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ANTIOXIDANT PROTECTION OF WHITE DRY WINE

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Abstract: General Background: The oxidative enzymatic processes in wine production significantly influence the quality and stability of white dry wines, affecting their sensory attributes and shelf life. Specific Background: Various technological methods utilized in winemaking can alter the intensity of these oxidative processes, subsequently leading to secondary oxidationreduction reactions that impact the final product. Understanding the chemistry of enzyme preparations is crucial for optimizing wine production technologies based on the specific types of enzymes used. Knowledge Gap: Despite the acknowledged importance of enzyme activity in wine production, there is a lack of comprehensive research addressing how different enzymatic preparations can be effectively employed to manage oxidative processes during winemaking. Aims: This study aims to elucidate the role of antioxidant protection in white dry wines, focusing on how various technological methods and enzyme preparations influence oxidative enzymatic activities. **Results:** The findings indicate that specific enzymatic treatments can significantly reduce oxidative stress in white dry wines, enhancing their antioxidant capacity and improving overall wine quality. Comparative analyses revealed notable differences in oxidative stability based on the enzyme types and technological approaches employed. Novelty: This research contributes novel insights into the interplay between enzymatic preparations and oxidative processes in winemaking, highlighting practical applications for improving wine production technologies. Implications: The implications of this study underscore the importance of tailoring winemaking techniques to incorporate appropriate enzyme preparations, thereby optimizing the antioxidant protection of white dry wines. Such advancements can lead to higher quality products with improved sensory characteristics and prolonged shelf life, benefiting both producers and consumers in the wine industry.

Keywords: Second Language Learning, Traditional Methods, CLT, TBLT.

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# Introduction

Resistance to environmental influences is determined by the state of the antioxidant protection (AP) components. The AP system includes both the SOD enzyme, catalase, [1]. peroxidase and antioxidants, represented by glutathione, pigments, phenolic compounds, which are present in wines [2].

**Introduction.** The aim of these studies is to determine the state (behavior) of enzymes of the AZ system during technological treatments adopted in winemaking.

Oxidoreductases (oxidation-reduction enzymes) catalyze the transfer of hydrogen atoms and electrons (dehydrogenases, oxidases, peroxidases, catalases) as well as oxidation-reduction reactions that occur during respiration and fermentation. They are divided into three groups: anaerobic [3,4]. dehydrogenases; oxygen-activating oxidoreductases and peroxidases.

The first (anaerobic dehydrogenases) do not react directly with oxygen, but transfer hydrogen or an electron to other acceptors according to the following scheme: DH  $_2$  + R  $\rightarrow$  D + RH  $_2$ 

Oxygen-activating enzymes are electron-transferring oxidoreductases and oxygenases. Oxidoreductases catalyze the reduction of molecular oxygen into either water or hydrogen peroxide, depending on the number of electrons transferred. When four electrons are transferred, we obtain water, and when two electrons are transferred, we obtain hydrogen peroxide.

$$RH_2 + 1/2 O_2 = R + H_2 O_2$$

$$RH_2 + O_2 = R + H_2O_2$$

Peroxidases are peroxide oxidizers and catalyze according to the scheme: AOH + KH  $_2 \rightarrow$  K +

### $AOH + H_2O$

Hydrogen donors can be phenols, amines and other organic compounds, and compounds of the AOH type can be hydrogen peroxide.

Catalase is also a peroxidase, oxidizing one hydrogen peroxide molecule with another hydrogen peroxide molecule to form two water molecules and an oxygen molecule: [5].

H  $_2$ O  $_2$  + H  $_2$ O  $_2$   $\rightarrow$  2H  $_2$ O + O  $_2$ 

Activation of molecular oxygen is more common by transferring four electrons to it with the formation of endogenous water, than by transferring two electrons to oxygen with the formation of hydrogen peroxide.

According to Bach, oxygen activation occurs with the help of oxygen (these are unsaturated, relatively low-molecular autooxidable organic compounds capable of interacting with molecular oxygen), breaking only one bond: [6].

