STUDY OF THE ANTIOXIDANT PROTECTION SYSTEM OF RED GRAPESS AND WINE

Irgasheva Gulmira Rakhimjanovna,
Ph.D., Tashkent Chemical and Technological Institute, Tashkent, Uzbekistan

Sapaeva Zamira Shavkatovna
Candidate of technical sciences, Associate professor,
Tashkent Chemical and Technological Institute, Tashkent, Uzbekistan

ABSTRACT
Currently, demand for light red wines has risen sharply. But the color of the wine loses its color and luster during technological treatments associated with the presence of oxygen. Therefore, the study of antioxidants, antioxidant protection system in the processing of red grapes and processing of wines in order to ensure the quality and presentation of red wines is relevant.
The work investigated the system of antioxidant protection (AOD) of products of primary and secondary winemaking. Describes the results of research on the determination of enzymes of the AOD system during all technological methods: crushing, dripping, sludge, fermentation, pasting, sludge, heat treatment, cold red wine and wine processing. Before and after each technological operation, the physicochemical parameters, the activity of the enzymes in the AOD system were determined, a cascade of oxygen reduction reactions and oxygen reduction reactions in wine are given.
As a result, on the basis of the obtained results, the technological canons of winemaking were corrected, for example, during the processing of red grapes, additional measures are clearly needed to provide antioxidant protection. Moreover, cold treatment of dry red wines leads to an increase in oxygen concentration, which threatens to oxidize the phenol - coloring matter in wine and causes a change in color and taste of wine, then this technological method for red wines is recommended in exceptional cases. Consequently, the treatment with heat of red wines provides an increase in the concentration of oxygen and at the same time activates all the component systems of AOD, there is a dismutation and the formation of organic peroxides. Over time, heat treatment enhances AOA, with that, AOA increases more intensively during heat treatment, slightly lower than AOA during technological processing with cold.

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 winemaking process. In the writings of W.J.du Toit noted the influence of oxygen on both the composition and the quality of wine, either positively or negatively [1].
Oxidation can be enzymatic or non-enzymatic, also called autooxidation. In wine, usually there is non-enzymatic, i.e. chemical oxidation. In the case of enzymatic oxidation, enzymes such as polyphenol oxidase and superoxide dismutase catalyze oxidation reactions.
The spectrum of antioxidants that make up the wine is represented by phenolic [2] components of mono-polymeric, oligo-polymeric and polymeric structures, anthocyanins, ascorbic acid, catechins, tannins, etc.,

which during technological processing participate in the addition of all forms of oxygen of aroma and taste, determining the quality of wines [3].

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

The relevance of research is to study the nature of the behavior of the AOD system, oxygen saturation, the presence of reactive oxygen species during the period of technological methods.

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 aim of the research is to determine the state of enzymes of the antioxidant defense system in the process of processing red grapes and technological processing of red wine material.

MATERIALS AND RESULTS

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

Physical and chemical indicators of grapes and wine materials were determined by methods generally accepted in winemaking.

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

The enzyme activity was determined: by the method based on the ability of superoxide dismutase to inhibit the reduction reaction of nitrotetrazolium blue. The catalase activity was determined by the reaction with ammonium molybdate, and the peroxidase activity was determined by the method based on the oxidation of pyrogallol in the presence of hydrogen peroxide to purpurogallin. The activity of the 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 supporting electrolyte.

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

The study of the antioxidant protection of red wines will make it possible to correctly build the technology of wine preparation. Crushing red grapes does not change the oxygen concentration and remains at 14 mg / dm^3. The activity of the enzyme catalase in red wort increased from 3.93 μmol / min / dm^3 to 4.35 μmol / min / dm^3. On the contrary, peroxidase activity decreased from 40.6 μmol / min / dm3 to 22.3 μmol / 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.

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An increase in SOD activity determines the presence of a superoxide oxygen radical, which intensifies the oxidation process and SOD protects against excessive oxidation [6].
Catalase, on the other hand, oxidizing one hydrogen peroxide molecule with another hydrogen peroxide molecule to form two water molecules and an oxygen molecule:

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

When the red wort flows down, oxygen saturation is observed, at which the oxygen concentration increased from 12 to 20 mg / dm^3. That is, the intense absorption of oxygen led to an increase in its concentration by 8 mg / dm^3. The activity of peroxidase increases, but the enzymes SOD and catalase lose some activity.
Peroxidases are oxidizers of peroxides, that is, peroxidation occurs according to the following scheme: Molecular oxygen during the oxidation process is gradually reduced to $2H_2O_2$. 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 [7].

