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SPECIFIC FEATURES OF FERMENTATION OF THE MUST FROM WHITE GRAPE VARIETIES IN THE CONDITIONS OF THE ODESSA REGION

O. Kananykhina, PhD, Associate Professor[1]
O. Tkachenko, Doctor of Science, Associate Professor[2]
T. Suhachenko, PhD, Senior Lecturer[2]
O. Titlova, PhD, Associate Professor[2]
[1]Department of Biochemistry, Microbiology and Physiology of Nutrition
[2]Department of Wine Technology and Sensory Analysis
Odesa National Academy of Food Technologies
Kanatna str., 112, Odesa, Ukraine, 65039

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Correspondence:

T. Suhachenko
E-mail: tanya.lozovskaia@gmail.com

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Abstract. One of the most important problems of the modern wine technology is the production of high quality and competitive products. It is commonly known that the quality of a wine largely depends on the yeast race. The main requirement for yeast is completeness of fermentation, which depends on the amount of yeast involved, aeration, initial content of various substances in the must, temperature, pH of the environment. Using imported materials in the specific conditions of national winemaking requires laboratory and industrial research to rationalize the use of local and foreign pure yeast cultures and to accelerate the process of grape must fermentation. So, this study considers how the yeast origin and additional nutrition effect on grape must fermentation and influence the physicochemical and organoleptic characteristics of wine materials obtained from the white grape varieties Aromatny and Zagrei of the generative breeding by the National Scientific Centre *Tairov Viticulture and Winemaking Institute*. Besides, it has been concluded whether using additional nutrition is practical. It has been established that the fermentation process was complete in both the control and the experimental samples. There were no facts of underfermentation. When supplementary nitrogenous nutrition was used, the fermentation activity of yeast increased and the fermentation process ended faster. The dynamics of the total number of cells of the Aromatic and Zagrei varieties was the same during the years 2015–2017 and did not depend on the fermentation scheme. It has been found that the fermentation process does not depend on supplementary nutrition, but the yeast race effects on the physicochemical parameters, namely on the volatile acids content. The results obtained have shown the effect of a yeast race and supplementary nutrition on the organoleptic characteristics of these varieties. In Southern Ukraine, to control the quality characteristics of wine materials, using active dry yeast *Saccharomyces cerevisiae* with a two-stage nutrition complex has a positive effect on the physicochemical parameters of the corresponding grape variety and allows revealing its varietal characteristics.

Keywords: must fermentation, white wine materials, yeast, organoleptic characteristics, varietal descriptors.

Introduction. Formulation of the problem

Manufacture of high quality and competitive wines is one of the most important problems of today's wine industry. According to the Oenological Codex, wine is a product obtained exclusively by complete or partial fermentation of whole or crushed grapes or grape must. It is commonly known that the quality of a wine largely depends on the yeast race used and its metabolic activity. Yeast is the most important component that can enrich wine with a complex of active enzymes and other biologically active compounds that form the organoleptic properties of a finished product.

The main requirement for yeast is completeness of fermentation, which depends on the amount of yeast involved, aeration, initial content of various substances in the must, temperature, pH of the environment. Careful selection of yeast strains significantly improves the controllability and reliability of fermentation processes.

It is safe to say that the choice of yeast also determines the characteristics of a wine, because, along with various grape varieties, there are many yeast races with their individual features.

Analysis of recent research and publications

International competition in the wine market, consumer demands for new wine styles, and growing concern about the environmental aspects of wine production are creating new challenges for innovation in wine fermentation. Throughout the entire production chain, alcoholic fermentation of grape must with yeast is a key process. Within this process, winemakers, by better management of yeast, can be true creators of the character and value of wine and thus strategically adapt it to the changing market [1-5].

Criteria of the yeast quality for wine production have been developed for years. Generally, these criteria fall into three categories:

– properties that influence the productivity of the fermentation process;
– properties that determine the quality and nature of a wine;
– properties related to the commercial production of wine yeast.

In each category, there are more important and less important properties. Some of them are necessary, and some are just desirable [6].

