PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

# BIOSSÍNTESE DE PROTEINASES E PECTINASES POR LEVEDURAS DE VINHO

# BIOSYNTHESIS OF PROTEINASES AND PECTINASES BY WINE YEASTS

# БИОСИНТЕЗ ПРОТЕИНАЗ И ПЕКТИНАЗ ВИННЫМИ ДРОЖЖАМИ

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## RESUMO

A relevância da pesquisa é determinada pela necessidade de utilizar o potencial biológico da levedura para melhorar a qualidade dos vinhos e garantir sua estabilidade contra a turbidez coloidal. O objetivo da pesquisa foi determinar a atividade de sistemas enzimáticos, proteinases e pectinases, sintetizados pela levedura durante fermentação alcoólica e autólise, dependendo de sua raça e da duração do contato do material vitícola com a biomassa de levedura. Métodos de pesquisa. Para determinar a atividade enzimática, leveduras de diferentes linhagens foram incubadas em meios contendo os seguintes substratos: albumina para proteinases e pectina de maçã para pectinases. Resultados da pesquisa. A biossíntese de proteinases e pectinases por várias raças de leveduras foi estudada, bem como a sua secreção em material vitivinícola. A pesquisa mostrou que a atividade das proteinases na biomassa celular e nos materiais do vinho varia muito, dependendo da raça da levedura, o que é explicado por suas características genéticas. A ausência de uma correlação entre a atividade das proteinases na biomassa de levedura e no material do vinho foi estabelecida. Pela primeira vez, foi demonstrado que a biomassa de células ativas de levedura seca possui um grande suprimento de proteinases em comparação com as leveduras iniciadoras de raças produzidas na Rússia. A atividade das pectinases na biomassa de levedura é significativamente menor que a atividade das proteinases, o que se deve a uma menor concentração do substrato - substâncias pectínicas. A pesquisa sugere várias interpretações dos mecanismos dos processos bioquímicos que ocorrem durante o contato da biomassa de levedura com os materiais do vinho. E, também contém dados experimentais sobre o efeito de proteinases e pectinases sintetizadas pela levedura de vinho na composição de biopolímeros de vinho. Significado prático. O estudo identifica as linhagens de leveduras que ativamente hidrolisam os componentes de alto peso molecular do vinho e complexos de biopolímeros, e prolongam a resistência dos vinhos à turbidez coloidal..

**Palavras-chave**: *levedura de vinho, proteinase, pectinase, compostos de alto peso molecular, complexos de biopolímeros.*

## ABSTRACT

The relevance of research is determined by the need to use the biological potential of wine yeast to improve the quality of wines and ensure their stability against colloidal turbidity. The purpose of the research is to determine the activity of enzyme systems, proteinases,and pectinases, synthesized by wine yeast during alcohol fermentation and autolysis, depending on their race and the duration of contact of the wine material with yeast biomass. To determine the enzyme activity, yeasts of different strains were incubated on media containing the following substrates: albumin for proteinases, apple pectin for pectinases. The biosynthesis of proteinases and pectinases by various races of wine yeast was studied as well as their secretion into wine material. The research has shown that the activity of proteinases in the cell biomass and wine materials varies widely depending on the race of the yeast, which is explained by their genetic characteristics. The absence of a correlation between the activity of proteinases in yeast biomass and wine material has been established. For the first time, it has been shown that the biomass of active dry yeast cells has a large supply of proteinases in comparison with the yeast starters of races produced in Russia. The activity of pectinases in yeast biomass is significantly less than the activity of proteinases, which is due to a lower concentration of the substrate - pectin substances. The paper suggests several interpretations of the mechanisms of biochemical processes occurring during the contact of yeast biomass and wine materials. The paper contains experimental data on the effect of





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proteinases and pectinases synthesized by wine yeast on the composition of wine biopolymers. The study identifies the yeast strains that actively hydrolyze the high-molecular components of wine and complexes of biopolymers, and prolong the resistance of wines to colloidal turbidity.