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

or schematically:

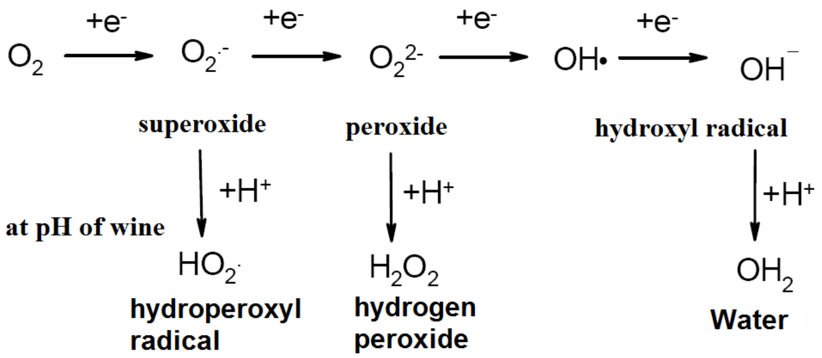

Fig. 1. Sequence of oxygen reduction reactions

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 a superoxide oxygen radical during the pressing period, while superoxide dismutase protects against excessive oxidation. Pressing activates this enzyme from 2.81 conventional units to 5.74 conventional units, which indicates a predisposition of red wort to oxidation. An increase in the activity of catalase from 3.95 µmol / min / dm^3 to 4.77 µmol / min / dm^3, which oxidizes one molecule of hydrogen peroxide, is also observed here. Apparently, pressing, as a technological method, gives an increase in peroxidation, thereby providing antioxidant protection of red wort.
During the settling of red wort, activation of peroxidase is observed and the accumulation of oxygen intensifies. The activity of SOD and catalase decreases from 4.76 µmol / min / dm^3 to 1.9 µmol / 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 prevent the ionization of molecular oxygen.
Fermentation of red wort has a different effect 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 / dm^3.
If we judge the oxidation of the must by the number of active enzymes that make up the antioxidant defense system, then when crushing red grapes, it is most susceptible to oxidation. The processing of red grape varieties is characterized by the maximum activity of SOD during draining, then during fermentation, crushing, during pressing and is absent only with the infusion of red must. That is, all operations in the processing of grapes, except for the infusion of the must, tend to oxidize the red must and the danger of

oxidation of one hydrogen peroxide molecule by another hydrogen peroxide molecule with the formation of two water molecules and an oxygen molecule is significant [8].

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

Red wines are characterized by a rich complex phenolic complex and are distinguished by a high antioxidant capacity, which is predetermined by a 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 due to the absorption of oxygen contained in the air and entering the wine during technological operations.

Pasting 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) was observed during cold treatment.

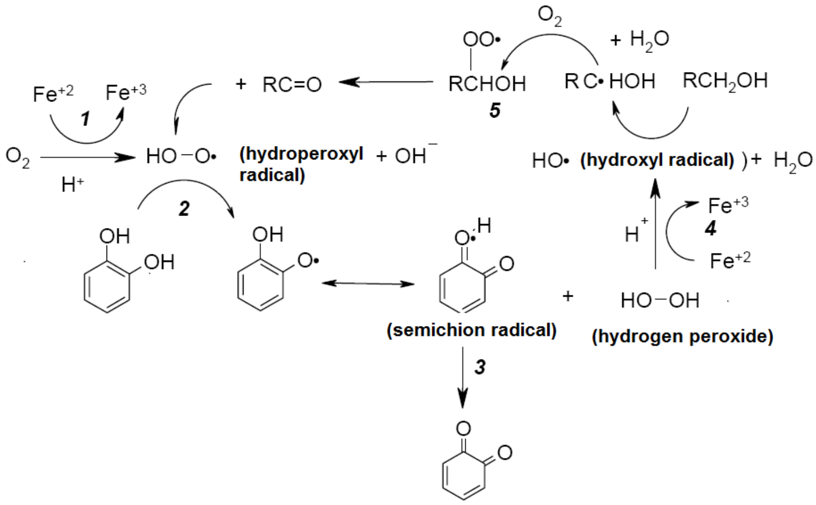

Fig. 2. Reduction reactions of oxygen in wine.

Hydrogen peroxide is a relatively weak oxidizing agent in relation to the components of wine, 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 the low-temperature treatment that inactivates it. An increase in its activity is provided by pasting with bentonite, and during this technological treatment the increase in catalase activity is about ten times higher than during heat treatment.

Peroxidase is active at the beginning and end of wine maturation. Glutione peroxidase activity in all samples increases, and it is maximum after pasting the wine and minimum (0.059 μmol / min / dm3.) During heat treatment, which confirms the existing hypothesis that heat treatment accelerates the maturation of wines. And this technique is recommended to accelerate the maturation and typing 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 growth is the same (1.74 conventional units). Superoxide dismutase catalyzes the dismutation reactions of the superoxide radical with the formation of hydrogen peroxide and oxygen. An increase in the activity of superoxide dismutase, catalase, peroxidase and molecular oxygen is observed during pasting, as well as during heat treatment, which is a vivid demonstration of the antioxidant protection of red wines [9].

