

**Physico-chemical characterization and polyphenolic composition of red wines produced from autochthonous grapes varieties (*Vitis vinifera*) in Turkey**

**Abstract**

**Aims:** Local varieties of “Hönüsü” and “Horozkarası” red grapes have been evaluated for the production of red wine and characterized<sup>33</sup> for their chemical and sensory characteristics.

**Study Design:** This research was initiated by the Food microbiology researcher and Applications Unit of Fermentation Laboratory.

**Place and Duration of Study:** Laboratory of Fermentation of the Food Engineering Department in Gaziantep University, October 2019 to May 2021.

**Methodology:** All wines were produced by a standard procedure of vinification. Three types of red wines were produced from combinations of red grapes. Ten phenolics were quantitatively detected in the red wines during processing. Brix, alcohol, pH and free SO<sub>2</sub> contents were also detected.

**Results:** The results showed that the type of technology affects significantly ( $p < 0.05$ ) the level of phenolic compounds formed during processing. Horozkarası” red grape contributed to the highest amount of phenolic compounds in red wines. Gaziantep wine provides valuable information about the production of red wine from Gaziantep red grapes.

**Conclusion:** The phenolic compounds of red wines were significantly ( $p < 0.05$ ) higher than that of musts. Many of the remarkable features of the phenolic profiles and Brix of grape varieties could help us to characterize Gaziantep wines. The mixture of must from “Hönüsü” and “Horozkarası” red grapes with 7:3 ratio contributes suitable sugar and phenolic compounds for red wine. The results from this study provide valuable information about the red wine produced from the ancient grape varieties of the Southeast region.

**Keywords:** Grape, Phenolic compound, Red wine, *Vitis vinifera*

## 1. INTRODUCTION

Grapes (*Vitis vinifera* L.) cultivation and wine production took place 7500 years ago in the northwest of Turkey, northern Iraq, Azerbaijan, and Georgia [1]. Gaziantep (a city in Turkey) is located in the Northeast of Turkey. Important ancient red grape varieties grown in Gaziantep are *V. sativa* subsp. *silvestris* types “Hönüsü” and “Horozkarası” [2]. Phenolic compounds contribute color, mouthfeel, bitterness, astringency and palatability to red wines, moreover, they also exert many favorable effects on human health, such as the inhibition of atherosclerosis, coronary heart disease and various cancer types [3]. The amount of phenolic compounds in wine depends on the grape variety, vineyard location, cultivation system, harvesting time, duration of the fermentation, vinification techniques, temperature, etc. The influence of vinification conditions and processing techniques on wine production is still poorly understood due to the variety of wines produced depending on regional processing conditions [1]. No research has been published and anthocyanin characterization of wines produced from Gaziantep grapes. In every wine-producing country, the selection of suitable grape variety selecting suitable grape varieties with the potential to produce unique, flavorful wines is a continuous and routine in Turkey's southern Anatolia region (including Gaziantep, Şanlıurfa, Kahramanmaraş, and Kilis), where, despite high grape productivity, suitable wine-producing varieties are in short supply. In this research, local varieties of “Hönüsü” and “Horozkarası” red grapes have been evaluated for the production of red wine and characterized for their chemical and sensory characteristics.

## 2. MATERIALS AND METHODS

Two red grapes (vernacular “Hönüsü” and “Horozkarası”) from *V. vinifera* subsp. *vinifera* L. cultivated in Gaziantep were harvested (45 kg “Hönüsü” and 30 kg “Horozkarası”) from

vineyards at the appropriate maturity, stored in separate plastic crates and transported to the winery in the Food Engineering Department (Gaziantep University, Gaziantep, Turkey). The appropriate maturity status of the grape is determined by using the quantitative parameters (red skin color, softened berries, Brix, pH and titratable acidity). The Brix values of mature Hönüsü and Horozkarası mature grapes were 21 and 17, respectively, and pH values were 3.45 and 3.30 respectively. A commercial *Saccharomyces cerevisiae* ProFerm-W and chemicals were obtained from Vinomarket (İzmir, Turkey). HPLC-grade chemicals and phenolic compounds were supplied by Sigma-Aldrich (Interlab, Adana, Turkey).

