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SPECIFIC FEATURES OF NITROGEN METABOLISM DURING FERMENTATION OF MUST FROM WHITE GRAPE VARIETIES GROWN IN THE ODESSA REGION

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Abstract. For wine quality management, nitrogen metabolism should be considered as a key process in the system "grape-wine". Nitrogen is one of the dominant elements a grapevine receives from the soil. It is important in many biological processes of the plant itself and of the microorganisms involved in fermentation. Nitrogen-containing compounds are nutrients necessary for yeast growth, in particular, for stable fermentation. This group of compounds directly and indirectly affects the aromatic and taste qualities of wine during its maturation and largely determines its stability. Nitrogen compounds are transferred to wine directly from grapes and yeast during fermentation. Since their role in the formation and maturation of wine is significant, it is highly important to regulate their metabolism in the fermenting must. On analysing literature references and summarising the information on the metabolism of nitric substances, a scheme has been developed reflecting how these substances influence the formation of the quality characteristics of grape wines. The paper presents the results of studying the metabolism of total nitrogen and amino nitrogen in grape must during its fermentation. The grape variety considered in the research was *Sukholimansky White* bred by the National Science Centre "Tairov Institute of Viticulture and Winemaking" and harvested in 2015–2017. It has been established that nitrogen metabolism during fermentation does not depend on the feedings added. However, the yeast race affects the physicochemical parameters, namely the content of volatile acids. It has been observed that during fermentation, the amine nitrogen concentration decreases by 90% and the total nitrogen concentration by 40–50%. Regarding the factors that effect on the quality characteristics of wines produced in the South of Ukraine, the physicochemical parameters of wine materials can be improved by using the active dry yeast Vitilevure Quartz and the nutritional supplements Actiferm 1 and Actiferm 2, in combination with aeration. This allows revealing fruity aromas, and achieving the right acidity and harmony of taste due to the presence of the descriptors (butter, apple, peach, apricot, and geranium) characteristic of the grape variety *Sukholimansky White*.

Keywords: nitrogen, nitrogen compounds, wine materials, must, variety *Sukholimansky*, metabolism.

Introduction. Formulation of the problem

For wine quality management, nitrogen metabolism should be considered as a key process in the system "grape – wine." Nitrogen is one of the dominant elements a grapevine receives from the soil, which is important in many biological processes of both the plant itself and the microorganisms involved in fermentation. Nitrogen-containing compounds are nutrients necessary for yeast

growth, in particular, for stable fermentation. This group of compounds directly and indirectly affects the aromatic and taste qualities of wine during its maturation and largely determines its stability.

The composition of a grape berry is the main factor that determines that of fresh must before fermentation. The kinetics of alcoholic fermentation and the aroma-forming complex of wine products depend on the amount of nitrogen in the must. Nitrogen compounds are transferred to wine directly from grapes and yeast during

fermentation. Since their role in the formation and maturation of wine is significant, it is highly important to regulate their metabolism in the fermenting must.

Analysis of recent research and publications

Analysis of scientific research shows that nitrogen deficiency in the must limits the growth of yeast and the fermentation rate. Besides, it significantly increases the risk of suboptimal (slow) fermentation, its stopping, and the formation of substances which adversely affect the organoleptic properties of wine. The content of this component in grape must can be regulated by additional introduction of mineral and organic forms that are assimilated by yeast. However, under factory conditions, various nitrogen supplements are often added without controlling the initial content of this macronutrient in the grape berries. This approach can lead to an unreasonable increase in production costs and can have a negative effect on the quality of finished products [1-3].

The temperature and aeration of must are factors that regulate the content of nitrogen compounds in must during fermentation. It is established that the fermentation temperature 15–20°C results in the smallest amount of nitrogen compounds in wine, which is a necessary condition for its further colloidal and microbial stability. Both lower and higher temperatures lead to an increase in nitrogen compounds in the wine material. This is due to the fact that low temperatures retard yeast reproduction, and at higher temperatures, yeast autolysis is accelerated.

Must fermentation with air access leads to the lowest content of nitrogen compounds under all temperature conditions.

