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ENZYMES INCLUDED IN THE ANTIOXIDANT PROTECTION SYSTEM DURING THE PROCESSING OF WHITE AND RED GRAPE VARIETIES

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ANNOTATION The enzymes included in the oxidative protection system of the technological cycle of processing white and red grape varieties were studied. It was established that some technological operations predetermine an increase in oxidative stress. When processing red grapes, the bound forms of SO2 increase, which reduces its antioxidant properties. Sulfitation of red must in the amount provided by the technological instructions is clearly insufficient to ensure antioxidant protection.

Key words: Enzymes, antioxidant protection, catalase, superoxide dismutase, peroxidase, oxidation, dismutation, sulfitation, oxygen.

In the technological chain of wine production, oxidation processes begin immediately after the grape harvest, which, in accordance with recommendations [1], must be protected with sulfur dioxide (SO_2).

The study of the state of the antioxidant protection system during the processing of grapes and during the production of wine materials is of particular interest and will allow us to regulate the degree of protection of the must and wine materials from oxidative stress, which is especially important when processing grapes into low-oxidized table wines.

A mixture of both white and red wine grape varieties, sulphited to 150 mg/dm^3, was subjected to analysis. Starting from the moment of grape acceptance until the wine material was obtained, the concentration of oxygen and the activity of the enzymes superoxide dismutase (SOD), peroxidase and catalase, which are part of the antioxidant protection system (APS), were determined before and after each technological operation.

SOD activity was determined by a method based on its ability to inhibit the reduction reaction of nitrotetrazolium blue; catalase activity was determined by a 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 purpurogallin. Results of analyses during the processing of white grape varieties.

Table 1

Indicators	crushing		drainer		press		infusion		fermentation	
	To	after	to	After	to	after	to	after	to	After
T^0	1 2.7	1 3.7	15.2	14.3	14.0	14.12	17.8	18.2	15.5	14.4
0_2 mg/ ^{dm3}	10.1	16	9.0	19	22.0	15	30	35	21	14
SOD us.ed	0.14	0.69	1.27	1.11	0.41	1.00	0, 14	0.62	0.53	0.45
Catalase m kmol/min/l	0.87	0.69	1.91	1.71	3.51	1.20	1.22	0.80	0.62	1.64
Gluthione peroxidase m kmol/min/l	60.2	65.6	51.5	55.8	53.4	48.9	49.1	58.3	70.2	52.8

Results of analyses during processing of red grape varieties.

Table 2

Indicators	crushing		drainer		press		infusion		Fermentation	
	to	after	to	After	to	after	to	after	to	After
T^0	24.3	24.4	24.3	25.2	24.4	24.6	26.3	25.5	25.6	25.3
0_2 mg/ ^{dm3}	14	14	12	20	5	3	10.3	24	20	33
SOD us.ed	2.93	4.60	4.84	2.98	2.81	5.74	4.76	1.90	1.95	1.78
Catalase M kmol/min/l	3,929	4.35	4.44	4.11	3.95	4.77	4.28	4.08	4.13	6.15
Gluthione peroxidase M kmol/min/l	40.6	22.3	21.1	25.0	41.8	19.3	22.4	25.0	24.2	13.3

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The most easily bound oxygen are ionic forms and mainly SO ⁻⁻ Sulfuric acid suppresses the action of oxidative enzymes and prevents the oxidation of polyphenols and other substances. SO_2 reacts directly with oxygen and protects polyphenols and other components from oxidation and the main function of SO_2 is the removal of hydrogen peroxide formed during the oxidation of polyphenols. [2].

In samples where oxygen saturation is noted, the reaction of the active form of sulfurous acid with oxygen can be represented by reaction 2, where 2 moles of bisulfite react directly with one mole of oxygen, forming two moles of sulfate:

$$2\ HSO_3^- + O_2 \rightarrow 2\ H^+ + 2\ SO_4^{2-} \ (1)$$

During fermentation, flowing down to the VSSSh, the activity of SOD is negative, that is, these technological methods do not enhance oxidation.

Crushing and pressing give SOD activity, therefore dismutation occurs. Increased SOD activity determines the presence of superoxide oxygen radical, which [3] intensifies the oxidation process and SOD protects against excessive oxidation.

Catalase is absent in both white and red worts during runoff and infusion, and is present during fermentation of both worts, but runoff and infusion determined the activity of catalase only in red wort.

Peroxidase is significantly inactivated during fermentation and pressing. Runoff and infusion activate peroxidase in both wines. This is probably due to the presence of solid parts of the grapes, in particular, the skin with enzymes deposited on its surface. Minor dismutation is present during the infusion of white wines; Academician Oparin explains this fact by the presence of an enzymatic process that takes place during the settling of the must. Only crushing makes a difference in peroxidase activity, so in the case of white grapes it appears, while in red grapes it drops rapidly (see Table 1). [4] Runoff and infusion are equally characterized by peroxidase and catalase activities, which have a related effect. Peroxidases are peroxide oxidizers and catalyze according to the scheme: $AOOH + KH_2 \rightarrow K + AOH + H_2O$. (2)

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

$$H_2O_2 + H_2O_2 \rightarrow 2H_2O + O_2 \ (3)$$

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If we judge the oxidation of the must by the number of active enzymes included in the AOP system, then when processing both white and red grapes, during the crushing process [5] the must is most susceptible to oxidation, and only white must during infusion. What must be taken into account when choosing a technological scheme in the production of wines of various types.

