INFLUENCE OF SELECTED MICROORGANISMS COMPARED TO NATURALLY OCCURRING MICROORGANISMS ON BIOGENIC AMINES CONTENT OF YOUNG WINES FROM MINIS VINEYARD

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Lucrarea are drept scop stabilirea influenței utilizării microorganismelor selectate asupra conținutului în amine biogene al vinului nou, produs în podgoria Miniş. Studiul s-a efectuat prin micro-vinificare, pentru vin roşu şi alb. După vinificare, vinul este stabilizat şi preparat pentru îmbătrânire, fiind analizat HPLC prin derivatizare cu OPA. Rezultatele obținute nu prezintă diferențe semnificative în cazul vinurilor produse de microorganismele selectate sau de microorganismele spontane, prezente în coaja bobului de strugure.

The aim of this paperwork is to establish in what degree the use of selected microorganisms influenced the biogenic amines content of young wine produced in the Romanian Minis vineyard. The study was carried out through micro-vinification experiments for both red and white wines. After the vinification took place, wine was stabilized and prepared for aging and samples were taken and analyzed via HPLC and pre-column OPA derivatization. The obtained results show no significant differences between the wines produced using selected microorganisms and wines that were produced using the spontaneous microorganisms present on the grape skin.

Keywords: biogenic amines, HPLC, OPA derivatization, micro-vinification

1. Introduction

Biogenic amines are chemical compounds containing an amino functional group and that derive from the corresponding amino acids during metabolic

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pathways. They have multiple roles in living organisms, some acting as neurotransmitters (namely dopamine, nor-adrenaline and adrenaline, histamine and serotonin) [1], others acting as cellular growth regulators (cadaverine, putrescine, spermine and spermidine) [2].

Besides having important roles in metabolism biogenic amines also have toxicological properties. For example, tyramine is presumed to play an active role in migraine, it causes hypertension, and it is also involved in depression mechanisms, schizophrenia and Parkinson disorder [3]. Serotonin was reported as being involved in the mechanisms of depression, schizophrenia, Alzheimer, Parkinson disease, anxiety, migraine, obesity, encephalopathy, panic attacks, circulatory disorders due to the fact that it intervenes in the thrombosis mechanisms and can also cause pancreatic disorders. [4,5,6,7,8]. Cadaverine and putrescine, also found in wines, are involved in allergic reactions and can also cause nausea and some digestive and skin disorders [8,9], while histamine was reported to cause headaches, diarrhea, hypotension, arrhythmia, urticaria, as well as other side effects [10].

Due to their side effects, the presence of biogenic amines in food must be monitored. It had also been proposed to be used as an indicator of food freshness and food quality [11, 12].

There are a number of studies concerning Roumanian wines [13-15].

Wine consumption and production is widely spread. That is why a study regarding the content in biogenic amines of wines obtained by using selected microorganisms during vinification compared to the vinification under the influence of natural occurring microorganisms seems appropriate.

2. Experimental

Materials and methods

Reagents as well as solvents and standards were purchased from Sigma-Aldrich (1), Fluka (2) and Merck (3), and used as received:

1. histamine, tyramine, 2-phenylethylamine, putrescine, cadaverine;
2. isopentyl-amine, 1-hexylamine, o-phthalaldehyde (OPA), potassium carbonate, potassium dihydrogenophosphate, di-sodium hydrogenophosphate;
3. ethanol, acetonitrile.

The experiment had two distinct phases:

Micro-vinification (I)

The first phase involved the making of the wine *via* micro-vinification, using selected microorganisms and also natural occurring microorganisms. The micro-vinification was conducted in batches of 15 L for each variation. Samples were collected for analysis first prior to the beginning of fermentation and then

after alcoholic fermentation was completed and the young wine was stabilized for aging with 80mg/L SO_2.

30 L of red and white must was collected from the Minis vineyard and divided into two batches of 15 L each. In one batch the spontaneous microorganisms were killed using 200 mg/L Velcorine, while the other was left unmodified. Then the batches were placed into glass jugs fitted with fermentation funnels as seen in Fig. 1 A and 1 B.

