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ANTIOXIDANT PROTECTION DURING RED GRAPE PROCESSING AND RED WINE PROCESSING

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Abstract:

Currently, the demand for light red wines has increased sharply. But the color of wine loses its color and shine during technological treatments associated with the presence of oxygen. Therefore, the problem of researching antioxidants, the antioxidant protection system during the processing of red grape varieties and wine processing in order to ensure the quality and presentation of red wines is relevant.

The work investigated the antioxidant protection system of primary and secondary winemaking products. The results of studies to determine the enzymes of the antioxidant defense system during all technological methods are described: crushing, draining, settling, fermentation, fining, heat treatment, cold treatment of red grapes and wines. Before and after each technological operation, physicochemical parameters were determined, the activity of enzymes included in the antioxidant defense system was determined, the cascade of oxygen reduction reactions and the reduction reactions of oxygen in wine were given.

Based on the results obtained, the technological canons of winemaking were adjusted, for example, when processing red grapes, additional measures are clearly necessary to ensure antioxidant protection. Moreover, cold processing of dry red wines leads to an increase in oxygen concentration, which threatens the oxidation of phenolic coloring substances in the composition of the wine and causes a change in the color and taste of the wine, then this technological technique for red wines is recommended in exceptional cases. Therefore, heat treatment of red wines provides an increase in oxygen concentration and simultaneously activates all the component antioxidant defense systems, i.e. dismutation and the formation of organic peroxides occur. Over time, heat treatment increases antioxidant activity, with a more intense increase in antioxidant activity during heat treatment, and slightly lower antioxidant activity during cold processing.

Keywords: Oxidation, oxygen, enzyme, antioxidants, antioxidant defense system, superoxide dismutase, catalase, peroxidase, oxygen saturation, activity, heat treatment, cold, fermentation

INTRODUCTION. Oxygen plays a key role in the metabolic and chemical reactions of the winemaking process. Wine cannot be completely protected from oxygen throughout the entire process of wine making technology. In the works of W. J. du Toit noted that the influence of oxygen on both the composition and quality of wine is either positive or negative [1].

Oxidation can be enzymatic or non-enzymatic, also called auto-oxidation. In wine, non-enzymatic, i.e., usually takes place. chemical oxidation. In the case of enzymatic oxidation, enzymes such as polyphenol oxidase and superoxide dismutase catalyze the oxidation reactions.

The spectrum of antioxidants that make up wine is represented by phenolic [2]. Components of mono-, oligo- and polymer structure, anthocyanins, ascorbic acid, catechins, tannin, etc., which during technological processing are involved in the composition of all forms of aroma oxygen and taste, determining the quality of wines [3]

Antioxidants are a shield against environmental toxins. [4]. The level of antioxidant depends on the grape variety, place of growth and method of processing [5]. Subsequently, we investigated the antioxidants of the antioxidant protection system during technological operations for processing grapes (crushing, draining, pressing, settling, fermentation) and processes that contribute to the production of wines of stable quality and high biological value that take place in secondary winemaking (fining, heat treatment, low temperature and heating). The antioxidant defense system is divided into primary and secondary, in which the antioxidants are enzymes and vitamins, respectively.

The relevance of the research lies in studying the nature of the behavior of the AOD system, oxygen saturation, and the presence of reactive oxygen species during the period of technological processes.

Since all easily oxidized components of wine are antioxidants, the possible presence of radicals and reactive oxygen species determines their interaction. Red wines are characterized by a high content of antioxidants, mainly of a phenolic nature, differing in both qualitative and quantitative composition.

The purpose of the research is to determine the state of enzymes of the anitoxidant protection system during the processing of red grapes and technological processing of red wine materials.

MATERIALS AND RESULTS. Technological methods of primary winemaking were carried out on grape processing lines of primary winemaking factories; fining, cold processing and heat treatment were carried out according to approved technological instructions in the mode of a secondary winemaking enterprise.

