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EFFECT OF MACERATION REGIME ON PHENOLIC COMPOUND QUANTITY AND COLOR QUALITY OF MADRASA WINE SAMPLES

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Abstract. Along with the well-known "Madrasa" natural wine, the kagor "Shamakhi" is made from the autochthonous red Madrasa grape variety native to the Mountainous Shirvan region of Azerbaijan. The type of wine produced depends on the processing characteristics of this variety, specifically, the mode of maceration of the solid parts of the grape with juice. In this regard, the study of the influence of the maceration period of the mash on the color quality of the wine and the amount of phenolic compounds is relevant and is of scientific and practical importance. The research aimed to explore the influence of the maceration regime of the mash on the amount of phenolic compounds and color indicators in autochthonous Madrasa wine samples. For 96 and 144 hours, maceration was performed at low heat (30°C), room temperature (20°C), and cellar temperature (10°C). The highest amount of phenolic compounds was observed during the maceration of the mash for 144 hours under room conditions (20°C) and 96 hours under cellar conditions (10°C).The highest indicator, 88 mg/dm^3, in total flavonoids was observed in samples obtained by maceration at 30 C temperature for 144 hours, and the lowest indicator, 62 mg/dm^3 under room conditions by maceration for 144 hours. In both processing methods, 7 phenolic acids were detected. Compared to others, the amount of catechin, gallic acid, and epicatechin was more. The amount of catechin varied from 34.90 to 39.80, galacturonic acid from 12.60 to 16.00, and epicatechin from 5.01 to 6.91 mg/dm^3. The amount of other four phenolic acids were found to be many times less abundant. In addition to natural adhesives such as bentonite and gelatin, polyvinylpolypyrrolidone (PVPP) was used to remove polyphenols, and the best results were obtained in this case. The total amount of phenolic compounds in the initial wine sample was 520 mg/dm³ and anthocyanins 83 mg/dm³, while those indicators were reduced by 14.4% and 20.5%, respectively, when treated with PVPP. We can note that bentonite is the second adhesive according to the degree of effect on phenolic compounds.

Key words: juice, mash, wine, maceration, filtering, phenolic compounds.

Introduction. Formulation of the problem

Although there are more than 10,000 grape varieties in the world, only 50% of them are suitable for wine production. Among these varieties, autochthonous grape varieties are trending. Because under local conditions, the wine obtained from grapes has a taste and aroma that is not compatible with other wines. The strength of autochthonous varieties is that they perfectly reflect their birthplace and terroir. Italy is considered the leading country in the world for the production of autochthonous wines. There, local producers make 500 types of wine from about 400 local grape varieties.

The best example of autochthonous grape varieties during the former Soviet Union was the Shirvanshahi variety cultivated in the Kurdamir region of Azerbaijan. The Shirvanshahi grape variety was found

to differ among aboriginal and introduced varieties due to the highest amount of phenolic components. It is impossible to produce in another location the wonderful "Kurdamir" wine made from this variety under these conditions [1]. Along with the well-known "Madrasa" natural wine, the kagor "Shamakhi" is made from the autochthonous red Madrasa grape variety native to the Mountainous Shirvan region of Azerbaijan. The type of wine produced depends on the processing characteristics of this variety, specifically, the regime of maceration of the grape solid parts with juice. The color substances are located in the bark, not in the leaf of the Madrasa variety, which is its distinguishing feature[2]. Therefore, pink wines are also made from this variety by processing the cluster in a soft regime (removing the berry skin). Besides, it is possible to produce high-quality red natural and high-extract dessert wines with the mash maceration. In this regard, the study of the influence of the mash maceration period on the color quality of the wine and the amount of phenolic compounds is relevant and is of scientific and practical importance.

Analysis of recent research and publications

Phenolic compounds represent a very wide group with structures ranging from a simple phenol (ring) compound to highly polymerized proanthocyanidins [3]. Phenolic compounds are found in fruits and vegetables, as well as in seeds, flowers, leaves, branches, and stems of plants [4].

These compounds have a number of important properties, such as their effects on the formation of taste, color, and smell in plants. Having antimicrobial and antioxidant effects, and an inhibitory effect on enzymes, they are also important in terms of human health [5].

Starting from the 90s of the last century, opinions about phenolic compounds began to change and it is accepted that it is a vital nutrient for health and nutrition [6].