## 2.1 Red Wine Production

Three types of red wines were produced from the “Hönüsü” grape (HRG) and the “Horozkarası” grape (HKRG) according to the process scheme given in Fig. 1.

**Extraction of juice and preparation of must.** The red grapes were separated from the stems, garbage and rotten, and crushed by hand without damaging the seeds. Three musts from crushed grapes (14.25 kg for each lot) were prepared together with juice, skin, and seeds in duplicate; must-1: 100% HRG, must-2: 85% HRG + 15% HKRG and must-3: 70% HRG + 30% HKRG. The Brix value of lots was adjusted by adding sucrose into musts. The final Brix values of musts-1, 2 and 3 were 22.78, 22.57 and 22.50 respectively.

Three different ratios of the two varieties were chosen due to variations in their characteristics. The skin of HRG has a color between red and brown, is thin, hardly crushed in the mouth, and is slightly juicy, has a sweet taste, seeds are easy to separate and grapes contain about 21% sugar. The HKRG is large, slightly hazy and dark purplish-black, has bitterness and strong astringency tastes, is medium juicy and contains about 17% sugar.

**Alcoholic fermentation.** The vinifications were carried out in a small-scale winery. Each mixture of musts (juice, skin, and seeds) was filled up to 75 % of clean 19 L plastic demijohns under aseptic conditions. The yeast nutrient (0.2 g/kg) and **potassium metabisulfite (PMB)** (25

mg/kg) were dissolved in their must juice separately and added to each must. *S. cerevisiae* (0.22 g/kg, dry yeast) is rehydrated in must juice, allowed for 10 min at room temperature (25°C), and added into each must. The airlock was fitted on each demijohn. They allowed fermentation in a dark room at 25°C for 5 days. The content of all plastic demijohns was mixed every day twice. The fermentation was monitored by measuring the Brix value in the fermented juice.

**Post-fermentation treatment (resting).** After fermentation, mixtures were filtered through disinfected cheesecloth (600 µm) to remove skin, seeds, mare, and coagulant. Subsequently, each wine was filled up to 90% of 10 L glass carboys containing the oak chips (2.5 g/L) and PMB (15 mg/L). The airlock was fitted on each carboy. They were placed in a dark room at 20°C. Oak chips were blooded into juices every day twice to release flavor. The rested wines for 7 days were filtered to remove oak chips and coagulants through disinfected cheesecloth.

**Maturation and aging.** Each wine was filled into 10 L disinfected glass carboys containing PMB (15 mg/L), and carboys were filled to reduce air. The airlock was fitted on each carboy. The wines were matured for 45 days at 20°C in the darkroom. The matured wines were separated from the sediments by filtration through disinfected cheesecloth. Wines were filled into dark green colored bottles (75 mL) and the bottles were capped with cork using a cork stopper closing machine (Atlantis Cam Ambalaj Ltd. Şti., İzmir, Turkey). The bottles were aged by storing them in the horizontal position for 3 months in the darkroom at 20°C.

## 2.2. Analysis

About 150 mL of the samples was removed in duplicate during fermentation (after 1, 3 and 5 days), after resting (7 days), after maturation (45 days) and after aging (3 months). Yeast counts and pH were detected as indicated by Erkmen [4]. The alcohol analysis was performed by the alcoholometry method [5]. Samples were also analyzed for water-soluble dry matter (Brix) and free SO<sub>2</sub> [5]. Sensory analysis was evaluated by a group of 12 expert panelists (6

females, 6 males, average age 35 in the sensory room. Panel tests were carried out in a separate special test panel room. Panel cabinets are separated by a glass partition that ensures that they were not in contact with anyone. The taste panel room has well-diffused daylight lamps to minimize optical effects and misconceptions. During the color analysis session, daylight was used in the cabins.

