines (histamine-HIS, cadaverine-CAD, putrescine-PUT, tyramine-TYR, tryptamine-TRYP and 2-phenylethylamine-2-PE) in opened bottles of wine kept at different storage conditions. Opened bottles of red and white wine were submitted to different temperature as well as different kind of stopper (screw cap, cork stopper) and use of vacuum devices. The studies were performed in order to ascertain if these conditions may change the original BA profile. 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 parameters is crucial and may be useful for the food industry, health professionals and consumers.

2. Materials and Methods

2.1. Reagents and standards

Chloroform, pyridine, isobutyl chloroformate (ICBF), and biogenic amine standards (histamine, cadaverine, putrescine, tyramine, tryptamine and 2-phenylethylamine) and internal standard (hexylamine) were obtained, mostly as hydrochloride salts, from Sigma Aldrich (Steinheim, Germany). Methanol (HPLC grade; purity ≥99.8%), 32 % hydrochloric acid, sodium hydroxide (purity 98–100.5%) were obtained from Fluka. Other chemicals were of analytical grade. The solution of alkaline methanol was prepared by dissolving KOH in methanol until saturation. Ultrapure water was obtained from a Milli–Q water purification system (Millipore, Bedford, MA, USA).

The amine standard solutions (1.0 mg/mL) were prepared individually by dissolving the pure compounds in deionized water. Concentrated solutions of amine standards were prepared by diluting the standard solution with water. The solutions were stored at 4 °C in silanized screw-capped vials with solid PTFE-lined caps (Supelco, Bellefonte, PA).

2.2. Samples

A total of 6 samples made from different variety of grapes were obtained from Polish vineyard places in different region of Poland. The wine samples were considered as follows: commercially not available white wine sample elaborated with 100 % Solaris grapes from West Pomeranian region (Poland), containing 12.9% (v/v) ethanol and pH 3.09; commercially available white wine sample elaborated with 100 % Solaris grapes from Kuyavian-Pomeranian region (Poland), containing 17% (v/v) ethanol and pH 3.43; commercially available white wine sample elaborated with 100 % Bianca grapes from Kuyavian-Pomeranian region (Poland), containing 12% (v/v) ethanol and pH 3.25; commercially available red wine sample elaborated with 100 % Regent grapes from Kuyavian-Pomeranian region (Poland), containing 13.5% (v/v) ethanol and pH 4.02; commercially not available red wine sample elaborated with 100 % Regent grapes from Masovian region (Poland), containing 12% (v/v) ethanol and pH 3.5; commercially not available red wine sample elaborated with 100 % Regent grapes from Masovian region (Poland), containing 12% (v/v) ethanol and pH 3.5; commercially not available red wine sample elaborated with 100 % Frontenac grapes from Masovian region (Poland), containing 13% (v/v) ethanol and pH 3.37.

A bottle of each wine was obtained directly from the manufacturer or the owner of the vineyard who produces the wine for his own use in accordance with the practice of wine-making.

Each of wine sample was analysed at the moment of opening and then was devided into six small bottles and subsequently stoppered. The variables selected for storage conditions were temperature and kind of stopper. Regarding temperature, wine bottles were maintained at room (22 °C) or refrigerated temperature (4 °C), while concerning the kind of stopper, three strategies were applied to stopper the bottles: a stopper cork, a stopper screw and a stopper which has a vacuum pump that extracts the air from the bottle (Vacu Vin). The samples were coded as A (Room temperature and cork stopper), B (Room temperature and screw stopper), C (Room temperature and vacuum), D (refrigeration temperature and cork stopper), E (refrigeration temperature and screw cork) and F (refrigeration temperature and vacuum).

An aliquot of 50 mL was taken from each bottle 0, 7 and 30 days after it was opened, and they were immediately frozen. Thirty days were set as the maximum reasonable time for an opened bottle to be consumed. This time was set due to the fact, that many people kept the opened bottles of wine for such a long time. The analysis of biogenic amines from each sample was carried out in duplicate.

2.3. Samples preparation

The procedure reported by Płotka-Wasylka [17] was applied to determine biogenic amines in wine samples. Five millilitres of sample were placed into a 25 mL screw cap plastic, spiked with IS (50 µL of an water solution containing the internal standard at 100 mg/L). A mixture of methanol (215 µL), piridine:HCl (1:1 v/v) and IBCF (60 µL) was rapidly injected into the sample tube. After 15 min, a 400 µL of chloroform was added and shaken by hand (1 min). 150 µL of bottom layer was taken for further analysis performed by GC-MS. The schematic diagram of the procedure is presented in Figure 1.

2.4. GC-MS analysis

The gas chromatograph 7890A (Agilent Technologies) equipped with an electronically controlled split/splitless injection port was interfaced to a inert mass selective detector (5975C, Agilent Technologies) with electron impact ionization chamber. GC separation was performed on ZB-5MS capillary column (30 m x 0.25 mm I.D., 0.25 μm film thickness) (Zebron Phenomenex). The injection was made in splitless mode (injection pressure 32 ps) at 230 ºC. Helium was the carrier gas with a constant pressure of 30 psi. The oven temperature program was as follows:50 ºC held for 1min, ramped to 280 ºC at 15 ºC/min and held for 9 min. Total run time was 25.3 min. The MS transfer line temperature was held at 280 ºC. Mass spectrometric parameters were set as follows: electron impact ionization with 70 eV energy; ion source temperature, 250 ºC. The MS system was routinely set in selective ion monitoring mode and each analyte was quantified based on peak area using one target and one or more qualifier ion(s) (Table 1). Agilent ChemStation was used for data collection and GC-MS control.

2.5. Quality assurance

The method linearity was determined by a regression analysis of the relative area (ratio between peak area of BAs to the peak area of the IS) versus the amine concentration. Thus, ten aqueous solutions containing all analytes with concentrations ranging from 0.05 to 1.0 mg/L and 1.0 to 10.0 mg/L were submitted to the whole analytical procedure. The results obtained showed that linearity were excellent for all the compounds with correlation coefficients (R^2) ranging from 0.9968 to 0.9989. The recovery was determined by comparing unspiked wine samples to spiked for two concentration levels (0.05 mg/L and 0.25 mg/L; n=4). The average recovery values ranged from 76 to 99 % as can be seen in Table 2. The intra-day precision was determined by analysing in the same day four replicates of wine samples spiked at two levels (0.05 mg/L and 0.25 mg/L); each replicate was submitted to the overall developed method. Inter-day precision was determined by analysis of samples on two different days over a period of three weeks. The relative standard deviation (RSD) for inter-day precision ranged from 5 % to 10 % and for intra-day precision ranged from 4 % to 12 % (Table 2). The limits of detection (LOD) and quantitation (LOQ) were calculated based on the ratio of 3.3 σ/S and 10 σ/S, respectively. Thus, σ is the standard deviation of the response, and S is the slope of the calibration curve. The LODs ranged from 1.4 to 4.2 μg/L and the LOQs ranged from 4.6 to 12.6 μg/L.