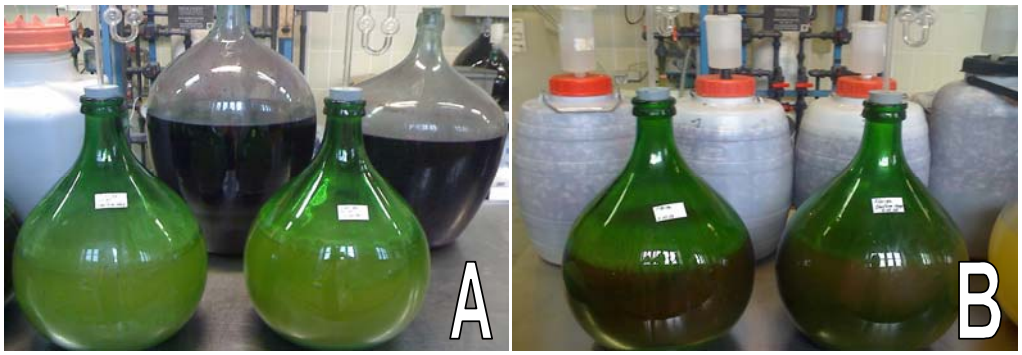

Fig. 1. Fit jugs for micro-vinification of white (A) and red (B) wines

For the white wine one jug was left to ferment under spontaneous microorganisms and the other was for fermentation in the presentce of *Oenoferm Klosterneuburg*, a strain of *Saccharomyces cerevisiae* at a concentration of 15g/100L.

For the red wine the procedure was similar to the white wine the only difference being the yeast used which was *Oenoferm Rouge* at a concentration of 15g/100L, which is a special selected strain of *Oenoferm Klosterneuburg*, in the selection of this strain a special importance was given to color preservation in the course of the fermentation of red mash and must.

Sample analysis using HPLC and pre-column OPA derivatization (II)

The second phase involved the HPLC analysis of the samples using pre-column OPA derivatization. The HPLC pre-column derivatization was performed automatically by the instrument, via the auto-sampler when 3 μL of ethanol solution sample is mixed with 4 μL of OPA and 4 μL of borate buffer and the 11 μL sample mix is injected. The analyses were carried out on a HP 1090 HPLC instrument, with fluorimetric detector using Licrocart 250-4 Licrosphere 100 Rp-18 columns. A solution of potassium dihydrogenophosphate 2.2681 g/L and di-sodium hydrogenophosphate 5.933 g/L in milipore water, adjusted at a pH of 7.2

was used as eluent A and acetonitrile was used as eluent B. the gradient of the two eluents over time is presented in table 1.

Table 1

Gradient of the A and B eluents over time

Time (minutes)	Eluent A (%Vol)	Eluent B (%Vol)
0	60	40
10	30	70
30	30	70
32	60	40

1-hexylamine was used as internal standard in a concentration of 0.210 g/L. The calibration curves were drawn for each of the analyzed biogenic amine.

The method involved pre-column derivatization of the samples with o-phthalaldehyde (OPA) and fluorimetric detection of the formed complex with an excitation wavelength at 330 nm and an emission wavelength at 450 nm.

Sample preparation

All solutions were left for 2 hours to heat at room temperature and then filtered on 10 μm filtering paper. A 3 mL sample from the filtered solution was collected and introduced in a centrifugation vial, then 0.050 ml internal standard (1-hexylamine, 0.210 g/L), 1 mL ethanol and 3 g K_2CO_3 were added. The vial was vigorously shaken, then centrifugated for 10 minutes at 4000 rpm. After centrifugation the ethanol solution containing the biogenic amines was siphoned into a second vial, then 1 mL of ethanol was added in the centrifugation vial, and it was strongly shaked and centrifugated again for 10 minutes. The ethanol solution was then siphoned into the second vial. The biogenic amines were extracted in the ethanol solution and this extract was used for the quantitative determination.