**Keywords**: *wine yeast, proteinase, pectinase, high-molecular compounds, biopolymer complexes.*

## АННОТАЦИЯ

Актуальность исследований определяется необходимостью использования биологического потенциала винных дрожжей для улучшения качества вин и обеспечения их устойчивости против коллоидных помутнений. Цель исследований заключается в определении активности ферментных систем – протеиназ и пектиназ, синтезируемых винными дрожжами в процессе спиртового брожения и автолиза в зависимости от их расы и продолжительности контакта виноматериала с дрожжевой биомассой. Для определения активности ферментов дрожжи различных штаммов инкубировали на средах, содержавших соответствующие субстраты: для протеиназ – альбумин, для пектиназ - яблочный пектин. Исследован биосинтез протеиназ и пектиназ различными расами винных дрожжей и их секреция в виноматериал. Показано, что активность протеиназ в биомассе клеток и виноматериале варьирует в широких пределах в зависимости от расы дрожжей, что объясняется их генетическими особенностями. Установлено отсутствие корреляции между активностью протеиназ в дрожжевой биомассе и виноматериале. Впервые показано, что биомасса клеток активных сухих дрожжей обладает большим запасом протеиназ в сравнении с разводками дрожжей отечественных рас. Активность пектиназ в дрожжевой биомассе значительно меньше, чем активность протеиназ, что связано с меньшей концентрацией субстрата – пектиновых веществ. Выдвинуты трактовки механизмов биохимических процессов, протекающих при контакте биомассы дрожжей и с виноматерилами. Представлены экспериментальные данные о влиянии протеиназ и пектиназ, синтезированных винными дрожжами, на состав биополимеров вина. Выделены штаммы дрожжей, активно гидролизующие высокомолекулярные компоненты вина и комплексы биополимеров, и пролонгирующие устойчивость вин к коллоидным помутнениям.

**Keywords**: *винные дрожжи, протеиназы, пектиназы, высокомолекулярные соединения, комплексы биополимеров.*

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## 1. INTRODUCTION

Under the action of yeast, a complex of biochemical transformations (processes) occurs, as a result of which a fundamentally new product is obtained from grape must - wine, the chemical composition of which is primarily determined by the direction and activity of metabolic processes that occur due to wine yeast.

Yeast is the producer of various chemical compounds, whose influence on taste, wine resistance to turbidity is difficult to assess unambiguously. There are significant differences between the races of yeast in their ability to transform the biopolymer complexes of wine; this is due to the different activity of enzyme systems produced by the cells of wine yeast and released into the wine material. Therefore, understanding the role played by yeast cell enzymes during must fermentation and subsequent contact of the wine material with the yeast biomass can help develop a rationally effective strategy for optimizing technological methods, modeling the composition and the sensory properties of wine.

Considering the significant influence of all the enumerated factors on the quality of wines, their uniqueness, and recognizability, it is relevant to study the activity of hydrolytic enzymes of different yeast races and their effect on high-molecular compounds of wine and their complexes. According to N. Buryan (2008), the determination of the activity of the enzymes of the pectin-cleaving complex in 292 races of Saccharomycete yeast showed that they differ in the activity of pectinesterase and polygalacturonase, that is, in their ability to cleave pectic substances.

According to the research of V.G. Gerzhikova (1997), S.A. Chursina (2009) and *et al.* (Abdullaeva, *et al.,* 2003; Shevchenko, *et al.,* 2009) there is a difference between the races of yeast by the activity of proteolytic enzymes, including their ability to cleave complexes of biopolymers. Knowledge of the activity of the enzyme systems of wine yeast used in the fermentation of grape must is of particular importance at the present time due to a large number of wine yeasts on the Russian market of supporting materials.

The purpose of the study is to establish

the activity of enzyme systems - proteinases and pectinases, synthesized by wine yeast in the process of alcoholic fermentation and autolysis, depending on their race and the duration of contact of the wine material with yeast biomass.

## 2. MATERIALS AND METHODS

The experiments used active dry yeasts of different races produced by German and French firms, as well as the races of the yeast produced in Russia of the species of Saccharomyces vini (according to the Lodder classification of Saccharomyces cerevisiae Hansen) - Kakhuri 7, Champagne 7-10C, Race 7, Rkatsiteli 6, Pinot 14, Sudak VI -5 grown on must agar and provided for research by the Federal State Institution of Science "RUSSIAN NATIONAL RESEARCH INSTITUTE Viticulture and Winemaking" Magarach "RAS"**,** Yalta.

