Investigation of the appearance and elimination of "pinking" phenomenon in white wines is presented in the paper. Several factors that can cause pinking were analyzed: the degree of grapes ripeness and enzymatic treatment. The effect of fining agents based on polyvinylpolypyrrolidone (PVPP) on the elimination of "pinking" was determined. It was proved that pinking in wines decreases with an increase in the sugar content in grape. This is explained by a decrease in the content of anthocyanins at the end of technical ripeness of grape. Experimental studies confirmed that the use of pectolytic enzymes with cellulase, hemicellulase, β-glucanase activities increases the intensity of straw color and the appearance of body tones, but does not affect pinking. Such treatment contributed to a slight increase in the content of phenolic compounds in wine materials, but did not affect the content of anthocyanins and "pinking" susceptibility. Treatment of wine materials with complex PVPP-based agents including sorbents and flocculants, is an effective way to eliminate the "pinking" phenomenon. This reduces "pinking" in wine materials from 70...90 % to 1...2 %. This is due to the presence of sorbents and flocculants in the fining agents, which react with phenolic substances that cause "pinking" in white wines, and remove them from the system. So, there are reasons to argue that processing grape at the end of technical ripeness will contribute to a lower "pinking" susceptibility of wine materials; pectolytic enzymes did not affect the appearance of this phenomenon in white wines, and PVPP-based fining agents together with bentonite, activated carbon, gelatin or casein, are an effective way to reduce the "pinking" susceptibility of wine

Keywords: "pinking", white wines, grape ripeness, enzymes, polyvinylpolypyrrolidone, phenolic substances, anthocyanins

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INVESTIGATION OF THE APPEARANCE AND ELIMINATION OF PINKING COLORATION IN WHITE WINES

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1. Introduction

In some white wines, a pink tinge with different intensities may appear during production and storage. In the scientific world, this phenomenon is called "pinking". This problem appeared about 30–40 years ago and winemakers around the world have been taken it seriously [1–3]. During this period, scientists from different countries were finding out the causes of pinking. However, the mechanism for the appearance of this phenomenon and the substances that cause it have not yet been determined, although there are some hypotheses. Understanding these processes is important because the appearance of pink tinges in the color of white wines indicates the beginning of oxidation, deviation from the requirements of current standards and loss of attractiveness to consumers.

The development of this defect can make it completely unfit for consumption and lead to the accumulation of harmful substances. Therefore, research of the causes and ways to eliminate the "pinking" phenomenon in white wines is relevant, and the results will allow us to approach an important winemaking problem – the oxidation of white table wines.

2. Literature review and problem statement

"Pinking" is directly related to grape and processes that occur during ripening, and how it is processed before the fermentation period.

The chemical that causes "pinking" remains unknown, and investigations of its structure continue, although this drawback is associated with the oxidation of phenolic complex compounds. There is no consensus among scientists on representatives of this class of substances.

In [2], it is indicated that this phenomenon may occur from the conversion of leucoanthocyanidins (flavan-3,4-diols) to their corresponding flavenes (flav-3-en-3-ol) in a highly reducing medium. After that, under the influence of oxygen, flavenes are rapidly oxidized to the corresponding color cations of flavylium – anthocyanins. However, it is unclear what factors in the winemaking contribute to this process.

Another hypothesis explains wine pinking by slow acid division of interflavan bonds of some proanthocyanidins present in grape skins to the corresponding intermediate product, which, as a result of oxygen action, is converted into flavylium cations [4]. The study was conducted with only one grape variety Siria. The issue of the appearance of anthocyanins in other grape varieties remains unclear.

Investigations of other scientists have shown that the skins of white grape varieties Chardonnay, Sauvignon Blanc and Riesling contain measurable traces of anthocyanins, mainly malvidin-3-O-glucoside [5]. However, the issue of the dependence of anthocyanins content on the degree of grape ripeness remains unclear. It is known that during ripening, the content of phenolic substances in grape changes, which affects the potential susceptibility of wine to "pinking".

