

Biotechnological processes for regulating the quality and safety of wine products

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Abstract. The production of a competitive wine industry in Russia is an important task for the successful development of the country's agro-industrial complex. The purpose of this research is to consider the ways of harmonious development of competitive Russian winemaking, based on the advanced biocotechnologies of developed countries. This paper presents biotechnological processes and classes of enzymes or individual representatives. Established that both the enzymes of the grape and the enzyme systems of wine yeast are involved in the processes of wine production. During delivery of grapes for processing, means of inactivation of the activity of oxidative enzymes such as sulfur dioxide, liquid nitrogen, carbon dioxide should be used. Improvement and acceleration of clarification of grape must have to be done with the use of pectinesterases, polymethylgalacturonases, polygalacturonases, pectin transesterases. In order to obtain wines stability to protein turbidity, the use of the technology of two-stage fermentation with deep protein transformation is recommended. Depending on the tasks of malolactic fermentation (MF) or its prevention, yeast strains that prevent the development of MF and strains that support the development of MF have been isolated. A relationship between the content of various groups of phenolic compounds and the activity of MF was established.

1 Introduction

In most countries of the world, wine is one of the most popular drinks containing alcohol. According to preliminary expert estimates, wine consumption is increasing, and an annual increase in sales is expecting in the next 5-10 years [1]. More than 70 countries produce wine, but France, Italy and Spain, which account for about 60% of the world's wine production, share the leadership. The main importers of wine are Germany, Great Britain, the USA, Belgium, Switzerland, the Netherlands, China, Japan, Canada, and Portugal.

The modern world wine industry is undergoing significant changes [2]: the leading countries are switching to the production of unique wine products, with the introduction of the principles of circular economy and environmental management, providing development of biotechnology [3-6]. Such manufacturers receive important competitive advantages and the ability significantly improve their market position [7]. In addition, modern trends are such

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that the world's leading companies are gradually switching to marketing, which based on the dissemination of ideas underlying their wine products, creating the needs [8, 9]. Thus, modern marketing of wine is the creation of a demand for wine [8, 10].

The grape and wine industry in Russia is currently developing quite rapidly and harmoniously with the support of the government. However, increasing its efficiency influenced by foreign economic activity, as well as measures to regulate the import and export of wine. Therefore, in order to be able to successfully compete in both the external and internal markets of Russia, it is necessary to develop a general strategy for the implementation of the principles of sustainable development of winemaking, based on the study, analysis and management of specific factors affecting the quality and safety of winemaking products, as well as the implementation of integrated food and environmental safety management systems aimed at the development of sustainable and bio-ecological technologies of winemaking and environmentally friendly methods of processing and disposal of secondary products of winemaking [5, 11, 12].

Consequently, for the effective development of the wine-making industry in Russia and ensuring the competitiveness of wines, it is urgent to switch to technologies that include modern achievements in biochemistry and biotechnology at all stages of wine production, including the use of new strains of microorganisms; introduction of scientific advances based on knowledge of biocatalytic actions of wine yeast enzyme systems, taking into account the antagonism of microorganisms at the stages of interruption / stopping of alcoholic or malolactic fermentation; using enzymatic catalysis, biological sorbents [13-17].

2 Materials and Methods

The research was carried out on the example of the production of red and white wines from technical grape varieties growing in the Anapo-Taman and Central zones of the Krasnodar region in the period from 2012 to 2020. Experimental samples of red wines in bulk were prepared in micro-winemaking by fermentation of grape must on a pulp with a "floating cap", white wines - using the technology of low-oxidized wines. We used active dry yeast (ADY) from manufacturers in Germany and France and pure yeast cultures of the selection of FSBSI NCFSCHVW, various enzyme systems, sorbents, including derived from inactivated yeast.

The aging of wines on yeast sediment (yeast biomass) was carried out in hermetically sealed containers with periodic manual stirring or using an anchor stirrer, simulating production conditions.

The study of the component composition of the products was carried out according to standard methods using modern equipment.

3 Results and Discussion

Wine is a product of biotechnology: biotechnological processes accompany every stage of the wine production. Winemaking technology based on the regulation of processes catalyzed by enzymes of raw materials, its microflora, cultural strains of yeast and bacteria - fermentation pathogens. Enzymes are a natural and essential element of the wine making process. Traditionally, the fermentation of fresh grape must was carried out by the metabolism of natural (wild) yeast presented on the skin of the grape, and biotransformation in the wine production process took place with the help of enzymes produced by this yeast. Advances in biotechnology and the discovery of the functions of enzymes allow us to consider modern winemaking as a high-tech industry. In the production of wine, the use of enzyme preparations at different stages of the technological process for solving various problems is now a widespread practice. Therefore, in order to achieve a high level of quality

of winemaking products, knowing the mechanisms of biochemical processes at different stages of production, it is proposed to form unique characteristics of wines by controlling biochemical processes at the main stages of production, depending on the set scientific and practical tasks. (Table 1).