The water used in the analysis was obtained from a Milli-Q water purification system (Millipore; Bedford, MA, USA). All solvents used were previously filtered through a 0.45 µm membrane (Millipore) and degassed before use. For all standards, stock solutions were prepared by dissolving the phenolic compounds at 4 different concentrations with the methanol-water solution (50:50 v/v). The phenolic standards and phenolic compounds from samples were determined by a modified HPLC method and the results were given in mg/L [6]. Two solvent gradient elutions were used. Solvent A is the acetic acid-water solution (2:98 v/v) and solvent B is the methanol-water solution (50:50 v/v). The standard curve was fitted by linear least-squares regression ( $r^2 > 0.98$ ). Quantification of the anthocyanin compounds (mg/L) was done concerning peak areas measured at 520 nm [6].

Chromatographic analysis was performed using a Shimadzu LC-20AB (Shimadzu Corporation, Kyoto, Japan) high-performance liquid chromatography (HPLC) equipped with a vacuum degasser (DGU-20A5), quaternary-pump LC-10AT, UV detector (SPD-20A), SIL-autosampler (20A HT) and VP column furnace (CTO-10AS). The LCsolution (v.1.25; 2002-2009 Shimadzu Corporation) was used to control the gradient settings, UV and data acquisition. The separation was performed using a C18 analytical column of 4.6 mm x 250 mm, 5 µm particle size (GL Sciences, Kyoto, Japan). A C18 guard column of 4.6 mm x 12.5 mm, 5 µm particle size (GL Sciences, Kyoto, Japan) was used to prevent contamination of the analytic column from any non-soluble residues coming from the samples. Peak areas were determined at 280 and 320 nm wavelengths for all phenolic compounds.

### **2.3. Statistical analysis**

All the experimental trials were conducted in triplicate sets. The wine production was repeated three times. At each repeat, parallel red wines were prepared. The results of the analyzes were given as the mean  $\pm$  standard deviation values of the three repeats. The wines were compared depending on process time and wine types by analysis of variance with ANOVA test using SPSS v.22 (IBM SPSS Corporation, Chicago, IL, USA) with a 95 % confidence level.

## **3. RESULTS AND DISCUSSION**

At the beginning of fermentation, the initial number of *S. cerevisiae* was 5.40, 5.38 and 5.36 log cfu/mL in musts-1, 2 and 3 respectively. The number of yeasts in musts was not significantly ( $p>0.05$ ) different from each other. Yeast counts of red wines-1, 2 and 3 were significantly ( $p<0.05$ ) increased to 7.68, 7.52 and 7.55 log cfu/mL, respectively, during 3 days of fermentation. *S. cerevisiae* was also increased by 2.14-2.24 log cfu/mL. *S. cerevisiae* was significantly ( $p<0.05$ ) decreased during settling, maturation and aging periods. After aging, the final number of *S. cerevisiae* were 2.38, 2.34 and 2.30 log cfu/mL for red wines-1, 2 and 3 respectively. Malolactic fermentation was performed slowly starting from fermentation to maturation. Since decompositions of malic acid were reduced approximately by 0.20 mg/L from about 0.95 mg/L (data not given).

### **3.1. pH and Brix**

The changes in pH during the processing steps of red wines were given in Table 1. The pH values of musts-1, 2 and 3 were 3.52, 3.47 and 3.42 respectively. On the first day of fermentation, the pH values of red wines-1, 2 and 3 were slightly increased to 3.63, 3.59 and 3.62 respectively. The pH values of the red wines were slightly decreased in the order to 3.47, 3.57 and 3.56 at the end of fermentation. After aging, the pH values of red wines-1, 2 and 3 were slightly increased to 3.64, 3.61 and 3.58 respectively. During fermentation, organic

acids, together with their salts, remain stable in the wine and maintain the pH of the wine in the range of 2.9-4.0, ensuring that the fermentation is carried out healthy. The low pH values were also essential for color, microbiological, chemical and oxidative stabilities [1]. The pH of the wine varies according to the type of acid contents on the ratio of tartaric acid to malic acid, the number of potassium ions and other wine components. If the tartaric acid content of the wine is high and the amount of potassium ions is low, the pH of the wine will be low [7]. Yeasts usually grow in an acidic medium. They are negatively affected at very low pH (<2.8) and high pH (>4.0) [7].

