Studying the biochemical transformations in sherry wines subjected to biological aging

Sofia Cherviak¹,*, Victoria Gerzhikova¹, Nadezhda Anikina¹, Nonna Gnilomedova¹, and Antonina Vesyutova¹

¹All-Russian National Research Institute of Viniculture and Winemaking "Magarach" of RAS, 298600 Yalta, Russian Federation

Abstract. The fundamental role in the process of biological aging is given to yeast which enrich the wine with substances that contribute to the formation of the characteristic organoleptic properties of sherry. We studied 55 samples of wine in the dynamics of biological aging. The purpose of this research was to study the transformation of wine components during biological aging and to determine their technological value. Generalization of experimental data showed that biological aging determines changes in the composition of wines and physical-chemical characteristics: reduction of the volume fraction of ethanol, the mass concentration of the components of the acid complex (malic, lactic and acetic acids), amine nitrogen and glycerin as sources of energy and carbon nutrition for yeast. Decrease in the content of phenolic substances, the value of the redox-potential of wine and optical density D₄₂₀ confirms the protective function of the sherry film that prevents non-enzymatic oxidation of wine components. The biological aging is also characterized by an increase in the mass concentration of aroma-forming substances: acetaldehyde, diacetyl, acetoin, dioxanes, dioxolans, lactones. Based on the data obtained, the role of the main components of wines in the process of biological aging and the ranges of their variation were determined.

1 Introduction

The fundamental role in the process of biological aging is given to yeast which, as a result of its development, as well as autolysis, enrich the wine with substances that contribute to the formation of the characteristic organoleptic properties of sherry [1-6]. The physiological state and metabolism of the yeast cell is influenced by the content of nutrient components, process inhibitors (sulfurous acid, lactic and acetic acid bacteria), as well as technological factors [1, 5, 7]. The oxidative metabolism of yeast results in the produced substances that provide the typical aroma and flavor properties of sherry wines [2, 8-15].

Presently, control over the biological aging is carried out by the accumulation of the mass concentration of acetaldehyde, as well as by the transformation of the organoleptic characteristics of the product. Studying the qualitative and quantitative changes in the

* Corresponding author: Sofi4@list.ru
component composition under the action of flor yeast to substantiate additional criteria for assessing the quality of Sherry-type white wine called "Fino" is relevant.

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2 Study objects and methods

The objects of our research were base wines from grapes of the ‘Aligote’ and ‘Rkatsiteli’ varieties, enriched with ethyl alcohol to provide the conditions of 16.0% vol. Biological aging of the samples was carried out by a film method using the sherry yeast race Jerez 20 C/96. The formation of a continuous sherry film on the surface of the wine was observed after 9-13 days. We analyzed 55 samples of wines in dynamics (before laying for biological aging, 1, 3, 5 months after sowing sherry film). Microbiological control and monitoring of the state of the yeast was performed every 2 weeks.

In wines, optical and potentiometric characteristics were determined [16, 17]. The organic acid profile and glycerol content were determined by the HPLC method on a Shimadzu LC20 Prominance chromatograph (Japan). The component composition of the aromatic complex of wines was carried out using high performance liquid chromatography by means of the Agilent Technologies system.

3 Results and Discussion

Generalization of the literature data and research results shows that the metabolism of sherry yeast which can adapt to conditions of a limited amount of nutrients is the basis of complex biochemical transformations of the components of wine, as well as the formation of specific organoleptic characteristics of sherry wine. The main sources of carbon and energy nutrition of sherry yeast include ethyl alcohol, the consumption of which in the process of biological aging is 1-3% vol., and glycerin, the content of which is reduced to trace levels. Yeast also assimilates organic (malic, lactic, acetic) and amino acids as substrates. Analysis of the literature data [2, 5, 8] and the results of studies of the dynamics of biological aging made it possible to establish the role of the main components of wine under the action of flor yeast, as well as to determine the ranges of their change (Table 1).

| Component       | Participation in the metabolism of sherry yeast                                                                 | Change * | Range of changes** |
|-----------------|---------------------------------------------------------------------------------------------------------------|----------|-------------------|
| Ethanol (% v/v) | Substrate of oxidation-reduction processes, source of carbon nutrition, source of energy for metabolite synthesis | ↓        | 16.5→13.0         |
| Acetaldehyde (mg/L) | The product of ethanol oxidation; is involved in the constructive exchange of sherry yeast in the form of Acetyl-CoA and obtainment of energy through the Krebs cycle and glyoxylate cycle | ↑        | 50→1000           |
| Glycerol (g/L)  | Is involved in the process of osmoregulation, gluconeogenesis, and synthesis of triacylglycerols               | ↓        | 7.0→trace amounts   |
| Acetic acid (g/L) | Is involved in the Krebs cycle in the form of Acetyl-CoA for obtainment of energy and synthesis of intermediate metabolites, as well as in the glyoxylic cycle and gluconeogenesis | ↓        | 0.4→0.1           |
| Lactic acid (g/L) | Is involved in the processes of gluconeogenesis and the Krebs cycle through Acetyl-CoA                        | ↓        | 1.3→0.9            |
| Amino acids (g/L) | Is involved in the formation of amino acids and protein biosynthesis                                         | ↓        | 150→90             |