2.6. Chemometric analysis

Cluster analysis (hierarchical and non-hierarchical clustering) is one of the most applied chemometric methods for multivariate data interpretation [19]. Hierarchical cluster analysis is thoroughly described as a unsupervised pattern recognition approach since non-hierarchical clustering is a typical supervised method. Both approaches make 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 non-hierarchical clustering approach the members of the pre-defined clusters are automatically given as well as the average values of the variables for each cluster. Missing data are replaced by the value LOD/2. The software package used was STATISTICA 8.0

3. Results and discussion

The monitoring of profile of biogenic amines occurrence and its content was evaluated in opened wine bottles along time. Wine bottles were storage under different conditions in terms of temperature and kind of stopper. The monitoring of biogenic amines occurrence and its level was performed in just opened bottles, seven days after opening and thirty days after opening.

3.1. Biogenic amines profile in just opened bottles

Information on the concentration of BAs determined in the different wine samples by GC-MS technique are summarised in Table 3. Generally, red wines have higher amounts of biogenic amines than white wines[5,20,21], but what was surprising the total concentration of biogenic amines in the white wines originated from Solaris grapes was higher than those produced from Regent grapes (red wines). And so, the total content of BAs in white wines ranged from 442 μg/L to 929 μg/L, while in red wines ranged from 669 μg/L to 2244 μg/L the set of just opened wine samples.

In fact, red wines elaborated from Regent grapes has similar total content of BAs: 669 and 671 μg/L (both for commercially available and non-commercially available samples, respectively), while in those obtained from Frontenac grapes was about three times higher (2244 μg/L). White wines obtained from the same type of grapes (Solaris) had different total concentration of BAs.

The most abundant biogenic amines in the six analysed wines were histamine and putrescine, as expected (Table 3). However, in one of white wine sample elaborated from 100% Solaris grapes which is not commercially available, the concentration of tryptamine is two times higher than concentration of putrescine. The content of all biogenic amines in red wines obtained from Regent grapes was similar, so it can be concluded that they have similar profile of biogenic amines. The wine produced from Frontenac grapes had totally different characterization taking into account the biogenic amines content. However, tyramine compound was under limit of detection in all of red wine samples.

Considering white wine samples, there was none similarity in its characterization of BAs profile.

Considering the commercial availability of the analysed wines, there was no relationship between the presence of biogenic amines in a given wine and their availability on the market.

3.2. Effect of the storage time and conditions

Considering different storage conditions of opened wine bottles, slight changes were observed in the profile of biogenic amines and the pH (Table 3).

In the all red wines, the total amount of biogenic amines showed a significant trend to decrease along time when were storage at room temperature. When samples were maintained at 4 °C, the total of biogenic amines content also decreased, however, changes in concentration were small. The type of stopper impacted on the concentration of all biogenic amines. Those samples that were kept in cork stopper showed a significant trend to decrease along time, while those wines kept in vacuum did not show significant changes in the total concentrations of biogenic amines. Samples kept in screw cork showed also trend to decrease along time, but these changes were not as big as in case when cork stopper was used. In all red wines was the same trend in changes of concentration in appropriate biogenic amines. These trends were as follows:

- 2-PE increase along time, but higher differences were visible between 7 and 30 days after opening, especially when wines were kept at room temperature;
- putrescine, tryptamine and histamine decreased along time in all conditions, but these changes were significant in case of histamine and putrescine maintained at room temperature;
- cadaverine content slightly decrease from the opening day to the seventh day, and then increased significantly from the seventh to the tenth day in all cases (Table 3a,b,c).

The changes in concentration of biogenic amines maintained at 4 °C were so low that they do not affect the total concentrations.

Like red wines, white wine show a clear and significant trend in the total content of biogenic amines, however, this trend was differ considering the type of biogenic amines. Moreover, while in red wines the higher concentration of biogenic amines was noted for just opened bottles of wine, in white wines, higher total concentration of BAs was observed in samples after seven days after opening in all storage conditions. The content of putrescine and cadaverine slightly increased among time, and these changes were not significant in case of refrigerated samples and kept in vacuum. A significant increase in histamine concentrations from the opening day to seventh day was observed, while from seventh day to thirtieth day the concentration significantly decreased. The same trend was observed in case of 2-phenylethylamine and tyramine (in one sample, while in other tyramine was not detected), as opposed to red wines (Table 3). Tryptamine significantly increased among time in all conditions.

Due to the fact that changes in concentration level of BAs in white wine samples kept in 4 °C were smaller than those kept at room temperature, thus the total concentration of BAs was higher in these samples. The lower concentration of biogenic amines was found in sample maintained at room temperature in vacuum.
In general, the evolution of biogenic amines in the six analysed wines show a clear common trend. It should be pointed out that the concentration of only one compound namely putrescine decreased in all wine samples, no matter if it was red or white wine. The way of other biogenic amines concentration changing was differ in white and red wines (Figure 2). For example in the case of histamine concentration, it was decreased in red wines, while in white wines, it was increased in first days and decrease from 7th to 30th day.
Moreover, it was observed that the different storage conditions employed in this experiment affect not only the biogenic amines profile, but also the pH. In red wines, the pH was higher in wines kept at room temperature than those kept in 4 °C. Considering the type of stopper used at different temperatures, pH was also differ. And so, when screw stopper was used, the pH was higher than in case of cork stopper, but lower than vacuum was applied.
In white wines, wines did not show a clear common trend.