The changes in Brix values during the processing steps of red wines were given in Table 1. Brix values were significantly ( $p < 0.05$ ) reduced during 5 days of fermentation. There was a slight decrease in the Brix values of the red wines during the settling, maturation and aging periods. After aging, the Brix values of red wines in the order were 6.11, 6.40 and 6.51. The amount of sugar in the must is important for yeast growth and metabolism, the amount of alcohol production and the taste of red wines. The Brix values of red wines were changed between 5.0 and 7.66 [6].

### **3.2. Alcohol, Free SO<sub>2</sub> and Sensory Score**

The alcohol gives power, warmth and sweetness taste, and plays an important role in the durability of wines. Wines with low alcohol levels are more sensitive to the effects of wild yeasts and bacterial spoilages. Phenolic compounds gain more and better soluble properties with the help of alcohol. At the end of fermentation, the alcohol values of red wines-1, 2 and 3 were 13.56, 13.46 and 13.26%, respectively, (Table 1). At the end of aging, the alcohol values of red wines-1, 2 and 3 were slightly decreased ( $p < 0.05$ ) to 12.66, 12.59 and 12.58 %. The reasons for the slight decrease of alcohol during processing may be due to the oxidation of alcohol with the addition of oxygen (O<sub>2</sub>) after each processing step, and the evaporation of alcohol during filtration between steps. Alcohol may also be converted to glycerin, acetic

acid, and acetaldehyde by microorganisms during processing [3]. Alcohol content in Öküzgözü red wines ranged between 10.65 and 13.92% [5]. Alcohol contents indicated between 12.5 and 13.5% for Cabernet Sauvignon red wines and 11.5 and 12.5% for Merlot red wines [8]. According to the Turkish Food Codex [9], the amount of alcohol in wine by volume should be between 9 and 15%.

PMB was used because of the risk of wild yeast spoilages and sulfur would prevent their growth during the red wine processes [3]. After the fermentation, the free SO<sub>2</sub> contents of the red wines-1, 2 and 3 were 20.56, 19.27 and 19.58 mg/L, respectively, (Table 1). After the aging, the free SO<sub>2</sub> contents of red wines-1, 2 and 3 were 22.0, 23.0 and 19.67 mg/L, respectively. Anli et al. [10] reported free SO<sub>2</sub> in red wines from 20 to 30 mg/L. SO<sub>2</sub> has a positive role in the prevention of red wine diseases and defects during processes, in the development of sensory properties of the wine, in the dissolution of phenolic compounds from cells of the grape tissue, in the protection of wine against enzymatic browning and the formation of wine stone in the presence of tartaric acid. According to TFC ([11], the amount of free SO<sub>2</sub> should not exceed 30 mg/L in wines.

The data on the sensory score of red wine prepared from 70%HRG+30% HKRG had a high standard with a score of 7.73 (Table 1). The sensory scores of the red wines prepared from 100% HRG and 85% HRG+15 HKRG were 5.95 and 6.70 respectively. The data also revealed a significant ( $p<0.005$ ) variation in sensory scores among three types of red wines.

### **3.3. Phenolic Compounds**

**Flavan-3-ols.** At the end of fermentation, (+)-catechin and procyanidin B2 contents of the red wines-1, 2 and 3 were significantly ( $p<0.05$ ) increased to 63.08, 64.00 and 70.26, and 274.42, 247.43 and 278.53 mg/L, respectively, (Table 2). Amounts of procyanidin B2 were 5 times higher than the (+)-catechin after fermentation. (+)-Catechin contents of the red wines-1, 2 and 3 were decreased ( $p>0.05$ ) to 62.12, 65.18 and 71.71 mg/L, respectively, after aging.

