NOVATEUR PUBLICATIONS
JournalNX- A Multidisciplinary Peer Reviewed Journal
ISSN No: 2581 - 4230
VOLUME 10, ISSUE 3, March -2024

ENZYMES IN TECHNOLOGICAL TREATMENT OF WINES ANTIOXIDANT SYSTEM INCLUDED IN OXIDATING PROCESSES

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Abstract
The intensity of oxidative enzymatic processes depends on technological methods. At the same time, conditions are created for the passage of secondary redox processes. Knowledge of the chemistry of enzyme preparations will allow you to correctly build a technology for preparing wines depending on their type. Resistance to environmental influences is determined by the state of the antioxidant defense (AD) components. The AZ system includes both the enzyme SOD, catalase, peroxidase and antioxidants represented by glutathione, pigments, phenolic compounds, which are present in wines
The purpose of these studies is to determine the state (behavior) of enzymes of the AD system in the process of technological treatments adopted in winemaking.
Oxidoreductases (redox enzymes) catalyze the transfer of hydrogen atoms and electrons (dehydrogenases, oxidases, peroxidases, catalases) as well as redox reactions that occur during respiration and fermentation. They are divided into three groups: anaerobic dehydrogenases; oxygen-activating oxidoreductases and peroxidases. [1]
The first (anaerobic dehydrogenases) do not react directly with oxygen, but transfer hydrogen or electrons to other acceptors according to the following scheme: $DH_2 + R \rightarrow D + RH_2$
Oxygen-activating enzymes are electron translating oxidoreductases and oxygenases. Oxidoreductases catalyze the reduction of molecular oxygen to either water or hydrogen peroxide, depending on the number of electrons transferred. When transferring four electrons, we get water, and when transferring two electrons, respectively, hydrogen peroxide.

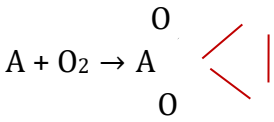
$$RH_2 + 1/2\ O_2 = R + H_2O$$
$$RH_2 + O_2 = R + H_2O_2$$

Peroxidases are peroxide oxidizers and catalyze according to the scheme: $A\ UN + KH_2 \rightarrow K + AON + H_2O$
Hydrogen donors can be phenols, amines and other organic compounds, and compounds like AOOH can be hydrogen peroxide.
Catalase is also a peroxidase, oxidizing one molecule of hydrogen peroxide with another molecule of hydrogen peroxide to form two molecules of water and an oxygen molecule:

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

Activation of molecular oxygen is more common transferring four electrons to it to form endogenous water than transferring two electrons to oxygen to form hydrogen peroxide. [2].
According to Bach, activation of oxygen occurs with the help of oxygen (these are unsaturated, relatively low-molecular autoxidable organic compounds capable of interacting with molecular oxygen) breaking only one bond:

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



To a substance that is difficult to oxidize, the enzyme peroxidase plays an important role.
Donor + H_2O_2 = oxidized donor + 2 H_2O
Superoxide dismutase (SOD)….

The above enzymes are part of the antioxidant defense system (AD). To study the state of antioxidant protection during the technological treatments adopted in winemaking, experiments were carried out in laboratory and production conditions and the oxygen concentration and activity of all three enzymes included in the AZ were determined. We chose technological methods for processing wines that are widely used in winemaking: fining, cold treatment and heat treatment.
Pasting was carried out
Cold treatment was carried out according to technological instructions...
The heat treatment was carried out according to the technological instructions...
The technological processing modes were kept identical, only there was a significant difference in the volumes of processed material.
Before and after technological treatments, the amount of molecular oxygen, activity of SOD, catalase, and peroxidase were determined in the samples.
Physico-chemical composition of the wine under study:
Specific gravity 0.987
Strength, %vol 10.8
Titratable acidity, mg/dm^3 5.2
Volatile acidity, g/dm^3 0.59
SO_2 mg/dm^3 96
Fe , mg/dm^3 12.5

Oxygen concentration was determined using the polarographic method [ ].
Superoxide dismutase activity was determined using a method based on the ability of the enzyme SOD to inhibit the reduction reaction of nitrotetrazolium blue [].
The activity of the catalase enzyme was determined…….
Peroxidase activity was determined by…….
Have you studied the behavior of AZ in white wines during fining, warm temperature?? and cold treatment. The results of the analyzes are shown in tables 1-2.
Indicators of antioxidant protection of dry white wine material (production tests)

Table 1

indicators	Pasting before after	Cold treatment before after	heat treatment before after
T^0	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 standard unit	2.78 6.79	1.13 0.185	0.68 0.22
Catalase µmol/min/l	5.79 1.51	4.29 0.22	0.80 1.49
Gluthione peroxidase µmol/min/l	0.531 0.124	0.106 0.018	0.779 0.106

Indicators of antioxidant protection of dry white wine material (laboratory tests) Table 2

indicators	Pasting before after	Cold treatment before after	heat treatment before after
T0 -	1 7 1 8	17 1 7.5	1 9 1 8
0_2 mg/dm ^{3}	0.7 8.2 _	2 .4 2 2.23	2.8 7.8 _
SOD standard unit	0.96 0.28	0.96 0.8	0.70 _ 5.68
Catalase	1 , 62 0.62 _ _	4, 4 0.2 4	1.33 1.62 _ _ _
Gluthione peroxidase µmol/min/l	0.14 1 0.053 _	, 230 0.018 _	0.0 9 0.054 _

When preparing certain types of wines, oxygen plays a decisive role in the process of their maturation, affecting the quantitative and qualitative composition of the main components of the wine, composing its taste, aroma and organoleptic characteristics [3].