* ↓ - reduction, ↑ - increase
** The table shows the average values for the studied selection of samples
As a result of oxidative metabolism of sherry yeast, the medium is enriched with new components that determine the specific organoleptic properties of sherry (Table 2). Due to the oxidation of ethanol, acetaldehyde is formed under the action of the alcohol dehydrogenase enzyme the amount of which can reach 1000 mg/l and higher. As a result of its high reactivity, acetaldehyde reacts with the components of wine forming specific products for sherry-type wine: 1,1-diethoxyethane with ethanol, dioxanes and dioxolanes with glycerin, and sotolon with α-ketobutyric acid. In addition, acetoin and diacetyl are formed enzymatically [1, 4, 8, 14].

**Table 2.** Products of metabolism of yeast in the process of biological aging

| Component or class of materials | Ways of formation | Technological value |
|--------------------------------|-------------------|---------------------|
| Acetaldehyde                   | Alcohol oxidation | Affects the organoleptic properties of sherry wine; characterizes the course of biological aging |
| Ketones (diacetyl, acetoin)    | Binding of excess acetaldehyde | Affect organoleptic characteristics; give milk and cheese tones |
| Aliphatic aldehydes            | Oxidation of higher alcohols | Affect organoleptic characteristics, give bread tones |
| Aliphatic acetals              | Formed from acetaldehyde and ethanol (or higher alcohols) | Contribute to the softening and harmonization of the pungent aroma of aldehydes |
| Cyclic acetals (dioxanes, dioxolanes) | Formed from acetaldehyde and glycerol | Affect the organoleptic characteristics of sherry wine |
| Lactones                       | Formed from keto acids on the way of synthesis of amino acids | Responsible for specific sherry tones in a bouquet of sherry wine |
| Volatile ethers                | Formed from ethanol and higher alcohols and volatile acids | Affect the organoleptic characteristics of wine |
| Non-volatile ethers            | Formed from ethanol, higher alcohols and organic acids | They are a sign of aging |
| Aromatic aldehydes             | They are a product of ethanolysis of lignin from oak wood | They are a sign of aging |
| Furans                        | Formed from sugars and amino acids | Give a nutty tone to the bouquet |
| Alcohols C6 – C12              | Formed from keto acids in the synthesis of amino acids | They are background flavor components |
| Higher alcohols                | Formed from keto acids in the synthesis of amino acids | They are background flavor components |

In addition to biochemical transformations, the optical and potentiometric characteristics of wine undergo changes under the action of flor yeast (Table 3): the optical density of D$_{420}$ and the oxidation reduction potential decrease which confirms the protective function of the sherry film which, by consuming dissolved oxygen, prevents non-enzymatic oxidation of wine components [5, 11]. As a result of coagulation with yeast cells and subsequent sedimentation, the content of phenolic complex components decreases.

**Table 3.** Changes in the physical and chemical properties of wine in the process of biological aging

| Indicator                                    | Technological value                                    | Range of changes* |
|----------------------------------------------|--------------------------------------------------------|-------------------|
| Optical density D$_{420}$                    | prevents non-enzymatic oxidation of wine components    | 0,113→0,083       |
| Oxidation reduction potential, mV            |                                                        | 250→220           |
| Mass concentration of phenolic substances, mg/l |                                                        | 263→218           |
4 Conclusion

Thus, as a result of the studies carried out, it has been shown that the process of biological aging of wines under a sherry film causes a change in the component composition of the wine and its physicochemical characteristics:
- reduction of the volume fraction of ethyl alcohol, the mass concentration of the components of the acid complex (malic, lactic and acetic acids), amine nitrogen and glycerol as sources of energy and carbon nutrition for yeast;
- decrease in the content of phenolic substances, the value of the oxidation reduction potential of wine and optical density D420;
- increase in the mass concentration of aromatic substances formed during the oxidative metabolism of yeast: acetaldehyde, diacetyl, acetoin, dioxanes, dioxolans, lactones.

Based on the data obtained, the role of the main components of wines in the process of biological aging was established, and the ranges of their variation were determined. The important role of acetaldehyde and products of its interaction with wine components in the formation of specific organoleptic properties of Sherry is shown.

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