After aging, procyanidin B2 contents of the red wines-1, 2 and 3 were significantly ( $p < 0.05$ ) increased to 298.30, 273.27 and 396.50 mg/L respectively. (+)-Catechin reacts easily with  $O_2$  in the air to form condensed tannins. As the chain length of procyanidin B2 increases, the color of the wine changes from yellow to brown. Hydrolyzes of procyanidin is associated with the separation of the central flavin unit from the oligomer by carbonation and subsequent oxidation to the colored compounds. During fermentation, tannins pass from the solid parts of the grape to wine. Hydrolyzable tannins from oaks had important effects on the taste and bouquets of the wine [12]. Gomez-Plaza et al. [13] reported that the amounts of (+)-catechin in Monastrell red wines ranged from 8.4 to 9.8 mg/L and procyanidin B2 ranged from 2.5 to 3.6 mg/L. Concentrations of (+)-catechin contents for Cabernet Sauvignon red wines ranged from 8.1 to 62.4 mg/L and for Merlot red wines ranged from 14.9 to 29.5 mg/L while procyanidin B2 contents for Cabernet Sauvignon red wines ranged from 1.7 to 54.7 mg/L and for Merlot red wines ranged from 2.7 to 25.1 mg/L [14]. Kelebek [15] found that the amounts of (+)-catechin ranged between 22.53 and 36.8 mg/L in Boğazkere red wines. The findings obtained in our study for (+)-catechin and procyanidin B2 are greater than the results indicated in the literatures. The red wines obtained in this research would be darker than the red wines indicated in literatures. Kocabey [16] reported the amounts of procyanidin B2 in Karaoğlan red wines between 492.75 and 713.48 mg/L. The reason for the difference in these phenolic contents would be due to the grape varieties and processes used in red wine production. Flavan-3-ols are mainly responsible for the senses of wines such as astringency, bitterness, and color, and play an important role in the stabilization of wine color during aging [16]. The color and density of red wines were increased during fermentation with the increasing amount of (+)-catechin and procyanidin B2 [13]. The results showed that (+)-catechin and procyanidin B2 were the major phenolic constituents in the red wines produced from HRG and HKRG.

**Flavonols.** Flavonols contribute bitterness and white to yellow color, and stabilize red wine color by reinforcing the pigmentation of anthocyanin [17]. At the end of fermentation, the myricetin content of red wines-1, 2 and 3 significantly ( $p<0.05$ ) increased to 4.80, 5.43 and 5.62 mg/L, respectively, (Table 2). During the settling, maturation and aging of the red wines, myricetin contents were significantly ( $p<0.05$ ) decreased. After the aging, the myricetin contents of the red wines-1, 2 and 3 were decreased to 1.12, 1.26 and 1.68 mg/L respectively. Concentrations of myricetin in Cabernet Sauvignon and Merlot red wines were 2.2 and 5.0 mg/L, respectively, [18].

At the end of fermentation, the quercetin content of red wines-1, 2 and 3 significantly ( $p<0.05$ ) increased to 3.40, 7.57 and 8.52 mg/L, respectively, (Table 2). After aging, the quercetin contents of the red wines-1, 2 and 3 were decreased to 2.86, 3.89 and 4.73 mg/L respectively. Kelebek et al. [19] found that quercetin in Öküzgözü red wines ranged from 0.76 to 2.01 mg/L.

At the end of fermentation, the rutin content of red wines-1, 2 and 3 significantly ( $p<0.05$ ) increased to 1.60, 2.00 and 2.21 mg/L, respectively, (Table 2). After the aging, the rutin contents of the red wines-1, 2 and 3 were decreased ( $p<0.05$ ) to 1.38, 1.58 and 1.61 mg/L respectively. Rutin is capable of chelating metal ions (such as iron) which causes the formation of oxygen radicals with their high antioxidant activity. The amount of rutin was 1.25 mg/L in Karaoğlan red wine at the end of 5 days of fermentation [15]. Flavonol contents of wines depend on the intensity of sunlight where the grape is cultured, the thickness of the grape skin, the type of grape and the technological processes applied in wine production. While wine-3 had the highest myricetin, quercetin and rutin contents at the end of aging, wine-1 had the lowest contents. Flavonols give bitterness to grapes and wines; we can infer that red wine-3 has a more bitter taste than wines-1 and 2. The change in flavonol contents of red wines can be explained by the accumulation of flavonols in the grape skins. Red wines

obtained from the Gaziantep region contained a higher amount of flavonols than most of the results indicated in the literature. The sunny and dry climate in the Gaziantep region is in good condition for the synthesis and accumulation of flavonols in grapes.