Active dry yeast (France, Institute of *Oenology*, Champagne) is represented by the following races:

- Lalvin RA17 (France) - wine yeast of the species Saccharomyces cerevisiae bayanus, intended for the disclosure of varietal aromas of white and red grapes;
- Lalvin EC 1118 Prise de Mousse (France) - wine yeast of the species Saccharomyces cerevisiae bayanus, which has good adaptability to high concentrations of ethanol and sulfur dioxide;
- Uvaferm GHM (Germany) - wine yeast of the species Saccharomyces cerevisiae, intended for the production of harmonious white wines;
- Lalvin Rhone 2056 (France) - wine yeast of the Saccharomyces cerevisiae species distinguished by its ability for stable fermentation in the temperature range from 15 to 28 ºC, characterized by resistance to ethanol and sulfur dioxide;
- Lalvin QA23 - wine yeast of the species Saccharomyces cerevisiae, antagonists of foreign strains of Saccharomyces cerevisiae during fermentation of white and red must;
- IOU Terroir - wine yeast of the species Saccharomyces cerevisiae, sulfo-and alcohol-resistant;
- IOC premium 9000 - wine yeast of the species Saccharomyces cerevisiae, active in a wide range of temperatures, sulfite-resistant.

For control, the strain IOC 1002 (France) was used, which is widely used in wineries.

Fermentation of grape must be carried out under anaerobic conditions at a temperature of 18–22 °C.

To establish the activity of hydrolytic enzymes in the wine material and yeast biomass, the technique (Avakyants and Belousova, 1992) was used, using appropriate substrates: albumin with a molecular mass of 12,000 Da for protein, and apple pectin for pectins. After determining the activity of proteinases in wine, an incubation mixture was prepared, which included 20 ml of analyzed wine, 4 ml of phosphate buffer with 5.5 pH and 1 ml of 5% albumin solution. Albumin was hydrolyzed with wine enzymes at 37 °C for 2 hours. Before and after hydrolysis in the sample of wine, the protein concentration was determined by the Lowry method. The amount of protein (in mg), hydrolyzed in one hour, was taken as a unit of proteolytic activity.

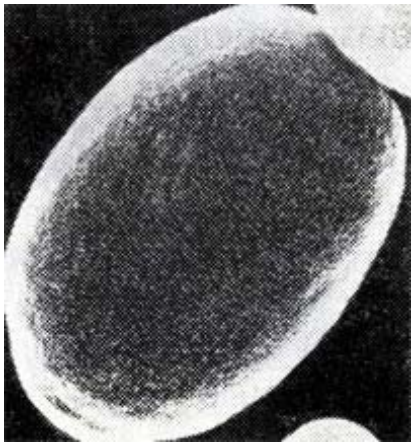
The study of the proteolytic activity of wine yeast was carried out by incubating them in a wine material containing 1% albumin, a phosphate buffer (g/dm$^3$: $K_2HPO_4$ – 0.4; $KH_2PO_4$ – 0.6; $MgSO_4$ x7$H_2O$ – 0.5; $ZnSO_4$x7$H_2O$ – 0.001; $CaCl_2$ – 0.05). The yeast was cultivated in 250 ml Ehrlenmeyer flasks containing 50 cm$^3$ of the nutrient medium at an optimum temperature of 30 °C during two hours. After carrying out the hydrolysis, 20 ml of the substrate were taken into an experimental tube, and 4 cm$^3$ of the trichloroacetic acid solution was added. The content was kept for another 20 minutes at a temperature of 30 °C. Then it was filtered in dry tubes. Before and after hydrolysis, the protein concentration was identified in the sample according to the Lowry method using *Folin–Ciocalteu* reagent. The amount of protein (in mg) hydrolyzed by yeast proteases per hour was taken as a unit of proteolytic activity.

To study the pectolytic activity of culture filtrates, the yeast was incubated on a pectin-peptone medium of the following composition (g/dm$^3$): pectin-3; peptone-3; $K_2HPO_4$ - 0.4; $KH_2PO_4$ - 0.6; $MgSO_4$x7$H_2O$ - 0.5; $ZnSO_4$x7$H_2O$ - 0.001; $CaCl_2$ - 0.05. Later, the composition of the medium was changed, namely: instead of pectin and peptone, the studied biomass of cells was used at the rate of 10 and 40 g/dm$^3$.Cultivation was carried out in 250 ml Ehrlenmeyer flasks containing 50 cm$^3$ of the nutrient medium at an optimum temperature of 28 °C. The acidity of the nutrient medium was adjusted to pH 4.0 with a 10% HCl solution. The activity of pectinases was determined in the culture filtrate on the 6th and

