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Formation of Biogenic Amines in Wine Production

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Abstract—Factors and conditions for the formation of biogenic amines in wine production were studied. It was shown that amino acids were decarboxylated during alcoholic fermentation and biological acidic reduction in the presence of enzymes of yeast and lactic acid bacteria. The pH value, the presence of phenolic compounds, tartaric and malic acid, the duration of the contact between the wine material and yeast lees, and the rate of their autolysis were found to influence the intensity of the decarboxylation process.

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INTRODUCTION

The quality and safety of food products have a tremendous impact on human health. The grape and its products contain a wide range of substances that integrate the system, as well as living microorganisms, such as yeast and bacteria. The physico-chemical properties of this heterogeneous system change significantly during the life process.

The main challenge in managing the system of living microorganisms is to preserve its energetic basis in changing environmental conditions. All of the functions of yeast and bacteria, from the means of obtaining energy to the synthesis of new structures or the cleavage of existing ones, are maintained due to biochemical processes. Their adaptation to the environment is provided in particular by the mutation of certain genes, which leads to a change in protein and enzyme content [1].

Toxic substances, such as biogenic amines and crotonaldehyde, are formed as a result of the biosystem life [2]. Biogenic amines are a nitrogen-containing organic compound with aliphatic (putrescine, cadaverine, spermine, and spermidine), aromatic (tyramine and phenylethylamine), or heterocyclic (histamine and tryptamine) structure [3]. Some of them have a high biological activity (histamine, serotonin, dopamine, and tyramine), while others enhance the toxic action of histamine on the human body [4–6].

The goal of the work was to find the relationship between the formation and transformation of biogenic amines in grape processing products and to study their interaction with media components.

METHODS

We studied grape, must, and table wine materials on the basis of white and red technical grape varieties that grow in the Krasnodar Territory. The wine materials were obtained in the micro winery of the State Scientific Organization North Caucasian Regional Research Institute of Horticulture and Viticulture (Russia) according to the method described in [7].

The cultivation and differentiation of wild east and lactic acid bacteria strains was carried out using ethylacetate-containing media [8] and Nahragar medium (DEV-Agar, Doehler, Germany), respectively.

The strains *Saccaromyces cerevisiae* Zymaflore X5 (Laffort Oenologie, France) and *Oenococcus oeni* Maxiflor (Oenologie Institute, France) were used as commercial strains of yeast and lactic acid bacteria.

The model medium for growing *S. cerevisiae* Zymaflore X5 was prepared according to [8] with the addition of 0.1% amine precursors (ornithine, lysine, histidine, phenylalanine, tyrosine, and tryptophan). The pH value was evaluated according to [9].

The content of organic acids and free amino acids was evaluated by the capillary electrophoresis method on a Capel 103R instrument (Lumex, Russia), and their absorption was registered at 270 and 254 nm, respectively [http://www.lumex.ru'application/13BR03.07-1.pdf].

The content of phenolic compounds in the table wine materials was evaluated by the colorimetric method on a UNICO 1201 spectrophotometer (Unico-SiS, Russia) [9].

The content of biogenic amines in must and wine was evaluated according to]10].

RESULTS AND DISCUSSION

Biogenic amines are formed as a result of the decarboxylation of free amino acids by microbial enzymes [3]. The formation and accumulation of biogenic amines in wine products can occur at different stages of the technological process. The necessary conditions for this purpose are the presence in the

Table 1. Composition of free amino acids in grape must of red and white varieties

Variety	Concentration, mg/ mL													
	Arg	Tyr	Phe	His	Leu	Met	Val	Pro	Thr	Try	Ser	Ala	Gly	Sum
Cabernet	196.5	7.7	3.1	8	7.8	44.2	23.1	973	71	71.2	34.4	64	3.9	1507
Merlot	80.5	6.2	0	0	4.2	52.4	4.1	878	28	4	26.6	42.3	0	1126.3
Saperavi	157	9.5	0	3.1	6.5	32.5	8.1	722.8	30	10.6	15.7	3.2	1	1000
Chardonnay	136	0	0	12	16.8	72.9	26	578	56.3	25	18	9.7	0	551
Aliquot	56	12	9	23	12	67	34	670	45	23	10	5	0	966
Sauvignon	36	45	12.9	35.4	56	92	70	1110	220	16.3	135	51	6.8	1883
Cabernet Sauvignon	157	9.5	0	3.1	6.5	33	8.1	423	30	10.6	15.7	3.2	1	700
Cabernet Franc	161	9	0	0	5.7	35.5	14.4	486	36.9	13.1	15	3.1	0	780
Cabernet EPA	188	7	4	3	7	77	29	387	49	21	33	9	0	813
Pinot blanc	76	0	12	45	18	14	10.5	505	48	20	65	76	7	897
Viorica	87	5.6	0	39.3	10.9	0	4.4	906	23	7.8	14.8	59.6	2	1158.4
Riesling	52	6.2	3.4	11	15	7	19	600	5	2	2.3	97	12	831

