Increasing amino acids and biogenic amines content of white and rosé wines during ageing on lees

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Abstract. The presence of biogenic amines in wine is more and more important both to consumers and producers alike, due to the potential threats of toxicity of humans and consequent trade implications. Biogenic amines are formed from amino acids by decarboxylation carried out by various enzymes located in yeasts and bacteria. During ageing of wines on lees, the release of different compounds, especially proteins, peptides, amino acids, breakdown products of yeasts, can impact on the quality of wine. The aim of this study is to describe the evolution of twenty-two amino acids, precursors of seven biogenic amines during ageing on lees for 12 months, respectively 18 months, using 12 commercial maturation products. The number of experimental samples is 26 (V1SB-V13SB, V1BB-V13BB), produced in Iasi vineyard, vintage 2020, from Sauvignon Blanc and Busuioaca de Bohotin grapes variety. Data indicated a major impact of the variables (commercial maturation products, autolysis process and grape variety) on wine's characteristics. Considerable amounts of some essential amino acids, such as L-alanine, L-leucine, L-lysine, L-valine and L-glutamic acid were found in samples treated with commercial products, which contained significant amounts of mannoproteins, amino acids and vitamins (samples V6SB, V4SB, V5SB, V5BB, V4BB and V6BB). Vitamins and nitrogenous compounds released by autolysis are used as a support in the decarboxylation process, thus forming biogenic amines.

1 Introduction

Sur lie wines are obtained by a traditional winemaking technique used in many countries, which involves the contact of wine with lees for a certain period of time. Typical yeast-aged wines are Muscadet wines from the Loire Valley in France, wines from the Gulf of Lion region, Pays d'Oc wines and famous Burgundy wines, as well as wines from Italy, California, Australia and South Africa [1].

The purpose of sur lie is usually based on wine complexity, increasing the aromatic complexity and adding extra body, enhancing the mouth feel and structure of wine [2].

During ageing on lees, the process of autolysis of the yeasts takes place. Yeast cells autolyse at the end of the alcoholic fermentation. Yeast autolysis is a slow process, carried out under the action of hydrolytic enzymes that are release from the cytoplasm (fatty acids, peptides, amino acids, nucleotides) but also cell walls (mannoproteins) into the wine [3].

Nitrogenous substances present in wine include compounds that contain nitrogen in a variety of forms. The dietary intake of nitrogenous substances present in grapes, must and wine is not significant, but it is of oenological importance in that they are a source of nitrogen necessary for the microflora involved in fermentation processes.

These substances have the ability to influence the taste characteristics of wine products, but they can significantly contribute to the quality of sparkling wines, by improving the quality of the foam.

Nitrogenous substances fall into two categories: inorganic nitrogenous substances and organic nitrogenous substances. In the case of nitrogen-containing organic substances, they are divided into substances in which nitrogen is part of the amino functional group (-NH2) and other substances containing nitrogen in other functional groups. Amines, proteins, proteins and amino acids are organic substances that have nitrogen in the amino functional group (-NH2) [4].

The amino acids in wine are initially derived from grapes, then released from yeasts during alcoholic fermentation and appear as a result of the autolysis process. In addition, grape-derived proteins are degraded into peptides and amino acids by enzymatic hydrolysis [5].

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The main amino acid in must is proline and it cannot be metabolized by yeasts in the fermentation process carried out under anaerobic conditions.

Yeasts have the ability to provide their amino acid requirements through direct assimilation, when they are present in wine or through a reamination reaction of those present in the must [6].

The presence of biogenic amines in food and wines is becoming increasingly important to both, consumers and producers due to potential threats of toxicity to humans and subsequent commercial implications.

Biogenic amines, in addition to polyphenols, are used as markers to prove the authenticity of wines. They are present in the form of salts and in a free state, becoming reactive with other compounds responsible for the aroma of the wine, thus having direct implications on the sensory profile, such as the loss of varietal character and the imprinting of unpleasant odours such as the smell of mould, of spoiled fish.

Many biogenic amines are important for the normal functioning of biological systems. In eukaryotic cells they are important precursors with a role as neurotransmitters, and others such as spermidine and putrescine are involved in critical biological functions. However, their presence in high concentrations in food and alcoholic beverages can be toxic. High concentrations of biogenic amines can cause headaches, nausea, hot flashes, heart palpitations and changes in blood pressure, and many other physiological problems [7].

Histamine and tyramine are considered the most toxic and relevant for food safety, cadaverine and putresceine potentiate negative effects by inhibiting enzymes involved in histamine detoxification [5].

Biogenic amines ingested from food are detoxified by amine oxidase enzymes present in the gastrointestinal tract and intestinal mucosa. Under normal conditions, ingested amines are metabolized by oxidation or conjugation reactions in the presence of amine oxidase enzymes: monamine oxidase (MAO), diamine oxidase (DAO), polyamine oxidase (PAO) and the enzyme N-methyltransferase (MOA).

Monitoring the level of biogenic amines in wines can be an important marketing advantage. A large data set such as vineyard, climatic conditions, terroir, harvest year, winemaking technology applied and oenological practices are needed to establish biogenic amine profiles as a wine signature and provide a scientific basis in quality control and safety in the winemaking process. In recent years, winemakers are making great efforts to reduce the content of biogenic amines and to improve the quality and safety of wines [4].

2 Material and methods

2.1 Samples and winemaking process

In order to conduct this study, grapes of Sauvignon blanc and Busuioaca de Bohotin varieties were harvested at the technological maturity from Iasi vineyard. Were obtained 26 experimental samples by adding 12 commercial products in different doses used in wine ageing on lees, as follows:

- V1S/V1B (4.02 g product/ 10 L wine);
- V2S/V2B (4.02 g product/ 10 L wine);
- V3S/V3B (4.02 g product/ 10 L wine);
- V4S/V4B (6.01 g product/ 10 L wine);
- V5S/V5B (4.02 g product/ 10 L wine);
- V6S/V6B (2.01 g product/ 10 L wine);
- V7S/V7B (2.01 g product/ 10 L wine);
- V8S/V8B (8.02 g product/ 10 L wine);
- V9S/V9B (3.01 g product/ 10 L wine);
- V10S/V10B (4.02 g product/ 10 L wine);
- V11S/V11B (3.01 g product/ 10 L wine);
- V12S/V12B (4.02 g product/ 10L wine);
- V13S/V13B (no inactive addition).