The combined use of cold pectinase maceration and juice pulp fermentation provides higher phenolic compounds, sensory indicators, and better stability [7].

The influence of different maceration periods (5, 10, and 15 days) on phenolic compounds and antioxidant activity in red wines was studied in the Karaoghlan grape variety grown in Malatya. It was found that a greater amount of phenolic compounds, as well as higher antioxidant activity, was provided by 15-day maceration [8].

The effect of thermal and pectinase maceration on phenolic compounds and the physicochemical composition of Strychnos cocculoides juice was studied. In enzymatic processing, in addition to the 26% increase in pulp yield, the physicochemical quality of the juice (the transparency of the juice increases by 38%), the amount of phenolic compounds and antioxidant activity increased [9].

The effects of apple ripening stages and mash maceration on the phenolic compounds and antioxidant power in turbid juice were studied. The research was conducted using chemical therapy. It was noted that depending on the ripeness of the apple, enzyme preparations can increase the concentration of phenolic compounds and the antioxidant properties in turbid grape juice [10].

Diffusion of phenolic compounds during model maceration in winemaking was studied and attention was paid to the role played by grape pulp and seed. It has been discovered that only the seed contains a sufficient amount of polyphenols [11].

Effect of different processing methods on phenolic compounds and antioxidant capacity in wine obtained from a grape variety Kalajik Kalasi [12] (Anli), effect of terroir on the amount of phenolic and aromatic compounds in wine [13] (Bayram), profiles of phenolic compounds of selected Queensland red wines at all stages of the winemaking process [14], as well as phenolic components of vintages made from Merlot and Cabernet Sauvignon grape varieties in Bordeaux were studied [15]. The effects of factors to the amount of aromatic and ather compound in the samples of Muscat wine are researched [16].

The effect of maceration time and conditions of mash on the physicochemical and organoleptic characteristics of pink wine samples was studied and the optimal regime was determined. However, the studies conducted were not at a sufficient level to fully cover the effect of mash maceration in different regimes on the amount of phenolic compounds and color indicators in Madrasa wine samples. As seen, there is a scientific issue that must be addressed.

The research aimed to study the influence of the mash maceration regime on the amount of phenolic compounds and color indicators in autochthonous Madrasa wine samples.

Objectives of the research

1. Study of the effect of the maceration regime on the total amount of phenolic compounds and flavonoids;
2. Study of the effect of the winemaking process and technological operations on the amount of phenolic acids;
3. Study of the effect of the maceration regime on color indicators (color intensity, color shades);
4. Study of absorbance values of wine samples at different wavelengths;

Research materials and methods

Grapes, juice, mash, pomace, and wine material were used as the study objects.

Grapes in form of raw materials, grape juice, pulp, wine material, wine as well as technological methods and tools were taken as the object of research. As raw material the aboriginal Madrasah grape variety was used. Grapes are harvested and processed in the state of technical ripeness, cleaned of diseased and damaged

parts. The grapes were harvested from Agsu district, located at the end of mountainous Shirvan region of Azerbaijan. After the grapes are crushed and separated from the comb, sulfite anhydride is injected at the rate of 30-50 mg per liter. Then the crushing is divided into weighted portions of 40 kilograms each according to the research to be conducted. The received weighted parts are stored in the following manner under different conditions and for a certain period of time:

1. Storing crush for 96 hours at a temperature of : A)10°C,B) 20°C, C) 30°C.

2. Storing crush for 144 hours at a temperature of: A) 10°C,B) 20°C, C) 30°C.

The physical and chemical composition indicators of the samples, which differ according to the maceration time and temperature, are analyzed. Diluent of wine samples is done using traditional dilution.

For the same purpose PVPP, gelatin, albumin and bentonite were used.

The analysis of the physicochemical composition indicators of wine samples was performed according to the general analysis methods available in enochemistry [17].

The amount of total phenolic compounds was determined by the Folin-Ciocalteu method. The total phenolic compounds corresponding to the absorbance of the samples were determined by a standard graph drawn using gallicacid. It is expressed as mg/l according to gallic acid [18,19].