Anthocyanins are macerated during grape processing into must, but the addition of sulfur dioxide masks them into colorless flaven-4-sulfonate. When the amount of sulfur dioxide in wines decreases, for example, during storage or under the oxygen action, there is an increase in the form of anthocyanin flavylium in wine due to the dissociation of flaven-4-sulfonate. When the colored form of anthocyanins reaches a certain concentration, the pink color can be visually detected in white wine. In [6], it is indicated that the minimum amount of anthocyanins required to visualize pink color in wine is 0.3 mg/dm^3. However, if this level of anthocyanins can be noticed by the tasters, then it is advisable to conduct research on agents that will reduce their concentration or completely remove them from wine.

In [7], scientists suggested that the "pinking" phenomenon may appear as a result of using enzymes with pectolytic activity, which have a macerating effect on phenolic compounds of grape. However, the level of increase in the content of phenolic compounds and their effect on the appearance of anthocyanins in white wines have not been determined.

In [8], the authors do not exclude the effect of metals with variable valence (copper or iron) on the oxidation processes of phenolic compounds. However, metals as oxidation activators of phenols oxidize them to the corresponding quinones, which have a yellow-brown color, instead of pink.

Some authors note that "pinking" does not usually affect other sensory features of wine [4], but in [9] there is another opinion that pinking is associated with oxidation, so, there is a change in the aroma and taste of wine. Obviously, "pinking" is the initial oxidation process and its continuation leads to the appearance of brown shades in color, although wine may darken, without the pinking stage.

Prevention and elimination of "pinking" involve the treatment of wine materials with agents that can prevent oxidation. The most common ways to prevent pink color are the use of ascorbic acid before bottling or treatment of wines by PVPP or potassium caseinate [1, 2], which can adsorb phenolic compounds responsible for color change. The research of these authors was aimed at determining the effect of certain substances on the elimination or prevention of "pinking". The combined effect of PVPP-based agents with other sorbents and flocculants widely used in winemaking has not been sufficiently studied in the scientific community.

3. The aim and objectives of the study

The aim of the work is to study the causes and ways to eliminate the "pinking" phenomenon in white wines, which will allow preventing changes in their organoleptic characteristics and loss of their attractiveness to consumers.

To achieve the aim, the following objectives are accomplished:

– to determine the influence of the degree of ripeness of Timorasso grape on the organoleptic characteristics of white wines, in particular, color, content of phenolic compounds, anthocyanins and "pinking" susceptibility;

– to determine the effect of enzymatic treatment on the content of phenolic substances, anthocyanins and color of white wine materials made from the grape of Zagrey variety;

– to study the effect of treatment with PVPP-based agents on reducing the "pinking" susceptibility of wine materials made from the grape of Rkatsiteli, Sauvignon Blanc and Muscat White varieties.

4. Research materials and methods

4. 1. Research hypothesis

The object of the study was the technology of white table wines.

The grape of white varieties has a low content of anthocyanins, depending on the degree of ripening. Enzymatic treatment can promote the extraction of phenolic compounds, including anthocyanins, into must, thereby leading to the appearance of "pinking" in wines. Complex PVPP-based agents together with sorbents and flocculants can reduce or eliminate the "pinking" phenomenon in white wines.

4. 2. Research materials

Materials of the study were grape of Rkatsiteli, Sauvignon Blanc, Muscat White, Timorasso, Zagrey varieties and dry white wines from them, produced in the winemaking enterprises of Odesa and Mykolayiv regions using enzymes and PVPP.

The characteristics of enzymes are given in Table 1.

Table 1

Characteristics of enzymes (Martin Vialatte, France)

Enzyme	Composition
Viazym Flux	Pectinases, cellulases, β-glucanase
Viazym Clarif ONE	Pectinases: polygalacturonase, pectin-methylesterase, pectinliase, pectin liase
Viazym MP	Pectinase, cellulase, hemicellulase, β-glucanase

The characteristics of PVPP-based agents are given in Table 2.

