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THE EFFECT OF DIFFERENT STORAGE CONDITIONS ON ANTHOCYANIN PROFILE OF MERLOT RED WINES

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ABSTRACT
Grape variety and health, soil and climate, agricultural techniques used, winemaking processes, storage time and temperature are the most important agents in the phenolic content of red wine. In this study, the effects of storage temperature and time on the distribution of anthocyanin compounds, and the total monomeric anthocyanin (TMA) content of Merlot wines were investigated at the beginning of storage and the following quarterly periods. The amount and distribution of delphinidin 3-glucoside, pelargonin 3-glucoside, malvidin 3-glucoside, cyanidin 3-glucoside, and peonidin 3-glucoside anthocyanins in wine samples were defined quantitatively by modifying HPLC method. When the change in TMA content in wines was evaluated, it was determined that there was a decrease of 13.77% at 4-5 °C, 24.28% at 8-10 °C, 43.93% at 12-14 °C, and 66.29% at 18-20 °C compared to the values before storage at the last of 24 months of storage.
Keywords: Merlot wine, HPLC, bottle storage, storage temperature, anthocyanins

FARKLI DEPOLAMA KOŞULLARININ MERLOT ŞARAPLARINDA ANTOSİYANİN PROFİLİ ÜZERİNE ETKİSİ

ÖZ
Üzüm çeşidi ve sağlığı, şarap yapım süreçleri, iklim ve toprak, kullanılan tarım teknikleri, depolama sıcaklığı ve depolama süresi kırmızı şarabın fenolik içeriğindeki en önemli etkenlerdir. Bu çalışmada, depolama sıcaklığının ve süresinin, Merlot şarabının antosiyanin bileşiklerinin dağılımı ve toplam monomerik antosiyanin (TMA) içeriği üzerindeki etkileri, depolamanın başlangıcında ve sonraki üç aylık dönemlerde incelenmiştir. Şarap örneklerinde siyanidin 3-glikozit, peonidin 3-glikozit, pelargonin 3-glikozit, delphinidin 3-glikozit ve malvidin 3-glikozit antosiyaninlerinin miktar ve dağılımı modifiye edilmiş HPLC yöntemi ile kantitatif olarak tanımlanmıştır. Şaraplarda 24 aylık depolama sonunda TMA içeriğindeki değişim değerlendirildiğinde depolama öncesi değerlere kıyasla 4-5 °C'de %13.77, 8-10 °C'de %24.28, 12-14 °C'de %43.93 ve 18-20 °C'de %66.29 oranında azalma olduğu belirlenmiştir.
Anahtar kelimeler: Merlot şarabı, HPLC, şişe depolama, depolama sıcaklığı, antosiyanin

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INTRODUCTION

Grape is the oldest perennial plant in the world, belonging to the genus *Vitis* of the Vitaceae family. Merlot is the main black grape variety that is included in the compound of the wines produced in the Saint-Emilion and Pomerol regions, which are important sub-regions of the Bordeaux wine region and have become the world variety. Its single wines and blends with Cabernet Sauvignon take attention with their quality (Anlı, 2011).

Wine is an alcoholic beverage produced by fermenting fresh grape must. The quality of red wine largely depends on the phenolic compositions that come from the grape and are formed during winemaking (Cheynier et al., 2006). Storage time and conditions also have a significant impact on wine quality. Temperature plays an important role at many stages throughout the development of wine. Wine can be exposed to high temperatures during fermentation, ripening, bottling, transportation, and storage. If the temperature is inadequately controlled at critical control points, high and/or fluctuating temperature accelerates the development process and creates an important risk for the sensory, physical, and chemical properties of the wine and, consequently, the shelf life (Ifie et al., 2018). Studies have shown that wines stored at low temperatures (e.g. <10 °C) have a longer shelf life due to a low risk of spoilage, and when optimal conditions are maintained, fresh-fruity aromas are preserved in a young wine, but it will take longer to develop (Robinson et al., 2010).