The main problems of the rational use of yeast in winemaking are acceleration of biochemical processes during fermentation and improvement of the quality parameters of wine materials. Almost all manufacturers of active dry yeast suggest using dry yeast preparations in winemaking with simultaneous introduction of a nitrogen-phosphorus vitamin supplement into the must.

As a result, sugars are completely fermented, the wine contains smaller amounts of volatile acids and ethers, quickly gets clarified, and has a clean flavour. However, a small amount of fermentation by-products is formed.

Nowadays, commercial yeast can be classified as universal, varietal, and that with additional functionality (like effective resistance to high contents of titrated acids in grapes; functioning well at lower temperatures), which makes it possible to choose the best yeast for a particular batch of wine. On the Ukrainian market, there is so-called "varietal" yeast, which can be used to ferment must from the corresponding grape varieties. When fermented, they are able to accumulate a certain set of secondary products and by-products emphasizing the varietal characteristics of a wine. The benefits of adding such yeast include: reliable and quick start of fermentation; steady fermentation throughout the whole cycle with a lower risk of inhibition.

Analysis of scientific research shows that nitrogen deficiency in must limits the growth of yeast biomass and the fermentation rate, which is a factor that significantly increases the risk of spontaneous fermentation, or even its stopping, and of the formation of compounds that negatively affect the organoleptic characteristics of wine. The content of this component in grape must can be compensated by additional introduction of its mineral and organic forms easily assimilated by yeast as supplementary feeding. However, in industrial conditions, various types of nitrogen supplements are very often applied without controlling their initial content. This approach results in unreasonable production expenses and can affect the quality of the finished product [7-10].

Popular practice of manufacturing organic, biodynamic, and natural wines involves fermentation of wine materials on wild microflora. Natural wine is fermented by a complex of yeast that are an integral part of the terroir. Yeast strains vary widely from place to place and contribute a lot to the aroma of a wine. Yeast belonging to a particular region is an important part of what gives the local wines their character. It is this production method that helps preserve and render the organoleptic characteristics of a certain grape variety and a certain area. However, it is very difficult to foresee the quality of the final product and to retain its stability. In this case, the fermentation process lengthens significantly. What is more, there is a risk that fermentation will stop because of the lack of nutrients, which depends entirely on the raw material quality.

Traditionally, only the *Saccharomyces* species of yeast is widely used in oenology. The hidden potential of all the yeast biodiversity is almost never used. In order to take advantage of the potential benefits of non-*Saccharomyces* yeast in wine production and to minimise possible spoilage, one should know the yeast populations in grapes and must, the effect of wine-making practices on this yeast, and the metabolic characteristics of non-*Saccharomyces* yeast. The choice of strains is very important, since not all strains within a certain species will have the desired characteristics. It has been established that the contribution of non-*Saccharomyces* yeast to wine quality can take various forms. There is evidence of the formation of glycerol *Candida stellata* and esters *C. pulcherrima* [11]. Other non-*Saccharomyces* yeasts are also widely recognised as useful when producing glucosidase, the enzyme that, during hydrolysis, can release volatile compounds associated with sugars, thus making the aromatic profile of a wine more complex [12-16].

Application of imported materials in the specific conditions of national winemaking requires laboratory and industrial research to rationalise the use of local and foreign pure yeast cultures and to accelerate the process of grape must fermentation. The results of this research should ensure obtaining high quality wines.

The purpose of the study is to investigate how the origin of yeast and the use of additional nutrition effect on grape must fermentation and on the physicochemical and sensory characteristics of white wine materials from the Aromatny and Zagrei grape varieties (generative breeding by the National Scientific Centre *Tairov Viticulture and Winemaking Institute*), and to determine whether additional nutrition is practical.

The research **objectives**:

1. To study the physiological state of microbiota.
2. To determine the physicochemical characteristics of white wine materials from the Aromatny and Zagrei grape varieties.
3. To carry out organoleptic evaluation of the wine materials.