From the siphoned ethanol solution a sample of 1 mL was collected and introduced in the HPLC vial.

HPLC pre-column derivatization was performed automatically by the instrument via the auto-sampler, 3 μL of the ethanol solution sample were mixed with 4 μL of OPA and 4 μL of borate buffer, then the 11 μL sample mix was injected into the HPLC column.

3. Results and discussion

The results of the HPLC analysis of the samples taken during the micro-vinification process are presented in table 2.

Table 2

Biogenic amines content of the analyzed samples

Sample name	Biogenic amines content (mg/L)						
	Histamine	Tyramine	Putrescine	2-phenyl etyl-amine	Cadaverine	Isopentyl-amine	Total amines
Red must	0.117	-	0.459	-	-	-	0.576
Red + Oen. rouge	0.130	-	0.545	-	-	-	0.675
Red natural	-	-	0.386	-	-	-	0.386
White must	0.418	-	0.946	1.513	0.107	3.782	6.765
White + Oen. klost.	0.394	0.699	0.580	1.148	0.093	0.554	3.468
White natural	0.479	0.772	0.445	1.201	0.093	0.927	3.917

From the experimental data one can see that the white wine samples have a higher content in biogenic amines compared to the red wine samples, in spite of the fact that white wines usually have lower content of biogenic amines than red wines.

It is notable that the white must has a total content in biogenic amines almost of more than 10 times of that of the red one. This situation is due to the condition of the grapes during the harvest. For the white grapes, the harvest took place after a few days of rain, when, based on the weather forecast, the vineyard decided that it was best to harvest, as the weather would not improve and they had to reduce the losses. Grapes were affected by *Botrytis* in a high degree and, therefore, it was expected to have high contents of biogenic amines in the must. This is also in accordance with the data found in the literature, as previous studies showed that damaged grapes lead to high content of biogenic amines, especially in the case of *Botrytis* contamination, where the content in isopentyl-amine and pheyl-etyl-amine is higher [16]. Red grapes, on the other hand, had been harvested in optimal conditions, two weeks prior to the white grapes, this being also reflected in the quantity of biogenic amines present in the must.

For the red wine it is notable the fact that the use of selected yeast for the fermentation did not have the desired effect, the total content in biogenic amines being higher in the case of the fermentation with selected yeast, compared to the spontaneous fermentation. One may notice that the histamine and putrescine content of the red wine slightly increased when selected yeast was used compared to the must, while all other amines were below the detection limit. When the fermentation took place under spontaneous microflora, the histamine level dropped below the detection limit and the putrescine content slightly decreased compared to the must. These findings could be explained by the fact that, in the case of natural occurring microorganisms, some of them metabolize histamine and putrescine [17]. Such microorganisms are no longer found in the selected yeast batch, where the must had been sterilized with Velcorine.

On the other hand, the situation in the case of white wine is totally different, total biogenic amines content being lower in the young wine compared with the must. The use of selected yeast also contributed to a final total biogenic amines content lower than the content found in the batch that was left to ferment under spontaneous microorganisms. Except of putrescine, all biogenic amines concentrations have been smaller when selected yeast had been used compared to the spontaneous process of fermentation. It is notable that histamine content decreased in the young wine that was obtained using *Oenoferm Klosterneuburg* compared to the young wine obtained by spontaneous microorganisms. Although isopentyl-amine content decreased in both batches, the young wine that used selected yeast has almost 40% less isopentyl-amine than the young wine obtained by fermentation under spontaneous microorganisms. The putrescine content of young white wine decreased compared to the must but, surprisingly the content in putrescine of the young white wine using *Oenoferm Klosterneuburg* is slightly higher than in the young wine obtained by fermentation under spontaneous microorganisms. One can also notice that although tyramine concentration was below the detection limit in the red must, tyramine levels increased after fermentation. Since yeasts do not produce tyramine during alcoholic fermentation, this increase could be explained by the hydrolysis of tyramine-hydroxycinnamic acid complexes that are present in grapes [14].