Table. Scientific and practical tasks in modern biotechnology of wine at the main stages of its production

Stage of wine production	Scientific and practical task	Biotechnological methods, forms, methods
Receiving and processing of fresh grapes	oxidation protection	inactivation of natural enzymes of grapes and enzyme systems of microorganisms of grapes, including yeast, control of oxidative reduction potential
Separation of grape must	provision a sufficient yield of must, control of the transfer of extractives and dyes to grape must	splitting of pectin substances by pectolytic enzyme preparations
Processing of grapes affected by gray rot	clarification of grape must, reduction of the content of toxins: pesticides, patulin, ochratoxin, histamine	use of compositions of pectolytic and amyolytic enzymes
Pressing and maceration (infusion on the pulp)	extraction of anthocyanins and tannins from grape skins, transformation of aroma-forming components	the use of β -glucosidase and other enzymes to break bonds between aromatic molecules and sugars
Alcoholic fermentation	ensuring the optimal kinetics of fermentation, the formation of individual organoleptic properties of wine and the specified physical and chemical parameters	use of certain yeast strains, yeast nutrition
Stage after alcoholic fermentation	formation of individual organoleptic properties of wine and specified physical and chemical parameters	transformation of the chemical and sensory characteristics of wine during baronage as a result of autolysis of wine yeast
Malolactic fermentation (MF)	Preservation of freshness of taste due to stabilization of wine acids, carrying out biological acid reduction	the use of yeast strains that prevent the development of MF, and strains that support the development of MF
High-quality pasting	transformation and decontamination of toxic compounds, obtaining a haze-stable product	the use of natural sorbents based on chitosan, yeast shells, plant raw materials

As can be seen from the data in the table 1, at the stage of acceptance and processing of fresh grapes, the main task is to prevent oxidation processes associated with the activation of enzymes of the Krebs cycle, especially the dehydrogenase of succinic (succinate dehydrogenase), citric (citrate dehydrogenase) acids. Already after 2-3 hours, their activity

leads to a decrease in the concentration of the most important antioxidant - succinic acid, to the oxidation of the labile fraction of polyphenols. It is proposed to use inactivation means to reduce enzymatic activity at this stage and prevent the development of oxidative processes: cooling fresh grapes to 4-6 ° C, adding sulfur dioxide or its derivatives 50-80 mg/kg, liquid nitrogen, carbon dioxide.

During the primary processing of grapes, an urgent issue is to ensure a sufficient yield of must, the value of which is significantly influenced by the varietal characteristics of the grapes and weather and climatic factors during the harvesting period. Depending on the factors that prevent sufficient juice separation, including the yield of gravity wort, treatment schemes with various pectolytic and amylolytic enzymes, as well as their combined use, were selected. This made it possible to increase the yield of fresh grape must (up to 10%), significantly increase the rate of self-clarification of the must, maintain the required color, and increase the transfer of extractives from the skins of grape berries. When processing grapes affected by gray rot and other diseases, satisfactory results were obtained with the combined use of preparations of amylolytic and pectolytic enzymes. As a result of the treatments, it is possible to reduce the content of harmful toxins, such as ochratoxin, histamine, etc., in grape must by 80% compared to the control without the use of enzyme preparations.

Alcoholic fermentation is a complex multistage process of enzymatic breakdown of organic substances, mainly carbohydrates. The fermentation process consists of a number of chemical reactions in which complexes of enzymes are involved, the most important are invertase (a class of hydrolases), pyruvate decarboxylase (a class of lyases), and alcohol dehydrogenase (a class of oxidoreductases), which catalyzes the formation of ethyl alcohol. In practice, however, there is much more going on than just the production of alcohol and carbon dioxide. Along with these main products, secondary and by-products are formed during the fermentation process, which are involved in the formation of the taste, aroma and color of the finished wine. Glycosidase enzymes (a class of hydrolases) work to maximize the aroma potential of a wine in a shorter time, increasing the concentration and speeding up the process. They help achieve a broader flavor profile and enhance it by releasing aromatic compounds associated with sugars in an odorless glycosidic form. It was found that the treatment with enzyme preparations to enhance the aroma is most effective for grape varieties with a high content of terpenes and a pronounced typical aroma (Riesling, Muscat and Gewurztraminer).

Enzyme activity is also of great importance in the processes occurring during the formation of wine after the completion of alcoholic fermentation. It is known that the quality of wine is significantly influenced by the conditions and duration of aging on yeast lees. The chemical and sensory characteristics of wine are transformed during such aging as a result of autolysis of wine yeast. Autolysis is the decomposition of yeast cells under the action of hydrolytic enzymes (hydrolase class), during which the bouquet of wine is enriched and useful amino acids, vitamins and mineral salts enter it. The period of aging on the lees varies depending on the specific characteristics of the wine desired. In order to shorten this period and, consequently, the risks of oxidation and microbial contamination, as well as the high production costs, the use of enzyme preparations rich in glucanases (hydrolase class) has been studied. The mechanisms of catalysis by glucanases of the decomposition of the glycosidic bonds of the cell walls of yeast cells, gradually destroying them and accelerating the autolysis of yeast.

