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THE INFLUENCE OF TANNIN PREPARATIONS ON THE CONTENT AND FORM OF ANTHOCYANINS OF MODEL WINE SYSTEMS IN THE CONDITIONS OF INDUCED OXIDATION

M. Bilko, PhD, doctoral student, associate professor[1], *E-mail*: aromat@ukr.net
M. Ishchenko, PhD, , associate professor [2], *E-mail*: gorbag@ukr.net
O. Tsyhankova, graduate student, assistant [1], *E-mail*: elenatsyhankova65@gmail.com
T. Yakovenko, master student[1], *E-mail*: stegka@ukr.net
T. Kyrpel, student[2], *E-mail*: tanya.kyrpel@gmail.com
[1] Department of biotechnology of fermentation and winemaking products
National university of food technologies, Volodymyrska str., 68, Kyiv, Ukraine, 01601
[2] Department of analytical chemistry
Taras Schevchenko National University of Kyiv, Volodymyrska str., 72, Kyiv, Ukraine, 01601

Abstract. The article considers how oenological tannins effect on the content of anthocyanins, phenolic substances, and their forms that influence the stability of the colour of rosé and red wines. The research material was wine model systems that underwent induced oxidation for a week at a temperature of 45±5 °C with limited access of air. Tannins of different botanical origin were used in the study: hydrolyzable ones – Oaktan FU (oak wood), Tannal W4 (Tara pods), CW Tannal (chestnut wood), and Tannal W2 (gallnuts); and condensed ones – Tannin WG (grape stones), Taniraisin (grape skin), Tannal QW (quebracho wood). The source of anthocyanins was an alcohol extract from the skins of European red grapes. It was obtained by 24 hours long extraction with ethyl alcohol, and with the addition of hydrochloric acid (0.01%). It has been determined that in the course of induced oxidation, the content of phenolic compounds tends to decrease in a wavelike manner in all samples. The anthocyanin content reduces intensively and becomes practically twice as low on the first day of oxidation. The total amount of phenolic compounds becomes 2 to 6 times lower depending on the variant of the experiment. It has been established that condensed tannins of grape origin contribute to the preservation of the total content of phenolic substances (including anthocyanins) in wine, unlike hydrolyzable tannins obtained from other plants. But their application leads to polymerization of anthocyanins, which will result in the appearance of yellow-brown pigments in the red colour of wine. Hydrolyzable tannins obtained from gallnuts and tara trees preserve the red colour of wine for a long time, unlike condensed tannins, due to their antioxidant capacity. Hydrolyzable tannins obtained from tara and quebracho trees will help obtain wine with rich red shades.

Key words: oenological tannins, anthocyanins, wine, model solutions.

ВПЛИВ ПРЕПАРАТІВ ТАНІНІВ НА ВМІСТ ТА ФОРМИ АНТОЦІАНІВ МОДЕЛЬНИХ СИСТЕМ ВИН В УМОВАХ ІНДУКОВАНОГО ОКИСНЕННЯ

М.В. Білько, кандидат технічних наук, докторант, доцент[1], *E-mail*: aromat@ukr.net,
М.В. Іщенко, кандидат хімічних наук, доцент[2], *E-mail*: gorbag@ukr.net
О.В. Циганкова, аспірант, асистент[1], *E-mail*: elenatsyhankova65@gmail.com
Т.М. Яковенко, магістрант[1], *E-mail*: stegka@ukr.net
Т.В. Кирпель, студент[2], *E-mail*: tanya.kyrpel@gmail.com
[1]Кафедра біотехнології продуктів бродіння та виноробства
Національний університет харчових технологій, вул. Володимирська, 68, Київ, Україна, 01601
[2]Кафедра аналітичної хімії
Київський національний університет ім. Т.Г. Шевченка, вул. Володимирська, 72, Київ, Україна, 01601