The resulting peroxide can be used before oxidation of substances that are difficult to oxidize by molecular oxygen. It is during the transfer of activated oxygen from

ABOUT

 $\begin{array}{c} A + O_2 \rightarrow A \\ T \end{array}$ 

# ABOUT

The enzyme peroxidase plays an important role in the oxidation of difficult-to-oxidize substances.

Donor + H  $_2$  O  $_2$  = oxidized donor + 2 H  $_2$  O

Superoxide dismutase (SOD)

The above enzymes are part of the antioxidant protection system (AP). To study the state of antioxidant protection during technological treatments adopted in winemaking, experiments were conducted in laboratory and production conditions and the oxygen concentration and activity of all three enzymes included in AP were determined. Technological methods for processing wines were selected, widely used in winemaking are fining, cold treatment and heat treatment.

Pasting was carried out. Cold treatment was carried out according to the technological instructions. [7]. Heat treatment was carried out according to the technological instructions.

#### Methods

The modes of technological processing remained identical, only in the volumes of processed material there was a significant difference.

Before and after technological treatments, the amount of molecular oxygen, SOD, catalase, and peroxidase activity were determined in the samples. [8].

Physicochemical composition of the wines under study:

Specific gravity 0.987 Strength, %vol 10.8 Titratable acidity, mg/ dm3  $^{5.2}$ Volatile acidity, g/ dm3  $^{0.59}$ SO  $_2$  mg/dm  $^3$  96 Fe, mg/  $^{dm3}$  12.5

### **Results and Discussion**

The oxygen concentration was determined by the polarographic method [9]. Superoxide dismutase activity was determined using a method based on the ability of the SOD enzyme to inhibit the reduction reaction of nitrotetrazolium blue [10].

The activity of the enzyme catalase was determined.....

Peroxidase activity was determined by .....

The behavior of AZ in white wines during fining, heat and temperature treatment and cold treatment was studied. The results of the analyses are presented in Tables 1-2.

**Table 1** Antioxidant protection indicators of white dry wine material (production tests)

Indicators	Pasting		Cold Tre	Cold Treatment		Heat Treatment	
	Before	After	Before	After	Before	After	
T <sup>0</sup>	18	16.5	17	18	18	17.5	
$0_2 mg/dm^3$	8.5	7.8	6.4	1.71	7.0	7.6	
SOD us.ed	2.78	6.79	1.13	0.185	0.68	0.22	
Catalase	5.79	1.51	4.29	0.22	0.80	1.49	
µmol/min/l							
Gluthione	0.531	0.124	0.106	0.018	0.779	0.106	
peroxidase							
µmol/min/l							

Table 2 Antioxidant protection indicators of white dry wine material (laboratory tests)

	1		5			
Indicators	Pasting		Cold Treatment		Heat Treatment	
	Before	After	Before	After	Before	After
T <sup>0</sup>	17	18	17	17.5	19	18
$0_2 \text{ mg/}^{dm3}$	0.7	8,2	2,42	2.23	2.8	7.8
Sod us.ed	0.96	0.28	0.96	0.8	0, 70	5.68
Catalase	1, 62	0, 62	4,4	0,24	1, 33	1, 62
Gluthione	0, 141	0, 053	0, 230	0, 018	0, 09	0,054
peroxidase						
µmol/min/l						

When preparing certain types of wine, oxygen plays a decisive role in the process of their maturation, influencing the quantitative and qualitative composition of the main components of the wine, forming its taste, aroma and organoleptic properties.

The analysis of the results shows that the concentration of molecular oxygen of production samples increases slightly during heat treatment, cold treatment and gluing led to a decrease in the amount of molecular oxygen from 6.4 mg/dm<sup>3</sup> to 1.71 mg/dm<sup>3</sup> and 8.5 mg/dm<sup>3</sup> to 7.7 mg/dm<sup>3</sup>, respectively.

From the data given in the table it is evident that the highest oxygen concentration of 8.5

mg/dm<sup>3</sup> was noted in the sample before fining. The minimum decrease in the concentration of molecular oxygen in the sample was after fining of the wine. This is explained by the fact that oxygen absorption ceased and at the same time it was during fining that its consumption?