medium of free amino acids and lactic acid bacterial yeast with decarboxylation activity and optimal conditions for their growth and development.

It was previously shown [11] that the maximal content of biogenic amines is typical for table wines, which are often produced using biological acidic reduction by lactic bacteria strains having decarboxylation activity. It was found that sparkling wines, which were obtained by classic champagnization in a bottle, contained 1.5–2 times more biogenic amines than table wines [11]. This is explained by the fact that the wine material is in prolonged contact with yeast during the champagnization process (secondary fermentation), which provides a significant accumulation of amino acids. It was also shown that the content of biogenic amines in liqueur wines is several times lower than that in table wines produced from the same grape varieties [11]. This is because the growth and development of the yeast involved in the formation of biogenic amines during the production of liqueur wines stops after two days. On the other hand, alcoholic fermentation for table wine production, which is enhanced by biological acidic reduction, continues for 5–7 days.

To study the influence of different types of malolactic fermentation on the intensity of decarboxylation of amino acids in wine material from white and red grape varieties, we evaluated the content of biogenic amines and amino acids, which can be their precursors (Tables 1 and 2). The qualitative and quantitative composition of amino acids in grape must from white and red grape varieties appeared to be rather different. The variations in the amino acid content can be explained by differing conditions for grape growing, as well as its variety and the method of must processing.

The content of arginine, methionine, threonine, proline, alanine, serine, and tryptophan in the wine material from the studied grape varieties was significantly greater than the content of other amino acids (Table 1). The content of the amine precursors was shown to vary depending on the grape variety. For example, the maximal content of tyrosine (45 mg/mL), i.e. the tyramine precursor, was found in must from Sauvignon grapes. No tyrosine was revealed in Chardonnay and Pinot Blanc grapes. Its content in the grapes of other varieties varied from 5.6 to

Table 2. Content of biogenic amines in must from different grape varieties

Grape variety	Concentration, mg/ mL			
	Put	Cad	His	PEA
Cabernet	2.8	0.2	0.2	0.5
Merlot	4.5	0.8	0.02	0.4
Saperavi	5.2	0.1	0.8	0.1
Chardonnay	3.0	1.8	0.2	0.3
Aliquot	6.5	1.1	0.3	0.3
Sauvignon	6.7	1.9	0.5	0.1
Cabernet Sauvignon	5.5	2	0.02	0.2
Cabernet Franc	5.9	1.6	0.3	0.1
Cabernet EPA	2.1	0.1	0.3	0.3
Pinot blanc	1.5	0.3	0.8	0.2
Viorica	6.1	2.1	0.9	0.1
Riesling	1.5	2.4	1	0.6

Table 3. Biogenic amines in dry table wines as a result of alcoholic and malolactic fermentation

Name of beverage	Concentration, mg/ mL						
	sum	average value					
		Put	Cad	His	PEA	Tyr	Try
Grape must	5.8	4.8	0.1	0.7	0.2	0	0
Table wine after alcoholic fermentation in the presence of *S. cerevisiae* Zymaflore X 5	14.1	9.2	0.2	3.4	0.4	0.4	0.5
Table wine after alcoholic fermentation in the presence of wild strain *S. cerevisiae*	29.8	15.8	1.5	9.8	1.2	0.8	0.7
Table wine after acidic reduction with *O. oeni* Maxiflore lactic bacteria	14	9.4	0.3	3.3	0.1	0.5	0.4
Table wine after acidic reduction with wild strains of lactic bacteria	26.8	13.4	1.2	9.2	0.6	1.4	1.0

12 mg/mL. The must from grapes of studied varieties contained phenylalanine, histidine, and tryptophan, while amino acids such as lysine and ornithine (precursors of cadaverine and putrescine, respectively) were absent.