Determinations of amino acids and biogenic amines were made after 12 months of maturation in glass bottles.

2.2 Methods used in the identification and quantification of biogenic amines and amino acids

The identification and quantification of amino acids and biogenic amines is carried out by using an UltiMateTM 3000 UHPLC chromatographic system (Thermo Scientific TM, Waltham, Massachusetts, USA), equipped with a pump system consisting of 2 pumps: the first is a quaternary UltiMateTM 3000 type, with a pressure of 1000bar, and the second pump is a binary UltiMateTM LPG-3400RS type, also with a pressure of 1000bar; thermostatic compartment of the UltiMateTM TCC-3000RS column, PAL LX sample auto injection system, which allows the analysis of biogenic amines and amino acids at a maximum temperature of 110 °C, in addition, the system is equipped with an interchangeable valve structure (VIM) [5].

A Kinetex C18 column with a length of 150 mm, internal diameter of 2.1 mm and a particle size of 1.7 μm was used for the separation of the compounds.

The separation of amino acids and biogenic amines was carried out under the following conditions: passing through a mobile phase consisting of two solutions that eluted in a gradient manner. Mobile phase A (FMA) consists of a mixture of 0.1% heptafluoroaminobutyric acid in water and mobile phase B (FMB) consists of a concentration of 0.1% heptafluoroaminobutyric acid in methanol [8].

During the identification of the amino acids included in the analysis, the concentrations of the two mobile phases varied, the elution was of the gradient type. Briefly, elution started with mobile phase A (MFA) and mobile phase B (MFB) mixture of 85:15% (V/V) for 2 minutes, then for another 3 minutes the composition was changed to 73% MFA and then to 50%MFA in another 4 minutes. For 2 minutes the elution was isocratic and re-equilibration in 3.5 minutes.

2.3 Statistical analyses

Data analysis - Statistical data analysis was performed using the analysis of variance (ANOVA) of Statistical V.7 software (Stat soft Inc.). Afterwards, we performed the multifactorial analysis between the composition of some oenological maturation products and the content of amino acids and biogenic amines in the experimental samples and applied the Fisher LSD test.

The Fisher LSD test was applied to be able to make a correction for multiple comparisons, it calculated the standard deviations extracted from each group included in the study according to the grouping criteria: maturation time and the type of chemical substances released from the oenological product during the ageing on lees period.

3 Results and Discussion

3.1 Biogenic amines of analysed samples

Biogenic amines are mostly formed from precursor amino acids in the presence of various wine microorganisms during alcoholic fermentation, yeast maturation, ageing and storage stages of wines.

Biogenic amines were identified and quantified after 12 months, respectively 18 months the addition of different dosages of the above-mentioned commercial products: ethanolamine, phenethylamine, histamine, tyramine, spermidine, cadaverine and putrescine.

Table 1 shows the content of biogenic amines in wines obtained from Sauvignon blanc produced at the Iasi-Copou vineyard, after ageing on lees 12 months, respectively 18 months in glass bottles.

Table 1. Biogenic amines identified in experimental samples obtained from Sauvignon Blanc, vintage 2020.

Nr.	Biogenic amines	mg/L												
		12 months												
		V1S	V2S	V3S	V4S	V5S	V6S	V7S	V8S	V9S	V10S	V11S	V12S	V13S
1	Ethanolamine	8,62	11,32	10,37	8,79	9,93	11,95	9,07	10,49	12,13	12,66	8,76	10,39	10,40
2	Phenethylamine	0,19	0,19	0,19	0,16	0,18	0,19	0,2	0,21	0,15	0,14	0,16	0,15	0,19
3	Histamine	0,32	0,61	0,37	0,3	0,85	0,35	0,26	0,18	0,21	0,17	0,09	0,28	0,23
4	Tyramine	9,18	9,37	8,33	7,76	8,62	11,4	8,32	8,08	8,6	7,9	9,14	8,80	9,17
5	Spermidine	1,38	1,46	1,59	1,59	1,36	1,74	1,52	1,42	1,26	1,25	1,33	1,38	1,38
6	Cadaverine	0,41	0,22	0,30	0,38	0,23	0,38	0,38	0,37	0,26	0,34	0,35	0,44	0,24
7	Putrescine	3,78	6,24	7,61	7,58	5,77	9,65	7,12	7,20	7,52	5,10	7,62	8,22	7,88
		18 months												
		V1S	V2S	V3S	V4S	V5S	V6S	V7S	V8S	V9S	V10S	V11S	V12S	V13S
1	Ethanolamine	8,97	9,50	10,66	11,35	11,23	14,25	13,63	13,85	14,18	13,27	12,09	12,62	10,47
2	Phenethylamine	0,84	0,80	0,69	0,72	0,71	0,76	0,66	0,71	0,70	0,79	6,13	0,68	0,64
3	Histamine	0,58	0,56	0,59	0,54	0,42	0,49	0,53	0,47	0,55	0,54	0,62	0,65	0,59
4	Tyramine	17,02	20,34	19,78	20,78	16,36	10,54	12,00	18,73	19,80	19,28	19,80	21,34	18,64
5	Spermidine	1,28	1,18	1,28	1,51	1,38	1,32	1,6	1,53	1,54	1,42	1,27	1,46	1,32
6	Cadaverine	0,56	0,67	0,69	0,72	0,63	0,44	0,34	0,85	0,66	0,47	0,54	0,87	0,72
7	Putrescine	27,96	26,97	30,38	27,07	25,24	13,08	15,08	30,09	32,14	25,80	27,85	30,6	31,69

Ethanolamine is an organic compound, from a chemical point of view it represents a primary amine, but also a primary alcohol because it contains a hydroxyl and an amino group. The biosynthesis of ethanolamine is made from the amino acid L-serine, through the decarboxylation process It appears as a viscous, colourless liquid compound with an odour like ammonia. Recent studies have confirmed the presence of ethanolamine, ethylamine and putresceine in grapes. In the case of the experimental samples obtained from the Sauvignon blanc variety, the ethanolamine content varied from 8.62 mg/L (sample V1SB) to 12.66 mg/L (sample V10SB) after 12 months of maturation on lees, registering an increase in all samples during the following 6 months of maturation on yeasts compared to the control sample, V13SB, values that varied from 8.97 mg/L (V1SB) to 14.18 mg/L (V9SB).