3.3.Chemometric assessment of biogenic amines impact in wines

Hierarchical and non-hierarchical cluster analysis was applied to a data set with different wines checked for presence of 6 specific organic compounds. The major goal of the study was to reveal latent relationships between the wine brands, the conditions for their storage and the amine content. Altogether 6 wine brands were studied (marked as A, B, C, D. E and F) for levels of 2-PE, PUT, CAD, TRYP, TYR, HIS and, additionally, time of opening the sample bottles (after 0, 7, and 30 days) which differs from one another by the type of stopper (cork, screw cap and stopper by vacuum pump). Temperature of storage (room temperature and 4^0 C) were checked in the experimentation.
This is a typical multivariate problem and, therefore, the chemical data were treated and interpreted by multivariate analysis.

3.3.1. Relationship between chemical variables

Hierarchical and non-hierarchical cluster analysis for all 6 wine brands was performed, each input set having dimensions [18x6]. As objects the different conditions applied to a specific brand (temperature of storage, type of stopper and time of opening) were involved and as variables – the concentrations of the 6 amines. It is important to note that in some cases not all 6 variables were used since some of the did not show any change with the variation of the experimental conditions and were actually not detected in the brand. It decreases the number of the variables used.
Clustering of chemical compounds (only for Wine A all 6 compounds were used as variable, for B, D, E, F – five variables were available and for Wine B – only four) is presented in Table 4.
The example of clustering is presented in Figure 3. The clustering for all wine samples is shown in Supplementary Materials (Figure 1SI - Figure 5SI).
It could be concluded that for all wines kept at 4^{0}C (refrigerator) the data structure is determined by two conditional factors: the one related to the correlation between 2-PE and CAD ("cadaverine factor") and the other related to the correlation between PUT, HIS and TRYP ("histamine factor"). TYR is not a significant variable. All these are red wines from REGENT and Frontenac grapes.
For wines kept at room temperature (white wines, SOLARIS and Bianca grapes) the first conditional factor related to wine quality is again "cadaverine factor" but correlated to putrescine; the second is "histamine" factor being correlated strongly to tryptamine. TYR and 2-PE are not significant variables.
The non-hierarchical clustering confirmed entirely the non-supervised hierarchical procedure.

3.3.2. Relationship between production and storage conditions for different wine brands

In order to understand the role of the biogenic amines as indicators for wine quality for different conditions of production and storage the same multivariate statistical analysis was applied to cluster the objects of the study.

The example of hierarchical dendrogram for wine sample (Wine A) presented in Figure 4. The hierarchical dendrogram for all wine samples is shown in Supplementary Materials (Figure 6SI - Figure 10SI).

The results of hierarchical clustering could be summarized as follows (Table 5).

The hierarchical classification is almost the same for each one of the brands studied. Cluster 1 includes all samples of bottles opened immediately, the second – those after 7 days of storage and the third – after 30 days of storage. It shows convincingly that the role of storage factor is substantial. It is important to note that samples C and F (for 7 and 30 days of storage) belong to cluster 1 along with the samples after immediate opening. This underlines the significance of the type of stopper as these samples are with stopper with vacuum pump. Once again the complete similarity of the brands D, E and F is confirmed.

In Figures 5, 6 and 7, the averages of each chemical variable for each of the identified clusters of wine samples are shown. The interpretation of the figures aims to reveal if some of the chemical compounds are specifically related to the groups of similarity, i.e. if specific markers could be found among the biogenic amines studied to control the wine quality.

For wine brands D – F which have absolutely one and the same classification pattern, cluster 1 (pattern 1 of immediately opened bottles) is indicated by high concentrations of CAD and 2-PE ; pattern 2 – (7 days of storage after opening) – by high levels of HIS, TRYP and PUT and pattern 3 (30 days of storage) – by lowest concentrations of all amines. Obviously, the wines lose their amine content after opening.

Very similar is the case with the other three wine brands. Wine B and C have very similar clustering with highest levels of TRYP and HIS for time after opening and lowest PUT and CAD. For the period after opening it was found that the concentrations of CAD and PUT increase and those of TRYP and HIS decrease. The impact of the other two chemical compounds (2-PE and TYR) is not significant.

Finally, wine A shows slightly different patterns as all 6 variables are significant. After opening cluster is characterized by highest levels of HIS, the 7 days after opening pattern – by highest levels of TRYP, TYR, 2-PE and HIS and the last cluster (30 days after opening) ; by high PUT levels.

5. Conclusions

It is a popular problem that wine consumers many often drink wine several days after opening the bottle of this alcohol and sometimes they keep it in room temperature. Moreover, in the restaurant sector wine is also usually be kept in opened bottles, thus it should be important to monitor the level of BAs in opened bottles along time and kept in different conditions. Therefore, the monitoring of BAs in wine should be of high importance. This work is focused on evaluation of the profile of selected biogenic amines in opened bottles of wine kept at different storage conditions. Summarizing the data obtained in this study following issues could be conclude:

- slight changes were observed in the profile of biogenic amines and the pH;
- the type of stopper impacted on the concentration of all biogenic amines;
- in all red wines was the same trend in changes of concentration in appropriate biogenic amines;
- white wine show a clear and significant trend in the total content of biogenic amines, however, this trend was differ considering the type of biogenic amines;
- the concentration of only one compound namely putrescine decreased in all wine samples, no matter if it was red or white wine;
- chemometric analysis confirmed that the samples were grouped according to their storage time and the storage conditions.

In general, these results suggest that the concentrations of biogenic amines in opened wine bottles suffered slight changes during storage. Thus, further analysis of chemical stability together with microbiology research are recommended to determinate which factors affect mainly in the evolution of biogenic amines during storage.

Supplementary Materials: The following are available online at www.mdpi.com/link, Figure 1 SI. Variable clustering for Wine B, Figure 2 SI. Variable clustering for Wine C, Figure 3 SI. Variable clustering for Wine D, Figure 4 SI. Variable clustering for Wine E, Figure 5 SI. Variable clustering for Wine F, Figure 6 SI. Hierarchical dendrogram for wine samples (Wine B), Figure 7 SI. Hierarchical dendrogram for wine samples (Wine C), Figure 8 SI. Hierarchical dendrogram for wine samples (Wine D), Figure 9 SI. Hierarchical dendrogram for wine samples (Wine E), Figure 10 SI. Hierarchical dendrogram for wine samples (Wine F).

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Author Contributions: J. Płotka-Wasylka conceived and designed the experiments, analyzed the data and wrote the paper; V. Simeonov performed the chemometric analysis and wrote the paper; J. Namieśnik had substantive supervision.