And if we consider that SOD activity is the first sign of the presence of active oxygen forms, then the most dangerous technological methods in the processing of white wines are pressing and infusion. In general, during the processing of white grapes, the following technological methods can serve as sources of "oxygen stress": crushing, where the increase in SOD activity was 0.55 conventional units; pressing - 0.59 conventional units, settling 0.48 conventional units. During the processing of white grapes, catalase activity appears only during fermentation and its activity is 1.02 mMol /min/l. Peroxidase oxidation is active during the infusion of white must, when the activity increase was 9.2 mMol/min/l, then, during crushing 5.4 mMol /min/l, a little during draining and it is not present at all during pressing and fermentation.

The behavior of the enzymes of the AOP system during the processing of red grape varieties is somewhat different in that the SOD activity is maximum during runoff 2.93 conventional units, then during fermentation 2.02 conventional units, crushing 1.67 conventional units and 0.82 conventional units during pressing and is absent only during the infusion of red must. That is, all operations during grape processing, except for the infusion of must, tend to the oxidation of red must.

If in white must the activity of catalase is noted only during fermentation of white grape must, then the processing of red grapes activated it during fermentation maximum 2.02 m kmol/min/l, then pressing 0.82 m kmol/min/l and during crushing 0.421 m kmol/min/l. Catalase activity both in quantitative and technological methods where its activity is manifested, prevails during the processing of red grapes. That is, during the processing of red grapes, 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 more significant than in white must.

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But peroxidase activity during the processing of red grapes appeared only in two cases: during runoff 3.9 m kmol/min/l and during infusion 2.6 m kmol/min/l**.** It can be concluded that during the processing of red grapes the must is less susceptible to peroxidation.

SO_2 performs its antioxidant function primarily in reaction with hydrogen peroxide. The main antioxidant action of sulfur dioxide in wine is due to the bisulfite ion, which reacts with H_2O_2 to form sulfuric acid, thus limiting further oxidation of phenolic molecules or ethanol. Undissociated sulfurous acid H_2SO_3 ionic ($HSO^1{}_3$ and $SO^2{}_3$) and bound forms have such properties to a small extent. In an aqueous system, SO_2 forms sulfurous acid, which dissociates in such a way that the bisulfite form ($HSO_3{}^-$) predominates in wine, as shown in the reaction:

$$H_2O + SO_2 HSO_3{}^- + H^+ \leftarrow\rightarrow SO_3{}^{2-} + 2H^+ \quad (4)$$

The antiseptic effect of SO_2 in red must is much less frequent than in white, since most of the SO_2 is spent on binding with coloring substances. Increasing the dose SO_2 reduces color stability by up to 50%. To protect coloring agents from oxidation, it is necessary to introduce free SO_2 at a level of 20-30 mg/l.

The results of the analyses (Table 2) show that red wines are not sufficiently protected from oxidative stress, which can be explained by the binding of SO_3, reducing its antimicrobial action. Part of the oxygen dissolved in wine is spent on its oxidation into sulfuric acid and is catalyzed. He, which are always present in wine in the form of iron ions. Sulfurous acid inhibits the action of oxidases. It is accepted to introduce SO_2 in red wine in smaller quantities (how much) because tannins and catechins, contained in sufficient quantities, have natural antimicrobial properties and secondly, SO_2 reacts with anthocyanids of wine and discolors them. It seems that for this reason in wine, especially in red, the interaction of SO_2 with oxygen is effectively blocked by polyphenols. At the same time, wine aldehydes (acetic, etc.), reacting with anthocyanins, prevent excessive oxidation. Oxidation of catechins by Fe (III) catalysis with the production of hydroperoxide (hydroperoxyl) radicals and quinone, is shown in Scheme 5.

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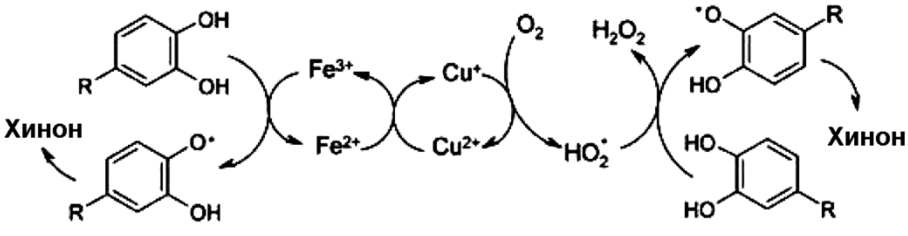
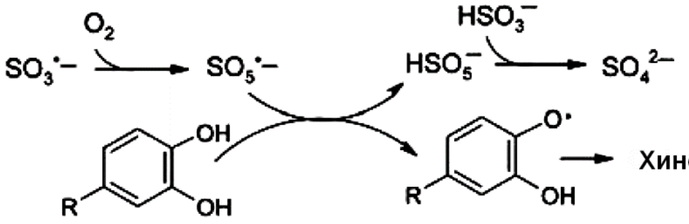