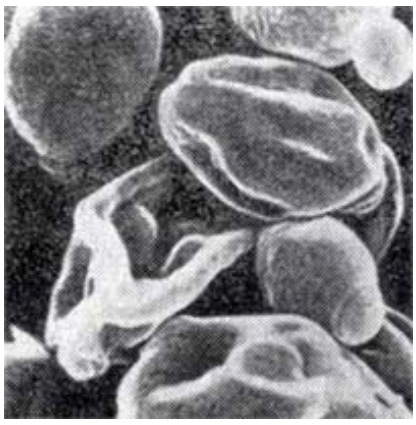
9th day of the cultivation of yeast. The activity of pectolytic enzymes was determined by the method of Kertesz (McCann and Roberts, 1996). Such an amount of enzyme was taken as a unit of pectolytic activity, which during the hydrolysis of pectin formed 1 mM of galacturonic acid per 1 min under experimental conditions (pH = 4, temperature 28 ° C). The activity of pectinases was determined by hydrolysis of apple pectin at 7.0-7.2 pH, optimal for the breakdown of wine pectin substances, and at an incubation temperature of 30-32 °C during one hour.

The activity of proteolytic and pectolytic enzymes in yeast biomass was carried out during the period of their active physiological state: more than 95% of the cells were vital, including about 70% of the pullulating ones; part of the cells was in the autolysis stage.

The cytological studies of wine yeasts in the process of autolysis were performed using a Leo 906E electron microscope (Zeiss, Germany). See Figure 1 (a, b).



a)



b)

***Figure 1.*** *Kakhuri 7 yeast cell: a - after 1 month of contact with wine; b - after 6 months of contact with the wine*

To isolate the complex of biopolymers from the wine material, the modified KM and KM-2P brand of carboxyl cation exchanger (Saint-Petersburg) were used. The composition of the biopolymer components was determined using the following methods: for protein substances (P) - the method of Lowry, phenolic substances (Ph) - colorimetrically using the *Folin–Ciocalteu reagent* (FCR), polysaccharides (P) - colorimetrically using the Diché reagent (Gerzhikova, 2002). The molecular weight of the protein was determined by gel chromatography using Sephadex followed by gel electrophoresis in a polyacrylamide gel (PAAG). To isolate the complex of biopolymers from the wine material, the KM and KM-2П brand modified carboxyl cation exchanger (Saint-Petersburg) was used. The peculiarity of these models lies in the fact that they are able to isolate the complexes of biopolymers (protein-polyphenolic-polysaccharide). The cation exchanger was first washed with warm (50-60 °C) distilled water and a weak (1.5%) solution of hydrochloric acid. A day later, the column was washed with distilled water until achieving a neutral reaction.

20 $cm^3$ of the tested wine was passed through a column filled with a cation exchanger. Distilled water with a 6-7 pH gradient was used for elution. 100 $cm^3$ of the eluate was collected. The composition of the components of biopolymers was determined by the following methods: protein substances (B) - by the Lowry method with preliminary deposition of proteins with trichloroacetic acid eluate, phenolic substances (F) – colorimetrically, by using Folin-*Ciocalteu* reagent, polysaccharides (P) – colorimetrically, by the phenol-sulfur method using Diché reagent (Gerzhikova, 2002). The molecular weight of the protein was determined by gel chromatography using Sephadex followed by horizontal polyacrylamide gel electrophoresis (PAGE) using a Bio-Rad system (USA). The gel for electrophoresis included agarous gel of various concentrations. For protein separation, the agarous gel was mixed with a small amount (1-3%) of polyacrylamide. The separated zones of proteins were fixed by deposition with a solution of trichloroacetic acid. For staining, Coomassie bright blue G-250 was used.

## 3. RESULTS AND DISCUSSION

Analysis of the experimental materials presented in Table 1 has shown that in the process of alcoholic fermentation (exponential phase -70-80% and stationary phase -20-30% of cell development), the activity of proteinases in

wine materials and cell biomass varies widely. This is due to the genetic characteristics of the races and their biosynthetic functions.

The highest activity of proteinases in wine material was revealed during the fermentation of the must by the races Lalvin QA23, Lalvin RA17, IOC1002, Rkatsiteli 6, Kakhuri 7.