The main anthocyanin pigments found in grapes and wines are malvidin, cyanidin, peonidin, delphinidin and petunidin (Han et al., 2008; Ivanova et al., 2011; Ivanova et al., 2015; Han et al., 2017). The most abundant and stable anthocyanin in *Vitis vinifera* grapes is Malvidin 3-glucoside (He et al., 2012; Blanco-Vega et. al., 2014; Lukic et al., 2019; Zhao et al., 2022). Malvidin 3-glucoside forms the basis of color in black grapes and the wines produced from them (Riberaeu-Gayon et al., 2000; Castillo-Munoz et al., 2010; Zhao et al., 2022).

The reduction in anthocyanin concentration is the most common effect of high temperature on non-volatile compounds in red wines. This process allows the unstable color component in the wine to be converted into a more stable form. It is known that monomeric anthocyanins are involved in the formation of polymeric pigments in the reactions occurring during the storage process, especially through the condensation reaction with tannin species (He et al., 2012a; Waterhouse and Zhu 2020; Zhao et al., 2022). It has been noted that this process rate increases at high temperatures (Gomez-Plaza et al., 2000; Scrimgeour et al., 2015; Ifie et al., 2018).

Studies have shown that monomeric anthocyanins in red wines decrease over time even at typical cellar storage temperature during storage, and in wine, polymeric pigments increase with the corresponding color change from purple to red-orange. In this context, it is seen that accelerate to exposure to heat the aging process. It is stated that temperature is a more effective factor in the fate and concentration of anthocyanins than storage time, alcohol, pH and SO_2 (Somers, 1971; Fulcrand et al., 2006; Maury et al., 2010).

Anthocyanins are among the most important antioxidants with health-promoting properties found in black grapes and red wine produced from these grapes. For this reason, the beneficial effects of red wine consumption on human health have attracted attention in recent years (Lingua et al., 2018). Studies have shown that the consumption of foods rich in polyphenols reduces the risk of oxidative stress-related diseases (Nardini and Garaguso, 2018). It also appears to be responsible for maintaining healthy cardiovascular and nervous systems, and the health benefits associated with anthocyanin compounds in wines are widely studied (Paixao et al., 2007; Walzem, 2008; Basli et al., 2012; Schrieks et al., 2013; Biasi et al., 2014; Tuberoso et al., 2017).

This study was carried out to evaluate the changes in anthocyanin compounds of red wines produced from the Merlot grape variety during storage in wine coolers. The effect of storage temperature on the anthocyanin compound distribution of wines was investigated by applying different storage temperatures to the wines.

MATERIALS AND METHODS

Materials

In this study, wine production was executed in Dimes facilities from Merlot grapes harvested from the vineyards of Dimes Vasfi Diren Farm in Tokat in 2015. The wine was produced under controlled fermentation conditions (20-23 °C) with the use of *Saccharomyces cerevisiae* as a starter culture yeast (Zymaflore RX60, Laffort; Zymaflore F83, Laffort; Lalvin ICV D 254, Lallemand) and filled into 750 ml dark glass bottles. Wine bottles were stored in light-proof Vestel VLP-4000 brand wine coolers at 85% relative humidity and at 4 different (4-5 °C, 8-10 °C, 12-14 °C, and 18-20 °C) temperatures. Changes in the anthocyanins composition of the wines were analyzed at the beginning of storage and one in three months.

Methods

pH

Wine pH was measured directly with a glass-electrode pH meter (Hanna HI 2221) (Ough and Amerine, 1988).

Total Acidity

For total acidity, a 10 mL wine sample was mixed with 20 mL distilled water and the resultant mixture was titrated with 0.1 N NaOH solution until a pH of 8.2. Results were expressed in g/L tartaric acid equivalent (Ough and Amerine, 1988; Anonymous, 1990).

Density

Density was determined using a pycnometer at 20 °C (Ough and Amerine, 1988).

