



The content of biogenic amines in Rondo and Zweigelt wines and correlations between selected wine parameters

Anna Stój^a, Justyna Płotka-Wasyłka^{b,*}, Vasil Simeonov^c, Magdalena Kapłań^d

^a Department of Biotechnology, Microbiology and Human Nutrition, Faculty of Food Science and Biotechnology, University of Life Sciences, Skromna 8, 20-704 Lublin, Poland

^b Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology, Gdańsk, Poland

^c Faculty of Chemistry and Pharmacy, University of Sofia, Sofia 1126, Bulgaria

^d Institute of Horticulture Production, University of Life Sciences, 28 Głęboka Street, 20-612 Lublin, Poland

ARTICLE INFO

Keyword:

Biogenic amines
Yeast
Lactic acid bacteria
Sequential inoculation
Red wines
Chemometric analysis

ABSTRACT

The purpose of this study was to evaluate the content of biogenic amines (BAs) in wines using dispersive liquid–liquid microextraction–gas chromatography–mass spectrometry (DLLME-GC–MS). An additional objective was to assess the correlations between selected parameters characterizing the samples such as the content of BAs, sugars, and organic acids, pH, and total acidity. Wines produced from the same grape variety in which alcoholic fermentation (AF) was carried out by different yeast strains and in which malolactic fermentation (MLF) was spontaneous, differed in the content of biogenic amines. The concentrations of putrescine, cadaverine and tryptamine were higher in the Rondo wines (237–405, 34.04–61.11, <LOD–12.456 µg/L, respectively) and Zweigelt wines (416–489, 72.67–88.43, <LOD–13.083 µg/L, respectively) subjected to spontaneous MLF than in the wines subjected to induced MLF. Chemometric analysis allowed us to determine correlations between selected wine parameters. The wine samples are well separated into two patterns depending on the grape variety. Despite the fact that information on BAs is not included in databases of wine composition, information on their concentration as well as knowledge of existing correlations between BAs and other wine parameters is crucial and may be useful for the food industry, health professionals and consumers.

1. Introduction

Biogenic amines (BAs) are products of decarboxylation of corresponding amino acids, or amination and transamination of aldehydes and ketones by microorganisms (Stadnik and Dolatowski, 2012). Taking into account the structure of BAs, they can be classified into aliphatic (putrescine, cadaverine, spermine and spermidine), aromatic (tyramine and 2-phenylethylamine) and heterocyclic (histamine and tryptamine) amines (Guo et al., 2015; Marques et al., 2008).

Having a knowledge of the level of BAs is important for the wine industry, because they are potentially toxic to consumers when the acceptable daily intake is exceeded (Arrieta and Prats-Moya, 2012; Costantini et al., 2019; Guo et al., 2015). It should be highlighted that in the case of wines, ethanol and acetaldehyde inhibit the activities of monoamine and diamine oxidases, the enzymes responsible for the detoxification of amines (Jeromel et al., 2018; Piasta et al., 2014; Woźniakiewicz et al., 2018). The physiological symptoms of excessive

consumption of BAs are headache, nausea, sweating, respiratory distress, heart palpitations and hypo- or hypertension (Jabłońska-Ryś et al., 2020; Lorenzo et al., 2017). Despite the toxicity of BAs, there are no regulations regarding the content of these compounds in wines. Some European countries recommend limits for histamine in wine ranging from 2 to 10 mg/L, but these limits are not mandatory (Esposito et al., 2019; Palomino-Vasco et al., 2019).

BA levels in wine depend on many factors including the grape variety, climatic conditions, agricultural practices, vinification techniques, the microorganism strains used in fermentation, ageing and wine parameters (pH, alcohol content and sulfur dioxide content). These factors influence the content of amino acid precursors as well as the growth of bacteria. They are usually correlated, and it is difficult to determine the individual influence of each of them (Arrieta and Prats-Moya, 2012; Costantini et al., 2019; Guo et al., 2015).

The grape variety affects the presence of some biogenic amines in musts. Ethanolamine, ethylamine and putrescine were found in initial

* Corresponding author.

E-mail address: juswasyl@pg.edu.pl (J. Płotka-Wasyłka).

<https://doi.org/10.1016/j.foodchem.2021.131172>

Received 13 May 2021; Received in revised form 24 August 2021; Accepted 15 September 2021

Available online 20 September 2021

0308-8146/© 2021 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

musts produced from Merlot, Syrah, Sangiovese, Cesanese d'Affile, Carmenere, Montepulciano and Cabernet Franc varieties (Del Prete et al., 2009). Six amines, including ethanolamine, tyramine, putrescine, cadaverine, phenylethylamine and spermidine, were present in musts of the Cabernet Sauvignon variety (Wang et al., 2014). Differences in BA content related to grape variety were reported in red wines originating from Greece and Chile (Soufleros et al., 2007; Pineda et al., 2012).

The key stages of wine production affecting the quality of wines are alcoholic fermentation (AF) and malolactic fermentation (MLF). These processes can occur spontaneously with the participation of yeast and lactic acid bacteria (LAB), respectively, naturally present on grapes or be induced by commercial starter cultures (Jeromel et al., 2018). LAB degrade malic acid to lactic acid and can also metabolize other substances, such as sugars, citric acid and amino acids, into undesirable substances such as acetic acid and biogenic amines (Soufleros et al., 2007). LAB can be inoculated before or after AF is complete (simultaneous/co-inoculation or sequential inoculation, respectively) (Bartowsky et al., 2015). The time of LAB inoculation can significantly affect the accumulation of biogenic amines during red wine production. The content of biogenic amines was lower in wines produced by simultaneous inoculation compared to traditional sequential inoculation after AF (Izquierdo Cañas et al., 2012; Jeromel et al., 2018). There is no consensus on which of the fermentation types is the main one to support the production of BAs in wines (Restuccia et al., 2018). The majority of data suggest that LAB make a greater contribution than yeast (Costantini et al., 2019; Guo et al., 2015), but there are also reports that amines formed during AF account for most of the BA content in the final wines (Ruiz et al., 2012; Wang et al., 2014). BA concentration is higher in red wines than in white wines due to MLF, which is common for most red wines, although it takes place in some white and sparkling wines as well (Esposito et al., 2019; Liu et al., 2020).