The highest activity of proteinases in the biomass of yeast cells was characteristic of the Sudak VI-5, Lalvin EC 1118, Champagne 7–10C, and Pinot 14 races.

A comparative analysis of the presented materials leads to the following conclusions:

- there is no correlation between the proteinase activity in the wine material and yeast biomass, which indicates a different biosynthetic function of the studied yeast races;
- yeast cells have different rates of secretion of proteinases from the cell to the medium, which is due to the different membrane permeability (Abdullaeva, *et al*., 2003; Avakyants and Belousova, 1992), due to which the concentration of proteinases in wine materials varies significantly;
- the biomass of active dry yeast cells has an abundant supply of proteinases in comparison with the yeast starters of races produced in Russia.

Pectinases catalyze the hydrolysis of pectic substances through depolymerization and de-esterification reactions. Pectinase is the collective name of a group of enzymes, the main of which are the following three:

- pectinesterase, catalyzing the breakdown of ester bonds in pectin;
- polygalacturonase, catalyzing the breakdown of galacturonic bonds in pectin and other polygalacturonides;
- pectinlyase, catalyzing the breakdown of galacturonid bonds by transillumination.

The obtained experimental data (Table 1) indicate that the activity of pectinases in a yeast cell is significantly less than the activity of proteinases. Perhaps this is due to a lower concentration of the substrate - pectic substances - in comparison with high-molecular nitrogenous substances. In addition, the low activity of pectinases can be a genetic feature of the Saccharomyces wine yeast in comparison with mushrooms of other species and genera (Gerzhikova, 1997; Pericin *et al.*, 1997; Smith *et al.*, 2003). The highest pectinase activity was observed in the biomass of the yeast races Rkatsiteli 6, Pinot 14, Sudak VI-5, Lalvin Rhone, Lalvin QA23, IOC1002.

The subsequent contact of the wine material with yeast biomass (at a temperature of 16-18°C) led to the lysis (autolysis) of the cells and secretion of enzymes from the cell to the medium. It is known (Ibragimov *et al.,* 2000; Cebollero *et al.,* 2008) that the release of various chemical cell components into the environment by wine yeasts is determined by the permeability of the cell membranes, which, in turn, depends on the physiological state of the yeast cells. In the specific conditions of the experiment, during the autolysis, most of the yeast in the initial period of time (up to two months) was in the stationary phase of development. At this phase, the integrity of the cells and their sizes were preserved. Starting from the third month, the number of depressed and dead cells increased, reaching 50-60% by the sixth month of observation. At this stage, cell deformation increased: they shriveled, but were not destroyed, the cell wall remaining intact. On the basis of the conducted research, all analyzed races of yeast, in terms of preserving their vital activity in the process of autolysis, can be divided into three groups:

- yeast cells which quickly, within 5 months, die during autolysis (86–90%) include such races as Kakhuri 7, Champagne 7–10C, and Sudak VI-5;
- yeast cells (up to 92%) which die within 6 months - Rkatsiteli 6, Pinot 14 Lalvin EC 1118, Lalvin Rhone;
- yeast cells which for a long time retain the active physiological state, such as Race 7, Lalvin RA17, Uvaferm GHM, IOC1002.

Based on the experimental data obtained, it is possible to make a conclusion about the rate of change in the permeability of the cell membranes of various yeast races during their autolysis. This can be explained by different reasons. It is possible that the yeasts of the first group consumed nutrients more actively and even reserve substances of the environment, in the metabolism to another physiological state, characterized by the consumption of their constituent components. The intracellular enzymes dissolve the membranes inside and around the cell, while the amino acids, fatty acids, and enzymes begin to secrete out. The entire metabolism gets out of control, and the cell dies. The death of yeast cells of this group may also be due to the absence of oxygen in the medium or its rapid consumption. The process is characterized by the separation of plasma from the thickened shell and the rapid deformation of the cells.

Another reason for the differing physiological state of the yeast under study may

be a difference in secondary metabolic products — higher alcohols, aldehydes, volatile acids, which can inactivate cell activity, especially in the stages of starvation and death. However, the mechanisms of these processes are still insufficiently studied and require carrying out new experiments.

The results obtained (Table 2) indicate that the activity of proteinases in the biomass of yeast varies in different ways. Two races of yeast can be distinguished in which the secretion of proteinase was different from other races. These are Champagne 7–10C, in the biomass of which activity remained at a similar level for 3 months, and IOC1002, in which the enzyme activity did not change significantly throughout the entire observation period.