Alcohol

The amount of alcohol was calculated from the density value determined by a pycnometer in the alcoholic liquid obtained as a result of distillation, with the help of special charts, first the weight (g/L) and then the volume (% v/v) (Ough and Amerine, 1988).

Individual Anthocyanin Compounds by Analytical HPLC

The amount and distribution of pelargonin 3-glucoside, cyanidin 3-glucoside, delphinidin 3-glucoside, malvidin 3-glucoside, and peonidin 3-glucoside anthocyanins in wine samples were determined quantitatively by modifying HPLC method (Anonymous, 2003). Anthocyanin standards, formic acid, and acetonitrile were obtained from Sigma-Aldrich. Stock solutions for all standards were prepared at 1 mg/mL and the standards were dissolved in 0.1% hydrochloric acid. Standard calibration solutions at 5 different concentrations were prepared for the calibration curve. Standards were stored in the dark at -18 °C. Quantitative analysis of anthocyanin compounds was determined by considering the chromatograms at wavelengths where their absorbance was maximum. 100 mL of wine samples used in the analysis were taken and filtered through a 0.45 μm (Millex-HV) membrane filter. 50 μL of the filtrates were taken and injected into the HPLC device (Table 1).

Table 1. HPLC Conditions

Equipment	Shimadzu
Deaerator	DGU-20 A5 Prominence (gradient valve)
Pump	1C-20 AT Prominence
Controller	CMB-20A Prominence
Detector	SPD-M10AVP DAD
Automatic Sample Injection Unit	SIL-10AXL
Column Furnace	CTO-10A
Column	Intersil ODS-3 Reverse Phase (5 μm-25x4.6 mm)
Solvent A	Water/Formic Acid/Acetonitrile (87:10:3)
Solvent B	Water/Formic Acid/Acetonitrile (40:10:50)

Table 2 shows the maximum absorbance, retention time and linearity (R^2) of anthocyanin compounds. Quantitative analyses of anthocyanin compounds were based on the chromatogram of the maximum absorbance wavelength.

Table 2. Retention times, maximum absorbance, and linearity (R^2) of the anthocyanin compounds

Antosiyanin Standards	Retention times (min)	Maximum absorbance values (nm)	R^2 values
Pelargonin 3-glucoside	3.749	518	0.9987
Delphinidin 3-glucoside	4.040	518	0.9978
Malvidin 3-glucoside	4.100	518	0.9996
Cyanidin 3-glucoside	4.576	518	0.9983
Peonidin 3-glucoside	4.657	518	0.9992

Total Monomeric Anthocyanin (TMA)

Developed by Guisti and Wrolstad (2003), by pH-differential method TMA contents of wine samples were determined. According to this method, it was incubated in (pH 4.5) 0.4 M CH_3COONa buffer and (pH 1.0) 0.025 M KCl buffer for 15 minutes at room temperature, the spectrophotometric absorptions of the extracts were measured at 700 nm and 520 nm, and the absorbance (A) values and TMA amount were calculated according to the equation 1 and 2 respectively.

$$A = (A\,\lambda 520 - A\,\lambda 700) pH\ 1.0 - (A\,\lambda 520 - A\,\lambda 700) pH\ 4.5 \quad (1)$$

$$TMA\ (mg/kg) = (A\ x\ MA\ x\ SF\ x\ 1000) / (\varepsilon\ x\ 2) \quad (2)$$

A: Absorbance
Molecular weight (MA) of Malvidin 3-glucoside: 493.5 gmol/l
Dilution factor (SF): 5
ε (molar absorption coefficient): 28,000

Statistical Analysis

All experimental analysis results were performed in triplicate and the results were given as mean value ± standard deviation. For the spectrophotometric and chromatographic analysis results obtained for the samples under different storage temperature conditions and storage time stages repeated one-way nested ANOVA analysis of variance and Kruskal Wallis comparison analysis were performed using the Minitab 17 program. In the experiment, there were 4 levels of temperature factor 4-5 °C, 8-10 °C, 12-14 °C, and 18-20 °C. Duncan's test ($P < 0.05$) was used to determine the different groups.