Research materials and methods

The objects of the study were the must and the wine materials from the varieties Aromatny (harvested in 2009, # 09328; mass concentration of sugars 191 g/dm^3; mass concentration of titrated acids 6.3 g/dm^3) and Zagrei (harvested in 2006, # 06296; mass concentration of sugars 173 g/dm^3; mass concentration of titrated acids 9.1 g/dm^3).

The experiment was carried out during the years 2015–2017 and hosted by the National Scientific Centre *Tairov Viticulture and Winemaking Institute*. This made it possible to create conditions for high-quality fermentation at a controlled temperature, and to determine the physicochemical and microbiological parameters. Also, the fermentation kinetics was studied in the Microbiological Laboratory of Odessa National Academy of Food Technologies.

The experiment focused on fermentation of clarified and sulphited must obtained from each grape variety according to the three schemes:

1. Fermentation on the endogenous microflora.
2. Fermentation with adding active dry yeast Vitilevure Quartz (Martin Vialatte, France). ADY *Saccharomyces cerevisiae galactose*: does not affect significantly the organoleptic properties of wine; wide temperature range 10°C to 32°C; quick start and steady kinetics of fermentation; alcohol accumulation up to 18%; resistant to pH as low as 2.8 and to high SO_2 dosages; low accumulation of bound sulphur and H_2S; low accumulation of volatile acids during fermentation (0.1–0.3 g/dm^3).
3. Fermentation with adding a pure yeast culture from the National Scientific Centre *Viticulture and Winemaking Institute* 86-10 K. Liquid culture of *Saccharomyces vini*: does not affect significantly the organoleptic properties of wine; wide temperature range 10°C to 28°C; killer factor; compact yeast deposit; alcohol accumulation up to 16%; low accumulation of bound sulphur and H_2S; low accumulation of volatile acids during fermentation (0.1–0.2 g/dm^3).

The scheme of the experiment is presented in Fig. 1.

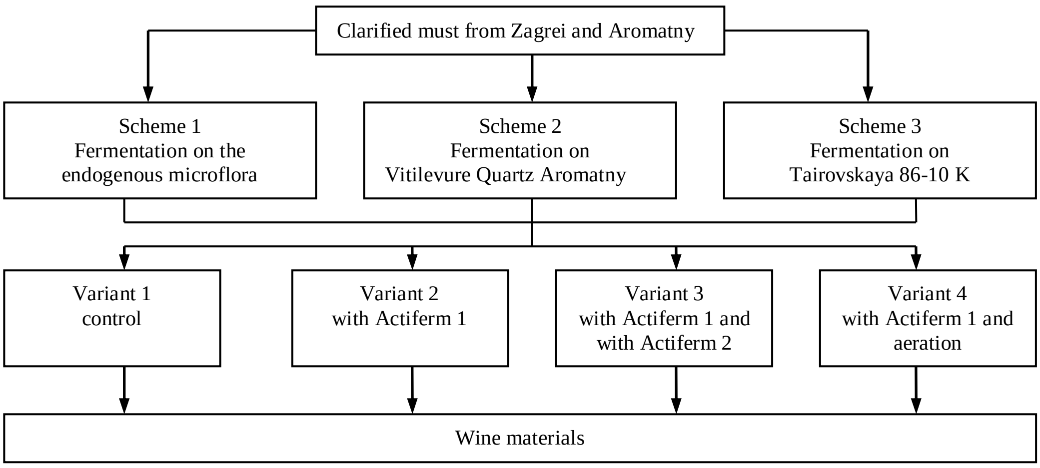

Fig. 1. Scheme of the experiment

The following nutritional supplements were used as additional sources of nitrogen: Actiferm 1 and Actyferm 2 (Martin Vialatte, France). They were added in each scheme at a dose of 2 g/daL at the beginning of fermentation and after 1/3 of sugars was consumed by the yeast.

The supplement Actiferm 1 promotes yeast reproduction and quick start of fermentation. It contains thiamine (vitamin B1), assimilable forms of nitrogen (ammonia and amino nitrogen), a support, and inactivated yeast. The dosage recommended by the manufacturer is 2 g/daL.