BAs are formed by microorganisms associated with different stages of winemaking (Guo et al., 2015; Smit et al., 2008). Two species isolated during spontaneous MLF, *Lactobacillus rhamnosus* and *Oenococcus oeni*, were producers of biogenic amines (Henríquez-Aedo et al., 2016). The ability to produce BAs is also found in species of bacteria that do not normally carry out MLF, but are spoilage bacteria indicative of a poor sanitary condition of grapes. Some authors suggest that it is spoilage bacteria that mainly contribute to the accumulation of BAs. Control of microbiota is, then, a promising strategy to diminish BA formation. This can be achieved through the use of selected starter cultures which do not produce BAs and, at the same time, reduce the growth of spoilage microorganisms. In addition, bacteria capable of degrading BAs can be used (Costantini et al., 2019; Guo et al., 2015). Among LAB isolated from must, wine and winemaking products, nine strains of the genus *Lactobacillus* and *Pediococcus* exhibited the greatest amine-degrading ability. Of those, 25% were able to degrade histamine, 18% tyramine, and 18% putrescine. None of the commercial MLF starter cultures had the ability to degrade any of the amines (García-Ruiz et al., 2011). Also two strains of *L. plantarum* degraded putrescine and tyramine (Capozzi et al., 2012).

Due to the toxicity of biogenic amines, it is necessary to analyze the factors influencing their production. This is especially desired and important at the present moment of intensive development of Polish winemaking. The key factors are AF and MLF. The purpose of this study was to determine and evaluate the content of biogenic amines in red wines produced from Zweigelt and Rondo grape varieties using dispersive liquid-liquid microextraction-gas chromatography-mass spectrometry (DLLME-GC-MS) and investigate the correlation between selected parameters characterizing the wine samples using chemometric tools. Rondo and Zweigelt wines were produced by five commercial yeast strains (*S. cerevisiae* or *S. cerevisiae* × *S. bayanus*) with and without inoculation with a LAB strain (*O. oeni*) (spontaneous vs. induced MLF). The specific relationships were determined by chemometric analysis.

Table 1
Concentrations of BAs (µg/L).

Sample	Concentration (µg/L), n = 4					
	2-PE	HIS	PUT	CAD	TYR	TRP
R1	9.09 ± 0.16 <LOD	639 ± 32 698 ± 34	237 ± 20 367 ± 23	36.00 ± 0.17 40.01 ± 0.19	25.05 ± 0.10 23.11 ± 0.11	12.120 ± 0.008 12.456 ± 0.011
R2	<LOD	698 ± 34	367 ± 23	40.01 ± 0.19	23.11 ± 0.11	12.456 ± 0.011
R3	13.82 ± 0.17	654 ± 33	281 ± 22	34.04 ± 0.17	24.67 ± 0.13	<LOD
R4	15.12 ± 0.13	701 ± 37	400 ± 26	57.00 ± 0.21	27.41 ± 0.18	<LOD
R5	19.00 ± 0.20	717 ± 32	405 ± 26	61.11 ± 0.24	27.33 ± 0.15	<LOD
R1 LAB	25.03 ± 0.19	668 ± 35	186 ± 14	25.00 ± 0.14	24.15 ± 0.10	11.981 ± 0.007
R2 LAB	14.09 ± 0.11	722 ± 37	336 ± 21	34.17 ± 0.17	22.65 ± 0.09	12.401 ± 0.009
R3 LAB	36.19 ± 0.23	672 ± 36	256 ± 20	27.02 ± 0.12	23.98 ± 0.13	<LOD
R4 LAB	41.23 ± 0.22	727 ± 38	373 ± 24	51.06 ± 0.19	26.35 ± 0.15	<LOD
R5 LAB	58.98 ± 0.25	741 ± 36	379 ± 23	54.43 ± 0.23	26.73 ± 0.15	<LOD
Z1	<LOD	517 ± 29	416 ± 25	76.34 ± 0.29	<LOD	13.083 ± 0.008
Z2	<LOD	539 ± 31	454 ± 27	81.34 ± 0.25	<LOD	11.922 ± 0.011
Z3	14.56 ± 0.21	548 ± 36	418 ± 24	72.67 ± 0.30	<LOD	<LOD
Z4	15.17 ± 0.14	601 ± 40	434 ± 28	76.49 ± 0.31	<LOD	<LOD
Z5	15.76 ± 0.16	633 ± 39	489 ± 25	88.43 ± 0.36	<LOD	<LOD
Z1 LAB	<LOD	529 ± 32	349 ± 22	69.48 ± 0.26	<LOD	12.989 ± 0.006
Z2 LAB	<LOD	549 ± 37	444 ± 24	71.16 ± 0.24	<LOD	11.764 ± 0.009
Z3 LAB	12.11 ± 0.09	567 ± 35	401 ± 26	66.89 ± 0.26	<LOD	<LOD
Z4 LAB	14.99 ± 0.15	619 ± 32	420 ± 25	69.64 ± 0.28	<LOD	<LOD
Z5 LAB	15.82 ± 0.21	651 ± 41	461 ± 29	81.29 ± 0.33	<LOD	<LOD

R1-R5-wines from the Rondo variety, in which alcoholic fermentation was carried out by different yeast strains and malolactic fermentation was spontaneous; R1 LAB-R5 LAB-wines from the Rondo variety, in which alcoholic fermentation was carried out by different yeast strains (but the same as in R1-R5 wines), and malolactic fermentation was induced. Z1-Z5-wines from the Zweigelt variety, in which alcoholic fermentation was carried out by different yeast strains and malolactic fermentation was spontaneous; Z1 LAB-Z5 LAB-wines from the Zweigelt variety, in which alcoholic fermentation was carried out by different yeast strains (but the same as in Z1-Z5 wines), and malolactic fermentation was induced. 2-PE, 2-phenylethylamine; HIS, histamine; PUT, putrescine; CAD, cadaverine; TYR, tyramine; TRP, tryptamine.

2. Materials and methods

2.1. Reagents and materials

All of the biogenic amine standards such as 2-phenylethylamine (2-PE), histamine (HIS), putrescine (PUT), cadaverine (CAD), tyramine (TYR) and tryptamine (TRP) and the internal standard (hexylamine, IS) were purchased, mostly as hydrochloride salts, from Sigma Aldrich (St. Louis, MO, USA). The derivatizing agent (isobutyl chloroformate, IBCF) was supplied by Sigma-Aldrich. Deionized water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Stock standard solutions of amines and IS (1 mg/mL) were prepared by weighing and dissolving in ultrapure water and stored at 4 °C in silanized screw-capped vials with solid PTFE-lined caps (Supelco, Bellefonte, PA).

Working standard solutions were prepared by appropriately diluting

stock solutions with deionized water. High-purity grade methanol used as a dispersive solvent was purchased from Fluka (Riedel de Haen. Burdick&Jackson). High-purity grade chloroform applied as an extractive solvent was obtained from Sigma. 0.1 M HCl was also supplied by Fluka. Other chemicals were of an analytical grade.