It is known from the literature that yeast uses nitrogen sources in a certain ratio, and the sequence of consumption of compounds (such as ammonium and amino acids) depends on the composition and concentration of nitrogen in the fermenting must. Generally, the key compounds of yeast nitrogen metabolism are glutamate or glutamine. Yeast uses the mechanism of so-called inhibited nitrogen catabolism (NCR – nuclear-cytoplasmic ratio). It promotes the selection and consumption of the nitrogen sources that provide the optimal growth due to appropriate transport systems and inhibiting the activity of inappropriate permeases. Ammonium, glutamine, glutamate, and asparagine are the “dominant” compounds, unlike others, such as arginine, alanine, aspartate, glycine, urea, and proline [4-7].

Permeases for the predominant nitrogen sources (ammonium and histidine amino acids, tryptophan and lysine) are induced by the NCR mechanism at the beginning of fermentation. The NCR mechanism stops its action when the “dominant” nitrogen sources are depleted, which promotes the induction of amino acid permeases. This allows accumulating nitrogen from poor sources. Ammonium permease, too, is induced under the conditions of nitrogen deficiency.

When the concentrations of assimilated nitrogen in grape must or in a synthetic medium range from low (less

than 100 mg/dm³) to medium (about 250–350 mg/dm³), all sources are quickly consumed and used by yeast. With a nitrogen concentration of about 600 mg/dm³, when the predominant nitrogen sources (especially ammonium) are not depleted, NCR takes place throughout all the fermentation process. The NCR mechanism is closely related to the yeast strains used. Under normal conditions of producing wine from must with low and moderate content of assimilable nitrogen, part of the amino acids is quickly accumulated and stored in vacuoles. The main changes in the structure of amino acid accumulation are only observed in must with a high content of assimilable nitrogen and/or when diammonium phosphate (DAP) is added [8,9].

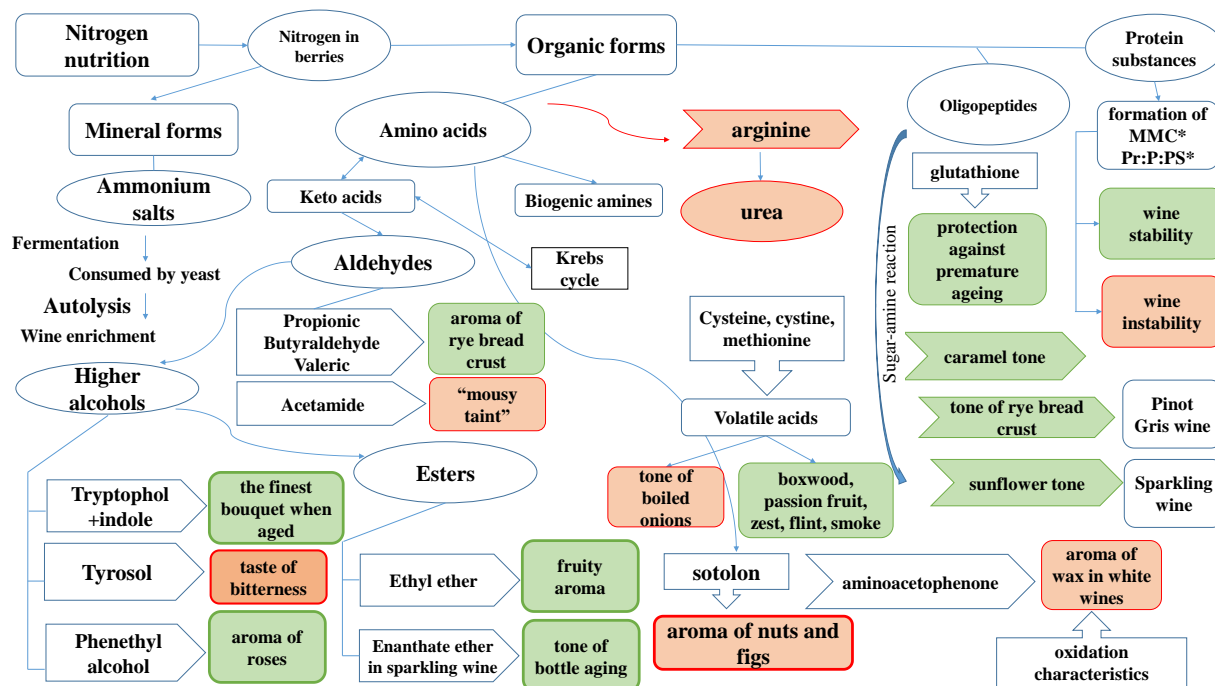
Thus, it can be concluded that yeast absorbs nitrogen from a wide variety of sources, because at different stages of fermentation, it consumes certain components of nitrogen-containing compounds, both organic and inorganic.