**Phenolic acids.** At the end of fermentation, gallic acid contents of the red wines-1, 2 and 3 significantly ( $p<0.05$ ) increased to 32.77, 23.94 and 40.54 mg/L, respectively, (Table 3). Gallic acid contents significantly ( $p<0.05$ ) decreased to 11.49, 15.51 and 24.78 mg/L in wines-1, 2 and 3, respectively, after aging. Gallic acid was released from the grape's skin during fermentation and decreased during the aging of the red wines due to the high concentration of alcohol. Gallic acid gives an astringent aroma to wines. The amounts of gallic acid in Öküzgözü wines ranged from 9.03 to 19.40 mg/L [15]. Gallic acid amounts ranged from 23.46 to 48.58 mg/L of Cabernet Sauvignon red wines and Spanish Fondillón wines [20]. In some of the literature, hydroxycinnamic acids (such as p-coumaric acid and chlorogenic acid) are reported as the dominant phenolic acids in wines [19], but in this study, hydroxybenzoic acid (such as gallic acid) was the dominant phenolic acid in red wines. Red wine-3 from 70% HRG +30% HKRG has a higher amount of gallic acid than red wines-1 and 2 from 100% HRG and 85% HRG + 15% HKRG respectively.

At the end of fermentation, the chlorogenic acid contents of the red wines-1, 2 and 3 significantly ( $p<0.05$ ) increased to 9.71, 10.90 and 11.20 mg/L, respectively, (Table 3). Chlorogenic acid contents were significantly ( $p<0.05$ ) reduced to 7.22, 7.24 and 7.32 mg/L in red wines-1, 2 and 3, respectively, after aging. There are no significant ( $p>0.05$ ) differences in chlorogenic acid values among red wines at the end of storage. It is responsible for the sour taste in wine, easily oxidizes in the presence of polyphenol oxidase and can also be converted to brown-colored compounds. It is mainly stored in grape pulp and is easily passed to wine during crushing and fermentation. Kocabey [16] indicated the amounts of chlorogenic acid from 1.46 to 1.67 mg/L in Karaođlan red wine.

At the end of fermentation, *p*-coumaric acid contents were significantly ( $p < 0.05$ ) increased to 11.27, 11.37 and 12.16 mg/L in the red wines-1, 2 and 3, respectively, (Table 1). After the aging, the *p*-coumaric acid contents of the red wines-1, 2 and 3 were significantly ( $p < 0.05$ ) decreased to 7.35, 7.15 and 7.33 respectively. Kelebek [15] stated that *p*-coumaric acid increased in the red wine during fermentation and the amount was 0.97 mg/L. Together with anthocyanin, phenolic acids contribute important characteristic qualities to red wines such as astringency and bitterness [21].

**Resveratrol and Tyrosol.** At the end of fermentation, resveratrol contents of the red wines-1, 2 and 3 were significantly ( $p < 0.05$ ) increased to 2.03, 2.14 and 2.26 mg/L, respectively, (Table 1). Resveratrol contents were slightly decreased ( $p > 0.05$ ) after maturation and aging. There are significant ( $p < 0.05$ ) differences in resveratrol content among the three red wines after aging. Gurbuz et al. [22] determined the resveratrol in Öküzgözü grape red wines as 4.40 mg/L. At the end of fermentation, the tyrosol content of red wines-1, 2 and 3 significantly ( $p < 0.05$ ) increased to 13.75, 19.74 and 23.83 mg/L, respectively, (Table 3). Tyrosol contents of red wines were significantly ( $p < 0.00$ ) decreased during resting, maturation and aging periods. Tyrosol contents of red wines-1, 2 and 3 after aging were 6.57, 11.05 and 11.49 mg/L respectively. Gris et al. [23] indicated the amounts of tyrosol in red wines from 23 to 47 mg/L. Tyrosol is produced by *S. cerevisiae* by oxidative decarboxylation of tyrosine amino acid during alcohol fermentation and tyrosol synthesis correlates with the amount of glucose. The presence of tyrosol in the wines depends on the type of yeast used in wine production and the glucose amount [24]. Resveratrol is found in the seed and skin of grapes. Its amount increases during red wine processes because grape skin and seeds contact the juice during the whole fermentation process. For these reasons, the amount of resveratrol and tyrosol was increased in red wines during the processes.