One of the most important stages of wine production is biological acid reduction or its prevention. Our studies made it possible to isolate yeast strains that prevent the development of malolactic fermentation (MF) and strains that support the development of MF. The relationship between the content of various groups of phenolic compounds and the activity

of MF has been established. All these factors must be taken into account when carrying out acid reduction with the help of bacteria.

4 Conclusion

It is considered that both grape berry enzymes and yeast enzyme systems are involved in the wine production processes at each stage. It is proposed to use means of inactivating the activity of enzymes of the Krebs cycle - sulfur dioxide or its derivatives, liquid nitrogen, carbon dioxide when delivering grapes for processing. In order to increase the yield of fresh grape must for the cleavage of pectin substances, it is advisable to use pectinesterases, polymethylgalacturonase, polygalacturonase, pectin trans-eliminase - an increase in the must yield - up to 10% reduction in the clarification time to 5-10 hours instead of 20-24 hours.

Depending on the tasks of the need for malolactic fermentation or its prevention, yeast strains that prevent the development of MF and strains that support the development of MF were isolated. A relationship has been established between the content of various groups of phenolic compounds and the activity of MF, which must be taken into account when carrying out acid reduction with the help of bacteria.

References

1. L.Ch. Chen, A. Kingsbury, J. Rural Stud., **72**, 104-115 (2019) <https://doi.org/10.1016/j.jrurstud.2019.10.015>
2. N. Jiménez-Asenjoa, D. A. Filipescu, Int. Bus. Rev., **28(4)**, 647-659 (2019) <https://doi.org/10.1016/j.ibusrev.2019.01.001>
3. F. Juan, V.-M. Joan, M.I F. Mendozabc, J. A. Aznar-Sánchez, A. Gallego-Schmid, Resour. Conserv. Recycl., **170**, 105618 (2021) <https://doi.org/10.1016/j.resconrec.2021.105618>
4. A. S. Brandão, A. Gonçalves, J.M.R.C.A. Santos, J. Clean. Prod., **295(1)**, 126407 (2021) <https://doi.org/10.1016/j.jclepro.2021.126407>
5. M. Zwingelstein, M. Draye, J.-L. Besombes, Ch. P., Gr. Chatel, Waste Manag., **102** (1), 782-794 (2020) <https://doi.org/10.1016/j.wasman.2019.11.034>
6. A. Soceanu, S. Dobrin, A. Sirbu, N. Manea, V. Popescu, Sci. Total Environ., **759(10)**, 143543 (2021) <https://doi.org/10.1016/j.scitotenv.2020.143543>
7. M. Lanfranchi, E. Schimmenti, M. G. Campolo, C. Giannetto, Wine Econ. Policy, **8(2)**, 203-215 (2019) <https://doi.org/10.1016/j.wep.2019.11.001>
8. R. Capitello, L. Agnoli, St. Chartersc, D. Begalli, J. Clean. Prod., **304**, 126991 (2021) <https://doi.org/10.1016/j.jclepro.2021.126991>
9. F. Taghikhah, A. Voinov, N. Shukla, T. Filatov, Environ. Sci. Policy, **109**, 116-124 (2020) <https://doi.org/10.1016/j.envsci.2020.04.001>
10. A.Castellini, A. Samoggia, Wine Econ. Policy, **7(2)**, 128-139, 2018 <https://doi.org/10.1016/j.wep.2018.11.001>
11. A.R. Borneman, S.A. Schmidt, I.S. Pretorius, Trends Genet., **29(4)**, 263-271 (2013) <https://doi.org/10.1016/j.tig.2012.10.014>
12. A. Galimberti, A. Bruno, G. Agostinetto, M. Casiraghi, L. Guzzetti, M. Labra, Curr. Opin. Biotechnol., **70**, 36-41 (2021) <https://doi.org/10.1016/j.copbio.2020.10.006>
13. B. Bovo, Ch. Nadai, Ch. Vendramini, W.J.F.L. Junior, M. Carlot, A. Skelinc, A. Giacomini, V. Corich, Int. J. Food Microbiol., **236(7)**, 56-64 (2016) <https://doi.org/10.1016/j.ijfoodmicro.2016.07.022>

14. G. Garrido-Bañuelos, A.B.J. Schückeld, A.J.J. Zietsman, W.G.T. Willatsde, John P. Moore, W.J. Du Toit, Food Chem., **278(25)**, 26-35 (2019)
<https://doi.org/10.1016/j.foodchem.2018.10.136>
15. G. Garrido-Bañuelos, A. Buic, J. Schückel, A.J.J. Zietsman, W.G.T. Willatsd, J.P. Moore, W.J. Du Toit, Food Chem., **278(25)**, 36-46 (2019)
<https://doi.org/10.1016/j.foodchem.2018.10.134>
16. A. De Iseppi, G. Lomolino, M. Marangon, A. Curioni, Food Res. Int., **137**, 109352 (2020)
<https://doi.org/10.1016/j.foodres.2020.109352>
17. G. Roca-Domènech, R. Cordero-Otero, N. Rozès, M. Cléroux, A. Pernet, R. Mira de Orduña, Food Res. Int., **113**, 401-406 (2018)
<https://doi.org/10.1016/j.foodres.2018.07.003>