Анотація. У роботі досліджено вплив препаратів танінів на вміст фенольних речовин та їхніх форм, які впливають на стійкість кольору рожевих та червоних вин. Дослідження проводили на модельних системах вин, які піддавали індукованому окисненню протягом тижня за температури 45±5°С з обмеженим доступом повітря. Використовували препарати таніну різного ботанічного походження та хімічного складу: гідролізовані – Оактан FU (деревина дуба), Танал W4 (стручки дерева Тара), Танал CW (деревина каштана) та Танал W2 (галові горішки); конденсовані – Танін WG (кісточки винограду), Танірейзн (шкірка винограду), Танал QW (деревина квебрахо). Як джерело антоціанів використовуали спиртову витяжку із шкірки європейських червоних сортів винограду, отриману екстрагуванням етиловим спиртом із додаванням хлоридної кислоти (0,01%) протягом 24 год. Встановлено хвилеподібну тенденцію зниження вмісту фенольних сполук в процесі індукованого окиснення у зразках модельних розчинів вин. Встановлено, що інтенсивне зменшення вмісту антоціанів відбувається на перший день окиснення практично у два рази, а загальний вміст фенольних сполук знижується у 1,5–6 разів залежно від варіанту досліду. Показано, що конденсовані таніни виноградного походження сприяють збереженню загального вмісту фенольних речовин, у тому числі й антоціанів, при виробництві рожевих та червоних вин у порівнянні із гідролізованими танінами іншого ботанічного походження, що пояснюється їхньою участю в реакціях копігментації. Але їхнє застосування приводить до полімеризації антоціанів, що сприятиме утворенню жовто-коричневих пігментів у червоному кольорі вин. Застосування гідролізованих танінів із галових горішків та стручків дерева Тара забезпечує збереження червоного кольору вин довгий час, на відміну від конденсованих танінів, завдяки своїй антиоксидантній здатності. Використання гідролізованих танінів, виготовлених із дерев Тара і Квебрахо, сприятимуть отриманню вин з насиченими червоними відтінками.

Ключові слова: енологічні таніни, антоціани, вина, модельні розчини.

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Introduction. Formulation of the problem

Anthocyanins determine the colour of rosé and red wines. While processing grapes, they are quickly extracted, but during the production of wine, they can be unstable and easily oxidized. When making wines, it is necessary to stabilize anthocyanins, and tannins are directly involved in this process [1].

Analysis of recent research and publications

Preparations of commercial ooenological tannins are widely used worldwide to produce various types of wines. Tannins have antioxidant properties and take part in copigmentation reactions together with anthocyanins, creating a strong covalent bond with them, providing protection against oxidation, and retaining ionized forms of anthocyanins [2,3,4].

When grapes are being processed, the extraction of water-soluble anthocyanins begins earlier than that of alcohol-soluble tannins, so natural tannins contained in grapes have no time to manifest their protective qualities towards the anthocyanins of grapes [5].

Scientists have proved that adding exogenous tannins effects positively on colour stabilization [6,7].

The protective effectiveness of tannins directly results from their botanical origin [8]. Raw materials for the production of tannins is the bark of the quebracho tree, oak, chestnut, acacia, gallnuts, seeds and skins of grapes, and others. The diversity of raw materials determines the complexity of the molecules of tannins, their different sizes, conformations, and properties [9,10].

Depending on their chemical composition, tannins fall into hydrolyzable, condensed, and mixed-type. Hydrolyzable tannins are compound esters of gallic or ellagic acids. Condensed tannins are composed of monomers, oligomers, and polymers of flavanols, mainly catechins, epicatechins, epicatetechin gallate, and [11,12].

A number of scientists have shown that hydrolyzable oak tannins positively influence the stability of anthocyanins. The effect other tannins have on the ability to participate in copigmentation reactions is currently a matter of extensive studies [13].

However, the literature does not provide enough information on the specific features of the effect that tannins of different botanic origin and chemical composition produce on anthocyanins when making rosé and red wines as for their protection against oxidation. This information gap makes it impossible to use a differentiated approach when choosing them.