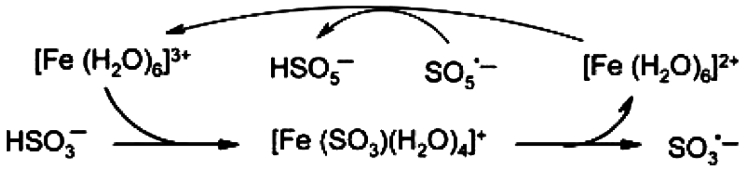
(5)

Removal of catechins using peroxymonosulfate radicals results in suppression of SO_2 autoxidation according to scheme 6.



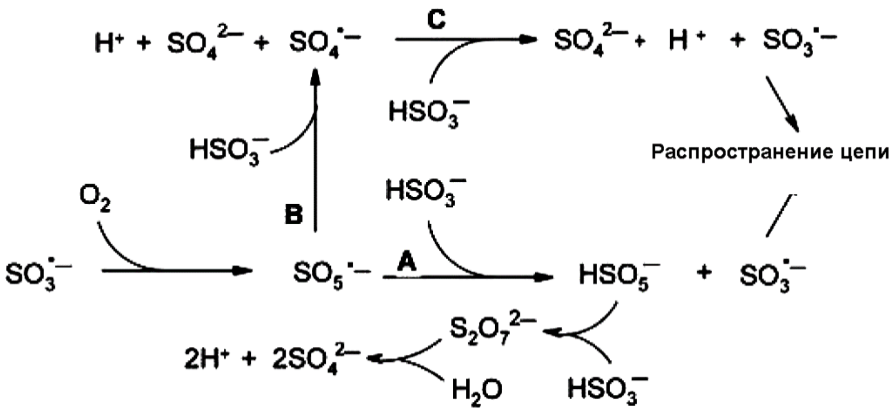
(6)

In the acidic conditions of grape must, bisulfite forms metal-sulfite complexes, according to scheme 7



(7)

The sulfite radical quickly reacts with oxygen according to scheme 8, forming the peroxomonosulfate radical ($SO_5^{\bullet -}$) [6].



(8)

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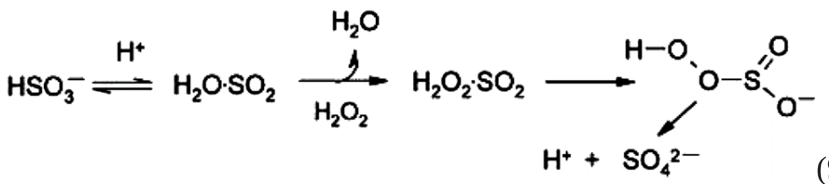
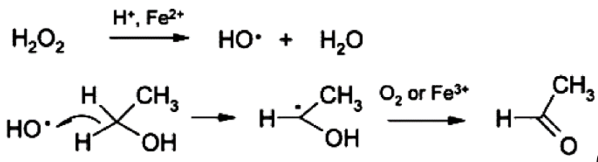
The reaction of hydrogen peroxide with SO_2 is represented as a nucleophilic displacement of water from the bisulfite ion. Scheme (9) of a possible mechanism reactions of hydrated SO_2 with hydrogen peroxide:



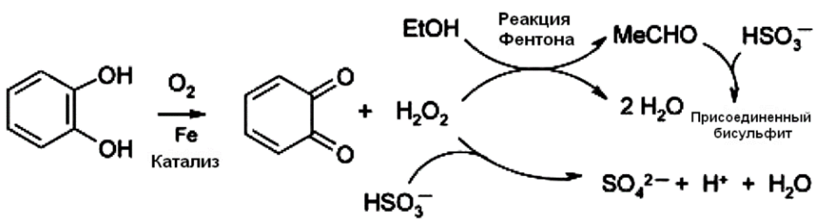
(9)

Oxygen is converted into hydrogen peroxide, which in the presence of iron forms a hydroxyl radical via the Fenton reaction (Scheme 10).



(10)

It is evident that SO_2 removes hydrogen peroxide, thus preventing its destructive action, as shown in Scheme 11 .



(11)

However, the oxidation product of the sulfite radical ($SO_3^{\bullet-}$), the peroxymonosulfate radical ($SO_5^{\bullet-}$), is a very strong oxidizing agent. In the presence of oxygen, SO_2 also promotes oxidation, which is prevented by the action of [7] polyphenols, which remove radicals.

Therefore, processing of red grapes appears to increase bound forms of SO_2, thereby reducing its antioxidant effects.

- SO2 removes hydrogen peroxide, and polyphenols block its interaction with oxygen, and only then the antioxidant effect is realized .

- Sulfitation of red wort in the amount specified by the technological instructions is clearly insufficient to ensure antioxidant protection.

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