Physico-chemical parameters of grapes and wine materials were determined according to generally accepted methods in winemaking.

The object of the research was red Pinot black grapes with sugar content 18% and acidity 5 mg/dm^3. Indicators of red wine material: specific gravity - 0.990 g/cm^3; strength - 11.2% vol; titratable acidity - 5.6 mg/dm^3; volatile acidity - 0.59 mg/dm^3; SO_2 content -100 mg/dm^3; Fe content -14 mg/dm^3.

Enzyme activity was determined using a method based on the ability of superoxide disuntase to inhibit the reduction reaction of nitrotetrazolium blue. Catalase activity was determined by reaction with ammonium molybdate, and peroxidase activity was determined by a method based on the oxidation of pyrogallol in the presence of hydrogen peroxide to purpurogalline. The activity of antioxidant activity was determined on a PU-1 polarograph by taking a voltammogram of the current of the test substance, and $NaClO_4$ dissolved in dimethylformamide was used as a background electrolyte. [6].

All groups of wine substances are involved in redox reactions - carbohydrates, phenolic and nitrogenous substances, organic acids. The intensity of oxidative enzymatic processes depends on technological methods, creating conditions for the passage of secondary redox processes. The state of antioxidant protection components determines resistance to environmental influences.

Studying the antioxidant protection of red wines will allow us to correctly develop wine preparation technology. Splitting up red grapes does not change the oxygen concentration and remains in the amount of 14 mg/dm^3 . The activity of the catalase enzyme in red wort increased from 3.93 m kmol/min/dm^3 to 4.35 m kmol/min/dm^3. Peroxidase activity, on the contrary, decreased from 40.6 m kmol/min/dm^3 up to 22.3 m kmol/min/dm^3. The activity of superoxide dismutase increased from 2.93 conventional units to 4.60 conventional units .

In general, crushing red grapes activates the enzyme superoxide dismutase (SOD) and catalase in the must. SOD and catalase reduce the level of primary reactive oxygen species (ROS) and contain iron ions as catalysts.

An increase in SOD activity is determined by the presence of oxygen superoxide radical, which intensifies the oxidation process and SOD protects against excessive oxidation [7].

Catalase, on the other hand, oxidizes one molecule of hydrogen peroxide with another molecule of hydrogen peroxide to form two molecules of water and an oxygen molecule:

$$H_2O_2 + H_2O_2 \rightarrow 2H_2O + O_2$$

When the red wort drains, oxygen saturation is observed, in which the oxygen concentration increased from 12 to 20 mg/dm^3. That is, the intensive absorption of oxygen led to an increase in its concentration by 8 mg/dm^3. Peroxidase activity increases, but the enzymes SOD and catalase lose some activity.

O_2 during the oxidation process. Free superoxide ($O_2^{\cdot -}$) and peroxide radicals (O_2^{2-}) are formed , which are directly reduced by oxidizing phenolic molecules that are stronger than O_2 [8].

$$O_2 + e^-, H^+ \rightarrow HO_2^{\cdot} + e^-, H^+ \rightarrow H_2O_2 + e^-, H^+ \rightarrow {}^{\cdot}OH\ (+H_2O) + e^-, H^+ \rightarrow (2)H_2O$$

or schematically:

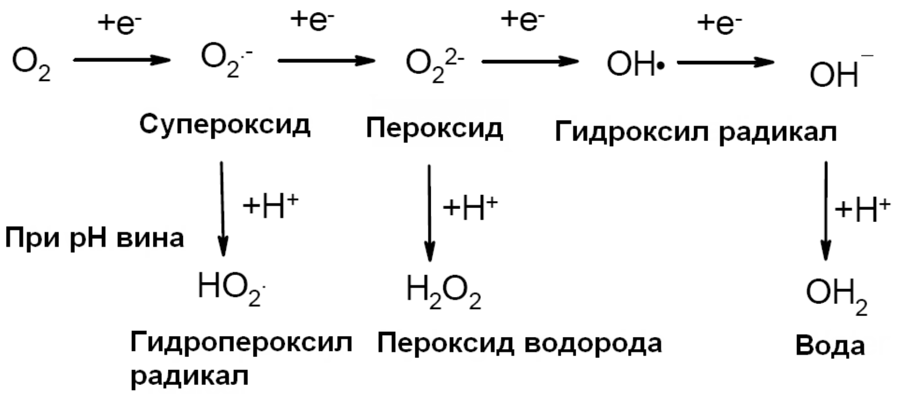

Fig.1. Sequence of oxygen reduction reactions

Pressing the red pulp reduces the accumulation of oxygen and the enzymatic activity of peroxidase, but the activity of the enzymes SOD and catalase increased. These data determine the presence of superoxide radical oxygen during the pressing period, and at the same time superoxide dismutase protects against excessive oxidation. Pressing activates this enzyme from 2.81 conventional units to 5.74 conventional units, which indicates the red wort's predisposition to oxidation. Here, an increase in catalase activity is observed from 3.95 m kmol/min/dm^3 to 4.77 m kmol/min/dm^3, which oxidizes one molecule of hydrogen peroxide. Apparently, pressing, as a technological method, enhances peroxidation, thereby providing antioxidant protection for red wort. [9].

When red wort settles, peroxidase activation is observed and oxygen accumulation intensifies. The activity of SOD and catalase decreases from 4.76 m kmol/min/dm ^{3} to 1.9 m kmol/min/dm^3 . The infusion increases the oxygen concentration in red wort from 10.3 mg/dm^3 to 24 mg/dm^3. Peroxidase activity intensifies peroxidation reactions. The peculiarities of the chemical composition of red wort make it possible to prevent the ionization of molecular oxygen.

Red wort fermentation has different effects on the enzymatic activity of the antioxidant defense system. High oxygen saturation is observed during fermentation, when the oxygen concentration has increased to 13 mg/ ^{dm3} .

If we judge the oxidation of the must by the number of active enzymes that are part of the antioxidant defense system, then during crushing, red grape varieties are most susceptible to oxidation . The processing of red grape varieties is characterized by maximum SOD activity during draining, then during fermentation, crushing, and pressing, and is absent only during red must infusion. That is, all operations during the processing of grapes, except for the must infusion, tend to oxidize the red must and the danger of the oxidation of one molecule of hydrogen peroxide by another molecule of hydrogen peroxide with the formation of two molecules of water and an oxygen molecule is significant [10].

But peroxidase activity during the processing of red grapes appeared only in two cases: during dripping and during infusion. It can be concluded that when processing red grapes, the must is less susceptible to peroxidation.

Red wines are characterized by a rich complex phenolic complex and are distinguished by high antioxidant capacity, determined by the high content of phenolic and coloring substances.

From the results of the analysis it follows that the redox processes occurring in red wines during the ripening period are caused by the absorption of oxygen contained in the air and entering the wine during technological operations.

Finishing with bentonite saturates red wine with oxygen. And the maximum (8.5 mg/dm^3) increase in the concentration of molecular oxygen was noted after treatment, and the smallest increase (only 0.62 mg/dm^3) observed during cold treatment.