To detect anthocyanin HPLC was performed using Agilent-1100 with a double pump, double wavelength, and diode array detector. 6 ml of the juice or wine to be analyzed was passed through the LC-18 Supelguard Cartridge and resination of anthocyanins was carried out in this way. Then, 18 ml H_2O-HCC (99.9/0.1; v/v) was passed through the cartridge to remove sugars in the medium. At the same time, resinous anthocyanins were mixed with this solvent. After mixing the anthocyanins with the solvent, the resulting mixture was concentrated to dryness in an evaporator at 25°C. Then, the anthocyanins stuck to the wall of the evaporator flask were dissolved in 1 ml of methyl alcohol/water/formic acid (40/55/5:v/v/v) solvent and injected into HPLC, and the amount and profiles of anthocyanins were determined. The profiles of anthocyanins were revealed by directly injecting pre-regulated peel extractors. HPLC-MS is used in the identification of anthocyanin compounds. Delphinidin, cyanidin, petunidin, peonidin, and malvidin-3-glucoside standards were used to detect the amount of anthocyanins. A solution of five different concentrations was prepared and injected into the HPLC for each standard, calibration curves were constructed, and the amount of compounds was calculated based on the obtained curves.

The optical densities (OD) of the color shades were measured spectrophotometrically in 1 mm cuvettes at 420 nm, 520 nm, and 620 nm compared to distilled water. Optical densities show the percentage of yellow at 420 nm, red at 520 nm, and blue at 620 nm.

$$OD_{420}=\frac{OD_{420}}{RS}x100;\ OD_{520}=\frac{OD_{520}}{RS}x100;$$
$$OD_{620}=\frac{OD_{620}}{RS}x100.$$

The sum of the absorbances of samples ($OD_{420}+OD_{520}+OD_{620}$) compared to distilled water at 420 nm, 520 nm, and 620 nm in 1 nm cuvettes expresses a color tone.

The statistical analysis is carried out by using the program of SPSS18 package [20].

Results of the research and their discussion

The effect of the "Mash maceration" regime (in other words, maceration temperature and duration) on the amount of total phenolic compounds and color was studied in wine samples. Mash maceration was carried out for 96 and 144 hours. Variants differed not only in terms of maceration time but also in terms of temperature. Thus, the samples for both variants were prepared by maceration at low heat (30°C), at room temperature (20°C), and under cellar conditions (10°C).

Following maceration, the mash samples are pressed separately according to the variant, fermented until the end by injecting a moderate amount (25-30 mg/dm^3) of SO_2, then kept cold at 8–11 C, diluted, and separated from the sediment.

The highest amount of phenolic compounds was found in the mash during maceration for 144 hours under room (at 20°C) and 96 hours under cellar conditions when studying the amount of phenolic compounds in the samples (10°C) (Figure 1).

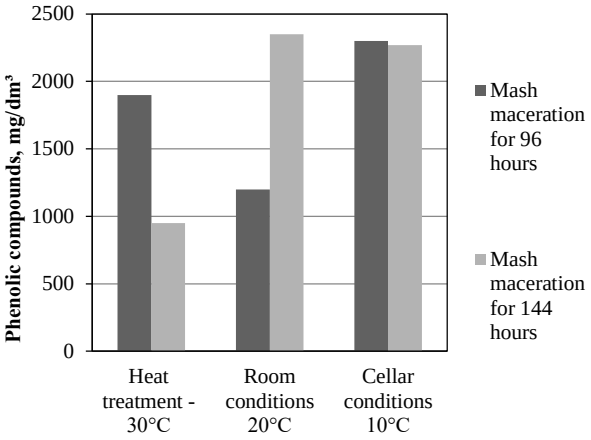

Fig. 1. Changes in the amount of total phenolic compounds depending on the "Mash Maceration" regime, n=6, p<0.05

The smallest amount of phenolic compounds was in the sample macerated for 144 hours at a temperature of 30°C, and it was 900 mg/dm^3. As seen, the amount of phenolic compounds differed depending on the temperature.

The highest level of total flavonoids amounted to 88 mg/dm^3 was observed in wine samples fermented by maceration at 30°C temperature for 144 hours (Figure 2). The lowest indicator was observed in the samples fermented by mash maceration for 144 hours under room conditions and was equal to 62 mg/dm^3.