The samples with the highest ethanolamine content are: V6SB (14.25 mg/L), V9SB (14.18 mg/L), V8SB (13.85 mg/L), V7SB (13.63 mg/L) and V10SB (13.27 mg/L). It should be noted that these samples are the ones that also recorded high values of the L-serine content, after the 12 months of ageing on lees, so in the following 6 months the enzymatic process of decarboxylation of the precursor amino acid L-serine took place, right therefore the ethanolamine content increased.

Tyramine is a biogenic amine, widely distributed in nature, which is found in a fungal species parasitic on cereals, more precisely in the rye horn. It is derived from the precursor amino acid L-tyrosine through the decarboxylation process.

The enzyme responsible for the decarboxylation of L-tyrosine is tyrosine-decarboxylase (TDC) present in bacteria, it was researched and isolated from the species *Enterococcus faecalis* later being also isolated from bacteria belonging to the genus *Lactobacillus*, strain *Lactobacillus brevis IOEB 9809* [13].

In the experimental samples obtained from the Sauvignon blanc variety, tyramine had values between 11.4 mg/L recorded in the V6SB sample and 7.76 mg/L in the V4SB sample, after 12 months of ageing on lees.

After 18 months, a large increase in tyramine content is observed, the highest values were recorded in samples V12SB (21.34 mg/L), V4SB (20.78 mg/L), V2SB (20.34 mg/L), V3SB (19.78 mg/L) compared to the control sample V13SB (18.64 mg/L). This increase is correlated with the decrease in the content of L-phenylalanine, so the TDC enzyme did not participate in the decarboxylation of the precursor amino acid and not L-tyrosine which registered quantitative increases in the following time interval [14].

Putrescine is a diamine, also called tetramethylenediamine, with an unpleasant odour. Researchers have several hypotheses regarding the origin of this biogenic amine. Some of them reported that it is produced by a strain of Oenococcus oeni bacteria, specifically *Oenococcus oeni IOEB 8419*, isolated from red wine (Marcobal A. et al., 2004). Later, following the studies, the existence of seven strains of *O. oeni* out of 44 taken in the study, responsible for the production of putrescine in the culture medium, was reported [11].

In experimental samples of Sauvignon blanc, high values of putrescein content were recorded after 18 months of maturation on lees, which varied between 31.69 mg/L (V13SB) and 13.08 mg/L (V6SB). The highest values were in the case of the following samples: V12SB (30.6 mg/L), V3SB (30.38 mg/L) and V8SB (30.09 mg/L), which was correlated with the large amounts of the precursor amino acid L-arginine during the first 12 months of ageing on lees, after which a decrease was recorded in all samples after another 6 months of ageing on lees.

So, in the case of experimental samples obtained from the Sauvignon blanc variety, the large amounts of putresceine recorded were possible through the decarboxylation of L-arginine, at a wine pH close to value 3. Thus, we can also speak of a complex system of enzymes, which converted arginine to putresceine, consisting of three active enzymes present in the structure of bacteria, arginine-deiminase (ADI), ornithine-transcarboxylase (OTC), carbamate-kinase (CK) involved in the decarboxylation of precursor amino acids [12].

Table 2. Biogenic amines identified in experimental samples obtained from Busuioaca de Bohotin, vintage 2020.

Nr.	Biogenic amines	mg/L												
		12 months												
		V1B	V2B	V3B	V4B	V5B	V6B	V7B	V8B	V9B	V10B	V11B	V12B	V13B
1	Ethanolamine	6,33	8,30	8,08	7,49	13,5	7,59	8,03	6,91	9,64	6,75	7,74	5,27	6,87
2	Phenethylamine	0,19	0,18	0,19	0,2	0,3	0,17	0,18	0,18	0,16	0,14	0,18	0,18	0,16
3	Histamine	0,74	0,85	0,88	0,85	1,16	0,99	0,79	0,76	0,21	0,95	0,8	0,86	1,07
4	Tyramine	9,81	9,48	9,71	9,62	13,9	9,86	9,51	9,02	8,98	10,5	10,3	0,98	10,43
5	Spermidine	0,89	1,66	1,29	1,91	3,27	1,12	1,18	0,76	1,13	1,12	1,17	1,43	0,90
6	Cadaverine	0,42	0,38	0,4	0,2	0,31	0,25	0,31	0,46	0,38	0,33	0,43	0,45	0,40
7	Putrescine	7,3	7,00	7,32	7,45	10,9	7,59	7,35	7,73	7,32	6,68	7,74	7,54	8,31
		18 months												
		V1B	V2B	V3B	V4B	V5B	V6B	V7B	V8B	V9B	V10B	V11B	V12B	V13B
1	Ethanolamine	20,02	19,83	19,41	5,05	4,34	2,74	2,57	3,2	2,88	4,63	5,64	4,13	5,77
2	Phenethylamine	0,86	0,77	0,81	0,38	0,68	0,73	0,68	1,02	0,80	0,77	0,74	0,78	0,80
3	Histamine	1,79	0,24	2,4	1,54	2,19	2,6	2,31	3,05	2,91	2,58	2,68	2,74	2,64
4	Tyramine	22,84	20,4	19,81	14,33	17,5	13,1	17,4	18,12	20,43	19,9	21,1	17,38	18,92
5	Spermidine	1,59	1,36	1,02	2,53	2,4	2,43	2,41	3,4	2,15	2,99	2,75	3,24	2,88â
6	Cadaverine	0,65	0,6	0,62	0,5	0,28	0,21	0,36	0,64	0,45	0,50	0,31	0,21	0,38â
7	Putrescine	25,85	30,2	42,58	43,7	52,7	31,2	38,6	38,75	42,72	38,8	41,9	34,71	34,3

Table 2 shows the content of biogenic amines in wines obtained from Busuioacă de Bohotin produced at the Iasi-Copou vineyard, after ageing on less 12 months, respectively 18 months in glass bottles.