2.2. Samples

The grapes of Zweigelt and Rondo varieties were obtained from 'Małe Dobre' and 'Dom Bliskowice' vineyards, respectively. The vineyards are located in the Lublin Province, Poland. The grapes were harvested manually in 2017. AF was carried out by five commercial yeast strains, *S. cerevisiae* or *S. cerevisiae* × *S. bayanus* for both the Zweigelt and the Rondo varieties. One part of the wines underwent spontaneous MLF without inoculation with LAB, and the other part were produced by induced MLF with *O. oeni* inoculation. *O. oeni* starter culture was added after the completion of AF (sequential inoculation) to the part of wines in which induced MLF was carried out. The experiments were performed in duplicate. In this paper, the following abbreviations for the samples are used: R1-R5 – wines from the Rondo variety, in which alcoholic fermentation was carried out by different yeast strains and malolactic fermentation was spontaneous; R1 LAB-R5 LAB – wines from the Rondo variety, in which alcoholic fermentation was carried out by different yeast strains (but the same as in R1-R5 wines), and malolactic fermentation was induced; Z1-Z5 – wines from the Zweigelt variety, in which alcoholic fermentation was carried out by different yeast strains and malolactic fermentation was spontaneous; Z1 LAB-Z5 LAB – wines from the Zweigelt variety, in which alcoholic fermentation was carried out by different yeast strains (but the same as in Z1-Z5 wines), and malolactic fermentation was induced.

The details of the winemaking process are presented in a previous article (Stój et al., 2020).

2.3. Determination of biogenic amines by DLLME-GC-MS

The procedure developed by Płotka-Wasyłka et al. (2016) was applied to determine BAs in the obtained wine samples. The sample preparation step was based on DLLME coupled with an *in situ* derivatization process, while the final determination technique was GC combined with MS. A schematic diagram of the procedure is presented in Fig. S1.

Optimized conditions were used for validating the method developed for quantitative analysis of selected BAs. The results obtained showed that linearity was excellent for all the compounds with correlation coefficients ranging from 0.9961 to 0.9992. The LODs ranged from 1.4 to 4.2 µg/L and the LOQs ranged from 4.6 to 12.6 µg/L. The procedure was characterized by good analyte recovery values, ranging from 76 to 105%. Information on selected validation parameters and recovery is presented in Table S1.

2.4. Equipment

2.4.1. GC-MS analysis of BAs

A gas chromatography (GC) 7890A (Agilent Technologies, Santa Clara, CA, USA) system equipped with an electronically-controlled split/splitless injection port was interfaced to an inert mass selective detector (5975C, Agilent Technologies) with an electron impact ionization chamber. Chromatographic separation was performed on a ZB-5MS capillary column (30 m × 0.25 mm I.D., 0.25 µm) supplied by Zebron Phenomenex. The injection was carried out in splitless mode at 230 °C. The interface was set at 250 °C. The volume of the injected sample was 2 µl. Helium was the carrier gas with a constant pressure of 30 psi. The oven temperature program was as follows: 50 °C held for 1 min, ramped to 280 °C at 15 °C/min and held for 9 min (total run time was 25.3 min). For improved selectivity and sensitivity, the analysis was performed in the selected ion monitoring (SIM) mode. Mass spectrometric parameters

Table 2
Contents of biogenic amines (mg/L) in red wines produced from different grape varieties determined in previous studies.

Type of wines	Biogenic amines											References							
	ETA	HIS	MEA	ETY	2-PE	ISM	TYR	TRP	PUT	CAD	SPD	SPM	AG	SER	ISO	IPA			
Spanish wines, Monastrell wines, young, Crianza and aged wines, table wines	9.9–35.8	nd	1.7–8.0	nd	nd	nd	1.1–17.8	nd–16.2	7.6–35.7	nd	nd	nd	nd	nd	nd	nd	Arrieta and Prats-Moya, 2012		
Greek wines, dry, semi-sweet and sweet wines, commercial wines, table wines	nd–2.11	nd–0.66	nd–1.61	nd	nd	nd	nd–8.15	nd–3.65	nd–5.23	nd–3.21	nd–1.56	nd–1.62	nd	nd	nd	nd	Soufferos et al., 2007		
Spanish wines, Toro wines, quality wines, organic and non-organic wines	1.72–14.94	nd	1.03–11.04	0.00–1.35	nd	nd	0.00–4.88	nq–0.60	0.51–25.03	nq–2.05	nd	nd	nd	nd	nd	nd	García-Marino et al., 2010		
Chinese wines, commercial wines	nd–8.22	nd	0.08–19.82	nd	nd	nd	nd–23.96	0.33–4.73	0.65–45.16	nd–2.24	nd–3.32	nd–1.51	nd	nd	nd	nd	Liu et al., 2020		
Italian wines, commercial wines	16.9–24.1	nd–0.9	nd–0.7	2.9–7.1	nd	nd	1.2–4.3	nd–0.9	4.5–16.1	nd	nd	nd	nd	nd	nd	nd	Manetta et al., 2016		
Type of wines	Biogenic amines											References							
	ETA	HIS	MEA	ETY	2-PE	ISM	TYR	TRP	PUT	CAD	SPD	SPM	AG	SER	ISO	IPA			
Italian wines, several grape varieties, commercial wines, table and quality wines	0.51–7.21	nd	1.54–5.53	nd	nd	nd	1.90–11.94	3.76–15.99	nd–0.45	nd	nd	nd	nd	nd	nd	nd	Martuscelli et al., 2013		
Spanish wines, Tempranillo wines, young, oak and aged wines	13.0–27.7	nd–10.3	nd–0.19	nd	nd	nd	nd–4.1	nd	5.88–42.6	nd–2.5	nd	nd	nd	nd	nd	nd	Palomino-Vasco et al., 2019		
Austrian wines, Cannonau wines, commercial wines	nq–8.11	0.20–1.66	4.14–11.3	nd–1.21	nd	nd	5.08–11.5	nq–0.05	11.4–32.8	1.00–2.42	nd–1.27	nd	nd	nd	nd	nd	0.07–0.14	Tuberoso et al., 2015	
Austrian wines, several grape varieties (including Zweigelt), quality wines	4.96–10.9	nd	0.11–0.22	0.13–0.42	2.25–5.00	0.03–0.44	12.6–29.5	0.27–1.23	1.09–2.5	nd	nd	nd	nd	nd	nd	nd	Konakovskiy et al., 2011		
Brazilian wines, several grape varieties, commercial wines	0.00–1.73	nd	0.17–1.37	nd	nd	nd	0.30–1.07	0.77–4.33	0.03–1.63	nd	nd	nd	nd	nd	nd	nd	Souza et al., 2005		

ETA, ethanalamine; HIS, histamine; MEA, methylyamine; ETY, ethylamine; 2-PE, 2-phenylethylamine; ISM, isoamylamine; TYR, tyramine; TRP, tryptamine; PUT, putrescine; CAD, cadaverine; SPD, spermidine; SPM, spermine; AG, agmatine; SER, serotonin; ISO, isobutylamine; IPA, isopentylamine.

were set as follows: electron impact ionization with 70 eV energy; ion source temperature, 250 °C. All the ion fragments with their relative intensities at the specific retention times were considered as a valid confirmation criterion and used for the identification of specific BAs. The ionic fragments of BA together with the relative ion intensities are given in Table S2. An Agilent ChemStation was used for data collection and GC–MS control.