For most of the races studied, the following relationship was characteristic: the activity of the proteinase gradually increased during the 2-3 months of contact of the yeast with the wine material, after which its decrease to values depending on the race of the yeast was observed. This trend can be explained on the basis of the following provisions (Gonzalez *et al*., 2003; Alexandre and Guilloux-Benatier, 2008). In the initial period of maturing, the high activity of intracellular proteolytic enzymes causes accelerated hydrolysis of proteins of the cytoplasm and, thus, rapid autolysis of the yeast cell, accompanied by the transition of proteinases into the wine material. A further decrease in proteinase activity in the yeast biomass may be due to the nutrient deficiency in the medium and a decrease in hydrolytic activity in the cell as a whole.

Thus, during the autolysis of yeast, the activity of enzymes involved in the hydrolysis of high-molecular compounds and other critical biosynthetic processes is sharply reduced. At the same time, the yeast cells of all the races studied have not shown complete inactivation of the proteinase.

The presence in the yeast of biochemical mechanisms that prevent cell death contributes to the long-term preservation of the viability of wine yeast in a nutrient-rich environment such as wine. The disintegration of the yeast cell is also inhibited due to the fact that the cell membrane consists of glucan and mannoproteins, for the destruction of which not only proteolytic but also saccharolytic enzymes are necessary, i.e., activation of proteases is not enough for complete autolysis of yeast in wine (Bayon *et al*., 2003; Leroy *et al.,* 1990; Todd *et al.,* 2000).

The mechanism of cell aging and death is still controversial and far from clear (Ibragimov *et al.,* 2000; Gonzalez *et al*., 2003; Todd *et al.,* 2000). The death of a yeast cell can occur instantaneously, for example when heated, and slowly with prolonged contact of the yeast in the wine material. In the first case, the cell structure after its inactivation does not change; with gradual dying off changes occur, which are called necrobiosis. Autolysis occurs especially intensively when the life of the cell is terminated, intracellular enzymes are preserved, and the cell membrane becomes more permeable to them. Obviously, it is precisely the process that takes place in the analyzed and experimental variants.

The cytological studies have shown that during autolysis of yeast in wine, the following changes in the cellular organization occur: the cell wall does not degrade, the cytoplasm turns from homogeneous into coarse-grained, the periplasmic space forms and clear areas appear in the cytoplasm. After 2-3 months of contact of the wine with the yeast biomass, the cytoplasmic membrane and other cell membranes begin to degrade, after which the ultrastructures of the cell become indistinguishable. In the process of autolysis, the microrelief and shape of the cell change significantly in wine: from smooth or around the cell first becomes lemon-like and then wrinkled, deformed, which is possibly due to plasmolysis. Apparently, the identified cytological changes are associated with changes in the enzymatic apparatus of the yeast cell (Alcaide-Hidalgo *et al.,* 2007, Alexandre *et al.,* 2001, Onodera and Ohsumi, 2004).

The research results in Table 3 show that the high activity of pectinases in the yeast biomass persists for 2 months of contact with wine material, after which its significant decrease is observed. However, in the biomass of some races of the yeast - Rkatsiteli 6, Lalvin EC 1118, Lalvin Rhone and IOC1002 - the activity of pectinase is quite high even after 6 months of aging of the wine material on yeast biomass. This suggests that the process of hydrolysis of polysaccharides, including pectic substances, takes a long period of time. Such yeast species should be recommended for the production of wines, the technologies of which provide for prolonged contact of wine materials with yeasts, for example, sparkling wines.

The highest activity of pectinases in the initial period of cell lysis was observed in the biomass of the yeast races Rkatsiteli 6, Pinot 14, Sudak VI-5, Lalvin Rhone, Lalvin QA23, and IOC1002. These races of yeast can be used to reduce the concentration of polysaccharides, including pectic substances, in the technology of table wine materials.

The influence of proteolytic and pectolytic enzyme systems of wine yeast on the concentration and composition of table wine biopolymers has been studied. It is known (Gerzhikova, 1997; Leber *et al.,* 2001; Chursina, 2009) that it is the biopolymers of wine - a protein complex (P) -polysaccharide (PS) -polyphenol (Ph) that is responsible for the formation of colloidal turbidity.