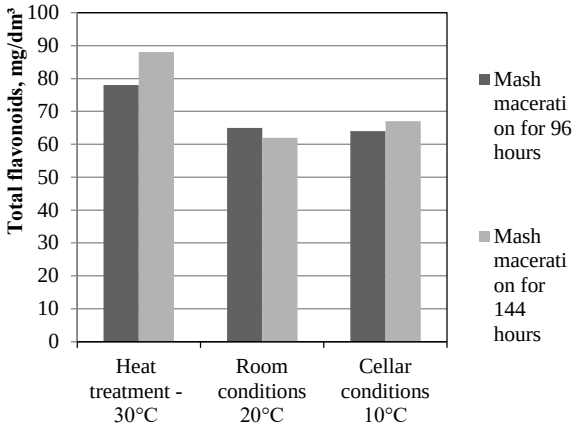

Fig. 2. Effect of maceration regime on the amount of flavonoids, n=6, p<0.05

Changes in the amount of phenolic acids were found to occur in the sequence of preparation of wine samples. According to the qualitative composition of phenolic acids, all 7 compounds are present in both processing methods. The amount of phenolic acids in both variants tended to decrease after technological operations in the sequence of wine preparation such as malolactic fermentation, diluting, and filtering. Among the phenolic compounds, catechin, gallic acid, and epicatechin were distinguished by their greater amounts. The amount of catechin varied between 34.90 and 39.80, galacturonic acid from 12.60 to 16.00, and epicatechin between 4.93 and 6.91 mg/dm^3. The amount of the other four phenolic acids was much lower than them (Table 1).

The greatest decrease in the mentioned processing sequence of wine sample preparation was observed after filtering. Filtration made the drink more harmonious by creating softness in the taste and gave it an advantage compared to the initial samples.

HPLC chromatogram identified anthocyanins in the Madrasa wine sample prepared by 96-hour mash maceration. Delphinidin-3,5-diglycoside; Cyanidin chloride; Delphinidin-3-0-glycoside; Pelargonidin-3,5-0-glycoside; Malvidin-3,5-0-glycoside; Pelargolidin-3-0-glycoside were detected in the wine sample during the study (Figure 3).

Table 1. Changes in the amount of phenolic acids during the production process of madrasa wine, mg/dm^3, n=6, p<0.05

Phenolic compounds	Maceration (96 h)				Maceration (144 h)			
	Fermentation	Malolactic fermentation	Dilution	Filtering	Fermentation	Malolactic fermentation	Dilution	Filtering
Gallic acid	12.60	12.80	14.11	14.17	12.71	13.80	16.00	15.94
Catechin	38.50	37.30	36.60	34.90	39.80	37.81	36.97	35.86
Caffeic acid	0.45	0.51	0.50	0.47	0.51	0.28	0.25	0.26
Epicatechin	6.85	6.60	5.81	5.01	6.35	6.91	6.00	5.73
P-coumaric acid	1.25	1.26	1.25	1.20	1.40	1.50	1.35	1.32
Ferulic acid	0.48	0.44	0.42	0.41	0.44	0.46	0.42	0.41
Hydroxycinnamic acid	3.15	3.70	3.21	2.95	3.52	3.40	2.20	2.00
Total	63.28	62.61	61.90	59.11	64.73	64.16	63.49	61.72

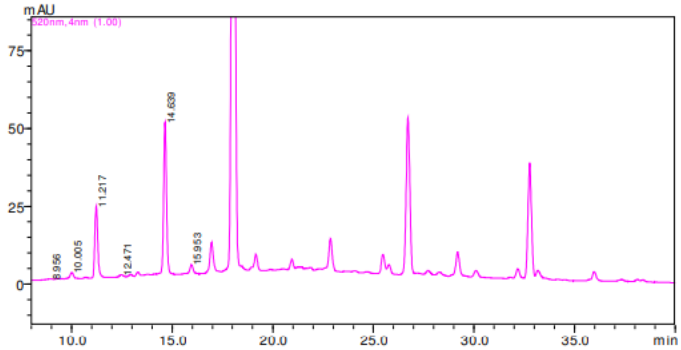

Fig. 3. HPLC chromatogram of anthocyanins in Madrasa wine obtained by mash maceration (96 hours)

(according to detection time 8.856, Delphinidin-3,5-diglycoside; 10.005, Cyanidin chloride; 11.217, Delphinidin-3-0-glycoside; 12.471, Pelargonidin-3,5-0-glycoside; 14.659, Malvidin-3,5-0-glycoside; 15.953, Pelargolidin-3-0-glycoside)

Color indicators (color density, color intensity, color tone) were studied in experimental samples (Figure 4, Figure 5, Figure 6).