RESULTS AND DISCUSSION

Chemical Analysis

In Merlot wine samples, the pH value was 3.68 at the beginning of the storage, the total acidity amount was 4.42 g/L, the density value was 0.9916 g/mL, and the alcohol content was 16.34% (v/v). The lowest and highest values for pH value in the samples of Merlot wines were measured as 3.59 and 3.81, respectively. After 24 months of storage, pH values increased to 2.85% at 4-5 °C, 3.13% at 8-10 °C, 3.13% at 12-14 °C, and 3.40% at 18-20 °C according to pre-storage values. The total acidity values in Merlot wines varied between 4.09 and 4.95. When the change in the total acidity level in wines is evaluated, it is seen that there is an increase to 5.20% at 12-14 °C, a decrease to 2.43% at 4-5 °C and 4.13% at 18-20 °C compared to the values before storage at the end of 24 months of storage. The density values in wines varied between 0.9913 and 0.9938. There was no statistically significant difference in the density levels of wines at the end of 24 months of storage compared to pre-storage values for all temperatures ($P \geq 0.05$). At the end of storage, the change in alcohol level in wines was examined and determined that it decreased at all temperatures. The lowest and highest values for alcohol value in wines were measured as 13.36 and 16.85, respectively. After 24 months of storage, the decrease in alcohol values compared to pre-storage values is 14.75% at 4-5 °C, 13.95% at 8-10 °C, 15.12% at 12-14 °C and 14.75% at 18-20 °C rate was found. These increases and decreases were found as statistically significant ($P < 0.05$). pH, total acidity and alcohol values of rose sparkling wines were investigated in the study carried out at 5 °C and 30 °C storage temperatures. According to the study, there was no differences were revealed in pH (2.97), total acidity (7.50 g tartaric acid/L) and alcohol content (12.5% v/v) after 3, 6 and 9 months of storage (Benucci, 2020).

Individual Anthocyanin Compounds by Analytical HPLC

Pelargonin 3-glucoside

The amount of pelargonin 3-glucoside in the wine samples was 65.20 mg/L at the beginning of the storage process (Table 3). When the change in pelargonin 3-glucoside values in wines is evaluated, it is seen that there is an increase in all temperatures at the end of storage.

Table 3. Pelargonin 3-glucoside analysis results

Storage period (months)	Pelargonin 3-glucoside (mg/L)			
0	65.20±1.156			
	4-5 °C	8-10 °C	12-14 °C	18-20 °C
3	197.12±1.796^{Eb}	198.46±0.492^{Eb}	224.30±1.252^{Gc}	172.12±0.755^{Ea}
6	322.22±1.436^{Hd}	303.20±2.669^{Fc}	256.04±0.749^{Hb}	211.92±1.346^{Fa}
9	157.28±2.716^{Dc}	183.32±1.102^{Dd}	123.01±0.542^{Db}	117.06±1.449^{Da}
12	75.16±1.230^{Ca}	124.42±0.766^{Cc}	102.74±0.439^{Cb}	105.42±0.446^{Cb}
15	53.05±0.400^{Ba}	53.71±0.251^{Ba}	57.69±0.655^{Bb}	52.60±0.617^{Ba}
18	24.64±0.836^{Ac}	12.77±0.236^{Aa}	15.56±0.626^{Aa}	28.33±1.285^{Ab}
21	206.34±1.623^{Fa}	211.70±0.556^{Eb}	217.85±2.016^{Fc}	ND
24	233.31±1.344^{Gd}	211.83±0.535^{Ec}	166.21±0.604^{Eb}	116.22±1.204^{Da}

Small letters in the same row indicate the difference between temperatures, and uppercase letters in the same column indicate the difference between months ($P < 0.05$).
ND: Not detected

After 24 months of storage, the increase in pelargonin 3-glucoside values compared to the values 0. month of storage; it was determined that 257.84% at 4-5 °C, 224.89% at 8-10 °C, 154.92% at 12-14 °C, and 78.25% at 18-20 °C. This increase was also found as statistically significant ($P < 0.05$).