For decades, adding inorganic nitrogen to must to help nitrogen deficiency has been common practice among world’s winemakers. Nowadays, the most common way to combat the lack of nitrogen (as inorganic compounds) during fermentation is adding nitrogen supplements in the form of ammonium salts (ammonium phosphate (NH₄)₂HPO₄ or ammonium sulphate (NH₄)₂SO₄). Inorganic nitrogen DAP, which is added at different stages during the yeast growth phase, increases the cell size, but has almost no effect on the number when added at later stages. Regarding the yeast life cycle rate, the addition of DAP has the same effect throughout fermentation. Therefore, the addition of DAP during the fermentation process reduces the duration of fermentation, and its effect is the greatest when it is added in the early stages.

Among organic forms, amino acids, as described above, are the main nitrogenous nutrients. As various amino acids are assimilated differently, it can be concluded that when adding amino acids as fertilisers, the concentration of each amino acid should be taken into account to ensure that the NCR mechanism remains constant until the end of fermentation. The ratio depends on the locality, on the concentration of various amino acids in grapes, and on whether the amino acids are assimilable or non-assimilable (the ratio of proline and arginine, respectively) [10-13].

On analysing literature references and summarising the information on the metabolism of nitric substances, a scheme (Fig. 1) has been developed. It reflects how these substances influence the formation of the quality characteristics of grape wines.

So, we have conducted a study to determine the dynamics of nitrogen (total and amine) in fermenting must obtained from the white grape variety *Sukholimansky* bred by the National Science Centre “Tairov Institute of Viticulture and Winemaking” and harvested in 2015–2017, according to different technological schemes (Fig. 2).



Notes: red colour – negative aromas; green colour – positive aromas;
 *MMC – macromolecular complex, Pr – proteins, P – polysaccharides, PS – phenolic substances

Fig. 1. Effect of nitrogen substances on the formation of quality and safety characteristics of wine

The purpose of the research is to investigate how nitrogen metabolism in fermenting must from the white grape variety *Sukholimansky* and the quality of the wine materials obtained depend on extra nutrition, and whether using it is practical.

The objectives of the research:

1. To investigate the effect of additional nutrition on the dynamics of total and amine nitrogen in the

course of fermentation of the must obtained from the white grape variety *Sukholimansky*.

2. To determine the physical and chemical parameters of the white wine materials from the white grape variety *Sukholimansky*.