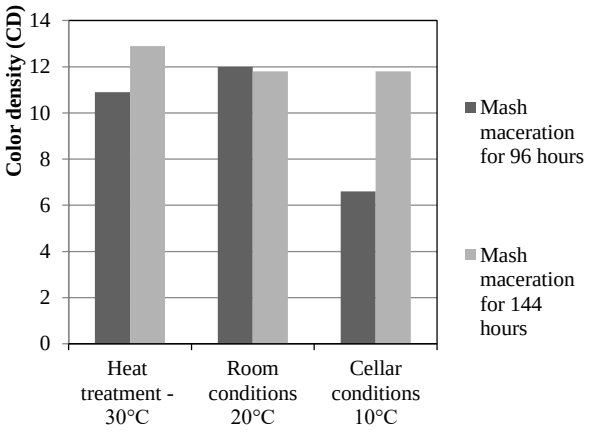

Fig. 4. Color density of samples, n=6, p<0.05

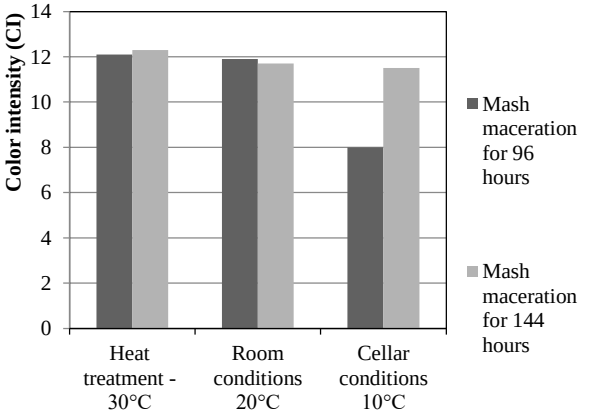

Fig. 5. Color intensity of wine samples, n=6, p<0.05

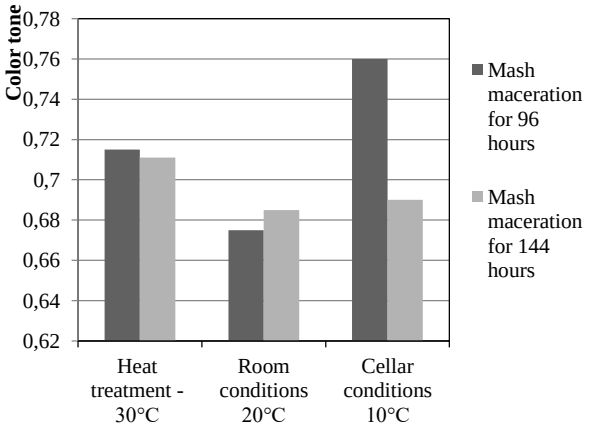

Fig. 6. Color tone variation in samples, n=6, p<0.05

The color density (CD) of the samples was 13 in samples kept in mash at low heat processing for 144 hours, and 6.8 in samples kept in mash under cellar conditions for 96 hours.

Color intensity (CI) varied from 8.0 to 12.3. The smallest value was observed in samples kept for 96 hours under cellar conditions, and the greatest values in the samples kept for 144 hours under heat treatment conditions.

The sample stored for 96 hours in the cellar manifested the highest value for color tone (CT) and the sample stored for 96 hours under room conditions showed the lowest value.

The proportion of different colors in the experimental samples was determined (in percentages) (Figure 7, Figure 8, Figure 9).