Table 2

Characteristics of PVPP-based agents (Martin Vialatte, France)

Agent	Composition and characteristics
PVPP	eliminates the phenomena of maderization and darkening of white wines, binds astringent tannins, significantly reduces bitterness in the taste, improves freshness and aroma
Polycase	Composition: PVPP and casein; Eliminates maderization phenomena, removes astringent, bitter oxidizing polyphenols, reduces color intensity, eliminates yellow-brown shades, refreshes the taste and does not cause of overfining
Polypresse AF	Composition: plant protein (gluten-free), PVPP, bentonite, gelatin and activated carbon; Eliminates oxidation effects, reduces the intensity of wine color, reduces the intensity of astringent tones in the aftertaste; removes oxidized polyphenols, and improves the appearance of wine
Polyclin	Composition: PVPP, plant proteins, cellulose and activated bentonites; Quickly brightens wines, removes oxidized phenolic substances, reduces color intensity, eliminates yellow-brown shades, refreshes the taste, reduces astringency and eliminates bitterness, restores varietal aroma of wine
Freshprotect	Composition: PVPP, bentonites, cellulose and gum arabic; reduces bitterness and grassy tones in wine
Colorprotect	Composition: bentonite, PVPP and plant proteins; increases the oxidation resistance of wines, improves the organoleptic properties of wines, eliminates the yellow tinge in the wine color

Fining agents presented in Table 2 contain sorbents or flocculants, which react with phenolic substances of wine and can eliminate faults associated with unusual shades of wine colors.

4. 3. Research methodology and methods

The first stage of research was to determine the influence of sugar content in grape on the "pinking" susceptibility of white table wine materials. The grape of Timorasso variety was used in the investigations. The technology of wine production provided for harvesting grape with a sugar content of 198...247 g/dm^3 and titrated acidity of 6...9 g/dm^3.

Four batches of grape with a sugar content of 198, 215, 226 and 247 g/dm^3 were delivered to the winery in plastic boxes. The grape was poured into a hopper-feeder and then fed to a roller crusher-destemmer. Next, the grape pulp was sulfated at the rate of 50–70 mg/dm^3 and sent for pressing by a membrane press to obtain the must in an amount of 60 dL/t. The must was pumped into a settling tank and stored for 18 h at a temperature of 14...16 °C.

Wine materials were removed from the sediment and pumped to a fermentation tank. Dry yeasts, previously reactivated by hot water at a temperature of 30...35 °C for 20 min, were added to the fermentation tank with the must that had a temperature of 20...25 °C. The temperature in the tank during fermentation was maintained within 15±2 °C. When the sugar content in the must reached 3 g/dm^3 or less, the must was removed from the yeast precipitate and sulfated again at the rate of 20...30 mg/dm^3 of free SO_2. Ready wine materials were stored at a temperature of 6–8 °C for 2 months.

The second stage of research was to determine the influence of treatment with pectolytic enzymes on the "pinking" phenomenon in white table wine materials. Wine materials were made from the grape of Zagrey variety with an initial sugar content of 202 g/dm^3.

The production of wine materials was carried out according to four schemes: the first scheme – control (without enzymatic treatment); the second scheme – treatment with Viazym Flux enzyme; the third scheme – treatment with Viazym Clarif ONE enzyme; the fourth scheme – treatment with Viazym MP enzyme. Enzymes were added before pressing in an amount of 0.2...0.4 g/10 kg of grape pulp.

The third stage of research was to select and justify PVPP-based stabilization agents to prevent or reduce "pinking". Wine materials from the grape of Rkatsiteli, Sauvignon Blanc and Muscat White varieties, which were susceptible to "pinking" or had pinkness, were treated by suspensions of fining agents in doses recommended by the manufacturer.

Wine fining was performed as follows.

First, the content of phenolic compounds and anthocyanins was determined in the wine samples before fining.

2 g samples of fining agents were dissolved in 100 cm^3 of distilled water, mixed thoroughly to a suspension state and left to swell for 2 hours.

Each sample of wine materials was poured into 250 cm^3 cylinders, then the prepared suspension was added at the rate of 30 g/hl, thoroughly mixed and left in the cylinders for 2...3 days. After that, the clear part of the wine materials was filtered through filter paper.

Weight concentrations of phenolic substances were determined in all the test samples by a colorimetric method using the Folin-Ciocalteu reagent. The weight concentration of anthocyanins was determined by a colorimetric method with color stabilization with ethyl alcohol solution acidified by hydrochloric acid [10].

The "pinking" susceptibility of wine material was determined by a hydrogen peroxide technique. According to this method, a hydrogen peroxide solution was poured into the wine samples and the mixture was exposed for 24 hours. The absorbance of wine materials was measured before and after oxidation by hydrogen peroxide. Wine is susceptible to pinking if the absorbance value at D_{500} increased by more than 5 %compared to the control [4].