3. To carry out sensory evaluation of the wine materials.

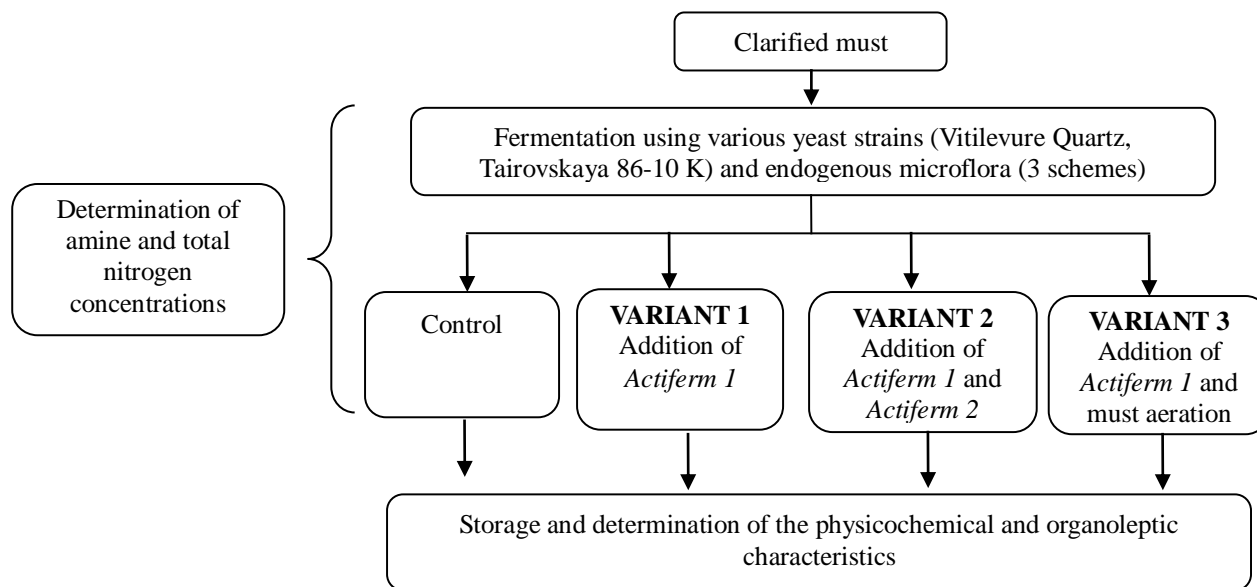


Fig. 2. Scheme of the experiment

Research materials and methods

The objects of the research were the must and the wine materials obtained from the white grape variety *Sukholimansky* bred in the National Science Centre "Tairov Institute of Viticulture and Winemaking": mass concentration of sugars 200 g/dm³; mass concentration of titrated acids 8.5 g/dm³.

The parameters of the dynamics of amine and total nitrogen were determined at a controlled temperature, that is, under conditions of high-quality fermentation.

The total amount of nitrogen was determined by the Kjeldahl method, and the amine nitrogen concentration by formol titration [16].

Sensory analysis of the wine materials was carried out according to the current regulations of Ukraine: Sensory analysis - Identification and selection of descriptors for the creation of a sensory profile using a comprehensive approach (ISO 11035:1994, IDT): DSTU ISO 11035:2005. Sensory analysis - Methodology - Flavour profile methods (ISO 6564:1985, IDT): DSTU ISO 6564:2005.

The experiment involved fermentation of clarified and sulphitated must obtained from the white grape variety *Sukholimansky* according to the three schemes:

1. Fermentation on endogenous microflora.

2. Fermentation with addition of active dry yeast *Vitilevure Quartz* (Martin Vialatte, France). ADY *Saccharomyces cerevisiae galactose*: affects the organoleptic properties of wine but insignificantly; wide temperature range 10 to 32°C; rapid reproduction and uniform fermentation kinetics; alcohol accumulation up to 18%; resistant to low pH (up to 2.8) and high doses of SO₂; low accumulation of bound sulphur and H₂S; low accumulation of volatile acids during fermentation - 0.1–0.3 g/dm³.

3. Fermentation with addition of the pure yeast culture from the National Science Centre "Tairov Institute of Viticulture and Winemaking" 86–10 K. Liquid preparation *Saccharomyces vini*: affects the organoleptic properties of wine but insignificantly; wide temperature range 10 to 28°C; killer factor; compact yeast sediment; alcohol accumulation up to 16%; low accumulation of bound sulphur and H₂S; low accumulation of volatile acids during fermentation – 0.1–0.2 g/dm³.

The food supplements used as additional nitrogen sources were Actiferm 1 and Actiferm 2 (Martin Vialatte, France), 2 g/dal of which were added to each scheme at the beginning of fermentation and after 1/3 of sugars had been consumed by yeast.

The supplement Actiferm 1 promotes yeast reproduction and quick start of fermentation. It contains thiamine, assimilable nitrogen (ammonia and amino form), a carrier, and inactivated yeast. The dosage recommended by the manufacturer is 2 g/dal.

The supplement Actiferm 2 increases yeast resistance to ethyl alcohol and accelerates the completion of the fermentation process. It contains

ammonia nitrogen (phosphate and sulphate) and inactivated yeast.

The control samples were fermented without any supplements.

The technology of processing grapes of the white variety *Sukholimansky* was similar to the experiment described in [15].

Results of the research and their discussion

As is generally known, in the course of reproduction, yeast intensively consumes ammonia nitrogen and that of the must amino acids. Yeast utilises 75–90% of glutamic and aspartic acids, valine, leucine, isoleucine, cysteine, arginine, tryptophan, tyrosine, phenylalanine, methionine. The content of proline, glycine, lysine, cysteine decreases but slightly [17]. So, in all the experimental schemes, the amine nitrogen content decreased by 90%. (Fig. 3). The process was the most intensive for 7 days on average, then the concentration stabilised.