Thus, it is a topical task to study how the use of tannin preparations, during oxidation of model wine solutions, changes the content and forms of anthocyanins.

The purpose of this work is to study the stability of anthocyanins in model wine systems during oxidation in the presence of commercial ooenological tannins of different botanical origin and chemical composition.

The objectives:

1) to investigate how tannins of different botanical origin and chemical composition change the total content of phenolic compounds (in particular, of anthocyanins) in the course of induced oxidation of model wine systems;

2) to establish how the tannin preparation used influences the distribution of anthocyanin forms after induced oxidation in model wine systems;

3) to recommend which tannin preparation should be selected to preserve the colour of rosé and red wines.

Research materials and methods

The objects of research were model wine systems based on tartrate alcohol-water solutions that simulated red (rosé) dry wine materials and contained a volume fraction of ethyl alcohol (10% vol.), mass concentraton of colourants (70 mg/l by the anthocyanin content), and of pH (3.0).

The source of anthocyanins was a grape skin extract of the Pinot Noir grape variety obtained by extraction for 24 hours with ethyl alcohol, with addition of chloride acid (0.01%). The mixture was filtered through Red Ribbon filter paper. The resulting solution was evaporated on a rotary evaporator at a temperature up to 35°C until a dry residue was obtained, which was further dissolved in deionized water with the addition of a HCl solution. The extract was then purified with solid-phase extraction on silica gel with grafted octadecyl groups and stored in a dark place at a temperature of 4°C [14].

Preparations of oenologic tannins were added into model solutions with a dose of 1.0 g/l, which is equivalent to the average content of phenolic compounds in red wines. The characteristics of tannins are given in Table 1.

The model solutions were subjected to induced oxidation carried out in a thermocouple at $t = 45 \pm 5$ °C for 7 days with limited access of air.

Prior to the induced oxidation and after it, the mass concentrations of phenolic compounds and anthocyanins in the model solutions were studied using techniques commonly practised in winemaking [15].

Table 1 – Characteristics of oenologic preparations of tannins

№	Tannin preparations	Raw material	Type of the tannin contained in the preparation
1	Tannal W4	tara tree	hydrolysed
2	Tannal CW	chestnut	hydrolysed
3	Oaktan FU	oak bark	hydrolysed
4	Tannin WG	grape stones	condensed
5	Tanirezn	grape skin	condensed
6	Tannal W2	gallnuts	hydrolysed
7	Tannal QW	bark of the quebracho tree	condensed

The percentage of copigmented anthocyanins, of polymer pigments, and the anthocyanin ionization index were determined by the methods of J. Levengood, R. Boolton [16] and T.S. Somers, M.E. Evans [17]. The method of determination is based on measuring the optical density of model solutions before and after sodium metabisulphite and hydrochloric acid are added, with the following calculation by the formulae (1–5).

Percentage of anthocyanine polymer fractions, %:

$$P = \frac{A^{HCl} - A^{20}}{A^{HCl}} 100; \qquad (1)$$

percentage of copigmented fractions of anthocyanins, %:

$$A_c = \frac{A^{SO_2}}{A^{HCl}} 100; \qquad (2)$$

percentage of ionized fractions of anthocyanins, %:

$$A_i = \frac{A^{20} - A^{SO_2}}{A^{HCl}} 100; \qquad (3)$$

total content of anthocyanins in terms of malvidin-3o-glucoside, mg/l:

$$An = (A^{HCl} - A^{SO_2}) \cdot 20; \qquad (4)$$

Anthocyanins ionization index:

$$I_i = \frac{A^{520} - A^{SO_2}}{A^{HCl} - A^{SO_2}} 100, \qquad (5)$$

where A^{HCl} is the optical density of the sample solution after adding 10 cm^3 of concentrated HCl to 0.1 ml of the sample;

A^{20} – optical density of the sample solution after dilution with a buffer solution at a pH of 3.3 by 20 times;

A^{SO_2} – the optical density of the solution of the sample of wine material or wine, at a wavelength of 520 nm, after 0.1 ml of a sulphur dioxide solution, at a concentration of 200 g/l, is added to 1.2 ml of the sample;

A^{520} – the optical density of the sample at a wavelength of 520 nm.