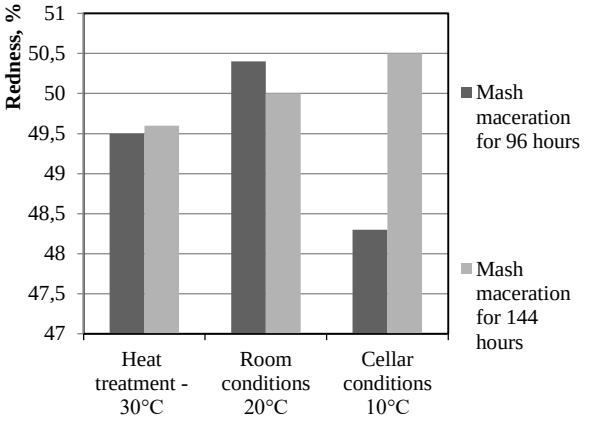

Fig. 7. Redness in the color of wine samples, %, n=6, p<0.05

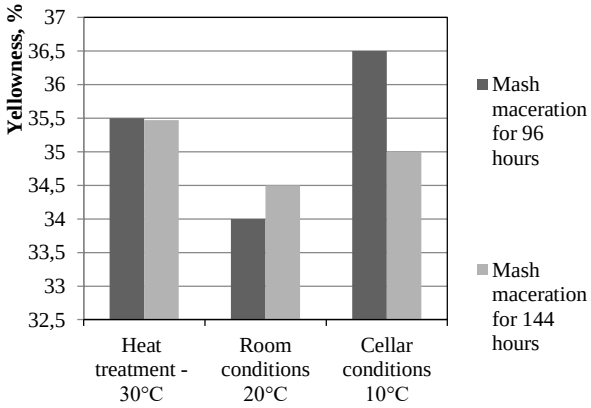

Fig. 8. Yellowness in the color, %, n=6, p<0.05

As seen, the redness rate was the highest, 50.49%, in the sample of wine prepared by keeping it in the cellar for 144 hours, and the lowest rate was 48.30%, when wine was prepared under the same conditions for 96 hours.

The percentage of yellowness ranged between 34.00 and 36.55%.

The lowest rate of yellowness was found in the wine sample made by keeping it under room conditions for 96 hours, and the highest rate of yellowness was found in the sample kept under cellar conditions for 96 hours.

The blueness percentage varied between 14.30 and 15.55% in the experimental samples.

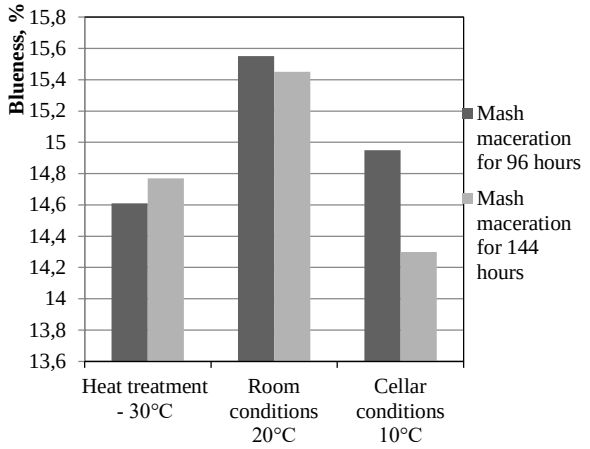

Fig. 9. Blueness in the color, %, n=6, p<0.05

As seen, the highest blueness -15.5% was observed in the sample of wine made by keeping it under room conditions for 96 hours, and the lowest blueness - 14.30% was observed in the sample kept under cellar conditions for 144 hours.

The study of absorbance values of wine samples at different wavelengths has also revealed interesting results (Figure 10, Figure 11, Figure 12, Figure 13).