During low-temperature processing, the antioxidant defense components behave somewhat differently. Thus, cold treatment gives a small increase in the concentration of molecular oxygen and peroxidase, but the activity of superoxide dismutase and catalase decreases. With the technological reception as cold treatment, there is a sharp drop in the activity of superoxide dismutase by 5.75 conv. units, loss of catalase activity by 1.45 conventional units.

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 enhances 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 pasting. Consequently, cold treatment increases the tendency of wine components to oxidize, while techniques such as pasting and heat treatment prevent wine components from oxidizing.

Antioxidant activity scavenges free radicals. No direct correlation between antioxidant activity and molecular oxygen concentration was found in this case. The absence of such a correlation indicates the presence of antioxidants of various nature.

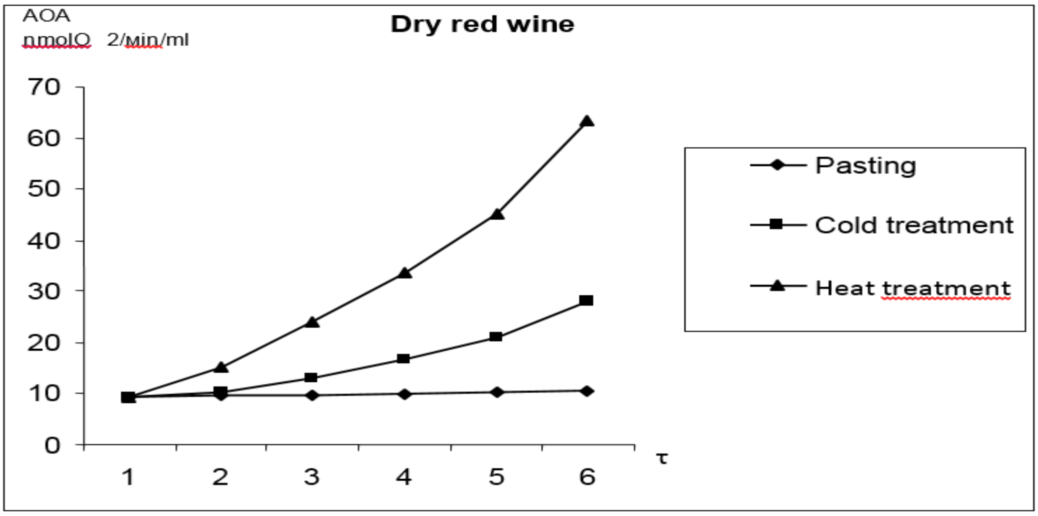

Fig. 3. Antioxidant activity of red wine during technological operations.

The results of studies to determine the antioxidant activity of red dry wine material during technological treatments are shown in the diagram (Fig. 3). Explicit 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 pasting a technological method used for clarifying wines and wine materials. At the same time, the quantitative component remains unchanged during the entire duration of treatment. A sharp increase in antioxidant activity gives the time factor during heat treatment of red wine. Slightly lower antioxidant activity when treated with cold

It should be noted that the studied enzymatic systems of antioxidant defense are quite complex and therefore the technological methods adopted in winemaking inadequately affect their activity. Thus, the maximum concentration of molecular oxygen (10.64 mg / cm3) was observed after heat treatment of dry red wine. The greatest change in the concentration of molecular oxygen was observed in red wine after cold treatment. The oxygen concentration decreased by 5.57 mg / cm3, and the greatest increase in oxygen concentration (4.63 mg / cm3) was noted in red wine after heat treatment.

Catalase activity is maximal (8.12 μmol / min / dm^3) in dry red wine after pasting it with bentonite. The increase in the activity of the enzyme catalase was the highest (2.2 μmol / min / dm3) in dry red wine treated with pasting agents.

Peroxidase is an enzyme related to catalase and inactivates H_2O_2 and other peroxide compounds [10]. Peroxidase showed the highest activity among other samples in dry red wine after cold treatment. Pasting of dry red wine gave the greatest increase in the activity of the peroxidase enzyme (0.438 μmol / min / dm^3).

1. AHO from reactive oxygen species is essential when crushing grapes, and somewhat lower when the must is drained

2. When processing red grapes, the must is less prone to peroxidation.
4. The processing of red grapes is clearly insufficiently provided with antioxidant protection
5. Pasting of red wines is characterized by high AOD;
6. Cold treatment inactivates all the studied enzymes of the AOD system, that is, this technological method gives a stable state of wine to oxygen stress;
7. Since cold processing of dry red wines leads to an increase in the 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 is recommended for red wines in exceptional cases.
8. All the constituent systems of AOD red wines are activated by heat treatment, i.e. there is dismutation and the formation of organic peroxides.