The supplement Actiferm 2 increases the resistance of yeast to ethyl alcohol and accelerates the completion of fermentation. Contains inorganic nitrogen (ammonium phosphate and sulphate), inactivated yeast. Actiferm 2 should be added in the middle of fermentation or after the density of the fermenting must has decreased by 30–40 points. The dosage recommended by the manufacturer is 2 g/daL.

The control samples were fermented with the same yeast strains, but without any supplements.

Grapes of certain varieties were stemmed on roll crusher-stemmers and sulphitated. The initial amount of sulphur dioxide was calculated in accordance with the optimum concentration of molecular SO_2 and the pH of the grapes, but not more than 150 mg/dm^3 of total sulphuric acid.

The must was obtained by crushing the grapes in a pneumatic press. For the study, the must was used in an amount of 65 daL out of 1 ton of grapes. Then, the must was allowed to settle at a temperature of 10–

12°C until it was completely clarified. The choice of substances for clarification was determined by trial fining.

The clarified must was decanted off the precipitate and sent to fermentation, which lasted until the sugars were completely fermented at 18–20°C in accordance with the experimental scheme shown in Fig. 1.

At the end of fermentation, the wine materials were removed from the yeast deposit and stored at 12–14°C.

In the clarified must, the mass concentrations of sugars and of titrated acids, and the active acidity were determined [14]. In the fermenting must, the number of cells in the pure culture yeast (PCY) and that in the active dry yeast (ADY) were determined. The parameters determined in each fermentation phase were: mass concentration of sugars (by changes in the density), quantitative changes in the yeast population, physiological state of the cells. The yeast cells were calculated in the Goryaev and Thoma counting chamber [14,15]. The organoleptic assessment was performed by the descriptive method (Flavour Profile method).

Results of the research and their discussion

The physiological state of the must microbiota of the variety Aromatny is presented in Fig. 2–4.

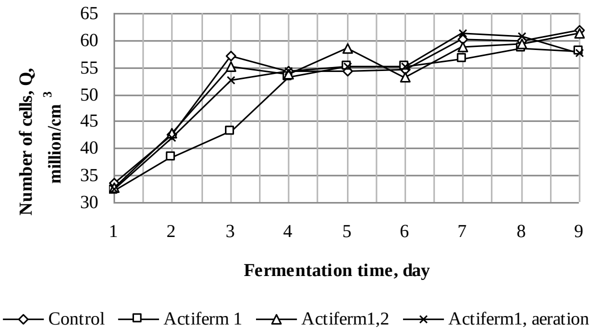

Fig. 2. Scheme 1. Fermentation on the endogenous microflora

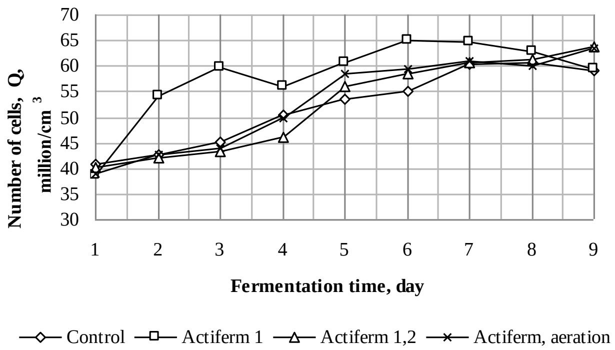

Fig. 3. Scheme 2. Fermentation with adding Vitilevure Quartz

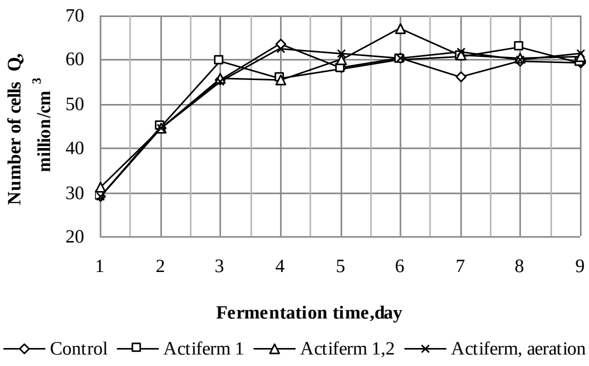

Fig. 4. Scheme 3. Fermentation with adding Tairovskaya 86-10К

Analysis of the data presented in Fig. 2–4 indicates that, during fermentation, a decrease in the mass concentrations of sugars was observed in all the samples. However, the fermentation rate depended significantly on the introduction of various feeding preparations. When using additional nitrogenous nutrition, the fermentation activity of yeast increased, and fermentation ended sooner. It can also be noted that the nature of fermentation activators has a substantial effect on the rate and effectiveness of alcoholic fermentation.