Delphinidin 3-glucoside

The amount of delphinidin 3-glucoside in the wine samples could not be measured at the beginning of the storage process. The highest value for the amount of delphinidin 3-glucoside in wine samples was determined as 226.55 mg/L at the 9th month of storage period and 4-5 °C storage temperature conditions and the lowest amount of delphinidin 3-glucoside was determined as 4.30 mg/L at the 24th month storage period and 18-20 °C storage temperature conditions (Table 4).

In a study to examine the effect of storage temperature and time on delphinidin 3-glucoside, Monastrell wines were stored at 15-20 °C for 12 months. It was stated that the delphinidin-3-glucoside value, which was 20.93 mg/L before storage, decreased to 31.49% at the end of 3 months, 38.80% at the end of 6 months, 45.48% at the end of 9 months and 50.26% at the end of 12 months (Gomez-Plaza et al., 2000).

Malvidin 3-glucoside

In the samples of Merlot wines, the amount of malvidin 3-glucoside was determined as 2.29 mg/L at the beginning of the storage process (Table 5). When the change in the 3-glucoside value of malvidin in wines was evaluated, it was determined that there was an increase of 22.27% in Merlot wines at 18-20 °C compared to the values before storage at the end of 24 months of storage. This increase was also found as statistically significant ($P < 0.05$).

Table 4. Delphinidin 3-glucoside analysis results

Storage period (months)	Delphinidin 3-glucoside (mg/L)			
0	ND			
	4-5 °C	8-10 °C	12-14 °C	18-20 °C
3	5.98±0.960^{Ab}	5.76±0.155^{Ab}	4.64±0.635^{Aa}	4.63±0.321^{Aa}
6	16.47±0.515^{Cd}	12.79±0.294^{Cc}	10.67±0.723^{Cb}	9.02±0.555^{Ca}
9	226.55±0.606^{Fd}	125.12±0.554^{Eb}	136.94±0.156^{Fc}	78.26±0.300^{Ea}
12	64.96±0.674^{Ed}	30.81±1.255^{Da}	45.22±0.525^{Ec}	41.35±1.300^{Db}
15	9.65±0.117^{Bd}	7.88±0.441^{Bc}	6.63±0.185^{Bb}	5.86±0.111^{Ba}
18	5.74±0.162^{Aa}	6.43±0.184^{Bb}	5.05±0.059^{Aa}	42.36±1.212^{Dc}
21	5.73±0.217^{Aa}	5.75±0.126^{Aa}	5.09±0.125^{Aa}	ND
24	34.73±1.211^{Dd}	28.81±0.223^{Dc}	21.55±1.074^{Db}	4.30±0.129^{Aa}

Small letters in the same row indicate the difference between temperatures, and uppercase letters in the same column indicate the difference between months ($P < 0.05$).
ND: Not detected

Table 5. Malvidin 3-glucoside analysis results

Storage period (months)	Malvidin 3-glucoside (mg/L)			
0	2.75±0.205			
	4-5 °C	8-10 °C	12-14 °C	18-20 °C
3	2.89±0.345^{Aa}	3.16±0.096^{Ba}	3.32±0.221^{Ba}	3.05±0.062^{Aa}
6	22.41±0.536^{Fc}	7.59±0.366^{Db}	7.13±0.026^{Db}	4.39±0.017^{Ba}
9	4.15±0.455^{Cb}	5.49±0.146^{Cc}	3.26±0.106^{Ba}	4.04±0.469^{Bb}
12	11.59±0.420^{Ec}	8.61±0.375^{Eb}	5.26±0.347^{Ca}	4.84±0.673^{Ba}
15	4.89±0.335^{Ba}	4.61±0.025^{Ca}	ND	ND
18	8.15±0.158^{Db}	14.69±0.276^{Fc}	28.02±0.885^{Ed}	3.11±0.110^{Aa}
21	2.50±0.065^{Aa}	3.75±0.200^{Bb}	3.64±0.023^{Bb}	ND
24	2.40±0.025^{Aa}	2.35±0.025^{Aa}	2.26±0.007^{Aa}	2.80±0.006^{Ab}