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Appendix A

Supplementary informations.

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Figures:

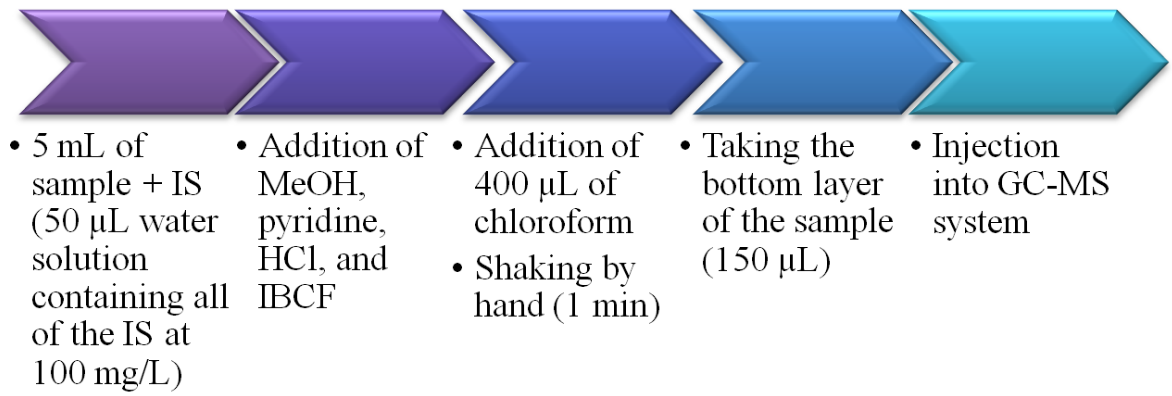

Figure 1. Schematic representation of DLLME-GC-MS procedure applied for biogenic amines determination in wine samples.

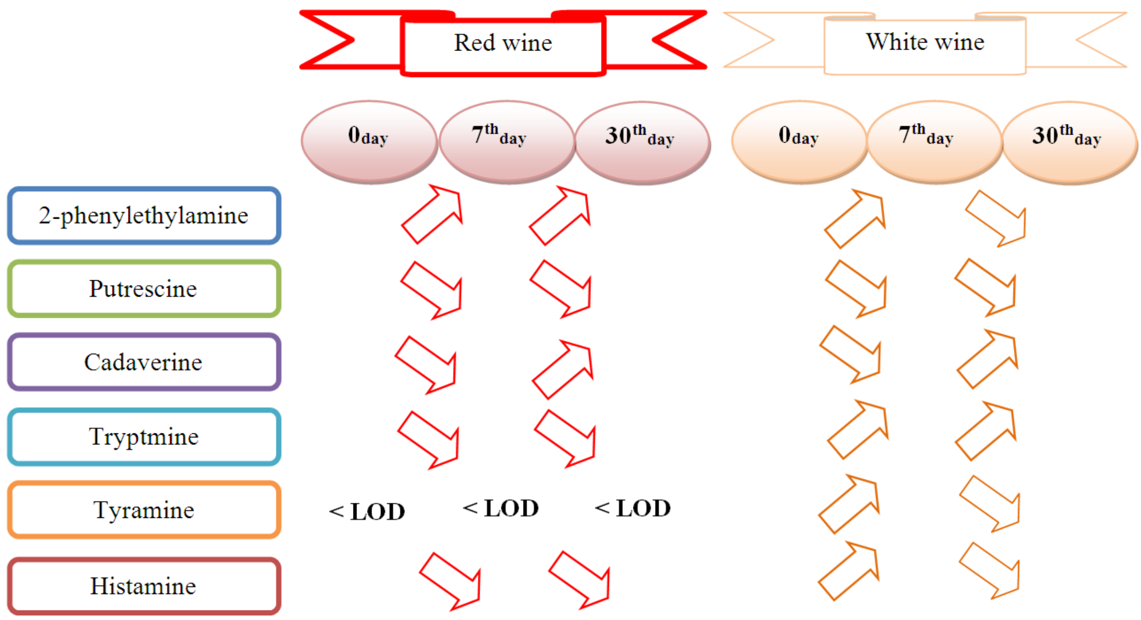

Figure 2. Schematic representation of the way of biogenic amines concentration changing along time.

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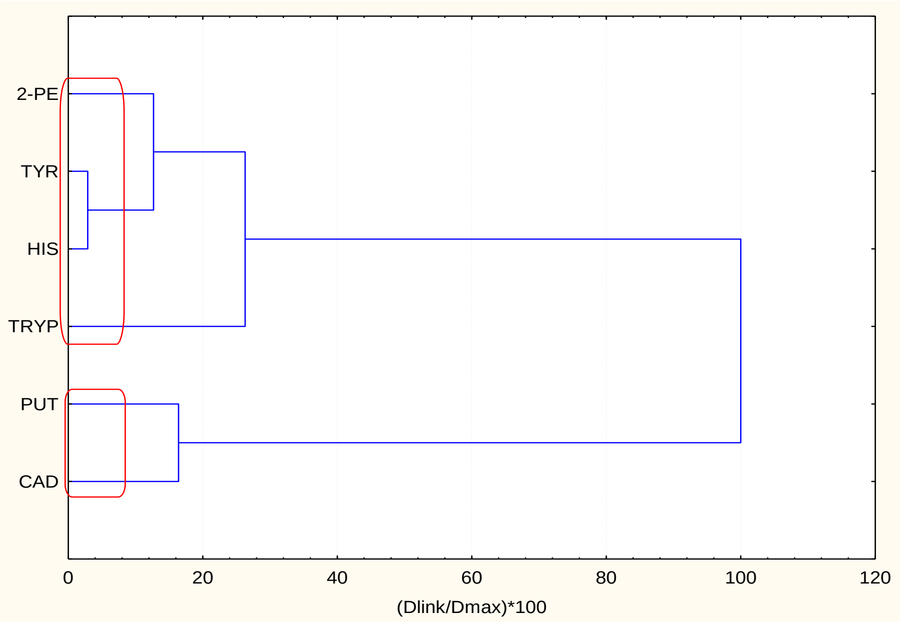

Figure 3. Variable clustering for Wine A.