2.5. Chemometric analysis

One of the most frequently-used chemometric methods for multivariate data interpretation is cluster analysis (hierarchical and non-hierarchical clustering) (Massart and Kaufman, 1983). In order to carry out the hierarchical clustering procedure, several steps were required: standardization of raw input data, application of squared Euclidean distances as similarity measures, Ward's method of linkage,

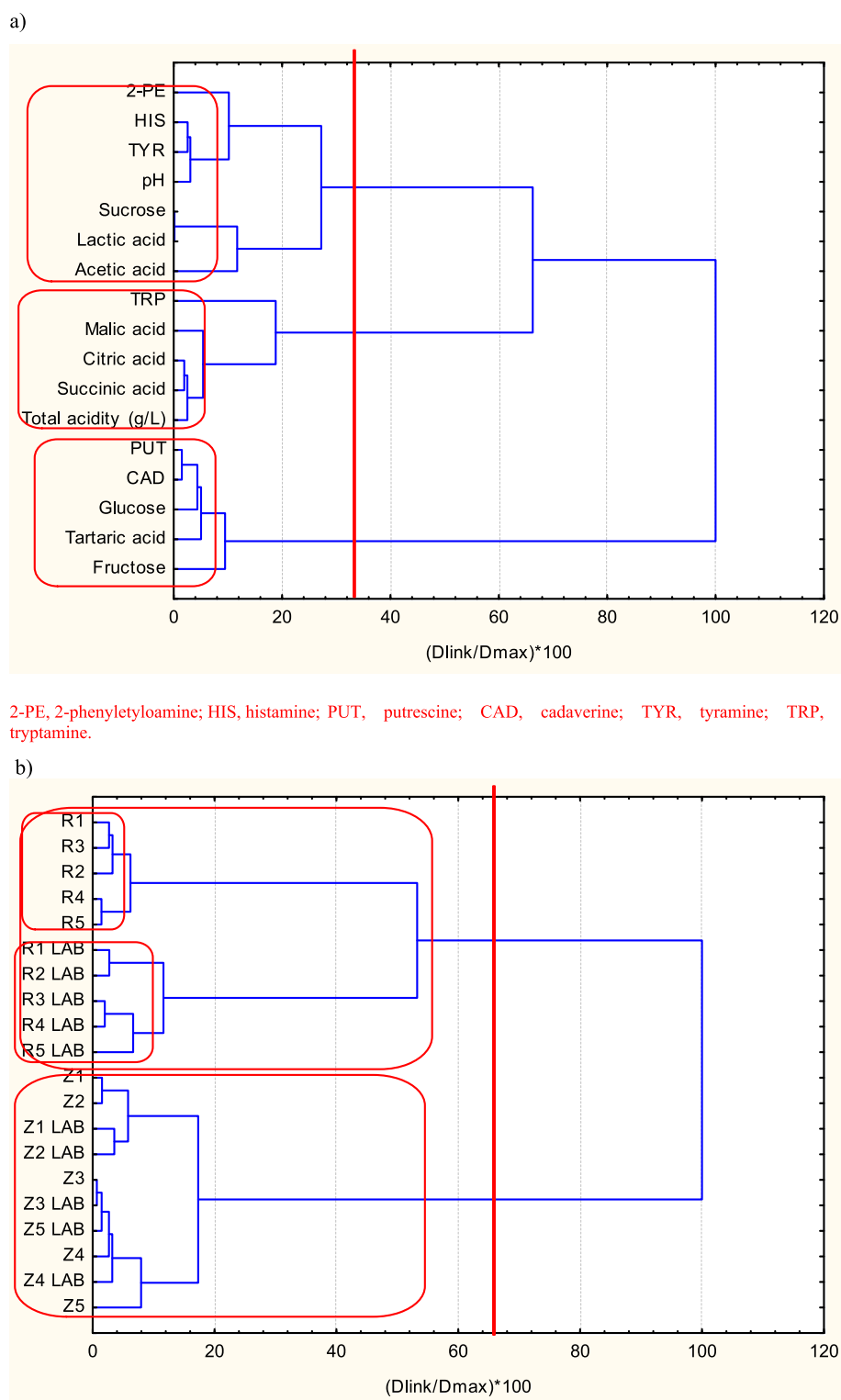


Fig. 1. Hierarchical dendrogram for linkage of 17 variables, (a); Hierarchical dendrogram for linkage of 20 objects, (b).

Sneath's test for cluster significance, and a hierarchical dendrogram as a graphical output. In addition, K-means non-hierarchical clustering of objects was performed. This is a typical supervised pattern recognition method, in which the objects or the variables are grouped into an *a priori* given number of clusters; this number should prove or reject preliminary hypotheses offered by experts or specific preliminary information. Moreover, factor analysis was performed using the Varimax rotation mode. Missing data were replaced by LOD/2 values. This substitution is obligatory for replacing missing data. The software package used was STATISTICA 8.0.

The input data set consisted of 20 objects (samples of wines produced from two grape varieties by different yeast strains, and subjected to induced or spontaneous MLF) described by 17 chemical components or a 20×17 matrix. The components were as follows: biogenic amines determined in this study and sucrose, glucose, fructose, tartaric acid, malic acid, lactic acid, acetic acid, citric acid, succinic acid, pH, and total acidity determined in a previous study (Stój et al., 2020).

3. Results and discussion

3.1. Occurrence of biogenic amines in wine samples

The contents of biogenic amines, such as 2-PE, HIS, PUT, CAD, TRP and TYR in the red wines produced from the two grape varieties tested in this study are shown in Table 1. The most abundant BAs were HIS and PUT. 2-Phenylamine, TYR and TRP were not detected in several of the wines. The concentrations of all the amines determined were many times lower than those reported by several other authors for red wines (Table 2). The content of HIS in the wines tested ranged from 517 to 741 $\mu\text{g/L}$ and was below the limits recommended (but not prescribed by law) in some European countries. Wines produced from the Rondo variety contained higher amounts of all the biogenic amines tested compared to the wines from the same grape variety tested by Plotka-Wasyłka et al. (2018). In the case of wines from the Zweigelt variety, the concentrations of BAs obtained in this study were lower than in a study by Konakovskiy et al. (2011).