Small letters in the same row indicate the difference between temperatures, and uppercase letters in the same column indicate the difference between months ($P < 0.05$).

In a study conducted to examine the effect of storage temperature and duration on malvidin 3-glucoside, Mencia wines were stored at 15 °C for 12 months, and it was stated that a significant decrease in malvidin 3-glucoside value occurred at the end of storage. At the beginning of storage, the value of malvidin 3-glucoside was determined as 126 mg/L; it was stated that this value decreased to 79 mg/L at the end of the 3rd month and 33 mg/L at the end of the 12th month (Garcia-Falcon et al., 2007).

Cyanidin 3-glucoside

In the samples of Merlot wines, the amount of cyanidin 3-glucoside was determined as 6.86 mg/L on 0. months of the storage process (Table 6). When the change in the cyanidin 3-glucoside level in wines is evaluated, it is seen that there is an increase in all temperatures at the end of storage.

Table 6. Cyanidin 3-glucoside analysis results

Storage period (months)	Cyanidin 3-glucoside (mg/L)			
0	6.86 ± 0.013			
	4-5 °C	8-10 °C	12-14 °C	18-20 °C
3	8.74 ± 0.255^{Ab}	6.83 ± 0.165^{Aa}	9.92 ± 0.765^{Ac}	6.66 ± 0.187^{Aa}
6	55.09 ± 0.668^{Dd}	25.30 ± 0.712^{Cb}	37.40 ± 0.239^{Dc}	22.68 ± 0.075^{Da}
9	66.30 ± 0.612^{Ed}	51.41 ± 0.420^{Dc}	32.19 ± 0.698^{Cb}	19.93 ± 0.485^{Ca}
12	67.41 ± 1.227^{Ed}	60.61 ± 0.674^{Ec}	14.31 ± 0.020^{Ba}	25.56 ± 0.081^{Eb}
15	162.65 ± 0.724^{Fd}	141.39 ± 1.527^{Fc}	107.44 ± 1.526^{Eb}	65.38 ± 0.765^{Fa}
18	19.50 ± 0.469^{Cb}	7.40 ± 0.046^{Aa}	36.01 ± 0.404^{Dd}	23.11 ± 0.561^{Dc}
21	11.31 ± 0.237^{Bc}	10.10 ± 0.097^{Bb}	8.00 ± 0.445^{Aa}	ND
24	10.54 ± 0.540^{Bb}	10.02 ± 0.065^{Ba}	9.49 ± 0.175^{Aa}	15.58 ± 0.059^{Bc}

Small letters in the same row indicate the difference between temperatures, and uppercase letters in the same column indicate the difference between months ($P < 0.05$).
ND: Not detected

The increase in cyanidin 3-glucoside values, after 24 months of storage compared to the values before storage, was 53.64% at 4-5 °C, 46.06% at 8-10 °C, 38.34% at 12-14 °C, and 18-20 °C 127.11 % in rate was found. This increase was also found as statistically significant ($P < 0.05$).

In a study to examine the effect of storage temperature and time on cyanidin 3-glucoside, Monastrell wines were stored at 15-20 °C for 12 months. The cyanidin 3-glucoside value which was 10.48 mg/L before storage, was determined as 9.77 mg/L at the end of the 3rd month, 9.25 mg/L at the end of the 6th month, 8.66 mg/L at the end of the 9th month and 7.40 mg/L at the end of the 12th month. It was reported that the cyanidin 3-glucoside value decreased to 6.77% at the end of 3 months, to 11.74% at the end of 6 months, to 17.37% at the end of 9 months, and to 29.39% at the end of 12 months (Gomez-Plaza et al., 2002).