First, the tested wine materials were cleared by fine filtration (0.45 μm or 0.65 μm), then the absorbance was measured in a 10 mm thick cuvette on a KFK-3 photoelectric colorimeter (Russia) in the visible range of 440–670 nm. Distilled water was used as a comparison solution. Then, 10 cm^3 of wine was weighed into a 50 cm^3 conical flask, 0.25 cm^3 of H_2O_2 solution was added, the mixture was left at room temperature for 24 h and the absorbance was measured in a 10 mm thick cuvette against distilled water.

Mathematical and statistical processing of the results was performed when determining the criteria of the Cochran's C-test, Fisher's and Student's t-test. The accuracy of the results was determined by the Cochran's test, and the adequacy of the mathematical model was tested by the Fisher's and Student's criteria. Statistical processing was performed in Microsoft Excel 2013 (USA), the values were estimated with mean and standard deviations.

5. Results of research of the indicators of grape and white wine materials

5. 1. Study of the influence of the degree of grape ripeness on the "pinking" susceptibility of white wines

In [1], it is noted that the degree of ripening affects the content of phenolic substances in grape. An increase in the content of phenolic substances can lead to an increase in anthocyanins, which belong to the group of phenolic compounds, and may cause a pink tinge of white wines [5].

Sensory analysis of the color of white wine materials made from grape with different initial sugar content showed that the samples had no large difference in shade (Table 3). White wines were light-straw in color; the sample with medium sugar content had yellowish tinges. No pink shades were observed in the samples.

Table 3

Results of organoleptic evaluation of white wines from Timorasso grape

Weight concentration of sugars in grape, g/dm^3	Transparency	Color	Aroma	Taste
198.0±1.0	Transparent	Light-straw with a tinge of green	Fresh with a tinge of greenery	Moderately acidic, fresh
215.0±1.0	Transparent	Light-straw	Fresh with a tinge of greenery	Moderately acidic, fresh
226.0±1.1	Transparent	Straw with yellowish shades	Floral-fruity, with tones of apples	Harmonious, pleasant, easy
247.0±1.2	Transparent with gloss	Straw	Flower-fruity, light tone of apples	Harmonious, fresh, but not alcoholic

According to the results of physicochemical studies (Table 4), as the sugar content in grape increases, the content of color substances at the beginning of technical ripeness increases, but the content of phenolic substances decreases. However, reaching a medium to high degree of ripeness (sugar content in grape of 226 g/dm^3 and more) leads to a decrease in the level of anthocyanins, which is in good agreement with the literature data [11, 12].

However, the concentration of anthocyanins in wine materials was 2.0...3.4 mg/dm^3, but the pink tinge was not observed by the tasters.

After the test for the "pinking" potential, the analysis of the results showed that with increasing sugar content of grape, the pinking susceptibility of wines decreases (Fig. 1). This is indicated by a small absorbance peak (less than 5 %) in the range of 540...670 nm in wine materials with a sugar content of 226.0 g/dm^3 (Fig. 1, *c*) and the absence of any peak at 247.0 g/dm^3 (Fig. 1, *d*).