In the first 4 days, extra nutrition added by schemes 1 and 2 stabilises the amine nitrogen concentration, but in general, has no effect on the final macronutrient content.

Thus, from the 4th to the 7th day of fermentation by schemes 1 and 2, there is a sharp decrease in the amine nitrogen concentration. With fermentation by scheme 3, the decrease in nitrogen was observed from the 1st to the 4th day (Fig. 3a–3c).

When must is aerated, consumption of nitrogen compounds by yeast increases. Along with the nitrogen consumption during fermentation, nitrogen substances, mainly amino acids, are released into the must [18].

It has been found that in all the experimental schemes, additional nutrition (Actiferm 1 and Actiferm 2) increases the initial content of total and amine nitrogen.

Analysis of the results shown in Fig. 4a allows concluding that fermentation on endogenous yeast microflora leads to a slight change in the total nitrogen concentration. This can be explained by the fact that at the end of fermentation, some of the yeast cells die, and their autolysis begins. During this period, the medium is enriched with polypeptides and amino acids, enzymes, purine and pyrimidine bases due to the autolysed cells [18].

During fermentation, part of the nitrogen compounds is precipitated by the alcohol formed. Besides, nitrogen-containing thanates are precipitated [17].

According to the chart in Fig. 4, the total nitrogen content during the fermentation period is reduced by 40–50%. Given that the same grape must was used in the experiments, and the fermentation was carried out under the same conditions, it can be assumed that this difference is due to the specific character of the wine yeast cells metabolism, in particular, due to the activity of protease enzyme systems [14].

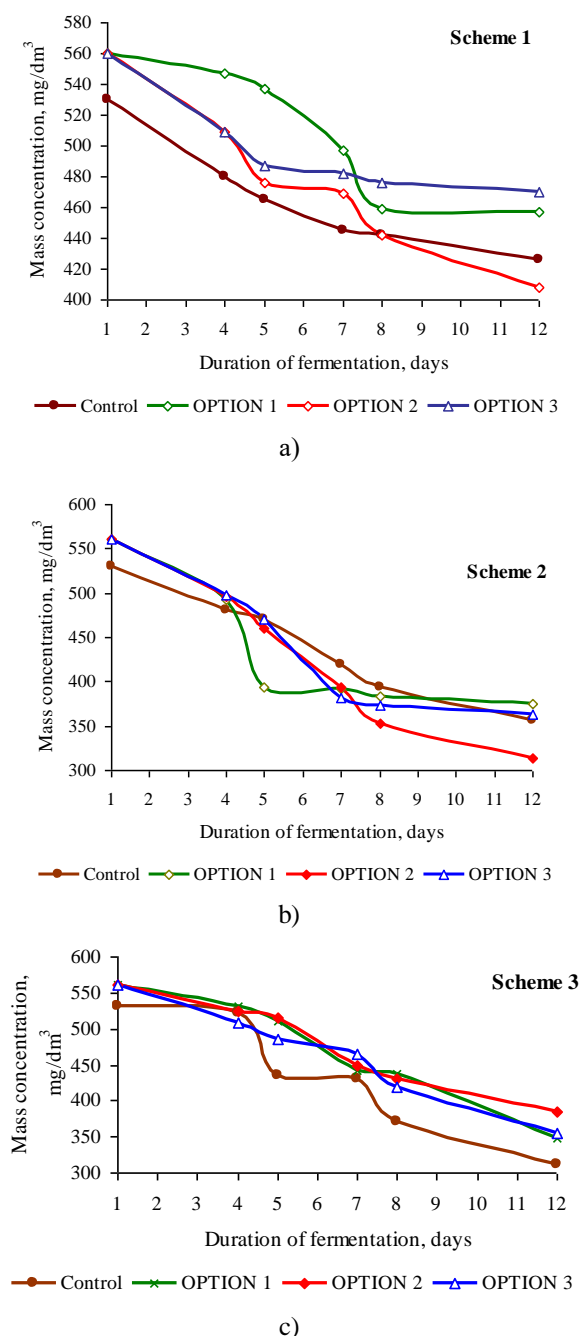


Fig. 4. Total nitrogen dynamics during fermentation of the must from the white grape variety *Sukholimansky*

The low nitrogen content in the must assimilated leads to a decrease in the yeast population, to reduced fermentation energy, and to the risk that fermentation might stop. Besides, it increases the content of undesirable thiols and higher alcohols in the fermenting medium, and decreases the mass fraction of esters and volatile fatty acids. Conversely, a high nitrogen concentration in the must contributes to the yeast cells accumulation due to the intensive fermentation rate and increased formation of acetyl ethyl, thiol ether, and volatile acids. There is also a risk of increased concentrations of urea, ethyl ester of

carbamic acid, and biogenic amines, and of protein turbidity [14].