The results obtained (Table 4) indicate that the resistance of wine to colloidal turbidity is determined not so much by the concentration of residual protein in the wine, as by its quantity in the complex of biopolymers and the total concentration of biopolymers. Studies have shown that the molecular weight of proteins in complexes of biopolymers varied within wide limits and depended on the race of yeast. It was established that in complexes of biopolymers of the Kakhuri 7, Shampagne 7-10C, Race 7, and Rkatsiteli 6 races, proteins with a molecular weight from 32 to 44 kDa prevailed; in the complexes of biopolymers of the Pinot 14 and Sudak VI-5 races, proteins from 18 to 23 kDa predominated. The complex of biopolymers of wine materials produced using different races of active dry yeast was more heterogeneous and included proteins with molecular masses: Lalvin RA17, Lalvin Rhone, Lalvin QA23 and Lalvin EC 1118 - 14 -15, 18-20, 34-37, 42- 45 kD; Uvaferm GHM -16-19, 27-28, 33-35 kDa; IOC1002 (control) - 14-15, 21-23, 34-37 kDa. This indicates a different resistance of wines to colloid turbidity depending on the used yeast race, which is confirmed by the data in Table 4.

It should be noted that the concentration of phenolic compounds in the complex of biopolymers did not change as significantly as the amount of protein and polysaccharides, with the exception of wine materials produced using Sudak VI-5 race, in which the number of phenolic compounds in the complex of biopolymers was the smallest.

## 4. CONCLUSION

Based on the analysis of the research results, the following patterns were established:

- wine yeast, which has the highest activity of enzyme systems - proteinases and pectinases - provides for the production of wine materials with lower concentrations of high-molecular substances;
- wine yeast, which has the highest activity of enzyme systems - proteinases and pectinases - can reduce the number of biopolymers in the wine material;
- the higher the activity of enzyme systems - proteinases and pectinases - in wine yeast, the greater their secretion into the wine material and the higher their activity in the wine material;
- the higher the activity of enzyme systems - proteinases and pectinases - in wine yeast and the more they are secreted into the wine material, the longer the resistance of the wine to colloid turbidity.

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## TABLES

***Table 1.*** *The activity of proteolytic and pectolytic enzymes during alcoholic fermentation, depending on the race of yeast*

| Yeast Race Name | Activity, nominal units | | | |
|---|---|---|---|---|
| | of proteinases | | of pectinases | |
| | in wine material | in yeast biomass | in wine material | in yeast biomass |
| Starters of pure yeast cultures | | | | |
| Kakhuri 7 | 21.4 | 218.6 | 7.65 | 34.8 |
| Champagne 7–10C | 18.8 | 254.7 | 11.4 | 44.2 |
| Race7 | 17.4 | 218.5 | 10.8 | 42.4 |
| Rkatsiteli6 | 22.0 | 212.7 | 15.2 | 51.7 |
| Pinot 14 | 18.6 | 248.5 | 12.8 | 47.8 |
| SudakVI-5 | 17.2 | 272.0 | 8.75 | 47.0 |
| Active dry yeast | | | | |
| Lalvin RA17 | 24.8 | 232.7 | 8.3 | 41.8 |
| LalvinEC 1118 | 22.6 | 256.4 | 10.2 | 45.6 |
| Uvaferm GHM | 15.9 | 223.3 | 8.8 | 46.0 |
| Lalvin Rhone | 16.4 | 238.4 | 11.1 | 48.2 |
| Lalvin QA23 | 27.6 | 226.4 | 10.0 | 47.0 |
| IOC1002, control | 22.8 | 227.6 | 10.3 | 47.6 |