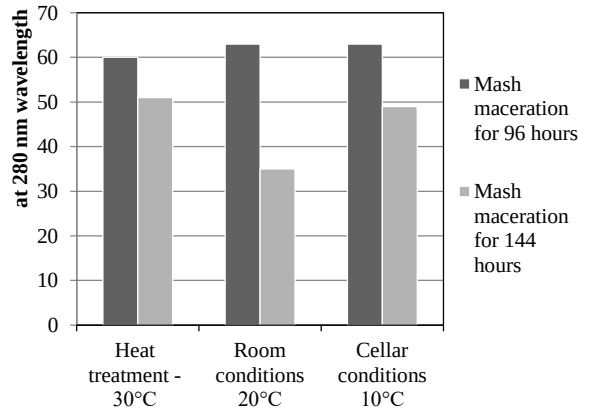

Fig. 10. Absorbance values of samples at 280 nm wavelength, n=6, p<0.05

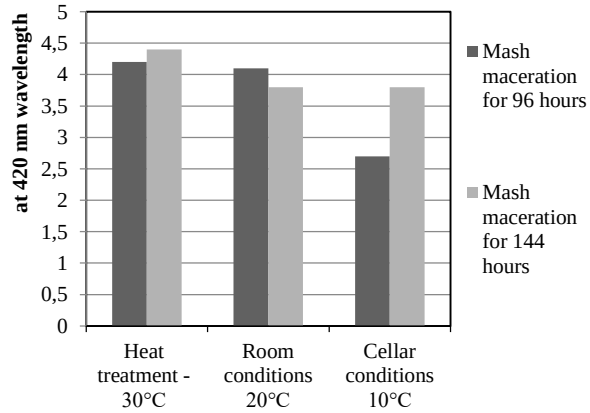

Fig. 11. Absorbance values of samples at 420 nm wavelength, n=6, p<0.05

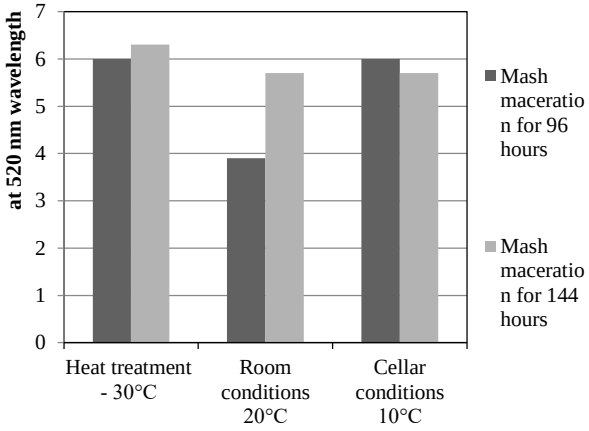

Fig. 12. Absorbance values of samples at 520 nm wavelength

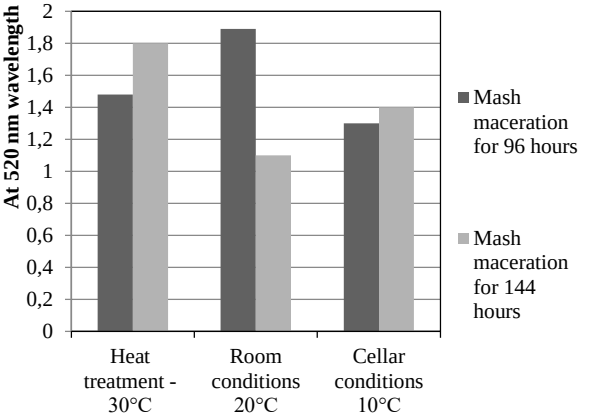

Fig. 13. Absorbance values at 620 nm wavelength, n=6, p<0.05

As can be seen from the absorbance values at the wavelength of 280 nm, the higher indicators were in the wine samples kept in the mash for 96 hours under room conditions and for 96 hours under cellar conditions; and the lowest indicator (35) was in the sample stored for 144 hours under room conditions.

The absorbance values at 420 nm wavelength ranged from 2.7 to 4.4 for the variants. The lowest value was observed in samples kept for 96 hours in the cellar, and the highest value was detected in samples stored for 144 hours at 30°C.

Absorbance values at 520 nm wavelength varied between 3.9 and 6.3 for the variants. The highest value was observed in samples subjected to hot processing for 144 hours, and the lowest value was observed in samples stored under room conditions for 96 hours.

The absorbance values at the wavelength of 620 nm were less than the previous ones and ranged between 1.1 and 1.9. The highest indicator was observed in the case of storage for 96 hours under room conditions. Interestingly, this indicator had the lowest value with the increase of storage time under those conditions. In other words, the absorbance value during 144 hours of storage was 1.1.

A chromatogram of organic acids was obtained and analyzed by the HPLC method in the Madrasa wine sample prepared by storing it in the mash (Figure 14).

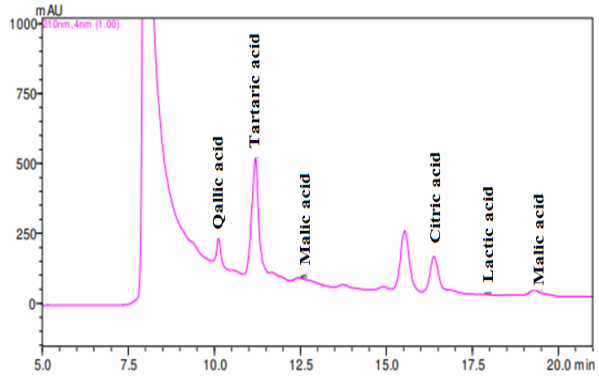

Fig. 14. Chromatogram of organic acids (HPLC) in the Madrasa wine sample obtained by mash maceration

As seen in the Figure, gallic acid, tartaric acid, malic acid, and citric acid were detected in the wine sample.