Ethanolamine appears in wines following the decarboxylation process of the precursor amino acid, L-serine. In the case of the experimental samples obtained from the Busuioacă de Bohotin variety, the values varied between 5.27 mg/L in the V12BB sample and 13.5 mg/L in the V5BB sample, after the 12 months of maturation on the lees. Sample V5BB was treated with a product containing yeast walls and a large amount of mannoproteins.

After the 18 months of ageing on lees, the ethanolamine values decreased in the case of all the samples, but the values of three samples were an exception: samples V1BB with 20.02 mg/L, V2BB with 19.83 mg/L and V3BB with 19.41 mg/L. All these samples were treated with products that are rich in proteins, that's why the amount of ethanolamine increased during the ageing on lees stage. Sample V5BB registered a decrease, reaching a value of 4.34 mg/L ethanolamine.

During ageing on lees and implicit autolysis process, the physico-chemical composition of the experimental samples changes, as a consequence of the hydrolysis of different molecules in the cell wall. One of the enzymatic processes is proteolysis, in which proteins are hydrolyzed into peptides and amino acids, and these pass from the yeast cell wall into the wine. When the bacteria run out of nutrients, which is typical for the maturation stage, they use amino acids for the energy they generate through decarboxylation reactions.

Prolonged contact with yeasts leads to the release of biogenic amines into wine [15].

Tyramine is formed from the precursor amino acid L-tyrosine by an enzymatic decarboxylation reaction.

In the experimental samples inserted in Table 2, the highest values of the biogenic amine tyramine were in the case of samples V5BB of 13.9 mg/L, V10BB of 10.5 mg/L, V11BB of 10.3 mg/L, all recorded after 12 months of maturation on yeasts, but which increased during the following 6 months, 17.5 mg/L in V5BB, 19.9 mg/L in V10BB and 21.1 mg/L in sample V11BB.

After 18 months of ageing on lees, samples V1BB (22.84 mg/L), V11BB (21.1 mg/L) and V2BB (20.4 mg/L) recorded the highest tyramine values. The increases are attributed to various hydrolysis and decarboxylation reactions of precursor compounds that occur following enzymatic autolysis.

Spermidine is of major importance in plant physiological processes. Spermidine values in the Bohotin Basil samples ranged between 3.27 mg/L in the V5BB sample and 0.76 mg/L in the V8BB sample after the 12-month time interval. The highest value was in the V5BB sample, treated with an oenological product that has a large number of mannoproteins in its composition, thus explaining the increase in the content of biogenic amines. After another 6 months of ageing on lees, spermidine registers the highest value in sample V8BB, 3.4 mg/L, and the lowest in sample V3BB, 1.02 mg/L. So, through the processes of hydrolysis and decarboxylation of nitrogenous compounds during proteolysis, a slight increase in biogenic amines is achieved in wines.

Quantitatively and qualitatively, putresceine is found in large quantities in wines. Concentrations of up to 200 mg/L have been found in red wines after the malolactic fermentation stage [16].

In the case of the experimental samples obtained from the Bosuioacă de Bohotin grape variety, the putresceine values varied between 10.9 mg/L in the V5BB sample and 8.31 mg/L in the V13BB sample, after the 12 months of maturation on lees in glass bottles. A large increase in putrescein content is observed after another 6 months of ageing on lees, the highest values were recorded in the samples: V5BB with a content of 52.7 mg/L, V4BB with 43.7 mg/L, V9BB with 42.72 mg/L, V11BB with 41.9 mg/L and V10BB with 38.8 mg/L.

By prolonging the contact with yeasts, there is a release of amines from yeast cells through enzymatic autolysis, a fact that was also noted by other studies in the oenological field [17,18]. Further research reported considerable increases in putrescein and tyramine content in Chardonnay and Pinot noir wines stored on lees in glass bottles. The increase in the amounts of biogenic amines is due to the enzymatic activity of the microorganisms present in the respective wines [19].

3.2 Multifactorial analysis between the composition of some aging oenological products and the content of amino acids and biogenic amines in experimental samples

Multifactorial analysis performs the subsequent evaluation of variations within data sets and the normality of data distribution according to grouping criteria.

The Fisher LSD test was applied to be able to make a correction for multiple comparisons, it calculated the standard deviations extracted from each group included in the study according to the grouping criteria: maturation time and the type of chemical substances released from the oenological product during the ageing on lees period.

For the individual evaluation of the chemical composition of each oenological maturation product in the two time periods, 12 months and 18 months, a statistically significant difference was found in the case of all the experimental samples regarding amino acids and biogenic amines, with the exception of some of them and namely: L-proline, L-leucine, ethanolamine, cadaverine and putresceine, in the case of the experimental samples obtained from the Bosuioacă de Bohotin variety. In contrast, for the experimental samples of Sauvignon blanc, testing and statistical interpretation between the two time periods, 12 months of contact with yeasts and 18 months, respectively, significant variations occurred for: L-glycine, 4-OH-proline, L-methionine, L-serine, L-threonine, L-phenylalanine, L-tyrosine, L-arginine, L-histidine, L-asparagine and L-cystine.

Biogenic amines show the same tendency between experimental samples, namely to show significant variations. The variation is significant and explained in terms of the changes that appeared in the experimental samples taken in the study, following the reactions that take place during the maturation process on yeasts: hydrolysis, esterification and decarboxylation of amino acids.