The optical density and absorption spectra were measured with a Shimadzu UV2100 spectrophotometer (Shimadzu Corp, Japan). The calculations were carried out after the optical density value complied with the condition l=1.0 cm and some allowances for dilution were made.

Results of the research and their discussion

On analysing the results of studying the changes in the mass concentration of phenolic compounds and anthocyanins in model wine samples during induced oxidation (Fig. 1,2), it has been possible to establish that the level of these compounds varies in a wavelike manner and tends to decrease in the control and the experimental samples. The anthocyanin content reduces intensively on the first day of oxidation, becoming almost twice as low, and that of phenolic compounds 1.5 to 6 times as low, depending on the variant of the experiment. At the end of oxidation, the content of phenolic compounds was almost the same in all experimental and control samples.

Throughout the whole oxidation process, sediment formation in all samples was observed. At the end of the oxidation, all model solutions had significant colloidal precipitates. They were caused by a violation of the aggregative stability of the system and by the precipitation of phenolic compounds – the latter was confirmed by their insignificant content in the liquid part of the model solutions on the 7th day of the process. It can also explain the wave-like change in the mass concentration of phenolic substances, depending on the time.

The content of phenolic substances in the samples varied significantly, depending on the tannins used during the research. Thus, the largest decrease in the concentration of phenolic substances was noted in the experiment with the use of hydrolyzable tannins from gallnuts and the tara tree, the smallest – when condensed tannin from grape skin was used.

The dynamics of anthocyanins in the course of induced oxidation had a similar tendency with the total content of phenolic compounds – a wave-like pattern tending to decrease. And only the control sample was characterized by a decrease in the concentration for each day of measurement (Fig. 2).

At the end of oxidation, their concentration in the samples varied depending on the experimental variant. The greatest loss in their concentration was observed in the control sample where tannins were not used. The least amount of anthocyanins in the experimental samples was noted in the variant with the addition of the hydrolyzable tannin W2 made from gallnuts (11.3±1.0 mg/l) and the condensed tannin QW from the bark of the quebracho tree (12.7±1.3 mg/l). The highest content was in the sample with the condensed grape skin tannin Taniraisin (25.3±2.2 mg/l).

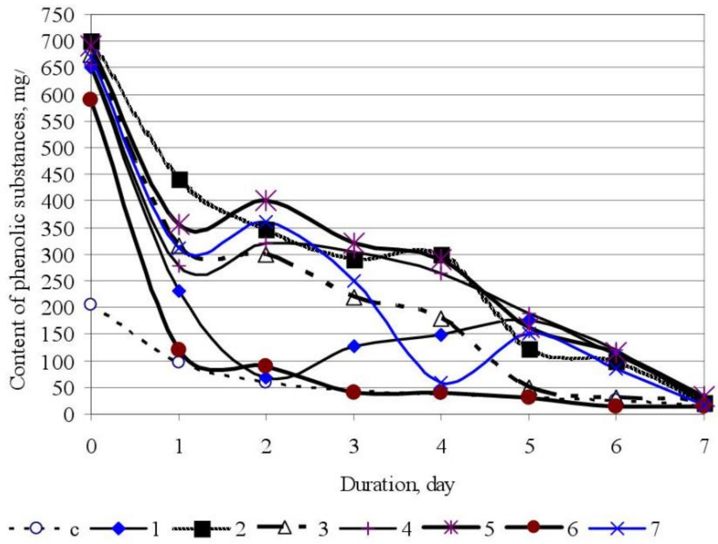

Fig. 1. Dynamics of the mass concentration of phenolic substances in the course of induced oxidation of model wine systems. Variants of tannins in the samples: c – control; 1 – Tannal W4; 2 – Tannal CW; 3 – Oaktan FU; 4 – Tannin WG; 5 – Taniraisin; 6 – Tannal W2; 7 – Tannal QW