3.2. Effect of the fermentation process on biogenic amine contents

Wines produced from the same grape variety (Rondo or Zweigelt) in which AF was carried out by different yeast strains (*S. cerevisiae* or *S. cerevisiae* \times *S. bayanus*) and MLF was spontaneous, differed in the content of biogenic amines. The concentrations of most of the biogenic amines (PUT, CAD and TRP) in the Rondo and Zweigelt wines subjected to spontaneous MLF (coded R1–R5 and Z1–Z5) were higher than in the wines subjected to induced MLF (coded R1 LAB–R5 LAB and Z1 LAB–Z5 LAB) (Table 1). For example, for PUT the results are as follows: 237–405 $\mu\text{g/L}$ for R1–R5, while 186–379 $\mu\text{g/L}$ for R1 LAB–R5 LAB; and 416–489 $\mu\text{g/L}$ for Z1–Z5, while 349–461 $\mu\text{g/L}$ for Z1 LAB–Z5 LAB. Higher contents of TYR were found in those Rondo wines in which spontaneous MLF was carried out (23.11–27.41 $\mu\text{g/L}$ in R1–R5 and 22.65–26.73 $\mu\text{g/L}$ for R1 LAB–R5 LAB). They were not found in detectable concentrations in Zweigelt wines, either those in which spontaneous MLF was carried out or those produced by induced MLF. In turn, lower contents of HIS were determined in Rondo and Zweigelt wines in which MLF was spontaneous (639–717 $\mu\text{g/L}$ for R1–R5 and 668–741 $\mu\text{g/L}$ for R1 LAB–R5 LAB; and 517–633 $\mu\text{g/L}$ for Z1–Z5, while 529–651 $\mu\text{g/L}$ for Z1 LAB–Z5 LAB). The concentrations of 2-PE in Rondo wines subjected to spontaneous MLF were lower compared to wines subjected to induced MLF, and no trend was observed in the concentration of this compound in Zweigelt wines. Other authors obtained similar results. García-Marino et al. (2010) found higher contents of BAs in organic than in non-organic wines, probably due to the fact that MLF occurs spontaneously in organic wines and they have a lower level of SO_2 . According to Marques et al. (2008), the use of commercial malolactic starters in wines caused a reduction in BA levels. BA concentrations were significantly lower in inoculated

wines when compared with non-inoculated wines, probably because MLF was carried out by indigenous malolactic bacteria. Those authors suggested that the application of well-selected malolactic starters could minimize BA production. Similarly, López et al. (2011) highlighted the importance of choosing the optimal *O. oeni* starter for the production of quality wines and controlling the amino acid concentrations in must and wine as a method of preventing the accumulation of biogenic amines.

3.3. Specific correlation between selected parameters of wine samples

Exploratory data analysis using several chemometric methods (hierarchical and non-hierarchical cluster analysis, factor and principal components analysis) was performed to reveal patterns of similarity (clusters) within the data set and to find latent factors responsible for the data structure. The analysis could contribute to better classification, modeling and interpretation of the data included.

3.3.1. Clustering of variables

A hierarchical dendrogram for clustering of the 17 wine sample descriptors (variables) is presented in Fig. 1a. As can be seen, three major clusters were formed, as follows:

- C1: PUT, CAD, Glucose, Tartaric acid, Fructose
- C2: TRP, Malic acid, Citric acid, Succinic acid, Total acidity
- C3: Acetic acid, Lactic acid, Sucrose, pH, TYR, HIS, 2-PE

It could be concluded that the chemical composition of the wine samples is determined by three major factors. The first cluster indicates the impact of sugars on wine composition, the second – the impact of total acidity, and the third – the impact of MLF products.

3.3.2. Clustering of objects

3.3.2.1. *Hierarchical cluster analysis.* Hierarchical cluster analysis of 20 wine samples was carried out under the same conditions as data pre-treatment and analysis. A hierarchical dendrogram for the linkage of 20 objects is presented in Fig. 1b. It is interesting to note that the clustering in Fig. 1b could be interpreted in two different modes:

- Cluster significance at 1/3Dmax (first level of Sneath's test) with three significant clusters:

- C1: (Z1–Z5 and Z1 LAB–Z5LAB)
- C2: (R1LAB–R5LAB)
- C3: (R1–R5)

or

- Cluster significance at 2/3Dmax (Sneath's test) with two significant clusters:

- C1: (Z1–Z5 and Z1 LAB–Z5LAB)
- C2: (R1–R5 and R1 LAB–R5LAB)

The general conclusion is that the wine samples are well separated into two patterns (in the former case, the R pattern is divided into two subclusters) depending on the grape variety: for the R pattern it is the Rondo type and for the Z pattern – the Zweigelt type. Some small sub-clusters, such as (Z1, Z2, Z1LAB, Z2LAB) and (Z3, Z3LAB, Z5LAB, Z4, Z4LAB, Z5) or (R1–R5) and (R1LAB–R5LAB), reflect some minor differences (as insignificant clusters). This additional division could be due to the different yeast strains involved and the type of MLF (with LAB inoculation or without LAB inoculation).

3.3.2.2. *K-means non-hierarchical clustering of objects.* K-means non-hierarchical clustering of objects is a typical supervised pattern recognition method in which objects or variables should be grouped into an

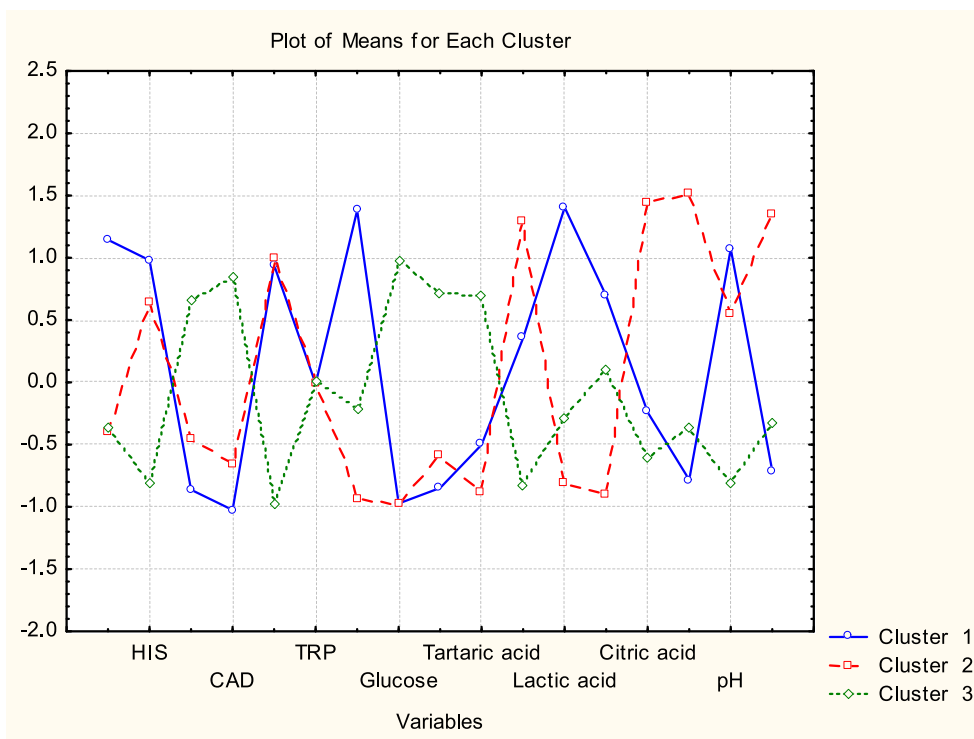


Fig. 2. Plot of means for each variable for each cluster identified (standardized values).