In Table 3, the mean values for specific grouping factors were inserted: the ageing on lees period and the types of chemical substances released during the maturation stage of the wine on the lees for the experimental samples of Sauvignon blanc and in Table 4 for the experimental samples of Busuioacă from Bohotin. A grouping of the chemical compounds yielded by oenological maturation products by autolysis was made, so that the evaluation was made according to this consideration.

From a compositional point of view, the product used in the V12SB and V12BB samples, POWERLEES® ROUGE, with a high content of mannoproteins with a stabilizing effect and β-glucanase that has the ability to produce an acceleration of the

extraction rate of the compounds released from the cell walls of yeasts by autolysis and their appearance in the wine mass. Thus, in the case of some amino acids such as L-leucine, 4-OH-proline and L-asparagine for the V12SB sample, statistically significant effects were found compared to the other types of oenological maturation products applied.

For the determined amino acids L-isoleucine and L-asparagine in experimental samples of Busuioacă de Bohotin, the levels were higher: 70.7 ± 25.6 mg/L for L-isoleucine and 76.4 ± 35.3 mg/L for L -asparagine compared to the control sample (V1BB) which shows average values of 21.4 ± 14.0 mg/L, and in the case of the V3BB sample treated with the oenological product from the BÂTONNAGE range, specifically BÂTONNAGE PLUS ÉLEVAGE®, which presents peptides, mannoproteins and amino acids in the composition.

Average values of 28.4 ± 14.4 mg/L were recorded for the amino acid L-isoleucine, and for L-asparagine 10.5 ± 6.3 mg/L in the case of the experimental sample V8BB treated with the product TRAP' METALS® , with a high content of PVI/PVP and chitosan, with variations of 14.7 ± 3.14 mg/L for sample V3BB.

In the case of the experimental samples of Sauvignon Blanc, the oenological products that have a high content of mannoproteins and have enzymes in their constitution, especially β-glucanase, produced variations of some amino acids [20].

Thus, in the case of the amino acid L-leucine, variations of 96.08 ± 65.73 mg/L were recorded, so it presented higher values than the other samples treated with other oenological maturation products and the control sample. Unlike L-leucine, the amino acid L-4-OH-proline had statistically different values of 7.14 ± 3.14 mg/L, confirmed by *P* values <0.5, but lower than the other administered products.

Discriminant analysis was applied to highlight the separation of results according to classification criteria. As more similarities were established between the variation behavior of the determined compounds, in the case of experimental samples obtained from two different varieties, it was possible to evaluate discriminant functions known as sources (ROOT), with statistical relevance for which p <0.05. To use the association function, the contribution of each determined nitrogen compound to group separation was considered.

Differentiation criteria were established based on several categorical variables set for the compounds under analysis. Thus, in order to carry out the discriminant analysis, the doses of added oenological products, the category of maturing oenological product, the types of macromolecules released during the yeast autolysis enzymatic process were taken into account. Due to the fact that between various types of oenological products administered after alcoholic fermentation, similarities were found regarding the macromolecular compounds present in their composition.

A normalization and association of several types of oenological products with similar properties was carried out, with the mention that the different characteristics were the basis for the formation of relevant criteria for separating product categories.

In the case of the differentiation criteria regarding oenological maturation product administered (Figs. 1 a)., b)., c).), the discrimination functions were determined based on the following compounds – valine, 4-OH-proline, cystine, serine, proline and cadaverine with high specificity and statistically significant (p<0.05). Histidine, glycine, phenylalanine, aspartic acid, tyramine and ethanolamine were also included in this analysis. On the other hand, when establishing the discrimination functions corresponding to the type of chemical compounds released by enzymatic autolysis in the wine mass (Fig. 1b).), of .01308 approximately F (102,172)=1.9256 p<.0001, the spectrum of substances taken into analysis was made up of 11 amino acids (leucine, asparagine, valine, glycine (p<0.05), 4-OH-proline, cystine, glutamine, phenylalanine (p<0.05), cysteine, methionine (p<0, 05), isoleucine, aspartic acid and serine, plus two biogenic amines, namely putrescein and tyramine (p<0.05).

The canonical differentiation functions were most strongly influenced by the doses applied from the maturing oenological products, thus 6 individual groups were identified with a specific degree of separation (.00735 approx. F (105,131) = 2.1741 p<.0000). For the amino acids valine (p<0.05), histamine (p<0.05), phenylalanine (p<0.05), 4-OH-proline, lysine, serine (p<0.05), proline (p< 0.05), cystine, glycine (p<0.05), aspartic acid, asparagine (p<0.05), tyrosine (p<0.05), cysteine (p<0.05), isoleucine (p< 0.05), arginine (p<0.05), glutamic acid, alanine, glutamine, and among the biogenic amines we mention cadaverine, spermidine and ethylamine.

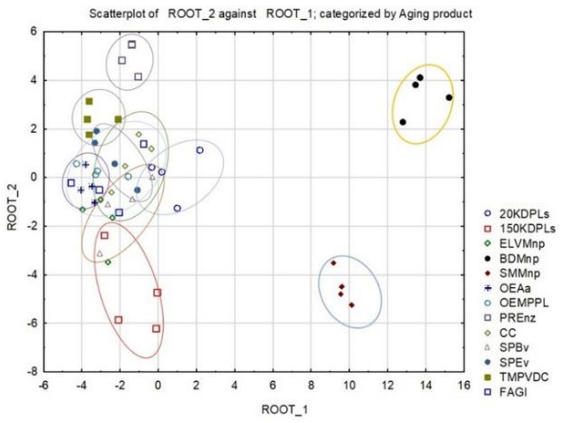

Figure 1 a). - Distribution of dependent variables according of the type of oenological product given in the ageing on lees stage.

Table 3. Evaluation of the average values of specific grouping factors of the experimental samples of Sauvignon blanc.