The data shown in Fig. 4b demonstrate that after the introduction of the additional nutrition Actiferm 1 (scheme 2), the yeast actively consumes nitrogen substances. A similar process was observed during fermentation by scheme 3 in the control variant, which is confirmed by the dynamics shown in Fig. 4c.

Analysis of the data indicates that yeast differs in its ability to consume nitrogen substances. However, to determine the nitrogen-lowering properties of yeast, further research is needed. It will allow determining the optimal period of contact between yeast biomass and a wine material, and thus, will help establish the time modes for the maximum nitrogen reduction.

In the experiments performed, the fermentation process was completed (complete fermentation of sugars was observed in the control and experimental samples). This was confirmed by studying the volume fraction of ethyl alcohol and the mass concentration of sugars.

The results of studying the physicochemical parameters have indicated an increased content of volatile acids in the samples obtained by schemes 1 and 3 (fermentation on endogenous microflora (0.8 g/dm³) and on the yeast race from the NSC "Tairov Institute of Viticulture and Winemaking" (0.71 g/dm³), respectively). The content of titrated acids and active acidity were the same in all the variants.

The sensory evaluation has shown that the wine materials samples obtained by scheme 1 (fermentation on endogenous microflora) do not comply with the current regulations (DSTU 4806:2007 and DSTU 4112.25-2002) as there are extraneous shades of microbial nature. The samples obtained by scheme 2 (fermentation on Tairovskaya 86-10K) had no specific characteristics, so it can be concluded that this race does not contribute to revealing the specific varietal characteristics.

The best wine materials were the samples obtained by scheme 2 (fermentation on Vitilevure Quartz, Variant 2 and Variant 3): there, descriptors were found that corresponded to the varietal characteristics of a certain grape variety.

The results of sensory analysis of the best samples are presented in Fig. 5-6 in the form of profile charts.

The sensory evaluation has shown that the aroma intensity in the studied wine materials made from *Sukholimansky* white grapes is generally low, except for the descriptors "fruity" (its intensity was estimated as 6 points), "acidity," and "harmony of taste" (Fig. 5). The Variant 3 sample also has the descriptor "grassy". Neither of the samples is characterised by the descriptors "bitterness" and "vegetable." Slightly higher values of the descriptors "floral" and "mineral" have been noted in the Variant 3 sample.

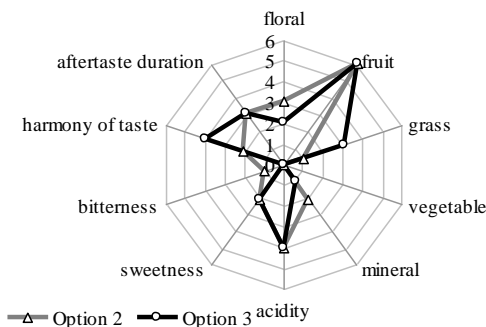


Fig. 5. Flavour of the wine materials *Sukholimansky White*

Respectively, in the aromatic descriptors of the wine materials from *Sukholimansky* white grapes, fruity shades prevailed (Fig. 6).