The dynamics of yeast culture growth in the samples studied had a more pronounced abrupt jump in the middle of the fermentation process when the feeding was added. The presence of additional sources of nitrogen contributed to the increased accumulation of a large amount of yeast biomass.

The fermentation process was carried out until completion. Complete fermentation of sugars was observed in the control and the experimental samples. No facts of underfermentation were registered. The control samples differed from the experimental ones in the volume of the yeast deposit.

The dynamics of the total number of cells of the Aromatny variety is identical over a period of 2015–2017 and does not depend on the fermentation scheme. The maximum number of cells is observed on the 3rd-4th day (50–85 million/cm^3) and then practically does not change. In 2017, the total number of cells on the 3rd-4th day of fermentation was 80–110 million/cm^3.

The dynamics of yeast cells' dying (D,%) and budding (B,%) during the must fermentation of the Aromatny variety is presented in Table 1 as a percentage of the total number of cells.

Table 1 – Dynamics of dying and budding of yeast cells

Scheme	Variant	Fermentation time, day								
		1	3	4	5	6	8	9	11	12
		M, %	M, %	M, %	M, %	M, %	M, %	M, %	M, %	M, %
		B, %	B, %	B, %	B, %	B, %	B, %	B, %	B, %	B, %
Scheme 1. Fermentation on the endogenous microflora	Control	2.5	3.5	3.0	10.0	12.0	18.0	18.2	32.5	43.5
		31.0	34.0	37.0	29.0	30.0	16.0	9.1	10.0	10.4
	Actiferm 1	3.0	4.6	7.4	9.0	11.3	12.6	22.1	33.3	45.6
		35.0	35.0	39.0	30.0	32.0	15.0	13.7	8.5	10.3
	Actiferm 1, 2	2.6	4.8	8.1	8.4	10.5	11.2	17.8	43.7	43.0
		37.0	36.0	24.0	30.0	36.0	13.0	16.5	10.9	10.9
	Actiferm 1, aeration	2.3	4.5	8.5	9.0	14.4	16.2	19.5	47.7	45.0
		38.0	30.0	24.0	35.0	38.0	13.0	8.9	11.5	10.3
Scheme 2. Fermentation with adding Vitilevure Quartz	Control	8.0	4.6	8.0	11.0	19.8	22.6	23.0	37.8	44.9
		29.0	28.0	27.0	31.0	34.0	12.0	12.8	9.8	8.9
	Actiferm 1	3.5	8.5	8.5	8.5	11.6	13.0	18.5	39.0	47.0
		25.0	25.0	25.0	24.0	24.0	12.0	12.4	13.1	10.5
	Actiferm 1, 2	7.3	4.0	8.0	9.0	13.0	16.2	18.1	35.9	36.0
		29.0	28.0	27.0	25.0	24.0	12.0	18.9	10.6	11.4
	Actiferm 1, aeration	7.5	4.7	8.2	8.8	15.7	19.3	26.2	35.0	54.3
		23.0	25.0	25.0	31.0	30.0	13.0	18.8	11.3	6.2
Scheme 3. Fermentation with adding Tairovskaya 86-10K	Control	7.0	8.1	12.5	16.0	30.0	36.0	24.8	42.6	51.2
		26.0	27.0	31.0	33.0	28.0	25.0	22.6	13.3	10.5
	Actiferm 1	6.3	8.2	15.0	16.0	22.4	27.8	25.4	33.3	38.6
		30.0	31.0	34.0	24.0	18.0	23.0	19.3	13.0	17.2
	Actiferm 1, 2	8.0	8.6	12.5	18.0	30.0	37.5	37.3	41.3	50.2
		36.0	37.0	39.0	25.0	19.0	20.0	15.9	10.3	12.7
	Actiferm 1, aeration	7.5	8.3	12.4	13.0	28.5	32.2	32.9	59.1	55.3
		35.0	36.0	37.0	33.0	26.0	16.0	20.2	10.4	13.4