Analysis of the results shows that the concentration of molecular oxygen of production samples during heat treatment increases slightly, but cold treatment and fining led to a decrease in the amount of molecular oxygen from 6.4 mg/dm ^{3} to 1.71 mg/dm ^{3} and 8.5 mg/dm ^{3} to 7.7 mg/dm ^{3} respectively.

From the data given in the table it can be seen that the highest oxygen concentration of 8.5 mg/dm ^{3} was observed in the sample before pasting. Minimal decrease in the concentration of molecular oxygen in the sample after wine fining. This is explained by the fact that the absorption of oxygen has stopped and, at the same time, it is during pasting that it is consumed???? ……

A slight increase in oxygen concentration is visible during heat treatment. The decrease in the concentration of molecular oxygen in the case of cold treatment is explained by the increased oxygen consumption of 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 of the wine. As the temperature rises, the dissolution of oxygen decreases, and an increase in strength increases the solubility of oxygen to 8-10 mg/dm ^{3} .

Part of the incoming oxygen binds to the components of the wine quite firmly 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 wine in the form of peroxide compounds and is conventionally considered to be "peroxide oxygen".

The decrease in the amount of oxygen during technological treatments 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 bulk of the oxygen is spent on the direct addition of phenolic substances, which condense and precipitate. A smaller part of the oxygen is spent on the oxidation of essential oils, organic acids, nitrogenous and other substances [4]

Against the background of changes in oxygen concentration, key enzymes of AD behave as follows:

The highest SOD activity was observed in the sample after pasting: 6.79 conventional units. Cold treatment gives a minimum SOD activity of 0.185 standard units. The maximum inactivation of this enzyme is achieved by heat treatment (by 0.46 conventional units). And the greatest activation of SOD is achieved by technological treatment, pasting with bentonite. During pasting, SOD activity increased by 4.01 conventional units. An increase in the level of activity of enzymes of the AOD system (in particular, SOD activity) determines the presence of superoxide oxygen radical, 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 of "oxidative" stress.

SOD and catalase are capable of reducing the level of primary reactive oxygen species (ROS) and they have high specificity for 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 ^{3}) and this apparently played a certain role in the activation of these enzymes during technological treatments. But 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 when producing low-oxidized table wines.

The main product of peroxidation is free radicals. The activity of catalase in the original wine, before technological treatment with fining agents, was maximum and amounted to 5.79 μmol/min , and a decrease in catalase activity by 4.28 μmol/min/l during fining indicates that there is an intensive consumption of peroxides for oxidation components of wine, but during 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, sharply decreases during fining and this inactivation is 4.28 μmol/min/l . and this is explained by the fact that pasting removes proteins and therefore enzymes, which are also proteins.

Among the AD enzymes studied, peroxidase had the lowest activity . Peroxidase oxidizes wort polyphenols into straw-yellow colored products [3]. Its maximum activity is 0.779 μmol/min/l . noted in the sample before heat treatment. During cold treatment, peroxidase activity is minimal and amounts to only 0.018 μmol/min/l . This enzyme showed a decrease in activity during all technological treatments, with the maximum loss of peroxidase activity being achieved by technological treatment of wines with fining agents (0.407 μmol/min/l .) . When treated with cold, this enzyme changes its activity slightly by 0.098 μmol/min/l.

Reduced activity of catalase and peroxidase by 4.28. μmol/min/l and 0.4 μmol/min/l, respectively, indicate 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 AOD system and, at the same time, activation of antioxidant enzymes is observed, which determines the level of antioxidant supply. An increase in antioxidant defense activity is a consequence of a decrease in free radicals.

Antioxidant activity is the ability of phenols and other biologically active compounds in wine to accentuate 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.
- oxygen content has decreased, but remains within the saturation range of 7.8 mg/dm ^{3}

During pasting [1], protein substances, including oxidative enzymes, are removed from the adhesive residues and, as a result, we observe a cessation of oxygen absorption.

Cold treatment results in the presence of all the studied enzymes of the OZ system A and a decrease in their activity. Among the technological treatments, low-temperature cold treatment gives the lowest residual catalase activity of 0.22 μmol/min/l . with a decrease in its activity by 4.07 μmol/min/l

This suggests that cold treatment leads

- reduction of ROS
- peroxide oxidation is most intense and approximately at the same level as fining. At the same time, the volume of wine did not matter.
- reduction in the rate of polyphenolic oxidation, but the lowest level among other treatments
- the free form of oxygen decreases sharply (oxygen at low temperatures can pass into a dissolved state)

Heat treatment:

- minimal presence of reactive oxygen species

!! - the presence of peroxides is the smallest, but the only technological treatment in which not only catalase is present, but increases the activity of catalase, i.e. technological processing with heat gives way to peroxide oxidation.

!!-Compared to other studied treatments, peroxidase activity is maximum, which leads to the most intense polyphenolic oxidation.

-and only heat treatment increases the presence of molecular oxygen. Heating for 30 seconds at a temperature of 85-90 ^{0} C leads to the destruction of oxidases

Research into the biochemical mechanisms of interaction between enzymes of the AD system is not only theoretical, but also practical, increasing its efficiency in the practical aspect (technological cycle)

So

1. Pasting and only pasting gives the presence of ROS, predetermining intense oxidation in a state of saturation with molecular oxygen, which results in a decrease in catalase activity by a maximum value (4.28 μmol/min/l).

2. Cold treatment gives a 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 processing of wines is characterized by high peroxidase activity, confirming the intensive process of oxidation of wine components.

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