While the amount of tartaric acid that passes from the juice to the wine is more in the juice, after fermentation, it crystallizes under the influence of alcohol and cold treatment, turns into wine stone and separates from the wine by precipitation. The amount of tartaric acid in wine can vary depending on the year, grape variety, alcohol content, and other factors.

In addition to natural adhesives such as bentonite and gelatin, synthetic auxiliary materials are used to remove polyphenols. The most important of them is PVPP. Besides removing polyphenols, PVPP is also used for clarifying purposes. Its mechanism of action on polyphenols is based on adsorbing polyphenols by forming hydrogen bonds.

The results of our experiments on pink wine samples with the most commonly used diluting agents in winemaking are given below (Table 2).

Table 2. Effect of some diluting agents on the amount of phenolic compounds in pink wines, n=6, p<0.05

Content indicators	Initial g/m mg/dm³	Dilution compounds			
		PVPP 1 g/dm³	Albumin 0.2 g/dm³	Gelatin 0.2 g/dm³	Bentonite 1.5 g/dm³
Phenolic compounds	520	445	480	472	464
Anthocyanins	83	66	71	67	68

Changes in the amount of phenolic compounds were observed during the studies conducted to eliminate turbidity substances in colored (pink and red) wines. The amount of polyphenols, including anthocyanins, removed from wine was found to increase with enhancing the dose of bentonite from 0.5 g/dm³ to 1.0 g/dm³ and 1.5 g/dm³. However, increasing the bentonite dose further did not produce significant results. Therefore, we used a dose of 1.5 g/dm³ of bentonite in further studies.

As seen, treatment with PVPP at a dose of 1 g/dm³ resulted in a greater reduction in the amount of phenolic compounds and anthocyanins. If the total amount of phenolic compounds in the initial wine material was 520 mg/dm³ and that of anthocyanins was 83 mg/dm³, those indicators were 445 mg/dm³ and 66 mg/dm³, respectively, after the treatment with PVPP.

We can note that bentonite is the second adhesive according to the degree of effect on phenolic compounds. As seen, a dose of 1.5 g/dm³ of bentonite led to a decrease of 75 mg/dm³ in the total amount of phenolic compounds and 14 mg/dm³ in the amount of anthocyanins. In terms of effect power, gelatin (phenolic compounds 472 mg/dm^3 and anthocyanins 67 mg/dm^3) came in second, followed by albumin (phenolic compounds 480 mg/dm^3, anthocyanins 71 mg/dm^3).

Conclusion

1. For 96 and 144 hours, maceration was performed at low heat (30°C), room temperature (20°C), and cellar temperature (10°C). The highest amount of phenolic compounds was observed during the maceration of the mash for 144 hours under room conditions (20°C) and 96 hours under cellar conditions (10°C).
2. The highest indicator, 88 mg/dm^3, in total flavonoids was observed in samples obtained by maceration at 30°C temperature for 144 hours, and the lowest indicator, 62 mg/dm^3 under room conditions by maceration for 144 hours.
3. In both processing methods, 7 phenolic acids were detected. Compared to others, the amount of catechin, gallic acid, and epicatechin was more. The amount of catechin varied from 34.90 to 39.80, galacturonic acid from 12.60 to 16.00, and epicatechin from 5.01 to 6.91 mg/dm^3. The other four phenolic acids were found to be many times less abundant.
4. After fermentation, followed by technological operations of wine preparation such as malolactic acid fermentation, diluting, and filtering the amount of phenolic acids tended to decrease. Filtration made the drink more harmonious by creating softness in the taste and gave it an advantage compared to the initial samples.
5. The absorption of wine samples at different wavelengths, as well as color indicators (color density, intensity, and tone), was characterized by different values.
6. In addition to natural adhesives such as bentonite and gelatin, polyvinylpolypyrrolidone (PVPP) was used to remove polyphenols, and the best results were obtained in this case. The total amount of

phenolic compounds in the initial wine sample was 520 mg/dm³ and anthocyanins 83 mg/dm³, while those indicators were reduced by 14.4% and 20.5%, respectively, when treated with PVPP. We can note that bentonite is the second adhesive according to the degree of effect on phenolic compounds.

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