According to the data presented in Table 1, the amount of buding yeast decreases on the 3rd-5th day. The dynamics of yeast cells dying is the same, regardless of the characteristics of the must fermentation scheme. Fermentation activators are added, among other reasons, to facilitate yeast reproduction and to maintain it in a satisfactory physiological state during fermentation.

It is known that inorganic nitrogen added at different stages during the yeast growth phase increases the size of the cells, but has almost no effect on their number when added at later stages. As for the rate of the yeast vital activity, the addition of DAP has the same effect throughout the whole fermentation process. The addition of DAP during fermentation leads to a decrease in its duration. It has the greatest effect when added at the early stages. However, in this experiment, when comparing the graphs of must density changes and the number of yeast cells, and while studying the physiological state of microbiota, there was no significant difference observed between the fermentation variants.

When studying the fermentation of must from grapes of the Zagrei variety, the results obtained completely correlated with those presented in Fig. 2–4 and Table 1.

According to the results of studying the physical and chemical parameters, an increased content of volatile acids was registered in the samples obtained according to schemes 1 and 3 standing for fermentation on the endogenous microflora (0.98 g/dm^3) and on the yeast race from the National Scientific Centre *Tairov Viticulture and Winemaking Institute* (0.88 g/dm^3), respectively. The contents of titrated acids and active acidity remained unchanged in all cases.

According to the results of organoleptic evaluation, it was established that the wine samples obtained by Scheme 1 (fermentation on the endogenous microflora) did not meet the requirements, as there were off-odours of microbial character. The samples obtained by Scheme 2 (fermentation on Tairovskaya 86-10K) had no specific characteristics, so it can be concluded that this race did not contribute to revealing specific varietal characteristics.

The best were the samples of the wine materials from the Aromatny and Zagrei varieties obtained by Scheme 2 (fermentation on Vitilevure Quartz) with the addition of the nutrients Actiferm 1 and Actiferm 2, as the descriptors were found that corresponded to the varietal characteristics of these grape varieties.

The results of organoleptic analysis of the best samples are presented, in the form of profile charts, in Fig. 5.

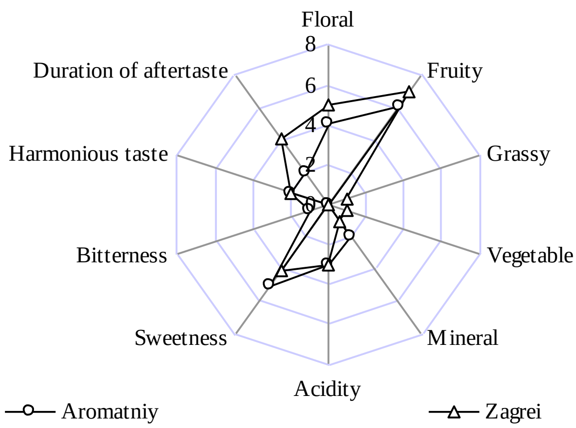

Fig. 5. Flavour Profile of the wine materials from the Zagrei and Aromatny grape varieties

During the sensory analysis of the varietal wine materials, it was established that those from the Aromatny grape variety, featured a caramel-fruit aroma with an elegant spicy accent and fresh taste, and those from the Zagrei grape variety had a pronounced aroma of ripe tropical fruit and a rich long aftertaste.

Approbation of results. The results of the study can be implemented in the relevant regulatory documents that standardise the manufacture of wine from the varieties Aromatny and Zagrei of the generative breeding by the National Scientific Centre *Tairov Viticulture and Winemaking Institute*.