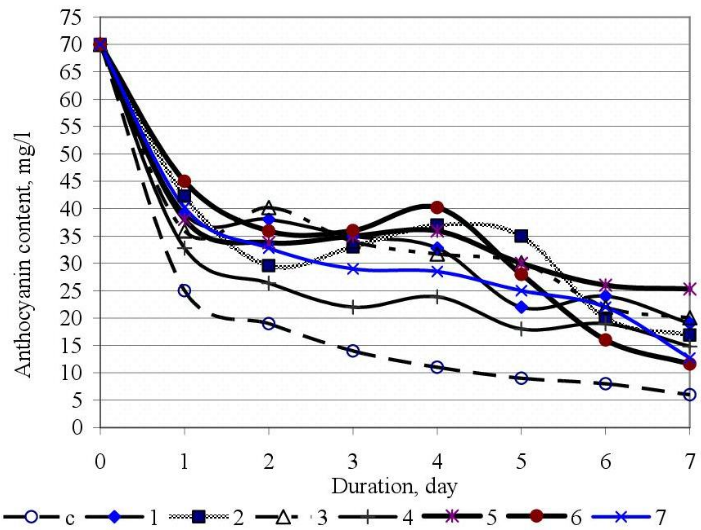

Fig. 2. Dynamics of the anthocyanin content in the course of induced oxidation of model wine systems. Variants of tannins in the samples: c – control; 1 – Tannal W4; 2 – Tannal CW; 3 – Oaktan FU; 4 – Tannin WG; 5 – Taniraisin; 6 – Tannal W2; 7 – Tannal QW

However, not only the total content of anthocyanins, but also the ratio of their forms, are essential for the formation of the tint and the colour retention of the wine [3].

Oxidation results in redistribution of anthocyanin forms that have different degrees of stability and contribute to the red colour of the samples [3,5]. We have noted that the use of tannins aids the copigmentation of anthocyanins after induced oxidation, compared with the control sample.

From Fig. 3, it is seen that polymeric forms of anthocyanins (77%) predominated in the control sample, a small percentage of copigmented (9%) forms can be explained by the presence of natural phenolic compounds of grapes.

Comparison of the results of the analysis of the samples has made it possible to establish that the samples with hydrolyzable tannins added, generally, make the fraction of copigmented anthocyanins predominant in model solutions, which increases the colour intensity and stabilizes the red colour of wines, since these forms of anthocyanins are stable and protected from oxidation [3]. It is known that copigmened forms of anthocyanins have a higher colour intensity, even if compared with free ionized anthocyanins [16].

It should be noted that the proportion of copigmented anthocyanins was almost 50% when hydrolyzable tannins from the tara tree, chestnut, and gallnuts were used, and only oak tannin had a slightly lower percentage of 42%.

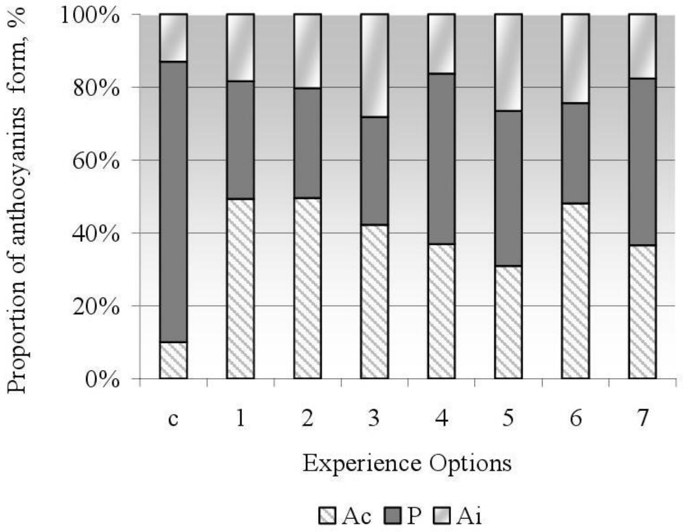

Fig. 3. Distribution of anthocyanin forms in model wine systems after induced oxidation. Anthocyanin fractions: Ac – copigmented, P – polymerized, Ai – ionized; variants of tannins in the samples: c – control; 1 – Tannal W4; 2 – Tannal CW; 3 – Oaktan FU; 4 – Tannin WG; 5 – Taniraisin; 6 – Tannal W2; 7 – Tannal QW