	Ageing on lees period		Compounds yielded by enzymatic autolysis from oenological maturation products						
Amino acids	12 months	18 months	PS/MN	PEP/MN/AO/AA	VT/AA	PVMD/ PVPD/ChT	GLu	MN/, ß-gl	Control
	1	2	1	2	3	4	5	6	7
GLY	20.48±3.66[2]	17.27±4.26[1]	17.73±2.21	20.52±4.89	16.61±2.72	18.29±7.56	16.34±0.85	19.64±0.20	19.52±0.56
ALA	97.67±20.17[2]	41.26±36.33[1]	95.63±15.12	79.57±48.63	54.56±14.10	53.81±26.36	57.17±20.71	55.01±20.72	60.31±42.63
VAL	32.88±6.66[2]	26.47±3.60[1]	30.99±0.37	33.47±8.10	25.40±5.06	27.03±4.71	27.78±2.85	26.44±5.05	28.02±1.47
4OH-PRO	11.55±1.41	12.59±3.84	13.08±1.99	**13.44±2.19[6]**	**12.57±1.31[6]**	**9.16±0.72[6]**	10.51±0.35	**7.14±3.14[2,3,4,6]**	**13.48±3.88[6]**
ILE	32.72±5.31[2]	16.13±4.89[1]	25.14±5.82	26.29±15.06	23.28±8.04	24.81±10.39	22.48±8.23	24.92±9.98	17.34±5.06
LEU	15.98±3.19	28.98±18.00	**19.88±7.74[6]**	**16.53±3.84[6]**	**14.78±1.36[6]**	**14.52±0.57[6]**	**15.30±1.58[6]**	**96.08±65.73[1,2,3,4,5,7]**	**15.18±2.02[6]**
MET	9.03±2.37[2]	7.38±1.31[1]	7.28±1.45	9.46±2.43	7.31±1.20	7.06±2.05	8.60±1.17	7.65±0.93	7.94±0.06
SER	23.61±4.61[2]	16.30±3.87[1]	20.49±1.27	23.59±5.56	**15.67±4.18[6]**	18.84±7.30	17.70±7.26	**18.08±5.30[3]**	18.92±6.47
TRE	23.08±5.20[2]	46.29±16.25[1]	28.48±8.09	34.80±12.45	30.27±11.81	31.07±14.39	35.22±11.06	38.89±25.72	43.96±20.55
CYS	5.74±2.39[2]	1.75±1.25[1]	3.68±1.58	4.23±3.26	3.04±0.79	5.95±1.98	4.81±1.78	1.98±0.12	1.66±0.79
PRO	94.22±14.6[2]2	68.06±27.05[1]	102.91±15.43	88.18±25.81	68.91±29.76	73.55±22.95	67.01±27.43	72.94±25.52	69.41±25.10
ASP	17.06±2.31[2]	8.43±4.40[1]	16.18±1.63	14.96±5.77	10.88±6.53	10.46±6.27	10.73±2.25	10.12±6.03	8.92±2.12
FEN	18.32±3.62[2]	12.51±0.94[1]	14.53±1.73	16.92±6.41	14.34±3.78	14.83±3.26	14.46±2.61	14.91±2.33	15.39±2.48
TRP	3.08±1.03[2]	1.24±0.59[1]	1.59±1.13	2.73±1.74	1.84±1.14	2.72±1.10	2.16±0.11	1.47±1.39	1.62±1.43
TYR	1.93±0.62[2]	3.57±0.60[1]	2.85±1.47	3.22±1.05	2.41±0.85	2.67±1.25	2.68±0.26	2.20±0.77	2.25±0.67
GLU	24.80±3.65[2]	11.09±2.99[1]	16.03±6.43	19.38±9.07	14.54±8.61	20.88±15.42	20.59±8.38	16.91±8.39	17.92±5.56
LYS	23.79±4.85[2]	19.77±4.37[1]	23.78±2.95	24.27±6.12	18.50±5.66	18.90±3.64	19.53±1.89	19.66±2.51	21.22±0.91
ARG	15.48±3.54[2]	6.79±2.84[1]	8.84±6.53	13.20±7.49	10.39±5.26	10.38±4.48	9.72±4.50	11.29±5.06	10.69±3.28
HYS	0.18±0.01[2]	4.15±4.78[1]	0.46±0.39	3.62±1.81	3.69±1.07	1.75±0.22	1.02±0.19	0.52±0.49	1.00±0.15
ASP_AC	21.35±3.35[2]	16.68±5.33[1]	19.85±1.79	**21.75±4.05[4]**	16.68±3.19	**11.98±2.08[2]**	17.57±4.86	19.61±1.70	18.11±1.37
GLU_AC	30.07±5.45[2]	13.74±6.39[1]	22.80±4.54	24.11±14.34	18.19±5.01	19.73±10.86	20.25±7.80	20.75±3.97	21.88±6.14
(CYS2)	0.11±0.05[2]	0.13±0.18	0.20±0.19	0.14±0.10	0.12±0.04	0.07±0.03	0.04±0.03	0.08±0.06	0.13±0.07
ETH	7.88±1.99	7.71±6.95	13.62±7.32	8.78±4.39[3,4,5]	5.23±2.98[2]	5.06±1.63[2]	6.26±2.78[2]	4.70±0.81	6.32±0.78
FENH	0.18±0.04[2]	0.76±0.14[1]	0.50±0.37	0.43±0.10	0.44±0.31	0.60±0.09	0.48±0.20	0.48±0.42	0.48±0.45
HISM	0.84±0.23[2]	2.28±0.74[1]	0.89±0.65	1.57±0.82	1.67±0.91	1.91±0,61	1.56±0.20	1.80±1.33	1.86±0.11
TYRM	9.39±2.81[2]	18.55±2.69[1]	15.63±6.99	14.46±4.72	12.46±3.65	13.57±6.43	14.70±1.10	9.18±3.59	14.67±6.01
SPMD	1.37±0.65[2]	2.40±0.72[1]	1.38±0.35	2.05±0.56	1.79±0.73	2.08±1.87	1.64±0.72	2.34±1.28	1.89±1.40
CAD	0.36±0.08	0.44±0.16	0.52±0.13	**0.39±0.12[3]**	**0.28±0.07[2],[4]**	**0.55±0.13[3]**	0.42±0.05	0.33±0.17	0.39±0.01
PUT	7.71±1.04[2]	38.15±7.01[1]	17.58±12.18	26.54±12.83	21.17±16.10	23.24±11.94	25.02±10.03	21.12±9.21	21.30±10.38

Table 4. Evaluation of the average values of specific grouping factors of the experimental samples of Busuioaca de Bohotin.