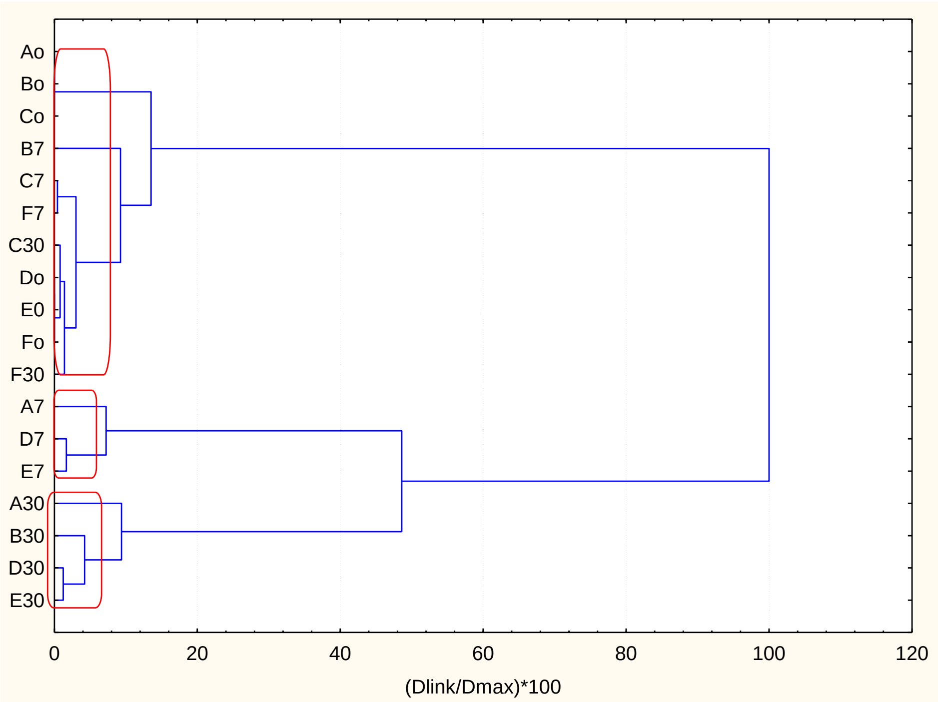

Figure 4. Hierarchical dendrogram for wine samples (Wine A)

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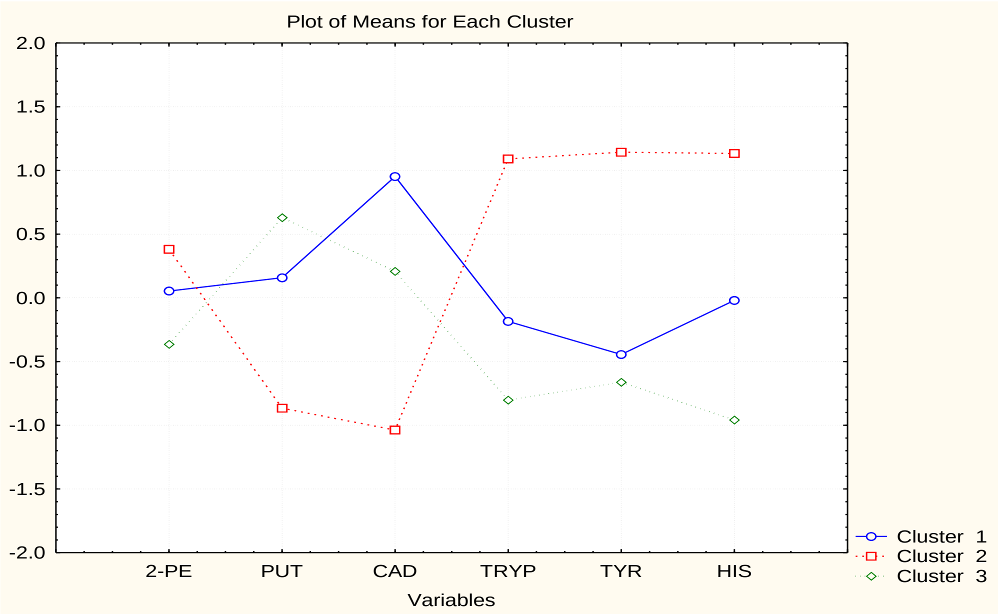

Figure 5. Averages of variables for each cluster (Wine A)

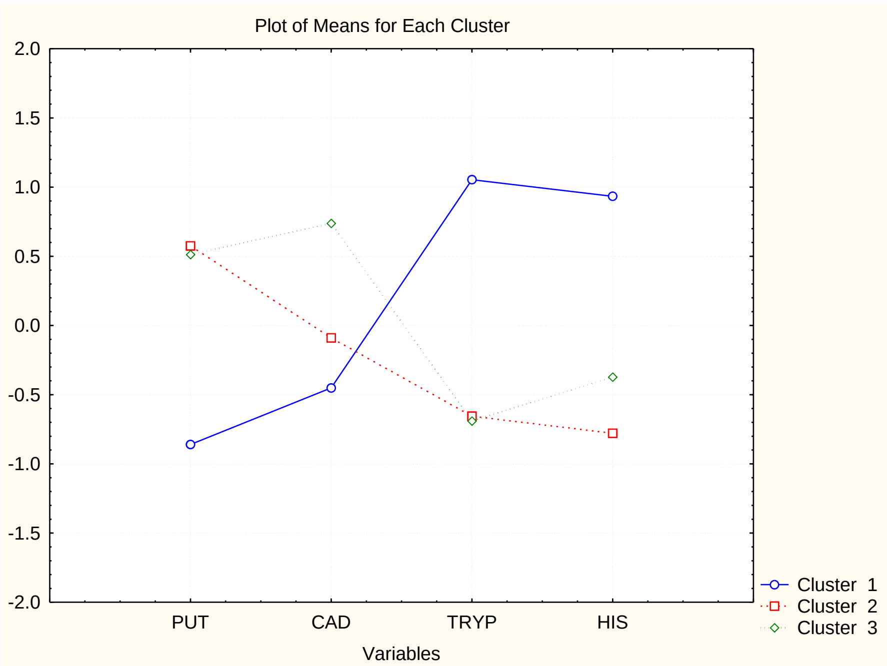

Figure 6. Averages of variables for each cluster (Wine B)