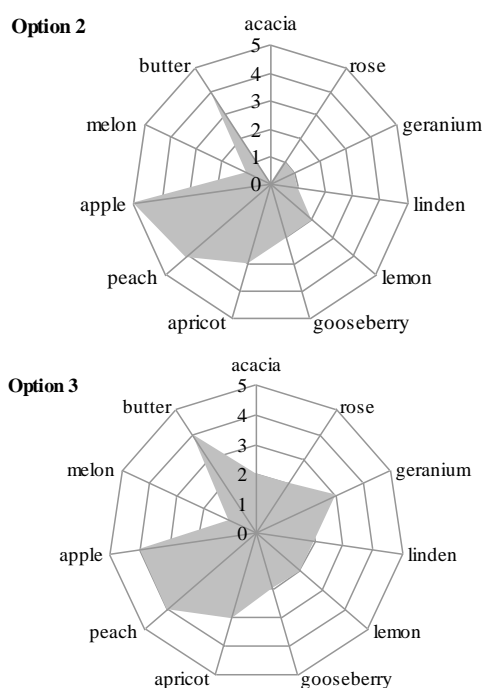


Fig. 6. Descriptors of the *Sukholimansky* white variety

Among the descriptors characterising the Variant 2 sample, the following ones should be noted: butter, apple and peach. For the Variant 3 sample, they are: butter, apple, peach, apricot, and geranium. These values of the aromatic descriptors completely correlate with the intensity descriptors that determine fruitiness, acidity, and harmony of taste.

Approbation of results

The research results can be included into the relevant regulations governing the production of wine materials from the white grape variety *Sukholimansky* bred in the NSC "Tairov Institute of Viticulture and Winemaking".

Conclusion

It has been found that nitrogen metabolism during fermentation does not depend on the feeding added, but the yeast race affects the physicochemical parameters, namely the content of volatile acids.

During fermentation, it was observed that the amine nitrogen concentration decreased by 90% and that of total nitrogen by 40–50%.

Regarding the effect on the quality characteristics of wine materials produced in the South of Ukraine, their physicochemical parameters can be improved by using the active dry yeast *Vitilevure Quartz* and the nutritional supplements *Actiferm 1* and *Actiferm 2*, in combination with aeration. This allows revealing fruity aromas, and achieving the right acidity and harmony of taste due to the presence of the descriptors (butter, apple, peach, apricot, and geranium) characteristic of the grape variety *Sukholimansky White*.

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ОСОБЛИВОСТІ МЕТАБОЛІЗМУ АЗОТУ СУСЛА З БІЛИХ СОРТІВ ВИНОГРАДУ ОДЕСЬКОГО РЕГІОНУ У ПРОЦЕСІ БРОДІННЯ

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Анотація. Питання управління якістю вина потребує розгляду метаболізму азоту в системі «виноград-вино» як ключового процесу. Азот – один із домінуючих елементів виноградної лози ґрунтового походження, який відіграє важливу роль в багатьох біологічних процесах як самої рослини, так і мікроорганізмів, що приймають участь у ферментації. Азотовмісні сполуки є необхідним поживним матеріалом для розвитку дріжджів, в тому числі в забезпеченні стабільного процесу бродіння. Ця група сполук прямо та опосередковано впливає на ароматичні та смакові якості вина в процесі його дозрівання та значною мірою визначає його стабільність. Сполуки азоту переходять у вино безпосередньо з винограду та із дріжджів в процесі бродіння. Оскільки їх роль в формуванні та визріванні вина дуже значна, існує проблема регулювання їхнього метаболізму в суслі що бродить. В результаті аналізу літературних джерел і узагальнення інформації про метаболізм азотних речовин було розроблено схему, що відображає їх вплив на формування якісних показників виноградних вин. В роботі наведено результати дослідження метаболізму азоту (загального та амінного) у складі виноградного сусла в процесі бродіння. У роботі використовували виноград сорту Сухолиманський білий селекції Національного наукового центру «Інституту виноградарства і виноробства ім. В.С. Таїрова» урожаїв 2015–2017 років. Встановлено, що метаболізм азоту в процесі бродіння не залежить від доданих підкормок, проте раса дріжджів впливає на фізико-хімічні показники, а саме на вміст легких кислот. В ході бродіння встановлено зниження концентрації амінного азоту на 90%, загального на 40–50%. В умовах Півдня України, з точки зору впливу на якісні характеристики виноматеріалів, використання сухих дріжджів Vitilevure Quartz та додаткового живлення у вигляді препаратів Актиферм 1 та Актиферм 2 з аерацією надає позитивний вплив на фізико-хімічні показники, що дозволяє проявити аромати фруктів, кислотність та гармонійність смаку, які обумовлені наявністю дескрипторів (вершкове масло, яблуко та персик, абрикос та герань) винограду сорту Сухолиманський білий.

Ключові слова: азот, азотні сполуки, виноматеріали, сусло, сорт Сухолиманський, метаболізм.

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