Peonidin 3- glucoside

In the samples of Merlot wines, the amount of peonidin 3-glucoside was determined as 1.75 mg/L at the beginning of the storage process (Table 7). When the change in the peonidin 3-glucoside values in wines is evaluated, it is seen that there is an increase in all temperatures at the end of storage.

Table 7. Peonidin 3-glucoside analysis results

Storage period (months)	Peonidin 3-glucoside (mg/L)			
0	1.75 ± 0.300			
	4-5 °C	8-10 °C	12-14 °C	18-20 °C
3	28.78 ± 0.597^{Ec}	20.58 ± 0.540^{Ca}	28.19 ± 1.213^{Fc}	22.85 ± 1.931^{Eb}
6	15.57 ± 1.197^{Ca}	22.40 ± 1.303^{Dd}	20.54 ± 0.474^{Dc}	17.43 ± 1.058^{Db}
9	19.55 ± 1.333^{Dd}	12.45 ± 1.400^{Ab}	16.49 ± 1.464^{Cc}	4.82 ± 0.695^{Aa}
12	38.38 ± 1.434^{Gd}	31.54 ± 1.440^{Ec}	8.46 ± 1.432^{Aa}	15.05 ± 0.170^{Cb}
15	79.50 ± 0.481^{Hc}	80.56 ± 1.525^{Gc}	71.33 ± 1.614^{Gb}	45.22 ± 0.593^{Fa}
18	6.72 ± 0.975^{Aa}	17.72 ± 1.219^{Bd}	10.96 ± 0.101^{Bb}	15.50 ± 0.442^{Cc}
21	12.78 ± 0.933^{Bb}	10.82 ± 0.851^{Aa}	10.75 ± 0.205^{Ba}	ND
24	37.46 ± 0.491^{Fd}	33.42 ± 0.430^{Fc}	26.42 ± 0.881^{Eb}	11.26 ± 0.248^{Ba}

Small letters in the same row indicate the difference between temperatures, and uppercase letters in the same column indicate the difference between months ($P < 0.05$).
ND: Not detected

It was determined that the increase in the peonidin 3-glucoside values after 24 months of storage was 2040.57% at 4-5 °C, 1809.71% at 8-10 °C, 1409.71% at 12-14 °C, and 543.43% at 18-20 °C in Merlot wines compared to the values before storage. This increase was also found as statistically significant ($P < 0.05$).

In a study in which Monastrell wines were stored at 20 °C for 7 months, peonidin-3-glucoside value before storage was determined as 22.22 mg/L. It was reported that at the end of the 3rd month it decreased by 83.03% to 3.77 mg/L and at the end of the 6th month it decreased by 86.05% to 3.10 mg/L (Zafrilla et al., 2003).

In the study conducted by Marquez et al. (2014), Merlot, Shiraz and Tempranillo wines were stored for 12 months at 18-20 °C and the values of peonidin-3-glycosides were measured at the beginning of storage and one in three months. After 12 months of storage, it was reported that the levels of peonidin-3-glycosides decreased to 91.26% in Merlot wine, 95.15% in Shiraz wine and 82.02% in Tempranillo wine.

Total Monomeric Anthocyanin

The effect of different storage temperature conditions and storage time stages on total monomeric anthocyanin in Merlot wines is given in Table 8. In the samples of Merlot wines, the amount of TMA was determined as 223.40 mg/L at the beginning of the storage process. The highest value for the total amount of monomeric anthocyanin in the samples of Merlot wines was determined as 257.15 mg/L under the 6-month storage period and 4-5 °C storage temperature conditions. The lowest total monomeric anthocyanin amount was determined as 75.30 mg/L under the conditions of a storage period of 24 months and a storage temperature of 18-20 °C.