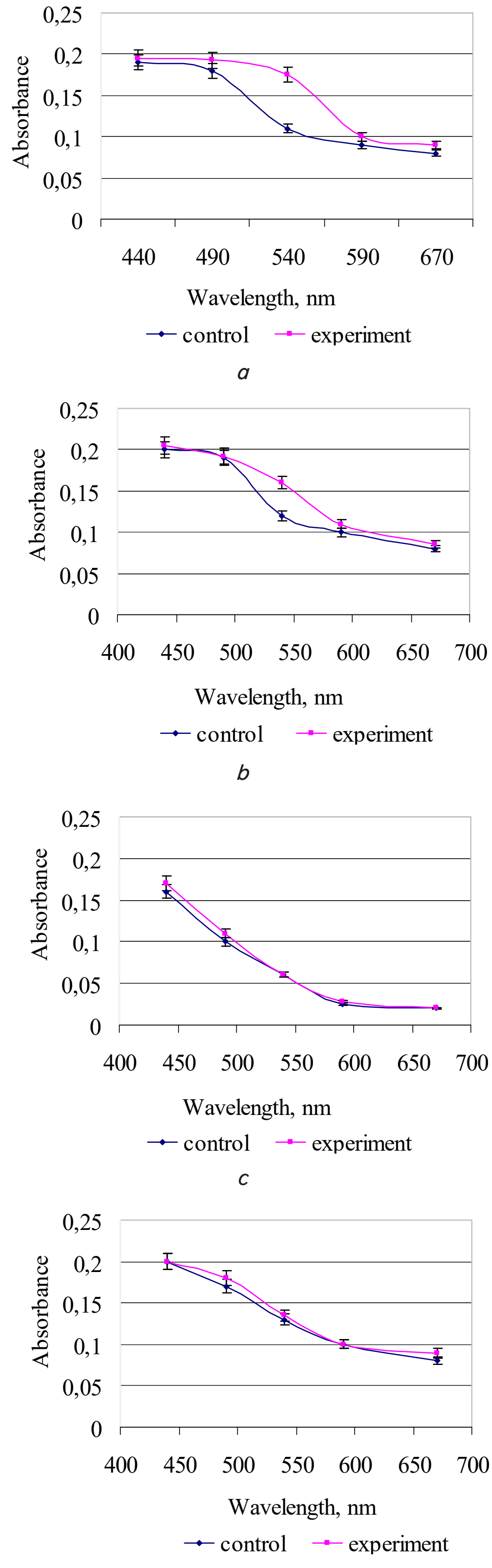

Fig. 1. Dynamics of absorbance before and after testing for "pinking" of wine material from Timorasso grape with a weight concentration of sugar in grape, g/dm^3: *a* – 198; *b* – 215; *c* – 226; *d* – 247

Table 4

Results of physicochemical studies of wines from Timorasso grape

Weight concentration		
sugars, g/dm^3	phenolic substances, mg/dm^3	anthocyanins, mg/dm^3
grapes	wine materials	
198.0±1.1	224.6±6.7	1.7±0.1
215.0±1.1	210.2±6.3	2.0±0.2
226.0±1.1	197.8±5.9	3.4±0.3
247.0±1.2	175.0±5.3	3.0±0.3

The sample with the lowest initial weight concentration of sugar (198.0 mg/dm^3) had potential susceptibility to pinking (Fig. 1, *a*). Our research somewhat differed from the hypothesis put forward by some authors [13].

5. 2. Investigation of the effect of enzymatic treatment on the content of anthocyanins in wine materials and pinking susceptibility

Enzymes are widely used in modern winemaking. Preliminary enzymatic treatment of pulp is one of the most effective methods of intensifying a number of technological processes as it helps to break the links between polysaccharides of the cell walls of grape skin and membranes of the cell organelles [14]. As a result, the yield of must per unit of raw material increases, maceration processes of grape pulp are intensified and, therefore, the content of terpene compounds, components of the phenolic complex, in particular, anthocyanins, in the must increases [15–18].

Using enzymes affects the change in yellow and red shades of wine materials. The red pigment indicates the presence of a low but still visible amount of anthocyanins. Some researchers note that most wines treated with enzymes were characterized by a change in red color intensity [18].

Comparison of the results of organoleptic analysis of the color of wine materials from Zagrey grape made using enzymatic treatment showed the presence of a more intense straw color. Viazym MP enzyme, which contains cellulase, hemicellulose and β-glucanase in addition to pectinases, gave body tones to the wine samples (Fig. 2). No pink shades were observed in the tested samples.