	Ageing on lees period		Compounds yielded by enzymatic autolysis from oenological maturation products						
Amino acids	12 months	18 months	PS/MN	PEP/MN/AO/AA	VT/AA	PVMD/ PVPD/ChT	GLu	MN/, ß-gl	Control
	1	2	1	2	3	4	5	6	7
GLY	18.6±2.46 [2]	13.6±3.91[1]	12.7±5.30	16.2±4.1	16.6±2.7	18.3±7.6	16.1±1.8	18.7±1.3	19.2±1.8
ALA	107.5±15.54	110.5±10.90	97.7±4.45	115.9±6.3	54.6±24.1	53.8±26.4	107.4±4.3	107.0±7.1	100.4±3.4
VAL	29.4±4.28	27.7±5.23	27.7±1.50	**31.6±1.3[3]**	**25.4±5.1[2]**	27.0±4.7	27.0±1.9	28.6±2.9	27.8±0.4
4OH-PRO	9.5±1.53[2]	7.8±1.81[1]	8.0±3.27	8.0±1.3	12.6±1.3	9.2±0.7	10.2±0.1	7.9±0.6	9.4±2.1
ILE	35.6±4.01	24.0±16.10	**27.0±10.10[6]**	**28.4±14.4[6]**	**23.3±8.0[6]**	**24.8±10.4[6]**	**24.3±12.2[6]**	**70.7±25.6[1,2,3,4,5,7]**	**21.4±14.0[6]**
LEU	14.8±2.18	16.6±4.44	17.0±1.63	17.3±4.1	14.8±1.4	14.5±0.6	15.4±4.0	13.3±0.8	11.5±2.8
MET	11.1±1.77[2]	4.8±3.31[1]	6.6±2.78	8.3±5.3	7.3±1.2	7.1±2.1	7.0±3.9	10.4±1.3	11.2±0.0
SER	24.7±4.09[2]	20.2±4.21[1]	19.2±5.96	23.9±5.6	15.7±4.2	18.8±7.3	22.8±1.1	22.6±1.0	22.3±0.3
TRE	21.4±3.51[2]	39.6±4.02[1]	26.3±8.03	33.0±11.6	30.3±11.8	31.1±14.4	30.6±16.6	26.0±11.3	31.4±14.4
CYS	1.4±0.92	0.7±0.73	0.8±0.61	0.8±0.7	3.0±1.8	6.0±2.0	1.6±0.7	0.9±0.0	0.8±0.1
PRO	98.8±9.33	102.4±9.32	**91.9±4.15[3]**	**99.0±6.2[3]**	**68.9±29.8[1,2]**	73.6±23.0	97.0±5.5	101.3±10.5	102.1±14.1
ASP	14.8±1.71	23.1±14.63	**12.5±1.45[6]**	**14.7±1.8[6]**	**10.9±6.5[6]**	**10.5±6.3[6]**	**13.9±0.4[6]**	**76.4±35.3[1,2,3,4,5,7]**	**14.0±0.2[6]**
FEN	22.8±2.59[2]	18.8±1.80[1]	19.3±2.19	21.9±3.0	14.3±3.8	14.8±3.3	19.8±2.5	20.5±1.5	20.0±0.2
TRP	3.4±1.42[2]	0.1±0.05[1]	1.1±0.48	1.5±0.9	1.8±1.1	2.7±1.1	2.0±0.8	2.0±0.6	2.1±0.2
TYR	2.6±0.51[2]	5.1±1.27[1]	4.1±2.05	4.4±2.1	2.4±0.9	2.7±1.2	3.6±1.8	3.8±1.8	3.4±1.7
GLU	13.5±1.50	14.5±6.82	11.2±3.86	13.4±4.6	14.5±8.6	20.9±15.4	19.1±6.5	18.7±6.2	12.3±2.8
LYS	31.7±3.12	30.8±5.61	31.7±1.46	**34.1±2.6[3]**	**18.5±5.7[2]**	18.9±3.6	31.8±3.6	32.4±5.8[3]	32.9±4.0
ARG	19.1±2.24[2]	12.3±4.51[1]	15.7±2.52	17.6±2.3	10.4±5.3	10.4±4.5	15.8±2.6	14.0±6.3	13.0±7.7
HYS	0.2±0.01[2]	1.0±0.52[1]	0.8±0.70	0.8±0.2	3.7±1.1	1.7±2.2	0.4±0.1	0.5±0.2	0.3±0.1
ASP_AC	24.2±2.71[2]	18.7±6.64[1]	15.2±8.50	21.3±5.7	16.7±3.2	12.0±5.1	23.7±1.7	23.6±3.0	23.8±3.7
GLU_AC	18.6±2.46	27.9±5.74	27.8±3.54	28.6±2.9	18.2±5.0	19.7±6.9	28.1±1.7	28.7±6.7	25.9±4.8
(CYS2)	107.5±15.54[2]	0.5±0.35[1]	0.5±0.43	0.4±0.1	0.1±0.0	0.1±0.0	0.2±0.08	0.2±0.05	0.6±0.1
ETH	29.4±4.28[2]	12.3±1.85[1]	**9.6±1.20[5]**	10.7±1.2	5.2±3.0	5.1±2.6	**13.2±1.5[1]**	11.5±1.6	12.4±2.9
FENH	9.5±1.53[2]	1.1±1.50[1]	0.5±0.16	0.8±0.2	0.4±0.3	0.6±0.6	0.4±0.1	0.4±0.1	0.4±0.1
HISM	35.6±4.01[2]	0.5±0.06[1]	0.5±0.10	0.5±0.2	1.7±0.9	1.9±0.6	0.4±0.2	0.5±0.3	0.4±0.1
TYRM	14.8±2.18[2]	18.2±3.39[1]	14.0±5.60	13.7±7.3	12.5±3.6	13.6±6.4	14.2±7.9	15.1±8.9	14.9±8.1
SPMD	11.1±1.77[2]	1.4±0.13[1]	**1.3±0.12[3]**	1.4±0.1	**1.8±0.7[1]**	2.1±1.9	1.4±0.2	1.4±0.1	1.3±0.0
CAD	24.7±4.09[2]	0.6±0.15[1]	0.5±0.20	0.5±0.2	0.3±0.1	0.6±0.1	0.5±0.1	0.7±0.3	0.5±0.3
PUT	21.4±3.51[2]	26.5±5.92[1]	16.2±13.01	17.1±13.9	21.2±16.1	23.2±5.9	19.8±5.4	19.4±5.8	19.8±6.8