It should also be noted that the must from Sauvignon grapes, in comparison with the other studied varieties, contained the maximal content of phenylalanine, threonine, and histidine (12.9, 220, and 35.4 mg/mL, respectively). The maximal content of tryptophan was found in the must of Cabernet grape 971 mg/mL), while its concentration in the must of the other studied varieties changed from 2 to 25 mg/mL.

The content of biogenic amines in the grape must depended on the grape variety (Table 2). For example, putrescine (Put), cadaverine (Cad), histamine (His), and phenylethylamine (PEA), but not tyramine or tryptamine, were found in the must of all studied varieties. The maximal content of amines was characteristic for the must from the varieties of Sauvignon (9.2 mg/mL), Aliquot (8.2 mg/mL), and Viorica

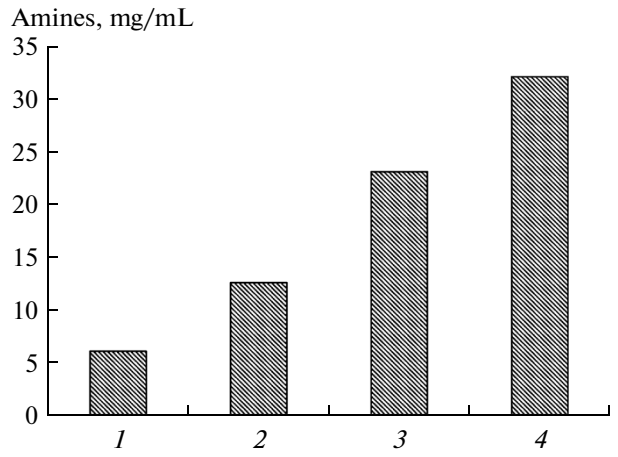

Fig. 1. Dependence of amino acid decarboxylation process on pH in fermentation medium. *1*, must; *2*, pH 3.2; *3*, pH 3.6; *4*, pH 3.9.

9.2 mg/mL). The minimal content of amines was detected in the must of the Cabernet, Cabernet EPA, and Pinot Blanc varieties (3.7, 2.8, and 2.8 mg/mL, respectively).

Thus, the grape must contained a rich set of amino acids, which could be decarboxylated to biogenic amines under favorable conditions. The results indicate that tryptophan and tyramine were absent in the grape must of the studied varieties. On average, the must contained 5.8 mg/mL of biogenic amines, with putrescine being the main part of them. The content of amines depended on both the grape variety and agrotechnical methods of the grape cultivation. The content of biogenic amines in the grape must can be explained by both the physico-chemical processes during grape berry ripening and the presence of wild strains of yeast and lactic acid bacteria on the grape surface.

Table 3 shows the results of studying the content of biogenic amines in table dry wines, which are formed during the alcoholic and malolactic fermentation. Alcoholic fermentation was carried out in the presence of the wild *S. cerevisiae* and commercial *S. cerevisiae* Zymaflore X5 yeast strains, and acidic reduction was performed in the presence of the commercial *O. oeni* Maxiflore strain of lactic acid bacteria. Fermentation of the grape must in the presence of the wild *S. cerevisiae* yeast strain led to the accumulation of putrescine, histamine, cadaverine, and 2-phenylethylamine (Table 3), while histamine and putrescine were formed in the presence of the *S. cerevisiae* Zymaflore X5 yeast strain. Spontaneous biological acidic reduction in the presence of the wild strain of lactic acid bacteria resulted in an accumulation in the wine of all amines, with a predominance of histamine, cadaverine, and putrescine.

The results of studying the influence of pH on the amino acid decarboxylation process in model media in the presence of the *S. cerevisiae* Zymaflore X5 yeast strain show that a change in the medium pH values to 3.6–3.9 leads to an increase in the total content of amines (Fig. 1). It should be noted that aging of the