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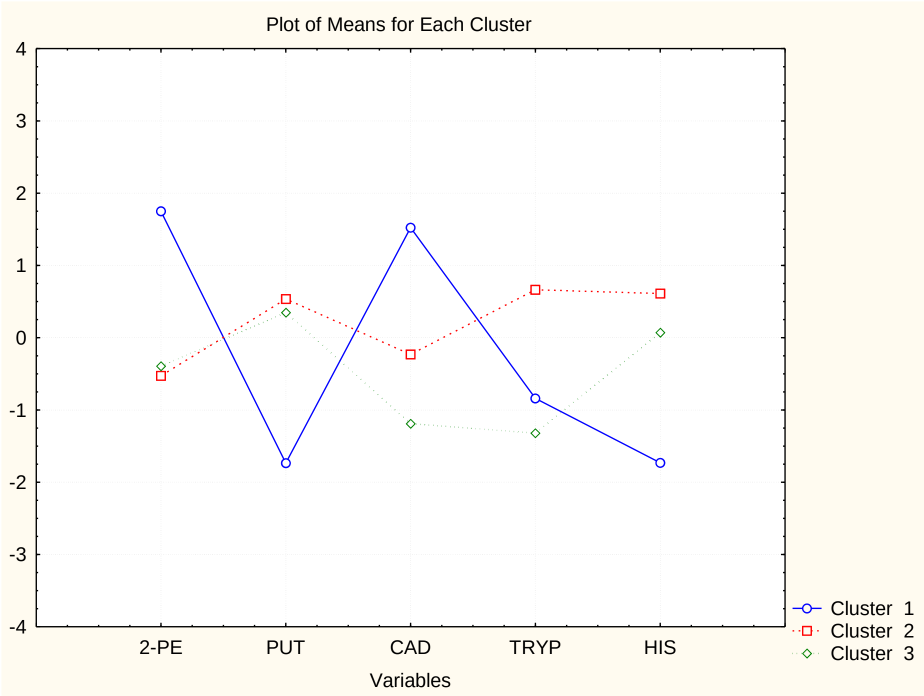

Figure 7. Averages of variables for each cluster (Wines –D – F)

Tables:

Table 1. Fragments, relative intensities and retention time (Rt) of BAs obtained by application of GC-MS technique.

Analytes	m/z SIM ions					Rt
Hexylamine	**146 (99.9)**	130 (76.7)	128 (14.8)			7.893
2-phenylethylamine	**130(99.9)**	104(79.6)	91(76.4)	221 (30.7)	148 (18.5)	10.016
Putrescine	**170 (99.9)**	130 (63.6)	288 (12)			11.773
Tryptamine	**130 (99.9)**	143 (59.2)	260 (19.1)	187 (4.1)		13.212
Tyramine	**120 (99.9)**	107 (27.7)	176 (4.6)	237 (2.2)	337 (1.4)	13.319
Cadaverine	**130 (79)**	84 (82)	129 (73)	302 (2)		13.505
Histamine	**194 (99.9)**	238 (16.7)	138 (25.8)			14.168

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Table 2. Information on average recoveries (%), intra-day repeatability (%RSD), inter-day repeatability (%RSD) and limits of detection (LOD, (µg/L) and limits of quantification (LOQ, (µg/L)) obtained with the optimized method in spiked wine samples, analyzed by GC-MS (n = 4 at each level).

Analyte	**Concentration levels**				**Interday (%RSD)**	**LOD** (µg/L)	**LOQ** (µg/L)
	0.05 mg/L		**0.25 mg/L**				
	Recovery (%)	**Intraday (%RSD)**	**Recovery (%)**	**Intraday (%RSD)**			
CAD	83	6	92	7	6	1.5	4.5
HIST	76	5	88	5	7	4.2	12.6
PUT	98	8	103	7	8	1.4	4.6
TRP	83	12	89	8	10	1.6	4.8
TYR	99	5	105	4	5	3.3	9.9
2-PE	88	6	97	6	6	3.2	9.6

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Table 3. Evolution of BA concentrations and pH in standard and high quality red wines and young white wine in different storage conditions.