4. Conclusions

The following conclusions can be deducted from the presented experimental data:

- In the case of red wine, the use of selected yeasts did not have the desired effect, total content in biogenic amines of the young wine being around 17% higher than the must while the fermentation under spontaneous microorganisms decreased the total biogenic amines content of the young wine with approximately 33%, compared to the must.
- In the case of white wine, the use of the selected yeasts had the desired effect decreasing the total biogenic amines content of the young wine with almost 49% compared to the must, while the young wine that fermented under spontaneous microorganisms only dropped about 42% compared to the must.
- In the case of histamine, the use of selected yeast determined an increase in concentration compared to the must and the young red wine obtained by spontaneous fermentation, while for the young white wine, the use of selected yeast leaded to a decrease of

histamine compared to the must and the spontaneous fermentation product.

- The putrescine content of the young red wine obtained with selected yeast was higher than the one obtained with spontaneous fermentation and also higher than the must. For the young white wine the putrescine content of the wine obtained with selected yeast was lower than the must, but higher than the wine obtained with spontaneous fermentation.

In conclusion, there are no general pattern that could be applied concerning the microorganism involved and the content in biogenic amines. Thus, a thorough analysis of biogenic amine content has to be performed for each year of production.

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R E F E R E N C E S

[1] *D. Purves, G.J. Augustine, D, Fitzpatrick, W.C. Hall, A. –S. Lamantia, S.O. Mc Namara, S.M. Williams,* Neurotransmitter and their receptors in "Neuroscience", 3rd edition, Sinauer Associates Inc., Sunderland, 2004, pp. 147-149

[2] *J. Sanchez-Lopez, G. Camanes, V. Flors, C. Vicent, V. Pastor, B. Vicedo, M. Cerezo, P. Garcıa-Agustın*, Plant Physiol. Biochem., 2009, doi:10.1016 /j.plaphy.2009.02.006

[3] *T.A. Branchek, T.P. Blackburn*, Curr. Opin. Pharmacol. **3**, 2003, 90–97

[4] *J. R. Lacasse, J. Leo*, PLoS Med 2 **12**: e392, 2005, doi:10.1371/journal.pmed.0020392

[5] http://www.healthandage.com/html/well_connected/pdf/doc08.pdf

[6] *P.R. Belcher, A.J. Drake-Holland, M.I.M. Noble*, Vascular Disease Prevention, **2**, 2005, 67-76

[7] *F. Lechin, B. van der Dijs*, JOP. J Pancreas, **5(4)**, 2004, 237-238

[8] http://www.rcpsych.ac.uk/pdf/semOAP_ch4.pdf

[9] http://www.eurotradekft.hu/Biogenic%20Amines.pdf

[10] *L. Maintz, N. Novak*, Am. J. Clin. Nutr. , **85**, 2007, 1185–1196

[11] *A. Halasz, A. Barath, L. S. Sarkadi, W. Holzapfel*, Trends Food Sci. Tech., **5**, 1994, 42-49

[12] *J.L. Mietz, E. Karmas*, J. Food Sci., **42**, 1977, 155-158

[13] *M.C. Todasca*, *N. Chira*, *C. Deleanu*, *S. Rosca*, U.P.B. Sci. Bull.,**69(4***)*, 2007, 3-11.

[14] *M.C. Todasca, L. Fotescu, F.A. Hincu, A. Hanganu, N.A. Chira, S. Rosca*, Rev. Chimie (Bucharest), **61**, 1042-1045

[15] *M.C. Buzas, N. Chira*, *C. Deleanu*, *S. Rosca*, Rev. Chimie (Bucharest), **54**, 2003, 831-835

[16] *M. Grossmann, I. Smit, O. Loehnertz, A. Ansorge*, Revista Internet de Viticultura Y Enologia, **5**(**3**), 2008, 1-7
[17] *V. Del Prete, A. Costantini, F. Cecchini, M. Morassut, E. Garcia-Moruno,* Food. Chem., **112**, 2009, 474-481, doi: 10.1016/jfoodchem. 2008.05.102.