**Anthocyanins.** The quantitative contents of identified anthocyanins in must and aged red wines are different and vary according to the grape variety used in red wine production (Table 4). The must-3 obtained from HRG (70%)+ HKRG (30%) has the highest amount of anthocyanins than the other musts. Malvidine-3-glucoside dominates among the musts and aged red wines. A higher amount of it is observed in the red wine-3 (46.50 mg/L), while the least amount is in the wine-1 (28.62 mg/L). The amount of malvidin-3,5-diglucoside was (39.13 mg/L) in the aged wine-3. The lowest content of cyanidin-3-glucoside (3.96 mg/l) was observed in aged wine-1. The quantitative content of the anthocyanins identified in wines made from 30% of HKRG is almost twice as high as compared to wine-1 and wine-2 made from HRG and 15% HKRG respectively. The results of the research showed that the quantity of each anthocyanin increases in the wines with the increasing amount of HRG.

Fermentation is the most important factor in the contribution of phenolic compounds to red wines. An intensive decrease of phenolic compounds was noticed in the red wines after maturation and aging. Some of the phenolic compounds are lost due to precipitation, while others undergo different polymerization reactions during the aging of the red wines that diminish astringency and increase suppleness in the red wine. Anthocyanins contribute diverse colors such as red, purple and blue [21]. In this research, phenolic compounds in red wines were increased during fermentation. Many remarkable features were observed, such as higher phenolic content in the red wine-3, while a lower content in the red wine type-1. Therefore, the bitterness, astringency and color intensity of wine-3 is expected to be higher than others. However, the other two wines also contain a higher amount of phenolic compounds compared with most of the literature results. The red wine-3 was produced from must containing a higher amount of HKRG. HKRG contributed a higher amount of phenolic compounds to red wine than HRG. Hence, the grape variety has a significant effect on the phenolic content of wines during fermentation. HRG contributed more sugar which was

essential in sufficient alcohol production in red wine. The phenolic compositions of the wines were determined largely by the phenolic composition of the grape.

Procyanidin B<sub>2</sub> was the first abundant phenolic in red wines, while (+)-catechin was the second abundant phenolic compound. Published results for red wines indicated that the main individual phenolic compounds in red wines were (+)-catechin and (-)-epicatechin [25]. Differences might be related to the 'terroir' of the zone, as previous research showed light, and water deficits, fewer temperature differences between daytime and nighttime, and infertile soil. Procyanidin B<sub>2</sub> and (+)-catechin is thought to be effective in the forming astringency and bitterness of Gaziantep red wines.

Red wine-3 had a higher amount of flavonols while red wine-1 had a lower amount. Flavonols generally cause bitterness; a more bitter taste was expected from wine-3. Wine-3 had the highest gallic acid (24.78 mg/L) after aging, while wine-1 had the lowest gallic acid (11.49 mg/L). Phenolic acids play a primary role in defining the sensorial characteristics of wines. They are largely responsible for the astringency and bitterness of wines [19]. The lower resveratrol and tyrosol contents were found in the red wine-1, while the red wine-3 had a higher level.