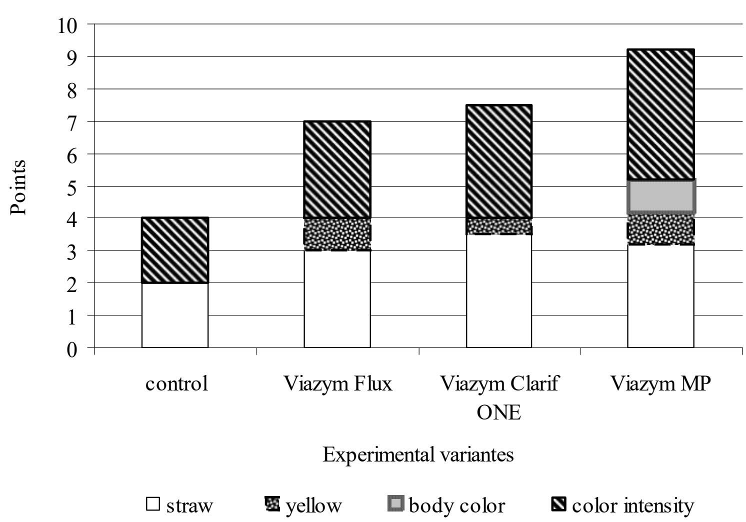

Fig. 2. Descriptive evaluation of the color of samples of white wine materials from Zagrey grape

The content of phenolic substances in the tested samples was 2...6 % higher than that of the control option (118.6±1.1 mg/dm^3), which is not a significant difference, and no anthocyanins were found. The "pinking" potential test also did not show the pinking susceptibility of wine materials. It should be noted that the initial sugar content in Zagrey grape was 190 g/dm^3.

5. 3. Effect of PVPP-based fining agents on the elimination of the "pinking" phenomenon in white wines

Literature data indicate a positive effect of some additives at the stage of wine stabilization or before bottling [1, 4, 19]. Thus, ascorbic acid having an antioxidant effect against phenolic compounds prevents their oxidation [20]. An alternative is to reduce the concentration or completely remove precursors from wines by fining. Additives that do not have a strong sorption effect, such as PVPP [1, 21, 22], sodium caseinate or their combinations with bentonite, were used for this purpose [23, 24]. Scientists note the positive effect of activated carbon on the pink color, but it also removes other compounds important for the aroma and overall quality of wines. PVPP has an adsorbing effect against phenolic compounds and forms hydrogen bonds with hydroxyl groups of polyphenols [24, 25].

The results of studies of white wine materials after fining with PVPP-based stabilizing agents revealed the most effective of them to reduce the "pinking" phenomenon. Thus, fining with Polycase agent, which contains casein in addition to PVPP and Polypresse AF agent, which contains bentonite, gelatin and activated carbon, did not show a susceptibility of wine materials to this fault (Fig. 3).

This can be explained by the presence of sorbents, in addition to PVPP, in the composition of agents that react with phenolic substances of wine materials, which cause "pinking", removing them from the system.

The difference in absorbance at a wavelength of 520 nm, which is responsible for capturing red tinges, did not exceed 5 %. The use of Colorprotect, containing bentonite, cellulose and plant protein, helped to reduce the "pinking" susceptibility of wine materials by an average of 50 %. Pure PVPP and Freshprotect agent significantly increased wine resistance to this fault, but did not provide protection against "pinking" susceptibility, as the difference in absorbance was more than 5 %.

It should be noted that the wine materials used in this experiment were susceptible to "pinking", and grape with a sugar content of no more than 200 g/dm^3 was used for their production.

6. Discussion of the results of research of the appearance and elimination of the "pinking" phenomenon in white wines

Investigations of the effect of different sugar content of Timorasso grape on the color of white wines (Table 3) didn't indicate significant differences between the samples. This is due to a decrease in the content of phenolic compounds, which are oxidized and can cause "pinking" in white wines.

However, the content of anthocyanins in Timorasso grape during ripening tended to increase with a peak of 3.4 mg/dm^3, but this concentration of anthocyanins was not detected by the tasters.

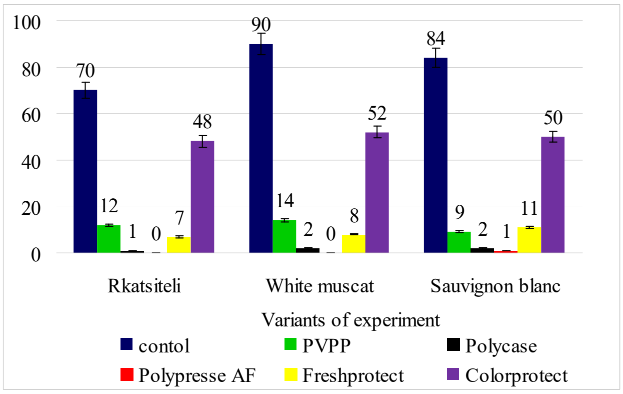

Fig. 3. "Pinking" susceptibility of wine materials before and after treatment with PVPP-based agents, %: D_0, D_1 — absorbance at a wavelength of D_{520} before and after fining, respectively