A	Commercially not available wine elaborated from 100 % SOLARIS grapes (Mean concentration (µg/L)±Standard deviation)																	
Analytes	Ao	A7	A30	Bo	B7	B30	Co	C7	C30	Do	D7	D30	E0	E7	E30	Fo	F7	F30
2-PE	43,02±0,21	51,11±0,32	40,16±0,19	43,02±0,21	47,45±0,34	42,23±0,45	43,02±0,21	44,09±0,38	43,96±0,45	43,17±0,32	48,11±0,42	42,12±0,38	43,17±0,32	46,32±0,23	42,34±0,28	43,17±0,32	43,12±0,28	42,99±0,43
PUT	62,12 ± 0,78	60,32±0,27	55,87±0,25	62,12 ± 0,78	60,76±0,65	58,65±0,56	62,12 ± 0,78	61,00±0,48	60,09±0,44	60,72 ± 0,73	59,32±0,67	57,43±0,54	60,72 ± 0,73	60,32 ±0,58	58,43±0,48	60,72 ± 0,73	61,09±0,37	59,19±0,35
CAD	32,08±0,45	31,36±0,32	31,08±0,28	32,08±0,45	33,09±0,54	32,23±0,46	32,08±0,45	33,31±0,57	32,87±0,48	32,76±0,49	32,11±0,39	31,67±0,43	32,76±0,49	31,98±0,32	31,87±0,31	32,76±0,49	32,99±0,45	32,57±0,32
TRYP	133,0 ± 1,6	156,0±2,1	178,4±2,5	133,0 ± 1,6	143,7±1,5	157,09±1,7	133,0 ± 1,6	136,3±1,6	137,0±2,1	132,8 ± 1,4	152,7±1,8	166,8±2,1	132,8 ± 1,4	147,2±2,1	155,3±2,0	132,8 ± 1,4	135,3±2,1	136,4±2,4
TYR	24,01 ± 0,18	35,65±0,11	27,43±0,12	24,01 ± 0,18	27,07±0,23	23,09±0,17	24,01 ± 0,18	25,09±0,16	24,89±0,17	24,32 ± 0,21	33,45±0,16	27,98±0,14	24,32 ± 0,21	31,09±0,17	28,21±0,19	24,32 ± 0,21	24,78±0,12	24,49±0,12
HIS	416 ± 13	552±15	482±13	416 ± 13	489±20	463±18	416 ± 13	452±17	438±20	421 ± 20	523±17	496±19	421 ± 20	503±21	484±20	421 ± 20	448±27	418±21
TOTAL	710	886	815	710	801	776	710	752	737	715	849	822	715	820	800	715	745	713
pH	3,09 ± 0,01	3,07±0,01	3,06±0,01	3,09±0,01	3,09±0,01	3,08±0,01	3,09 ± 0,01	3,07±0,01	3,08±0,01	3,09 ± 0,01	3,07±0,01	3,09 ± 0,01	3,09 ± 0,01	3,07±0,01	3,07±0,01	3,09 ± 0,01	3,07±0,01	3,08±0,01
B	**Commercially available wine elaborated from 100 % SOLARIS grapes (Mean concentration (µg/L)±Standard deviation)**																	
2-PE	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
PUT	759±21	700±26	612±23	759±21	711±24	634±21	759±21	745±23	730±19	756±23	738±24	650±20	756±23	746±23	700±26	756±23	751±20	748±23
CAD	12,00±0,12	11,89±0,09	11,91±0,11	12,00±0,12	12,13±0,14	12,01±0,11	12,00±0,12	11,87±0,14	11,85±0,09	11,80±0,14	11,76±0,11	11,00±0,13	11,80±0,14	11,78±0,13	11,69±0,16	11,80±0,14	11,79±0,11	11,79±0,13
TRYP	30,15±0,17	51,21±0,21	74,32±0,23	30,15±0,17	40,09±0,23	54,12±0,25	30,15±0,17	33,78±0,18	35,01±0,18	31,05±0,15	47,84±0,65	65,09±0,56	31,05±0,15	45,67±0,15	52,08±0,23	31,05±0,15	35,67±0,18	36,10±0,13
TYR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
HIS	128,0±2,0	234,26±4,1	163,43±4,1	128,0±4,0	189,98±4,4	160,54±3,9	128,0±4,0	169,0±3,9	146,8±4,0	127,7±4,1	227,1±4,3	201,0±3,8	127,7±4,1	201,0±5,0	185,5±4,6	127,7±4,1	143,9±3,7	128,7±3,9
TOTAL	929	997	861,66	929	953	860,67	929	960	923,67	927	1025	927	927	1004	949	927	942	925
pH	3,43 ± 0,01	3,41 ± 0,01	3,42 ± 0,01	3,43 ± 0,01	3,40± 0,01	3,41± 0,01	3,43 ± 0,01	3,42 ± 0,01	3,40± 0,01	3,39± 0,01	3,42 ± 0,01	3,43 ± 0,01	3,41± 0,01	3,40± 0,01	3,39± 0,01	3,42 ± 0,01	3,40± 0,01	3,41± 0,01
C	**Commercially available wine elaborated from 100 % BIANCA grapes (Mean concentration (µg/L)±Standard deviation)**																	
2-PE	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
PUT	260±10	201±13	131±16	260±10	221±11	157±13	260±10	245±11	228±16	259±11	237±14	156±11	259±11	231±13	178±10	259±11	250±14	245±10
CAD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
TRYP	10,11±0,10	23,01±0,11	37,91±0,17	10,11±0,10	21,00±0,14	35,13±0,14	10,11±0,10	13,45±0,13	15,14±0.11	10,14±0,11	22.00±0.14	35,09±0,11	10,14±0,11	19,76±0,16	33,12±0,14	10,14±0,11	11,99±0,11	13,56±0,10
TYR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
HIS	172,1±3,2	258,1±4,0	197,7±3,9	172,1±3,2	241,9±4,5	210,1±4,1	172,1±3,2	209,0±3,7	189,1±4,1	171,9±3,3	240,5±4,2	210,4±3,5	171,9±3,3	227,8±4,0	214,7±4,6	171,9±3,3	189,0±4,7	176,9±3,7