A slight increase in oxygen concentration is seen during heat treatment. A decrease in molecular oxygen concentration in the case of cold treatment is explained by an increase in oxygen consumption by wine in the presence of various oxidoreductases, in this case SOD and catalase (see Table 1). A sharp decrease in oxygen during cold treatment is due to its participation in oxidative reactions and, at the same time, its transition to a dissolved state.

The solubility of molecular oxygen depends on the temperature, strength, and content of extractive substances in wine. As the temperature increases, oxygen dissolution decreases, and an increase in strength increases oxygen solubility to  $8-10 \text{ mg/dm}^3$ .

Part of the incoming oxygen binds with the components of the wine quite strongly and is not removed from it when bubbling the wine with inert gases ( $CO_2$  or  $N_2$ ). Presumably, this part of the oxygen is in the wine in the form of peroxide compounds and is conventionally considered to be "peroxide oxygen".

The reduction in the amount of oxygen during technological processing is due to its participation in the oxidation of wine components. The higher the oxygen concentration and the processing temperature, the more intensively it is used.

In samples where oxygen consumption increases sharply, it can be explained by the fact that the main part of oxygen is spent on the direct addition of phenolic substances, which condense and precipitate. A smaller part of oxygen is spent on the oxidation of essential oils, organic acids, nitrogenous and other substances [11]

In the context of changes in oxygen concentration, key enzymes of the AZ behave as follows:

The highest SOD activity was noted in the sample after fining, which was 6.79 conventional units. Cold treatment gave a minimum SOD activity of 0.185 conventional units. The maximum inactivation of this enzyme was provided by heat treatment (by 0.46 conventional units). And the greatest activation of SOD was provided by technological treatment - fining with bentonite. During fining, SOD activity increased by 4.01 conventional units. An increase in the level of activity of the enzymes of the antioxidant protection system (in particular, SOD activity) determines the presence of the superoxide radical of oxygen, which [5] intensifies the oxidation process and indicates an increase in the content of radical compounds in the environment and the possibility of creating conditions for "oxidative" stress.

SOD and catalase are capable of reducing the level of primary active forms of oxygen (ROS) and they are the ones that have high specificity to ROS and contain metals as catalysts in the active center. In the original wine, the concentration of iron ions is quite high (12.5 mg/dm<sup>3</sup>) and this apparently played a certain role in the activation of these enzymes during technological processing. And in practice, this suggests that, first of all, it is necessary to pay attention to the concentration of metals in wines, since their presence will catalyze oxidation processes, which is especially undesirable in the production of low-oxidized table wines.

The main product of peroxidation is free radicals. Catalase activity in the original wine, before technological processing with fining agents, was maximum and amounted to 5.79  $\mu$ mol/min, and a decrease in catalase activity by 4.28  $\mu$ mol/min/l during fining indicates that there is an intensive consumption of peroxides for oxidation of wine components, but during the processing it is inactivated. The activity of catalase, which protects against the harmful effects of hydrogen peroxide, which appears as a result of the activity of flavoprotein and other enzymatic oxidative systems,

decreases sharply during fining and this inactivation is 4.28 µmol/min/l. and this is explained by the fact that fining removes proteins and, consequently, enzymes, which are also proteins.

Among the studied AZ enzymes, peroxidase had the lowest activity. Peroxidase oxidizes must polyphenols into products colored in straw-yellow color [3]. Its maximum activity of 0.779  $\mu$ mol/min/l was noted in the sample before heat treatment. During cold treatment, peroxidase activity was minimal and amounted to only 0.018  $\mu$ mol/min/l. This enzyme showed a decrease in activity during all technological treatments, with the maximum loss of peroxidase activity resulting from the technological treatment of wines with fining agents (0.407  $\mu$ mol/min/l). During cold treatment, this enzyme changed its activity insignificantly by 0.098  $\mu$ mol/min/l.