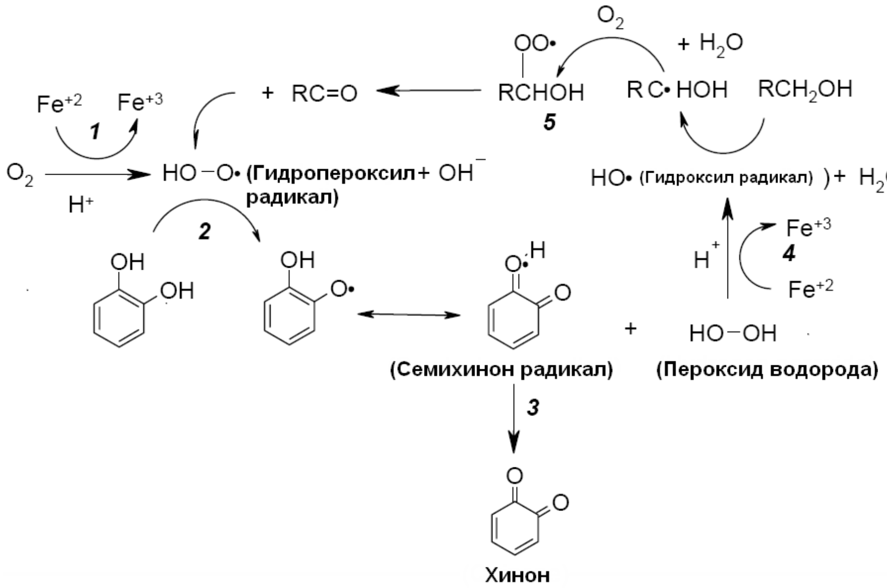

Fig.2. Reduction reactions of oxygen in wine.

Hydrogen peroxide is a relatively weak oxidizing agent relative to wine components, but again, in the presence of iron, it is reduced to a hydroxyl radical, a very strong oxidizing agent.

The highest catalase activity was determined before cold treatment. It is low temperature treatment that inactivates it. An increase in its activity is achieved by pasting with bentonite, and during this technological treatment the increase in catalase activity is approximately ten times higher than during heat treatment.

Peroxidase is active at the beginning and end of wine maturation. Gluthione peroxidase activity in all samples increases, and is maximum after wine fining and minimal (0.059 μmol/min/dm^3) during heat treatment, which confirms the existing hypothesis that heat treatment accelerates the ripening of wines. And this technique is recommended to accelerate the ripening and typification of wines.

The enzyme superoxide dismutase is most active before cold treatment and it is this technological method that sharply reduces its activity (by 5.75 conventional units), and during pasting and during heat treatment its increase is the same (1.74 conventional units). [11] Superoxide dismutase catalyzes the dismutation reactions of the superoxide radical to form hydrogen peroxide and oxygen. An increase in the activity of superoxide dismutase, catalase, peroxidase and molecular oxygen is observed during fining, as well as during heat treatment, which is a clear demonstration of the presence of antioxidant protection in red wines [12].

During low-temperature treatment, the antioxidant protection components behave somewhat differently. Thus, cold treatment gives a slight increase in the concentration of molecular oxygen and peroxidase, but the activity of superoxide dismutase and catalase decreases. With a technological method such as cold treatment, a sharp drop in superoxide dismutase activity by 5.75 conventional units is observed. units, loss of catalase activity by 1.45 conventional units.

The technological method – *pasting* – gives the greatest increase in the activity of catalase and peroxidase with an increase in all the studied indicators of antioxidant protection.

Heat treatment increases the activity of the antioxidant defense system. However, the increase in the concentration of molecular oxygen during heat treatment is approximately two times less than during fining.

Therefore, cold treatment enhances the tendency of wine components to oxidize, while technological methods such as fining and heat treatment protect wine components from oxidation.

Antioxidant activity scavenges free radicals. A direct correlation between antioxidant activity and molecular oxygen concentration was not found in this case. The absence of such a correlation indicates the presence of antioxidants of various natures