Table 8. TMA analysis results

Storage period (months)	Total Monomeric Anthocyanin (mg/L malvidin 3-glucoside)			
0	223.40±1.869			
	4-5 °C	8-10 °C	12-14 °C	18-20 °C
3	226.17±4.050^{Dd}	219.56±0.062^{Dc}	216.08±0.374^{Hb}	175.32±0.312^{Ga}
6	257.15±0.000^{Fd}	220.67±0.000^{Dc}	204.36±3.739^{Gb}	165.59±0.125^{Fa}
9	246.35±0.436^{Ed}	226.44±0.436^{Ec}	178.45±0.125^{Fb}	144.61±0.374^{Da}
12	245.25±0.436^{Ec}	194.14±0.125^{Cb}	158.67±0.062^{Ea}	156.33±1.246^{Ea}
15	222.08±0.125^{Cc}	228.90±0.062^{Fd}	152.19±0.125^{Db}	104.82±0.312^{Ca}
18	199.78±0.000^{Bd}	170.08±0.125^{Ac}	140.60±0.312^{Cb}	89.89±0.000^{Ba}
21	225.42±0.125^{Dd}	172.24±0.187^{Bc}	137.92±0.000^{Bb}	90.68±0.249^{Ba}
24	192.64±0.249^{Ad}	169.16±0.062^{Ac}	125.27±0.187^{Ab}	75.30±0.436^{Aa}

Small letters in the same row indicate the difference between temperatures, and uppercase letters in the same column indicate the difference between months ($P < 0.05$).

It was determined that the increase in the TMA values after 24 months of storage was 13.77% at 4-5 °C, 24.28% at 8-10 °C, 43.93% at 12-14 °C, and 66.29% at 18-20 °C in Merlot wines compared to the values before storage. This increase was also found as statistically significant ($P < 0.05$).

The findings obtained in the study are also compatible with the literature data. In a study in which Monastrell wines were stored at 20 °C for 7 months, the total anthocyanin value before storage was determined as 386.0 mg/L. It was reported that at the end of the 3rd month it decreased by 76% to 91.8 mg/L and at the end of the 6th month it decreased by 85% to 57.7 mg/L (Zafrilla et al., 2003). Hermosin-Gutierrez et al. (2005) reported that Shiraz, Cencibel and Cabernet Sauvignon wines were stored at 16-18 °C and lost 60%, 62% and 68%, respectively, in total anthocyanin amounts at the end of 9 months. In another study examining the effect of storage temperature and time on total anthocyanins, it was stated that a significant decrease in the amount of total anthocyanin occurred in Violeta wine depending on the temperature increase. After 2 months of storage, 40% reduction was observed in wines stored at 15 °C. A reduction of 50% was observed in wines stored at 25 °C, 88% in wines stored at 35 °C, and 96% in wines stored at 50 °C (Lago-Vanzela et al., 2014).

The result obtained from the studies is a significant decrease in the level of free anthocyanins due to the formation of polymeric derivatives. It is stated that storage temperature plays an important role in the degradation of wine pigments and polymerization reactions and is the primary environmental factor affecting the changes in the color properties of red wine. Studies have shown that storage length is another important factor affecting wine color, since many of the changes that occur during the storage of wines are time-dependent (Zafrilla et al., 2003; Hermosin-Gutierrez et al., 2005; Lago-Vanzela et al., 2014; Marquez et al., 2014; Ifie et al., 2018).

CONCLUSION

The findings obtained in the study showed that the storage temperature and time were effective on the total monomeric anthocyanin content and the distribution of anthocyanin compounds in the wines produced from the Merlot variety. It was determined that the total amount of anthocyanins in the wines decreased gradually as the storage temperature increased. This indicates that storage temperature is an important factor for preserving color compounds in wine and preventing early development effects.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest between them.

AUTHOR CONTRIBUTION

The authors declare that they have contributed equally to the article.

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