Fig. 3. Two-way joining of objects and variables.

priori given number of clusters. This number should prove or reject preliminary hypotheses offered by experts or specific preliminary information. It was interesting to see whether the hypothesis that there existed three patterns of similarity between the wine samples would be proven by non-hierarchical clustering. The members of each of the identified clusters were:

- C1 (5 members): R1LAB–R5LAB: Rondo wine, induced MLF
- C2 (5 members): R1–R5: Rondo wine, spontaneous MLF

C3 (10 members): Z1–Z5; Z1LAB–Z5 LAB: Zweigelt wine.

It is readily seen that wine type (Rondo wine, Zweigelt wine) is the major descriptor for the samples but, additionally, for Rondo wine, the type of MLF is another specific descriptor. It is very important to investigate if the chemical parameters contribute to this separation of the samples by wine type. The plot of averages for each chemical variable for each of the identified clusters is shown in Fig. 2. The two-way joining of objects and variables is shown in Fig. 3. The separation into

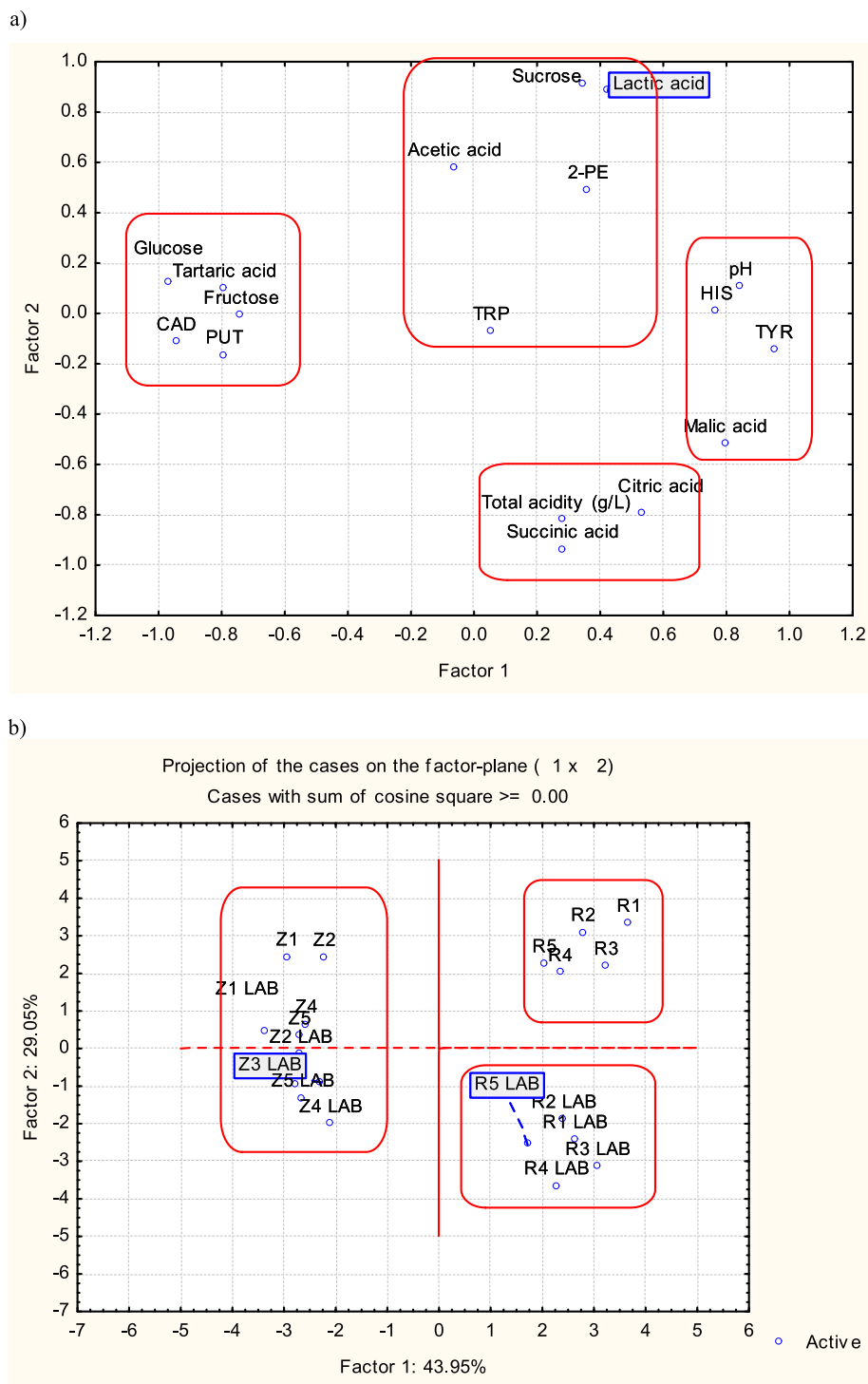


Fig. 4. Factor 1 vs. Factor 2 loadings plot, (a); Factor scores plot for 20 objects, (b).

two parts (conditionally named “red” and “green”) is obvious (it corresponds to the second way of clustering commented upon in the discussion on hierarchical clustering). In general, the two wine types (Rondo type and Zweigelt type) are distinguished by differences in their amine, sugar and organic acid contents.

Cluster 1 (R1LAB–R5LAB) is characterized by the highest levels of 2-PE, HIS, TYR (the same high level as for cluster 2), TRP (all three clusters have the same level of this BA), sucrose, lactic acid and pH. It could be concluded that this wine pattern is specifically described by the levels of three amines (2-PE, HIS, TRP), one organic acid (lactic acid) and pH, i.e.

it is predominantly described by amine characteristics and less by the impact of acidity. This pattern could be conditionally named “amine wine type” (probably due to induced MLF). It should be mentioned that another group of characteristics for this cluster are the lowest levels of PUT, CAD, glucose, succinic acid, total acidity (of note, pH and total acidity are negatively correlated). Pattern 2 (R1–R5) indicates four descriptors with specifically high levels: malic acid, citric acid, succinic acid and total acidity, and the lowest levels of sucrose, glucose, tartaric acid, lactic acid and acetic acid. This cluster is a conditional “acidic wine type” with a lower sugar content (probably due to spontaneous MLF).