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TOTAL	442	482	367	442	484	402	442	467	432	441	499	402	441	479	426	441	451	435
pH	3,25±0,01	3,24±0,01	3,26±0,01	3,25± 0,01	3,22±0,01	3,24±0,01	3,25± 0,01	3,24±0,01	3,23±0,01	3,25±0,01	3,24±0,01	3,26±0,01	3,25±0,01	3,23±0,01	3,24±0,01	3,25±0,01	3,24±0,01	3,26±0,01
D	Commercially available wine elaborated from 100 % REGENT grapes (Mean concentration (μg/L)±Standard deviation)																	
2-PE	19,23±0,16	20,15±0,18	30,12±0,21	19,23±0,16	20,09±0,18	28,01±0,20	19,23±0,16	19,78±0,15	23,09±0,17	19,43±0,19	20,01±0,21	28,31±0,21	19,43±0,19	19,91±0,22	26,09±0,18	19,43±0,19	19,56±0,20	21,19±0,22
PUT	298,2±6,8	291,1±7,0	211,9±6,8	298,2±6,8	293,6±7,1	230,3±6,7	298,2±6,8	296,2±3,0	286,8±3,7	297,3±7,2	293,1±8,0	234,81±7,6	297,3±7,2	295,2±8,1	254,2±7,1	297,3±7,2	296,5±7,1	290,4±8,1
CAD	35,89±0,43	30,81±0,38	45,87±0,41	35,89±0,43	32,22±0,35	38,45±0,37	35,89±0,43	34,12±0,36	36,09±0,38	36,01±0,51	32,89±0,60	42,89±0,49	36,01±0,51	32,90±0,54	37,12±0,60	36,01±0,51	35,15±0,52	36,12±0,54
TRYP	4,32±0,11	2,32±0,12	2,30±0,16	4,32±0,11	2,78±0,17	2,89±0,18	4,32±0,11	4,27±0,12	4,22±0,10	4,22±0,15	2,78±0,17	2,80±0,16	4,22±0,15	3,11±0,18	3,09±0,19	4,22±0,15	4,12±0,17	4,09±0,19
TYR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
HIS	311,3±7,7	300±8,1	250,2±7,9	311,3±7,7	306,0±6,5	276,0±7,1	311,3±6,7	310,3±8,1	308,9±7,9	309,3±6,9	301,1±6,5	265,9±8,1	309,3±6,9	305,5±8,5	280,1±7,8	309,3±7,9	308,1±8,1	306,1±7,9
TOTAL	669	644	540	669	655	576	669	664	659	666	650	575	666	657	602	666	663	657
pH	4,02±0,01	4,06±0,01	4,09±0,01	4,02±0,01	4,07±0,01	4,10±0,01	4,02±0,01	4,06±0,01	4,09±0,01	3,99±0,01	3,97±0,01	3,94±0,01	3,99±0,01	3,96±0,01	3,94±0,01	3,99±0,01	3,97±0,01	3,95±0,01
E	Commercially not available wine elaborated from 100 % REGENT grapes (Mean concentration (μg/L)±Standard deviation)																	
2-PE	21,17±0,20	22,18±0,22	29,09±0,19	21,17±0,20	21,98±0,22	27,78±0,19	21,17±0,20	21,56±0,21	22,87±0,19	21,43±0,21	22,34±0,23	30,09±0,19	21,43±0,21	21,9±0,23	27,67±0,27	21,43±0,21	21,56±0,19	22,09±0,24
PUT	289,9±8,5	280,98±9,1	202,78±6,8	289,9±8,5	282,78±8,1	221,9±7,9	289,9±8,5	286,2±8,1	278,2±8,5	285,4±7,7	281,09±8,0	220,19±6,9	285,4±7,7	283,12±8,1	245,23±7,8	285,4±7,7	284,1±7,8	279,09±8,1
CAD	31,09±0,21	26,16±0,23	41,78±0,19	31,09±0,21	28,12±0,25	36,14±0,21	31,09±0,21	29,97±0,20	31,76±0,19	31,16±0,24	27,98±0,19	37,78±0,30	31,16±0,24	28,34±0,19	33,45±0,21	31,16±0,24	30,01±0,27	31,46±0,22
TRYP	3,21±0,11	2,01±0,10	2,10±0,11	3,21±0,11	2,45±0,13	2,44±0,12	3,21±0,11	3,15±0,10	3,12±0,11	3,11±0,17	1,70±0,16	1,73±0,17	3,11±0,17	2,11±0,16	2,09±0,16	3,11±0,17	3,02±0,18	2,98±0,15
TYR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
HIS	326,0±6,9	315,0±6,6	265,1±7,9	326,0±9,0	321,8±6,3	291,1±7,1	326,0±6,9	325,3±7,4	322,0±7,1	324,9±7,4	315,01±6,9	280,9±7,9	324,9±7,4	319,0±7,1	295,1±6,8	324,9±7,4	323,78±8,1	321,1±8,0
TOTAL	671	646	541	671	657	579	671	666	657	666	648	571	666	654	604	666	662	657
pH	3,50±0,01	3,52±0,01	3,57±0,01	3,50±0,01	3,53±0,01	3,57±0,01	3,50±0,01	3,52±0,01	3,56±0,01	3,49±0,01	3,48±0,01	3,46±0,01	3,49±0,01	3,47±0,01	3,45±0,01	3,49±0,01	3,46±0,01	3,44±0,01
F	Commercially not available wine elaborated from 100 % FRONTENAC grapes (Mean concentration (μg/L)±Standard deviation)																	
2-PE	24,31±0,22	25,46±0,23	30,23±0,19	24,31±0,22	24,98±0,22	30,17±0,27	24,31±0,22	24,67±0,31	26,01±0,27	24,17±0,27	24,15±0,32	29,56±0,26	24,17±0,27	24,76±0,24	28,43±0,26	24,17±0,27	24,35±0,19	25,00±0,21
PUT	482±13	471±16	389±14	482±13	474±12	416±14	482±13	479±18	471±15	481±11	477±13	416±15	481±11	479±12	435±11	481±11	480±14	476±11
CAD	96,01±0,91	90,2±1,2	107,2±1,4	96,01±0,91	94,7±1,0	102,1±2,0	96,01±0,91	95,7±1,4	96,7±1,2	95,87±0,78	91,80±0,56	101,1±1,1	95,87±0,78	95,09±12±0,98	100,0±1,1	95,87±0,78	95,82±0,98	95,97±0,95
TRYP	3,04±0,10	2,10±0,11	2,19±0,13	3,04±0,10	2,56±0,13	2,54±0,11	3,04±0,10	2,98±0,11	2,96±0,12	3,00±0,13	2,45±0,13	2,53±0,10	3,00±0,13	2,74±0,16	2,81±0,11	3,00±0,13	2,99±0,11	2,96±15
TYR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
HIS	1639±48	1578±51	1415±47	1639±48	1602±45	1454±43	1639±48	1625±48	1613±51	1637±51	1592±48	1465±51	1637±51	1612±47	1498±50	1637±51	1631±47	1625±42
TOTAL	2244	2167	1944	2244	2198	2005	2244	2227	2210	2241	2187	2014	2241	2214	2064	2241	2234	2225
pH	3,37±0,01	3,38±0,01	3,40±0,01	3,37±0,01	3,37±0,01	3,39±0,01	3,37±0,01	3,38±0,01	3,40±0,01	3,36±0,01	3,35±0,01	3,34±0,01	3,36±0,01	3,34±0,01	3,33±0,01	3,36±0,01	3,35±0,01	3,34±0,01

Table 4. Cluster composition for variables for 6 wine brands

Brand	Cluster 1 Cadaverine factor	Cluster 2 Histamine factor
Wine A	PUT CAD	2-PE TYR HIS TRYP
Wine B	PUT CAD	TRYP HIS
Wine C	PUT	TRYP HIS
Wine D	2-PE CAD	PUT HIS TRYP
Wine E	2-PE CAD	PUT HIS TRYP
Wine F	2-PE CAD	PUT HIS TRYP

Table 5. Cluster content for all wine brands

Brand	Cluster 1	Cluster 2	Cluster 3
Wine A	A0, B0, C0, D0, E0, F0, B7, C7, F7, C30, F30	A7, D7, E7	A30,B30, D30, E30
Wine B	A0, B0, C0, D0, E0, F0, C7, F7, C30, F30	A7, B7, D7, E7 E30	A30,B30, D30, E30
Wine C	A0, B0, C0, D0, E0, F0, C7, F7, C30, F30	A7, B7, D7, E7	A30,B30, D30, E30
Wine D	A0, B0, C0, D0, E0, F0, C7, F7, C30, F30	A7, B7, D7, E7	A30,B30, D30, E30
Wine E	A0, B0, C0, D0, E0, F0, C7, F7, C30, F30	A7, B7, D7, E7	A30,B30, D30, E30
Wine F	A0, B0, C0, D0, E0, F0, C7, F7, C30, F30	A7, B7, D7, E7	A30,B30, D30, E30