Earlier studies [6] indicated that 0.3 mg/dm^3 of anthocyanins was sufficient to visualize the pink tinge in wines. The difference in the obtained data can be explained by different storage conditions of wine materials and dosages of sulfur dioxide, which has a masking effect against anthocyanins. In Timorasso wine materials, the free form of sulfur dioxide was set at 20...30 mg/dm^3. However, the authors of [6] did not control this indicator.

In [2], it was pointed out that one of the ways of appearance of pink tinge in white wines is the conversion of leucoanthocyanidins (colorless compounds) to flavylium cations – anthocyanins. The "pinking" susceptibility of wine materials made from grape at the beginning of technical ripeness (Fig. 1, *a*, *b*) is due to the high content of phenolic compounds, which are directly related to leucoanthocyanidins – representatives of the phenolic complex.

The organoleptic investigations of the color of white wine materials made from Zagrey grape with enzymatic treatment showed the absence of pink shades in them, which can be explained by the minimal macerating effect on the phenolic substances of grape (Fig. 2). Earlier studies [7] showed that pectolytic enzymes with additional glycosidase activity change the tinge of wine color, mainly towards the yellow component. Although the values of the red component also changed. In [15], it was noted that enzymes with additional cellulose activity, in parallel with maceration of phenolic compounds, extract grape oxidases, which perform oxidative transformation and are partially removed from the system at the stage of must clarification.

The results shown in Fig. 3 confirm the effectiveness of PVPP-based agents in reducing the "pinking" susceptibility of white wines. Pure PVPP does not eliminate this phenomenon, unlike agents containing sorbents such as bentonite, activated carbon, casein and gelatin flocculant. Apparently, they react with fractions of phenolic compounds, which cause the appearance of "pinking" and remove them from the system. In particular, potassium caseinate is able to adsorb phenolic compounds responsible for color change [1], PVPP together with bentonite absorbs phenolic substances more efficiently than pure PVPP [22, 23].

The "pinking" susceptibility of white wines from Timorasso grape with a sugar content of 198...247 g/dm^3 was determined without taking into account the temperature conditions of ripening, which can also affect the content of phenolic compounds in grape.

It should be noted that research is limited by the storage conditions of wine materials, namely for two months in a cold warehouse without oxygen access. Under other conditions, the results may differ from those given in the paper.

The low content of anthocyanins in white wine materials up to 3.4 mg/dm^3 leads to difficulties in determining them by a colorimetric method. More accurate results can be obtained by chromatographic methods. Despite the limitations and complications, the results of these studies can be used to improve the quality of white wines.

As; the content of phenolic compounds, including anthocyanins, in grape depends on its ripeness, and wines – the use of sorbents, the appearance of "pinking" phenomenon will depend on the time of grape harvesting and the choice of complex PVPP-based agents.

Further research should be aimed at finding methods of grape processing in order to minimize the substances of the phenolic complex that contribute to "pinking", treatment, conditions and storage regimes of white wines.

7. Conclusions

1. The degree of grape ripeness the "pinking" susceptibility of wine materials. The "pinking" susceptibility of wines decreases with an increase in the sugar content in Timorasso grape from 198 to 247 g/dm^3. Medium and high degree of ripeness (sugar content in grape of 226 g/dm^3 and more) leads to a decrease in anthocyanins and absence of the "pinking" susceptibility of wine materials.

2. The use of pectolytic enzymes increases the content of phenolic compounds in wine materials from Zagrey grape with a sugar content of 190 g/dm^3 by up to 2...6 %. The addition of enzymes doesn't affect the increase in color fractions of phenolic substances (anthocyanins) and, therefore, the "pinking" susceptibility of wine materials.

3. Elimination of the pinking phenomenon in white wines made from Rkatsiteli, Muscat White and Sauvignon Blanc grape by 98–100 %can be achieved by treatment with complex PVPP-based agents, including bentonite, gelatin, activated carbon (Polypresse AF) or casein (Polycase).

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