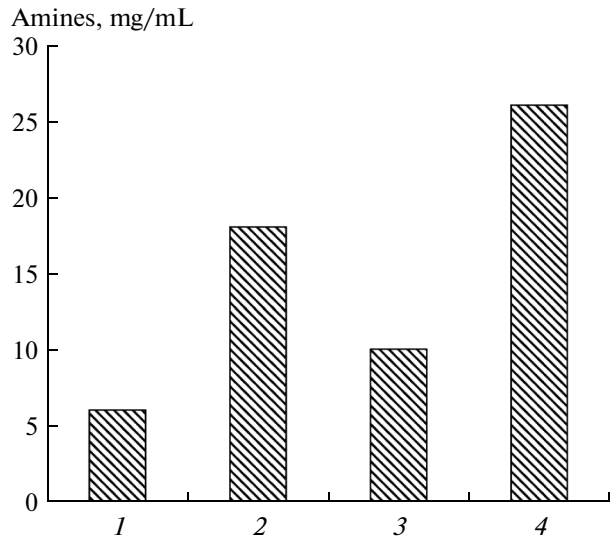

Fig. 2. Influence of duration of the contact between wine material and lees of yeast *S. cerevisiae* Zymaflore X 5 on accumulation of biogenic amines. *1*, must; *2*, wine material; *3* and *4*, wine material after incubation with yeast lees for 14 days and two months, respectively.

fermented wine materials in the presence of the *S. cerevisiae* Zymaflore X5 yeast lees for 14 days led to a decrease in the total content of amines (Fig. 2), which were probably coprecipitated. Aging of the fermented wine materials in the presence of the yeast lees for two months provided an accumulation of histamine, cadaverine, and putrescine. The total amount of amines increased by 20–30%, which is probably due to the favorable media for amino acid decarboxylation during yeast cell autolysis.

Thus, it was found that amino acid decarboxylation and amine formation in model media depended on its pH and the duration of yeast autolysis.

Exogenous enzymes influence the extraction and sedimentation processes and the formation of the wine quality at the stage of the infusion of grape must on pulp (crushed grape mass) and the initial stage of the alcoholic fermentation. The use of pectolytic enzymes at the stage of grape processing reduces the content of biogenic amines as a result of hydrolysis and sedimentation processes [11].

Biological acidic reduction with the use of the commercial *O. oeni* Maxiflore strain of lactic acid bacteria in wine material containing up to 2500 mg/mL of phenolic compounds slowed down the decarboxylation process of amino acids (Fig. 3).

The decarboxylating activity of enzymes of *O. oeni* lactic acid bacteria decreased at pH 2.9–3.3 (Fig. 4). The pH value of 3.6–3.7 was optimal for this process.

Figure 5 shows the influence of tartaric and malic acids on amino acid decarboxylation in a dry table wine material, Riesling, in the presence of the *O. oeni* lactic acid bacterium. The intensity of amino acid

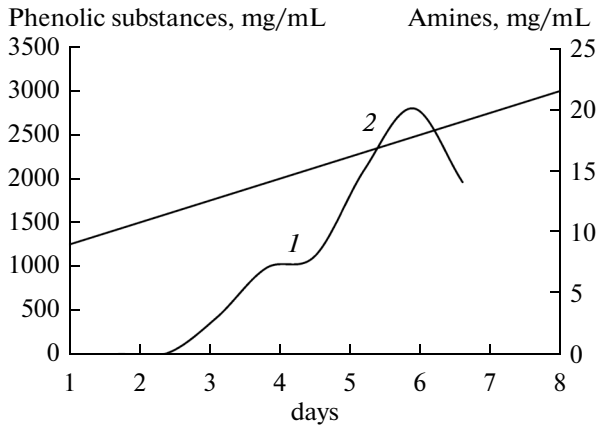

Fig. 3. Dependence of the content of biogenic amines (*1*) on the content of phenolic substances (*2*).

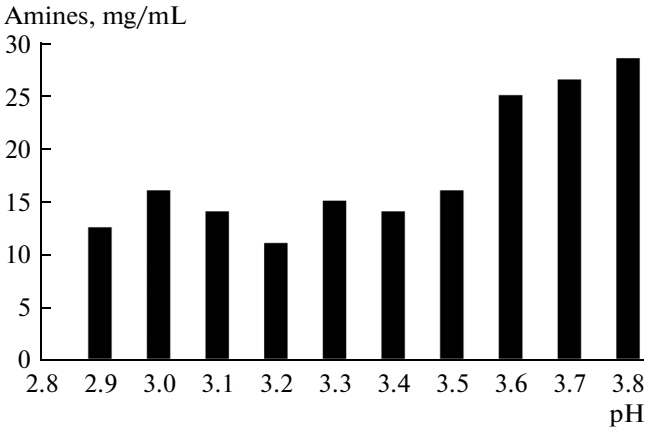

Fig. 4. Dependence of amino acid decarboxylation process in the presence of enzymes on the pH of the medium of lactic acid bacterium *O. oeni*.