The decrease in catalase and peroxidase activity by  $4.28 \ \mu mol/min/l$  and  $0.4 \ \mu mol/min/l$  respectively indicates that free radical oxidation is being neutralized. Approximately the same behavior of catalase is observed during cold treatment.

Consequently, fining large volumes of wine with inorganic substances, in particular bentonite, leads to the presence of active enzymes of the antioxidant protection system and at the same time activation of enzymes of antioxidant activity is observed, which determines the level of antioxidant provision. Increased antioxidant protection activity is a consequence of the reduction of free radicals. *Antioxidant activity is the ability of phenols and other biologically active compounds of wine to scavenge free radicals, which leads to the suppression of oxidation.* 

- the appearance of ROS, as evidenced by the high activity of SOD
- a decrease in catalase activity leads to a decrease in peroxidation
- Low peroxidase activity; its slight decrease is a sign of slow oxidation of phenolic substances.
- the oxygen content has decreased but remains within the saturation limit of 7.8 mg/ dm<sup>3</sup>.
   During gluing [12], protein substances, including oxidative enzymes, are removed along with the adhesive deposits and, as a result, we observe the cessation of oxygen absorption.

Cold treatment provides the presence of all the studied enzymes of the AOP system and a decrease in their activity. Among the technological treatments, low-temperature cold treatment gives the lowest residual catalase activity of 0.22  $\mu$ mol/min/l, with a decrease in its activity by 4.07  $\mu$ mol/min/l

This indicates that cold treatment leads

- reduction of ROS
- peroxidation is the most intense and approximately at the same level as fining. In this case, the volume of wine did not play a role.
- decreased rate of polyphenol oxidation, but the lowest level among other treatments
- the free form of oxygen is sharply reduced (oxygen at low temperatures can pass into a dissolved state)

Heat treatment:

Minimal presence of active forms of oxygen, the presence of peroxides is the least, but the only technological treatment in which not only is catalase present, but also gives an increase in the activity of catalase, i.e. technological treatment with heat, gives way to peroxidation. In comparison with other studied treatments, the activity of peroxidase is maximum, which leads to the most intensive polyphenol oxidation. only heat treatment increases the presence of molecular oxygen. Heating for 30 seconds at a temperature of 85-90 <sup>0</sup> C leads to the destruction of oxidases. Research into the biochemical mechanisms of interaction of enzymes of the AZ system has not only theoretical but also practical significance, increasing its effectiveness in the practical aspect. (technological

cycle.) And so

- 1. Gluing and only gluing gives the presence of ROS, predetermining intensive oxidation in a state of saturation with molecular oxygen, the consequence of which is a decrease in catalase activity by the maximum value (4.28 μmol/min/l).
- 2. Cold treatment gives the maximum decrease in the concentration of molecular oxygen and an approximately equal decrease in catalase activity, which indicates an equal flow of peroxidation during fining and cold treatment.
- 3. High-temperature treatment of wines is characterized by high peroxidase activity, confirming the intensive oxidation process of wine components.

### Conclusion

In conclusion, this study elucidates the critical role of antioxidant enzymes in managing oxidative processes during the production of white dry wine. Key findings reveal that various technological treatments, including fining, heat, and cold treatment, significantly impact the concentration of molecular oxygen and the activity of enzymes such as superoxide dismutase (SOD), catalase, and peroxidase. Specifically, fining with bentonite markedly enhances SOD activity while concomitantly reducing catalase activity, indicating a consumption of reactive oxygen species (ROS) and highlighting a shift in oxidative dynamics. Furthermore, cold treatment leads to a pronounced decrease in molecular oxygen levels and correlates with a reduction in both SOD and catalase activities, reflecting a stabilization of wine quality through diminished oxidative stress. These findings imply that careful management of enzyme activity and oxygen levels is crucial for optimizing the antioxidant protection in white wines, thus enhancing their sensory qualities and shelf life. Future research should focus on exploring the biochemical mechanisms governing enzyme interactions during various winemaking processes and evaluating the long-term effects of these treatments on wine quality. This comprehensive understanding will contribute to the development of improved vinification techniques that preserve the integrity and flavor profiles of white dry wines.

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