Cluster 3 includes all Zweigelt wine samples (marked as Z samples). The specific descriptors are marked by the highest levels of the amines PUT and CAD, the sugars glucose and fructose, and one acid–tartaric acid. Cluster 3 wines have the lowest levels of TYR, malic acid, citric acid and pH. It could be assumed that they represent a conditional “mixed wine type”.

3.3.3. Factor analysis (Varimax rotation mode)

The factor loadings of three latent factors are shown in Table S3. They explain over 85 % of the total variance of the system and could contribute to a better interpretation of the data structure. The first latent factor, which explains 43.27 % of the total variance, shows the relationships between variables with high positive loadings (HIS, TYR, malic acid, pH), on the one hand, and the negatively associated variables (CAD, PUT, glucose, fructose, tartaric acid), on the other. A tentative name for this mixed factor, typical of the Zweigelt type wine samples, could be “sugar/amine”. The second latent factor describes 27.81 % of the total variance with high positive factor loadings (sucrose, lactic acid, acetic acid) and high negative loadings (citric acid, succinic acid and total acidity). This factor, characteristic of the R1–R5 pattern of wine samples, could be conditionally named “acidity”. The third latent factor, which explains another 14.38 % of the total variance, is typical of R1LAB–R5LAB wine samples and could be conditionally named “amine”.

A graphic plot of the factor loadings display for factors 1 and 2 is presented in Fig. 4a. It describes the data in Table S3 completely.

A principal components plot for the factor scores (the new coordinates of the system after introduction of the new latent factors) is shown in Fig. 4b. The three identified patterns of object similarity are seen.

All the exploratory data analysis methods applied confirm the formation of three patterns of similarity between the wine samples and the formation of three patterns of similarity between the chemical variables related (as specific descriptors) to the objects’ patterns.

4. Conclusions

This is the first study to evaluate the biogenic amine content of red wines produced with several commercial yeast strains of *S. cerevisiae* or *S. cerevisiae* × *S. bayanus*. The results show that the yeast strain and the type of MLF (spontaneous or induced) affect the content of biogenic amines in wines. BA contents, except for HIS, were lower in wines made by sequential inoculation of yeast and LAB. More research is needed on coinoculated wines. In addition, it is worth looking for other strains of *O. oeni* that produce less HIS. Chemometric analysis allowed us to determine some relationships between the parameters characterizing the wine samples. The general conclusion of the chemometric analysis is that the wine samples are well separated into two groups depending on the grape variety. It is readily seen that the wines have different characteristics depending on the grape used for production (Rondo wine, Zweigelt wine). Additionally, for Rondo wine, the type of MLF is another specific parameter that affects the characteristics of the wine.

The Rondo wine which was produced using induced MLF was characterized by the highest levels of 2-PE, HIS, TYR, and this was also visible for the Rondo wines produced with the application of spontaneous MLF. It could be concluded that the profile of wine is also specifically described by lactic acid and pH, i.e. it is described predominantly by amine characteristics and less by the impact of acidity. Rondo wines produced with the application of spontaneous MLF were characterized by high levels of malic acid, citric acid, succinic acid, a high total acidity and the lowest levels of sucrose, glucose, tartaric acid, lactic acid and acetic acid. All Zweigelt wines were characterized by the highest levels of PUT and CAD, glucose and fructose, and tartaric acid. They contained the lowest levels of TYR, malic acid, and citric acid and had the lowest pH. It can be concluded that the application of specific winemaking conditions may help obtain the desired

characteristics of wine.

CRediT authorship contribution statement

Anna Stój: Validation, Resources, Writing – original draft, Writing – review & editing. **Justyna Plotka-Wasyłka:** Conceptualization, Methodology, Software, Validation, Resources, Writing – original draft, Writing – review & editing, Supervision. **Vasil Simeonov:** Methodology, Software, Writing – original draft, Writing – review & editing. **Magdalena Kaplan:** .

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2021.131172>.

References

- Arrieta, M. P., & Prats-Moya, M. S. (2012). Free amino acids and biogenic amines in Alicante Monastrell wines. *Food Chemistry*, *135*(3), 1511–1519.
- Bartowsky, E. J., Costello, P. J., & Chambers, P. J. (2015). Emerging trends in the application of malolactic fermentation. *Australian Journal of Grape and Wine Research*, *21*, 663–669.
- Capozzi, V., Russo, P., Ladero, V., Fernández, M., Fiocco, D., Alvarez, M. A., ... Spano, G. (2012). Biogenic amines degradation by *Lactobacillus plantarum*: Toward a potential application in wine. *Frontiers in Microbiology*, *3*, 122.
- Costantini, A., Vaudano, E., Pulcini, L., Carafa, T., & Garcia-Moruno, E. (2019). An overview on biogenic amines in wine. *Beverages*, *5*(1), 19.
- Del Prete, V., Costantini, A., Cecchini, F., Morassut, M., & Garcia-Moruno, E. (2009). Occurrence of biogenic amines in wine: The role of grapes. *Food Chemistry*, *112*(2), 474–481.
- Esposito, F., Montuori, P., Schettino, M., Velotto, S., Stasi, T., Romano, R., & Cirillo, T. (2019). Level of biogenic amines in red and white wines, dietary exposure, and histamine-mediated symptoms upon wine ingestion. *Molecules*, *24*(19), 3629.
- García-Marino, M., Trigueros, Á., & Escribano-Bailón, T. (2010). Influence of oenological practices on the formation of biogenic amines in quality red wines. *Journal of Food Composition and Analysis*, *23*(5), 455–462.
- García-Ruiz, A., González-Rompinelli, E. M., Bartolomé, B., & Moreno-Arribas, M. V. (2011). Potential of wine-associated lactic acid bacteria to degrade biogenic amines. *International Journal of Food Microbiology*, *148*(2), 115–120.
- Guo, Y.-Y., Yang, Y.-P., Peng, Q., & Han, Y. (2015). Biogenic amines in wine: A review. *International Journal of Food Science & Technology*, *50*(7), 1523–1532.
- Henríquez-Aedo, K., Durán, D., García, A., Hengst, M. B., & Aranda, M. (2016). Identification of biogenic amines-producing lactic acid bacteria isolated from spontaneous malolactic fermentation of Chilean red wines. *LWT-Food Science and Technology*, *68*, 183–189.
- Cañas, P. M., Pérez-Martín, F., Romero, E. G., Prieto, S. S., & Herrerros, M. d. L. L. P. (2012). Influence of inoculation time of an autochthonous selected malolactic bacterium on volatile and sensory profile of Tempranillo and Merlot wines. *International Journal of Food Microbiology*, *156*(3), 245–254.
- Jabłońska-Ryś, E., Sławińska, A., Stachniuk, A., & Stadnik, J. (2020). Determination of biogenic amines in processed and unprocessed mushrooms from the Polish market. *Journal of Food Composition and Analysis*, *92*, 103492. <https://doi.org/10.1016/j.jfca.2020.103492>.
- Jerome, A., Kovačević Ganić, K., Mihaljević Žulj, M., Maslek, M., Puhelek, I., & Jagatić Korenika, A. M. (2018). Biogenic amine production during spontaneous and inoculated MLF of Zweigelt Wines. *Agriculturae Conspectus Scientificus*, *83*(3), 239–242.
- Liu, Y., Han, F., Liu, Y., & Wang, W. (2020). Determination of biogenic amines in wine using modified liquid-liquid extraction with high performance liquid chromatography-fluorescence detector. *Food Analytical Methods*, *13*(4), 911–922.
- López R., López-Alfaro I., Gutiérrez A.R., Tenorio C., Garjjo P., González-Arenzana L., & Santamaría P. (2011). Malolactic fermentation of Tempranillo wine: contribution of the lactic acid bacteria inoculation to sensory quality and chemical composition. *International Journal of Food Science & Technology*, *46*, 2373–2381.
- Lorenzo, C., Bordiga, M., Pérez-Álvarez, E. P., Travaglia, F., Arlorio, M., Salinas, M. R., ... Garde-Cerdán, T. (2017). The impacts of temperature, alcoholic degree and amino acids content on biogenic amines and their precursor amino acids content in red wine. *Food Research International*, *99*, 328–335.
- Konakovskiy, V., Focke, M., Hoffmann-Sommergruber, K., Schmid, R., Scheiner, O., Moser, P., ... Hemmer, W. (2011). Levels of histamine and other biogenic amines in high-quality red wines. *Food Additives and Contaminants*, *28*(4), 408–416.