***Table 2.****Proteinase activity in biomass of different yeast races in the process of autolysis*

| Yeast Race Name | Proteinase activity, nominal units, for period, months | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 6 |
| Starters of pure yeast cultures | | | | | | |
| Kakhuri 7 | 218.6 | 232.4 | 206.8 | 188.2 | 144.8 | 118.5 |
| Champagne 7–10C | 254.7 | 252.7 | 246.4 | 232.8 | 189.7 | 164.3 |
| Race 7 | 218.5 | 234.7 | 252.1 | 267.6 | 244.0 | 210.2 |
| Rkatsiteli 6 | 212.7 | 222.7 | 244.2 | 211.4 | 188.6 | 178.1 |
| Pinot 14 | 248.5 | 267.3 | 277.5 | 245.4 | 210.7 | 187.4 |
| SudakVI-5 | 272.0 | 312.8 | 318.6 | 288.5 | 166.8 | 244.5 |
| Active dry yeast | | | | | | |
| Lalvin RA17 | 232.7 | 246.2 | 277.6 | 255.4 | 272.3 | 232.8 |
| Lalvin EC 1118 | 256.4 | 288.6 | 290.6 | 290.2 | 216.4 | 208.6 |
| Uvaferm GHM | 223.3 | 234.6 | 246.3 | 254.8 | 256.6 | 244.5 |
| Lalvin Rhone | 238.4 | 262.8 | 254.3 | 255.0 | 238.2 | 209.2 |
| Lalvin QA23 | 226.4 | 229.4 | 234.8 | 256.8 | 249.2 | 236.7 |
| IOC1002, control | 227.6 | 245.6 | 244.8 | 256.2 | 250.7 | 248.4 |

***Table 3.****Changes in the activity of pectinases in yeast biomass during the aging of wine material*

| Yeast Race Name | Proteinase activity, nominal units, for period, months | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 6 |
| Starters of pure yeast cultures | | | | | | |
| Kakhuri7 | 34.8 | 34.0 | 35.6 | 32.4 | 27.8 | 21.0 |
| Champagne 7–10C | 44.2 | 44.4 | 41.2 | 35.2 | 30.7 | 18.9 |
| Race7 | 42.4 | 40.8 | 33.7 | 27.4 | 20.2 | 17.6 |
| Rkatsiteli6 | 51.7 | 52.0 | 51.3 | 45.6 | 36.8 | 32.0 |
| Pinot 14 | 47.8 | 46.2 | 42.8 | 38.3 | 29.6 | 26.3 |
| SudakVI-5 | 47.0 | 36.0 | 44.2 | 40.3 | 32.6 | 28.4 |
| Active dry yeast | | | | | | |
| Lalvin RA17 | 41.8 | 36.2 | 33.7 | 27.8 | 22.3 | 19.5 |
| Lalvin EC 1118 | 45.6 | 45.0 | 43.8 | 40.3 | 36.2 | 35.8 |
| Uvaferm GHM | 46.0 | 44.8 | 41.3 | 35.2 | 31.8 | 20.6 |
| Lalvin Rhone | 48.2 | 48.4 | 45.7 | 40.5 | 38.2 | 35.0 |
| Lalvin QA23 | 47.0 | 45.3 | 41.7 | 41.0 | 36.2 | 24.4 |
| IOC1002, control | 47.6 | 45.8 | 44.7 | 41.6 | 37.2 | 34.0 |

***Table 4.*** *Effect of races of wine yeast on the biopolymers of white grape table wine*

| Name of the wine material | Mass concentration of biopolymers, mg/dm$^3$ | | | | Duration of wine stability, months |
|---|---|---|---|---|---|
| | Total Sum | P | Ph | PS | |
| Sauvignon white table wine, control | 18.7 | 7.8 | 4.2 | 6.7 | |
| Starters of pure yeast cultures | | | | | |
| Kakhuri7 | 16.2 | 6.2 | 4.6 | 5.4 | 3 |
| Champagne 7–10C | 12.5 | 4.7 | 4.0 | 3.8 | 6 |
| Race7 | 14.4 | 4.7 | 4.3 | 5.4 | 4 |
| Rkatsiteli6 | 12.1 | 5.8 | 3.0 | 3.3 | 4 |
| Pinot 14 | 8.5 | 1.8 | 3.2 | 3.5 | 8 |
| SudakVI-5 | 7.8 | 1.3 | 2.7 | 3.8 | 8 |
| Active dry yeast | | | | | |
| Lalvin RA17 | 8.1 | 1.1 | 3.3 | 3.7 | 7 |
| Lalvin EC 1118 | 8.3 | 1.1 | 3.6 | 3.5 | 8 |
| Uvaferm GHM | 8.2 | 1.3 | 3.3 | 3.6 | 7 |
| Lalvin Rhone | 7.4 | 0.9 | 3.2 | 3.3 | 10 |
| Lalvin QA23 | 9.1 | 1.4 | 4.0 | 3.7 | 8 |
| IOC1002, control | 8.1 | 0.9 | 3.7 | 3.5 | 10 |