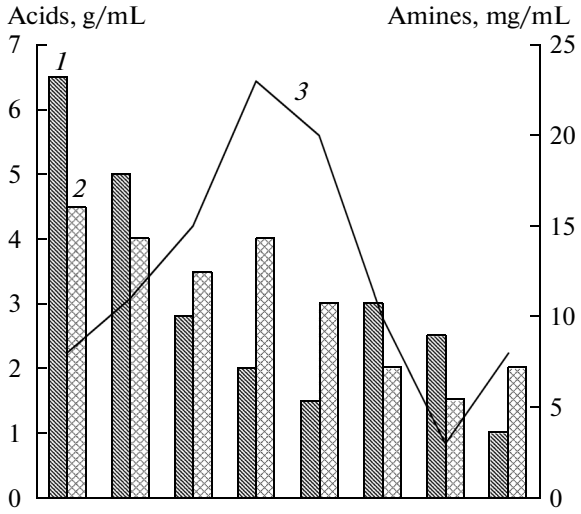

Fig. 5. Effect of tartaric (*1*) and malic (*2*) acids on the content of amines (*3*) during the incubation of wine material with the enzymes of the lactic acid bacterium *O. oeni*.

decarboxylation by the enzymes of this bacterium increased when the content of tartaric and malic acids was 2–4 mg/mL, with the ratio of these acids being 0.5–0.7. This can be explained by a feature of the acidic reduction process in which the presence of malic acid in the substrate induces the formation of the adaptive malic enzyme in the bacterial cell [8]. Consequently, the predomination of malic acid in the medium accelerated the metabolism of the bacteria and activated its enzymes.

Thus, amino acid decarboxylation in wine material can occur during both alcoholic fermentation and biological acidic reduction in the presence of enzymes of both yeast and lactic acid bacteria. The intensity of decarboxylation depends on the pH of the medium, the content of phenolic compounds and tartaric and malic acids, the duration of the contact between the wine material and yeast lees, and the intensity of their autolysis.

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REFERENCES

1. Emel'yanenko, P.A., Dunaev, G.V., Kudlai, D.G., Lyubashenko, S.Ya., Matvienko, B.A., Polteev, V.I., Rotov, V.I., Ryagin, S.T., Ryaguzov, V.S., Tarasevich, I.V., and Chepurov, K.P., *Veterinarnaya mikrobiologiya* (Veterinary Microbiology), Kozlovkii, E.V. and Emel'yanenko, P.A., Eds., Moscow: Kolos, 1982.
2. Serjak, W.C., Day, C., Van Lanen, J.M., and Boruff, C.S., *Appl. Microbiol.*, 1954, vol. 2, no. 1, pp. 14–20.
3. *Biokhimiya* (Biochemistry) Severin, E.S., Ed., Moscow: GEOTAR-Media, 2003.
4. Bauza, T., in *Les amines biogenes du vin: Metabolisme et toxicite*, Bauza, T., Blaise, A., Teissedre, P., Cabanis, J., and Kanny, G., Eds., Paris: OIV, 1995, pp. 42–67.
5. Bertoldi, D., Larcher, R., and Nicolini, G., *Industrie Delle Bevande*, 2004, vol. 33, pp. 437–444.
6. Brodequis, M. and Dumery, B., *J. Int. Sci. de la Vigne et du Vin*, 1989, vol. 23, no. 1, pp. 1–6.
7. *Sbornik osnovnykh pravil, tekhnologicheskikh instruktsii i normativnykh materialov po proizvodstvu vinodel'cheskoi produktsii* (The Rules, Technological Instructions, and Regulatory Materials for the Production of Wine Products), Sarishvili, N.G., Ed., Moscow: Pishchepromizdat, 1998.
8. Bur'yan, N.I., *Prakticheskaya mikrobiologiya vinodeliya* (Applied Microbiology of Winemaking), Simferopol': Tavrida, 2003.
9. *Metody tekhnokhimicheskogo kontrolya v vinodelii* (Technochemical Control Methods in Winemaking), Gerzhikov, V.G., Ed., Simferopol': Tavriya, 2009.
10. Kushnereva, E.V., *Biogennye aminy v vinodel'cheskoi produktsii: prichiny obrazovaniya i metody identifikatsii* (Biogenic Amines in Wine Production: Causes of Formation and Methods of Identification), Krasnodar: Ekoinvest, 2012.
11. Kushnereva, E.V., *Vinod. Vinograd*, 2012, no. 5, pp. 13–14.

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