- Manetta, A. C., Di Giuseppe, L., Tofalo, R., Martuscelli, M., Schirone, M., Giammarco, M., & Suzzi, G. (2016). Evaluation of biogenic amines in wine: Determination by an improved HPLC-PDA method. *Food Control*, *62*, 351–356.
- Marques, A. P., Leitão, M. C., & San Romão, M. V. (2008). Biogenic amines in wines: Influence of oenological factors. *Food Chemistry*, *107*(2), 853–860.
- Martuscelli, M., Arfelli, G., Manetta, A. C., & Suzzi, G. (2013). Biogenic amines content as a measure of the quality of wines of Abruzzo (Italy). *Food Chemistry*, *140*(3), 590–597.
- Massart, D. L., & Kaufman, L. (1983). *The interpretation of analytical chemical data by the use of cluster analysis*. Amsterdam, The Netherlands: Elsevier.
- Palomino-Vasco, M., Rodríguez-Cáceres, M. I., Mora-Díez, N., Pardo-Botello, R., & Acedo-Valenzuela, M. I. (2019). Biogenic amines profile in red wines regarding aging and storage conditions. *Journal of Food Composition and Analysis*, *83*, 103295. <https://doi.org/10.1016/j.jfca.2019.103295>.
- Piasta, A. M., Jastrzębska, A., Krzemiński, M. P., Muziol, T. M., & Szlyk, E. (2014). New procedure of selected biogenic amines determination in wine samples by HPLC. *Analytica Chimica Acta*, *834*, 58–66.
- Pineda, A., Carrasco, J., Peña-Farfal, C., Henríquez-Aedo, K., & Aranda, M. (2012). Preliminary evaluation of biogenic amines content in Chilean young varietal wines by HPLC. *Food Control*, *23*(1), 251–257.
- Plotka-Wasyłka, J., Simeonov, V., Morrison, C., & Namieśnik, J. (2018). Impact of selected parameters of the fermentation process of wine and wine itself on the biogenic amines content: Evaluation by application of chemometric tools. *Microchemical Journal*, *142*, 187–194.
- Plotka-Wasyłka, J., Simeonov, V., & Namieśnik, J. (2016). An in situ derivatization – dispersive liquid–liquid microextraction combined with gas-chromatography – mass spectrometry for determining biogenic amines in home-made fermented alcoholic drinks. *Journal of Chromatography A*, *1453*, 10–18.
- Restuccia, D., Loizzo, M. R., & Spizzirri, U. G. (2018). Accumulation of biogenic amines in wine: Role of alcoholic and malolactic fermentation. *Fermentation*, *4*(1), 6.
- Ruiz P., Izquierdo P.M., Seseña S., García E., & Palop M.L. (2012). Malolactic fermentation and secondary metabolite production by *Oenococcus oeni* strains in low pH wines. *Journal of Food Science*, *77*, M579–M585.
- Smit, A. Y., Du Toit, W. J., & Du Toit, M. (2008). Biogenic amines in wine: Understanding the headache. *South African Journal of Enology and Viticulture*, *29*(2), 109–127.
- Soufleros, E. H., Bouloumpasi, E., Zotou, A., & Loukou, Z. (2007). Determination of biogenic amines in Greek wines by HPLC and ultraviolet detection after dansylation and examination of factors affecting their presence and concentration. *Food Chemistry*, *101*(2), 704–716.
- Souza, S. C., Theodoro, K. H., Souza, É. R., Motta, S. D., & Glória, M. B. A. (2005). Bioactive amines in Brazilian wines: Types, levels and correlation with physico-chemical parameters. *Brazilian Archives of Biology and Technology*, *48*(1), 53–62.
- Stadnik, J., & Dolatowski, Z. J. (2012). Biogenic amines content during extended ageing of dry-cured pork loins inoculated with probiotics. *Meat Science*, *91*(3), 374–377.
- Stój, A., Kapusta, I., & Domagała, D. (2020). Classification of red wines produced from Zweigelt and Rondo grape varieties based on the analysis of phenolic compounds by UPLC-PDA-MS/MS. *Molecules*, *25*(6), 1342. <https://doi.org/10.3390/molecules25061342>.
- Tuberoso, C. I. G., Congiu, F., Serrelli, G., & Mameli, S. (2015). Determination of dansylated amino acids and biogenic amines in Cannonau and Vermentino wines by HPLC-FLD. *Food Chemistry*, *175*, 29–35.
- Wang, Y.-Q., Ye, D.-Q., Zhu, B.-Q., Wu, G.-F., & Duan, C.-Q. (2014). Rapid HPLC analysis of amino acids and biogenic amines in wines during fermentation and evaluation of matrix effect. *Food Chemistry*, *163*, 6–15.
- Woźniakiewicz, M., Woźniakiewicz, A., Nowak, P. M., Kłodzińska, E., Namieśnik, J., & Plotka-Wasyłka, J. (2018). CE-MS and GC-MS as “green” and complementary methods for the analysis of biogenic amines in wine. *Food Analytical Methods*, *11*(9), 2614–2627.