Even though the spectrum of compounds was higher in the discriminant analysis using differentiating variables, such as the dose of oenological product used in the stage of ageing on lees and the category of chemical substances released by autolysis in the wine mass, the discriminating power of the models was more limited , in the sense that it was possible to separate a zone corresponding to mannoproteins, β-glucanase, which demonstrates the influence of the applied maturation product on the profile of corresponding biogenic amines and amino acids [21].

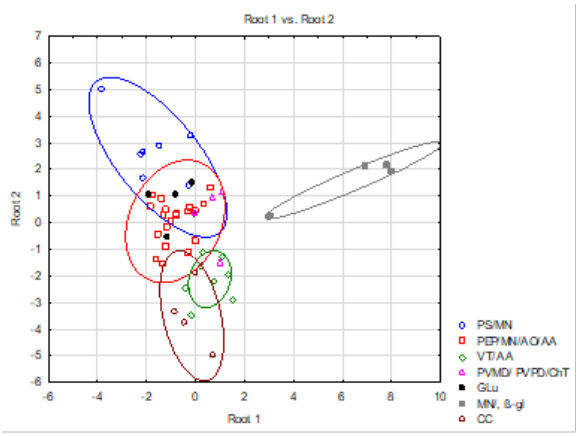

Figure 1 b). – Distribution of dependent variables according of the type of chemical compounds given in wine samples in the ageing on lees stage.

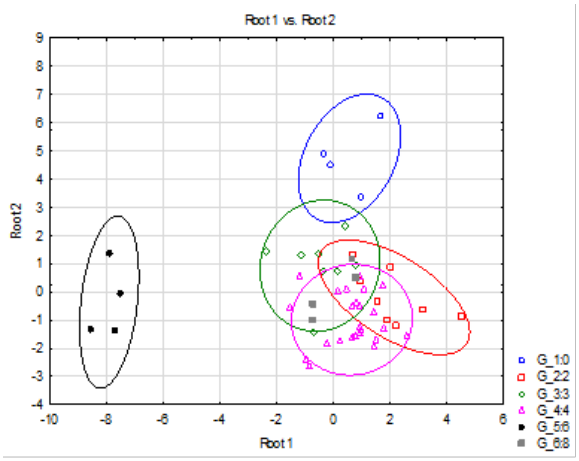

Figure 1 c). – Distribution of the dependent variables according to the dose of oenological products applied to wine samples in the ageing on lees stage.

On the other hand, when evaluating the doses administered in order to mature the experimental samples on yeasts, it was revealed that the doses of 6 g oenological product/10L wine produced distinctive separation zones at the level of amino acids and biogenic amines (Fig. 1 c). Figure 1 a). confirms several areas of separation, even if the analysis is based on the contribution of a smaller number of amino acids, but on several categories of chemical substances released through autolysis: mannoproteins (BÂTONNAGE BODY®, SUPER-MANN®), enzymes (POWERLEES® ROUGE) and polysaccharides, nitrogen compounds (BÂTONNAGE PLUS 150 KD®).

4 Conclusions

During ageing on lees, a series of chemical changes occur, several processes take place that contribute to the final sensory characteristics. The physico-chemical composition evolves as a consequence of important changes that develop from esterification, hydrolysis, redox reactions, slow and continuous diffusion of oxygen, removal of carbon dioxide and spontaneous clarification.

The products used for maturation stage showed a low influence on the biogenic amines and amino acids contents.

Experimental samples V12SB, V4SB, V2SB, V3SB recorded the highest tyramine concentration values after 18 months of maturation on yeasts, an increase is observed between the two-time intervals in which the analyses were carried out, 12 months of contact with lees, and subsequently after another 6 months. The increase is correlated with the decrease in the content of L-phenylalanine, so the TDC enzyme did not participate in the decarboxylation of the precursor amino acid and did not influence the content of L-tyrosine which registered quantitative increases in the following time interval.

In the case of the experimental samples of Sauvignon blanc, high values of the putresceine content were recorded after 18 months of maturation on the lees, the highest were in the case of the V12SB, V3SB and V8SB samples, changes that were correlated with the large amounts of the amino acid precursor L-arginine during the first 12 months of maturation on yeasts, after which there was a decrease in all samples after a further 6 months of maturation. So, the large amounts of putresceine arise from the decarboxylation reaction of arginine.

Regarding the amino acid content of the experimental samples, the highest values for L-alanine and L-proline can be observed both in the case of those obtained from the Sauvignon blanc variety and those obtained from the Busuioaca de Bohotin variety after 18 months of ageing on lees.

In order to evaluate the variation according to the ageing on lees period, significant changes in the amino acid content were recorded in the case of the experimental samples obtained from the Sauvignon Blanc variety, as well as from the Busuioacă de Bohotin variety. They did not only show variations in the sense of their degradation and their passage into biogenic amines, so that the amino acids L-threonine, L-tyrosine, L-histidine and L-cystine showed positive values.

On the other hand, when evaluating the doses administered in order to mature the experimental samples on yeasts, it was revealed that the doses of 6g oenological product/10L wine produced distinctive separation zones at the level of amino acids and biogenic amines in the case of V4SB and V4BB samples, respectively.

The wines stand out for their good structure, fullness and physico-chemical stability over time, all of which are due to the prolonged contact with the oenological products in the ageing on lees stage.

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