Condensed tannins result in less copigmenting than hydrolyzable tannins. A difference has been noted in the fractions of copigmented forms, depending on the nature of the condensed tannin. It is perhaps due to the number of regions in the tannin molecule that contribute to direct or indirect condensation. Thus, the smallest content of copigmented forms of anthocyanins (36%) was noted when grape skin tannin was used. Oenotannin from grape stones and the quebracho tree resulted in slightly higher copigmenting – 38%.

The highest percentage of polymer fractions, which could later be responsible for the intense brick-yellow shades in the colour of wines, was noted when condensed tannins of grape origin and from the quebracho tree were used (options 4, 5, and 7).

The ionized forms of anthocyanins, which are carriers of red shades of the colour of wines, prevailed in the samples using hydrolyzable oak tannin and phenotanin, which is well consistent with the literature data [4].

The ionization percentage, or index, of anthocyanins in the colour of wines indicates the percentage of anthocyanins that are present in the samples in the form of flavylium and are red (Fig. 4). It should be noted that in all samples, after oxidation, there was a high percentage of this indicator, except for the control sample, where the proportion was 47%. The largest values were noted when hydrolyzable tannin of the tara tree and condensed tannin from the quebracho tree were used.

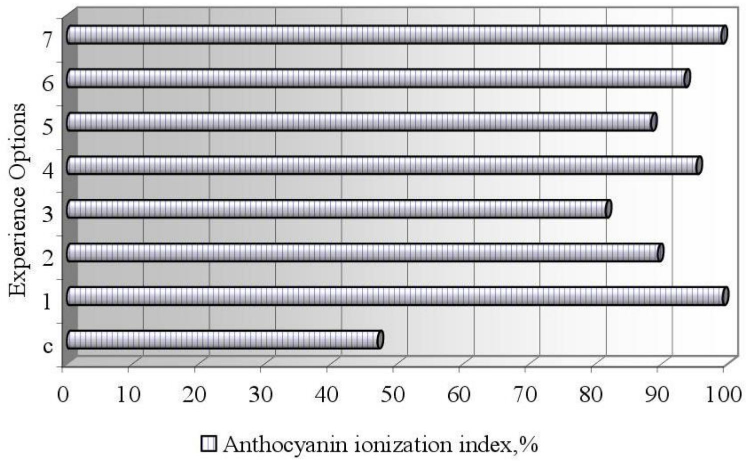

Fig. 4. Percentage of ionized anthocyanins in model wine systems after induced oxidation. Variants of tannins in the samples: c – control; 1 – Tannal W4; 2 – Tannal CW; 3 – Oaktan FU; 4 – Tannin WG; 5 – Taniraisin; 6 – Tannal W2; 7 – Tannal QW

Conclusions

The research of how oenologic tannin preparations influence the stability of the red colour of rosé and red wines (with model wine systems as an example) has allowed establishing the following:

1. Application of tannins in red wines technology contributes to the formation of copigmented fragments of anthocyanins, which positively tells on the stability of their colour.

2. Condensed tannins of grape origin aid in the preservation of phenolic substances and anthocyanins when making wines unlike hydrolyzable tannins of a different botanic origin. However, their application leads to polymerization of anthocyanins, which results in the formation of yellow-brown pigments in the red colour of wines.

3. Application of hydrolyzable tannins from gallnuts and the tara tree, unlike condensed tannins, retains the red colour of wines for a longer time because they take part in the reactions of copigmentation.

4. The use of hydrolyzable tannins made from the tara and quebracho trees will help to obtain wines with intense shades of the red colour.

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