Conclusions

In the course of the research, according to their purpose, the following has been established:

1. The Tairovskaya 86-10K yeast race, active dry yeast Vitilevure Quartz, and fermentation on the endogenous microflora ensure complete fermentation of wine materials obtained from the varieties Aromatny and Zagrei in the conditions of Odessa region.

2. Introducing additional nutrition and using aeration do not affect the completeness of the fermentation of wine materials from the Aromatny and Zagrei varieties. However, when using Actiferm 1 and Actiferm 2 nutritive complexes, the volume of yeast deposit increases.

3. A yeast race affects the physicochemical characteristics, namely the content of volatile acids. The highest concentration of volatile acids was registered in the samples obtained by fermentation on the endogenous microflora (0.98 g/dm^3).

4. Using active dry yeast of the species *Saccharomyces cerevisiae* in combination with two-stage nutrition effects on the physicochemical parameters and helps the corresponding varietal characteristics reveal themselves to the maximum.

So, in the conditions of Odessa region, no evidence has been registered of the risk that fermentation could stop with different variants of the process. Meanwhile, the choice of the yeast race and using additional technological methods are the tools to control the quality characteristics of wine.

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ОСОБЛИВОСТІ ПРОЦЕСУ БРОДІННЯ СУСЛА З БІЛИХ СОРТІВ ВИНОГРАДУ В УМОВАХ ОДЕСЬКОГО РЕГІОНУ

О.М. Кананихіна, кандидат технічних наук, доцент[1], *E-mail*: k_elni@ukr.net
О.Б. Ткаченко, доктор технічних наук, професор[2], *E-mail*: obtkachenko@gmail.com
Т.С. Сугаченко, кандидат технічних наук, станший викладач[2], *E-mail*: tanya.lozovskaia@gmail.com
О.О. Тітлова, кандидат технічних наук, доцент[2], *E-mail*: titlova@ukr.net
[1] Кафедра біохімії, мікробіології та фізіології харчування,
[2] Кафедра технології вина та сенсорного аналізу
Одеська національна академія харчових технологій, 112, вул. Канатна, Одеса, Україна, 65039

Анотація. Однією з найважливіших проблем сучасної технології виробництва вин є отримання високоякісної і конкурентоспроможної продукції. Як відомо, якість виноматеріалів в значній мірі залежить від раси дріжджів. Основною вимогою до дріжджів є повнота виброджування, яка залежить від кількості внесених дріжджів, аерації, вихідного вмісту різних речовин в суслі, температури, pH середовища. Використання імпортних матеріалів в особливих умовах національного виноробства потребує проведення лабораторних і промислових досліджень з метою раціонального використання вітчизняних і закордонних чистих культур дріжджів і прискорення процесу бродіння виноградного сусла. У роботі досліджено вплив походження дріжджів і доцільність використання додаткового живлення на процес бродіння виноградного сусла та фізико-хімічні показники та органолептичні характеристики білих виноматеріалів сортів винограду Ароматний та Загрей генеративної селекції Національного наукового центру «Інститут виноградарства і виноробства ім. В. Є. Таїрова». Встановлено, що процес бродіння відбувся до кінця в контрольних і експериментальних зразках. Фактів недоброду не зафіксовано. При використанні додаткового азотистого харчування, бродильна активність дріжджів збільшувалася, і сам процес ферментації закінчувався швидше. Динаміка загальної кількості клітин сортів Ароматний, Загрей ідентична впродовж 2015-2017 рр. і не залежить від схеми бродіння. Було встановлено, що процес бродіння не залежить від доданих підкормок, проте раса дріжджів впливає на фізико-хімічні показники, а саме на вміст летких кислот. Отримані результати показали вплив раси дріжджів та підкормок на органолептичні показники зазначених сортів. В умовах Півдня України, з точки зору впливу на якісні характеристики виноматеріалів, використання активних сухих дріжджів виду *Saccharomyces cerevisiae* з комплексом двоетапного живлення надає позитивний вплив на фізико-хімічні показники та дозволяє проявити сортові характеристики відповідного сорту винограду.

Ключові слова: бродіння сусла, білі виноматеріали, дріжджі, органолептичні показники, сортові дескриптори

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