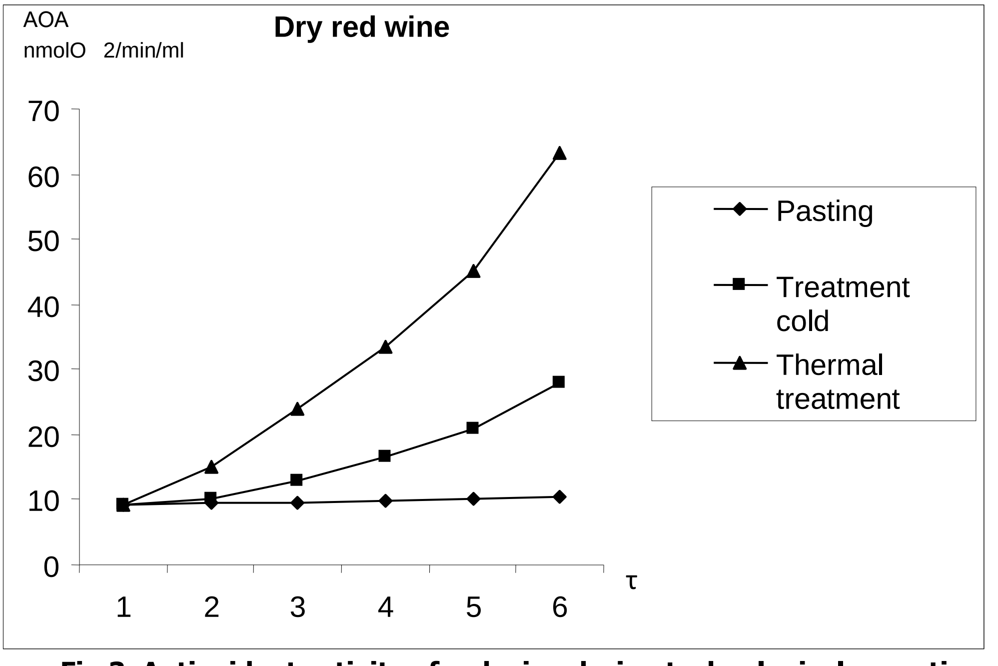

Fig.3. Antioxidant activity of red wine during technological operations.

The results of studies to determine the antioxidant activity of dry red wine material during technological treatments are shown in the diagram (Fig. 3). Clear antioxidant activity is characteristic of heat treatment (64nmol02/min/ml), slightly lower antioxidant activity during cold treatment (28nmol02/min/ml) and minimal antioxidant activity is characteristic (10nmol02/min/ml) for fining a technological method used for clarification of wines and wine materials. At the same time, the quantitative component remains unchanged throughout the entire duration of treatment. A sharp increase in antioxidant activity is provided by the time factor during heat treatment of red wine. Slightly lower antioxidant activity during cold treatment.

It should be noted that the enzymatic antioxidant protection systems under study are quite complex and therefore the technological methods adopted in winemaking do not adequately affect their activity. Thus, the maximum concentration of molecular oxygen (10.64 mg/cm^3) was observed after heat treatment of dry red wine. The greatest change in molecular oxygen concentration was observed in red wine after cold treatment. The oxygen concentration decreased by 5.57 mg/cm^3, and the largest increase in oxygen concentration (4.63 mg/cm ^{3}) was observed in red wine after heat treatment.

Catalase activity is maximum (8.12 μmol/min/dm ^{3}) in dry red wine after fining it with bentonite. The increase in catalase enzyme activity was greatest (2.2 μmol/min/dm^3) in dry red wine treated with fining agents.

Peroxidase is an enzyme related to catalase and inactivates H_2O_2 and other peroxide compounds [13]. Peroxidase had the maximum activity among other samples in dry red wine after cold treatment. Fining dry red wine gave the greatest increase in the activity of the peroxidase enzyme (0.438 m kmol/min/dm ^{3}).

1.AOD from reactive oxygen species is significant when crushing grapes, and somewhat lower when draining the must

2.When processing red grapes, the must is less susceptible to peroxidation.

3. Processing of red grapes is clearly insufficiently provided with antioxidant protection

2. Fining of red wines is characterized by high AOD;

3. Cold treatment inactivates all the studied enzymes of the AOD system, that is, this technological method provides a stable state of wine to oxygen stress;

4. Since cold processing of dry red wines leads to an increase in oxygen concentration, which threatens the oxidation of phenolic-coloring substances in the composition of the wine and causes a change in the color and taste of the wine, this technological method for red wines is recommended in exceptional cases.

5. All component AOP systems of red wines are activated by heat treatment, i.e. dismutation and the formation of organic peroxides occur.

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