REFERENCES

1) W.J. du Toit, J. Marais, I.S. Pretorius3 and M. du Toit. Oxygen in Must and Wine: A review. Department of Viticulture and Oenology, Stellenbosch University, S. Afr. J. Enol. Vitic., Vol. 27, No. 1, 2006.
2) Boskou Dimitrios. Sources of natural phenolic antioxidants. Trends in Food Science & Technology 17 (2006) 505–512.
3) Jean-Michel Salmon. Interactions between yeast, oxygen and polyphenols during alcoholic fermentations: Practical implications. LWT 39 (2006) 959–965. Swiss Society of Food Science and Technology.
4) Clingeleffer, P.R., et. al. Production Efficiency and Relationships Among Crop Load, Fruit Composition, and Wine Quality. in ASEV 50th Anniversary Annual Meeting. 2000. Seattle, Washington.
5) Waterhouse, A.L., and V.F. Laurie. 2006. Oxidation of wine phenolics: A critical evaluation and hypotheses. Am. J.Enol. Vitic. 57:306-313.
6) Rosenfeld, E., Beauvoit, B., Rigoulet, M., & Salmon, J. M. (2002). Nonrespiratory oxygen consumption pathways in anaerobically grown Saccharomyces cerevisiae: Evidence and partial characterization. Yeast, 19, 1299–1322
7) Z.Sh.Sapaeva,Sh.M.Gulyamov S.T.Tuychieva, B.A.Abdullaeva, G. R. Irgasheva. Bioximicheskie elementi termokislorodnoy obrabotki vin[Biochemical elements of thermo-oxygen treatment of wines]. Kimyo va kimyo texnologiyasi №1 S.61-64.
8) Ageeva N.M. i dr. Antiradikalnoe deystvie krasnix vin[Anti-radical effect of red wines]// Vinodelie i vinogradarstvo. 2009. №3. S. 24-25.
9) G.R.Irgasheva. Izuchenie superoksiddismutaznoy aktivnosti pri okleyke vin[Study of superoxide dismutase activity when wrapping wines]// Trudi nauchno-texnicheskoy konferensii molodix uchenix «Garmonichno razvitoe pokolenie - dvijuщaya sila». Tashkent. 2010. S. 80-81.
10) W.J.du Toit, J.Maraus, I.S.Pretorius and M.du Toit / Oxygen in mast and Wine: A review. S. Afr.J.Enol.Vitie,Vol.27, No.1, 2006.
11) Irgasheva G.R., Sapaeva Z.Sh., Abdullaeva B.A. Issledovanie sistemi antioksidantnoy zaщiti krasnogo vinograda i vina[Investigation of the antioxidant defense system of red grapes and wine] // O‘zMU xabarlari. 2018. №3/2. S.360-364.
12) Abdullaeva B.A., Irgasheva G.R., Toshpulatova F.I., Tuychieva S.T., Shamsutdinova V.X. Research of laws of process of oxygen electroreduction in conditions steady-state and unsteady-state diffusion // International Conference on European Science and Technologi. Munich, Germany October 30-31, 2012. V.I. P. 100-104.
13) Abdullaeva B.A., Irgasheva G.R., Toshpulatova F.I., Tuychieva S.T., Shamsutdinova V.X. Comparative analysis of spectrophotometric and voltammetric methods for the determination of antioxidant activity //

7-th World Conference on Intelligent systems for Industrial Automation - WCIS. Tashkent, Uzbekistan November 25-27, 2012. V.II. P. 109-112.

14) Sapaeva Z.Sh., Abdullaeva B.A., Irgasheva G.R., Gulyamov Sh.M., Tuychieva S.T. Ximizm antioksidantnoy zaщiti pererabotki vinograda [The chemism of the antioxidant defense of grape processing] // «Kimyoviy texnologiya. Nazorat va boshqaruv». Xalqaro ilmiy –texnikaviy jurnali, Toshkent, 2012, №4. S. 10-14.
15) Bondarev M.M., Suchkov V.B. Tversin Yu.A. Issledovanie antiokislitelnoy aktivnosti belix i krasnix vin[Research on the antioxidant activity of white and red wines] // Vinodelie i vinogradarstvo. - M, 2008. -№ 3. –S. 24-25.
16) Nikolaev I.V. i dr. Otsenka antioksidantnoy yomkosti konyakov[Evaluation of the antioxidant capacity of cognacs] //. Vinodelie i vinogradarsto. – M, 2009. -№ 2. -S13-15.