Polymeric pigments resulting from the reactions between anthocyanins and other phenols are responsible for the red color and antioxidant capacity of wines, while non-anthocyanin phenols, such as flavan-3-ols and flavonols, are responsible for astringency, bitterness and color, and health properties of wines [19]. The amounts of phenolic compounds are shown diversity in the wines produced from the grapes grown in different regions of Turkey as well as other countries. The reasons for these are related to grape variety, cultural and plant protection practices, ecological conditions, yeast strain used in wine production, resting in the oak and aging. The results showed that HRG and HKRG are suitable for high-quality red

wine production. Since these grapes contribute enough sugar for alcohol production, a higher amount of phenolic characteristics to wines provide better acidity and Brix.

Oak can also derive aroma and flavor to red wines. A certain amount of air exposure (oxidation) is necessary for the maturation of red wine. In this study, the red wines were exposed to air during filtration and transferred to a new step. Moderate uptake of O<sub>2</sub> during aging can accelerate and/or trigger specific reactions influencing sensory properties. Chemical reactions between wine and wood phenolics enhance the decrease of wine astringency and stabilize the color. Gradual exposure of wine to O<sub>2</sub> during the process can impart a softer mouthfeel to the wine and a more reddish, rather than purple, hue color [26].

#### **4. CONCLUSIONS**

**HKRG contributes higher amounts of phenolics than HRG.** Gaziantep wine had high phenolic content and this is associated with high antioxidant capacity and dark color. The phenolic compounds of red wines were significantly ( $p < 0.05$ ) higher than musts. Many of the remarkable features of the phenolic profiles and Brix of grape varieties could help us to characterize Gaziantep wines. The mixture of must from HRG and HKRG with 7:3 ratio contributes suitable sugar and phenolic compounds for red wine. The results from this study provide valuable information about the red wine produced from the ancient wine grape variety of the Southeast region. Typical Gaziantep HRG and HKRG red wines were analyzed for the first time to determine phenolic compounds and anthocyanins. These results could be of great interest to nutritionists and dietitians for the assessment of dietary phenolic compounds intake.

#### **REFERENCES**

1. Ozgur A, Cangi R, Uzun T. Effects of brined vine leaf picking on cane quality and bud fertility at Narince grape cultivar. 2021;10(1):1-10.

2. Erkmen A. The Transcription and Analyze of Aintab Judicial Record Number 156 (H.1312-1314; P. 167-250, 1-93, 1-8). MSc Thesis. Gaziantep University, Social Science, Institute, History Department Gaziantep, Turkey, 2005.
3. Erkmen O, Bozoglu TF. Fermented vegetables and fruits”, In: Erkmen O, Bozoglu TF, editors. Food Microbiology Principles into Practice. Microorganisms in Food Preservation and Processing. Vol. 2, Chichester: John Wiley and Sons, Ltd., 2016, pp. 313-348.
4. Erkmen O. Yeasts and molds counting techniques, In: Erkmen O. editors. Microbiological Analysis of Foods and Food Processing Environment., London: Elsevier Inc., pp.43-52. <https://doi.org/10.1016/C2021-0-01219-0>
5. OIV. Compendium of International Methods of Wine and Must Analysis. International Organization of Vine and Wine, Paris, 2020.
6. Andrade RHS, Nascimento LS, Pereira GE, Hallwass F, Paim APS. Anthocyanic composition of Brazilian red wines and use of HPLC-UV-Vis associated to chemometrics to distinguish wines from different regions. *Microchem J.* 2013;110:256-262. <https://doi.org/10.1016/j.microc.2013.04.003>
7. Zhang D, Zhang Y, Lin K, Wang B, Shi X, Cheng w. Comparison of sugars, organic acids and aroma components of five table grapes in Xinjiang. *Earth Env. Sci.* 2021; 792:012029.
8. Kondrashov A, Sevcik R, Benakova H, Kostirova M, Stipek S. The key role of grape variety for antioxidant capacity of red wines. *European e-J Clin Nutr Metabol.* 2009;4:41-46. <https://doi.org/10.1016/j.eclnm.2008.10.004>
9. TFC. Wine Communique. Ministry of Agriculture and Rural Affairs. Turkish Food Codex (TFC), Notification No: 2008/67, Ankara: Ministry of Agriculture and Rural Affairs, 2009.

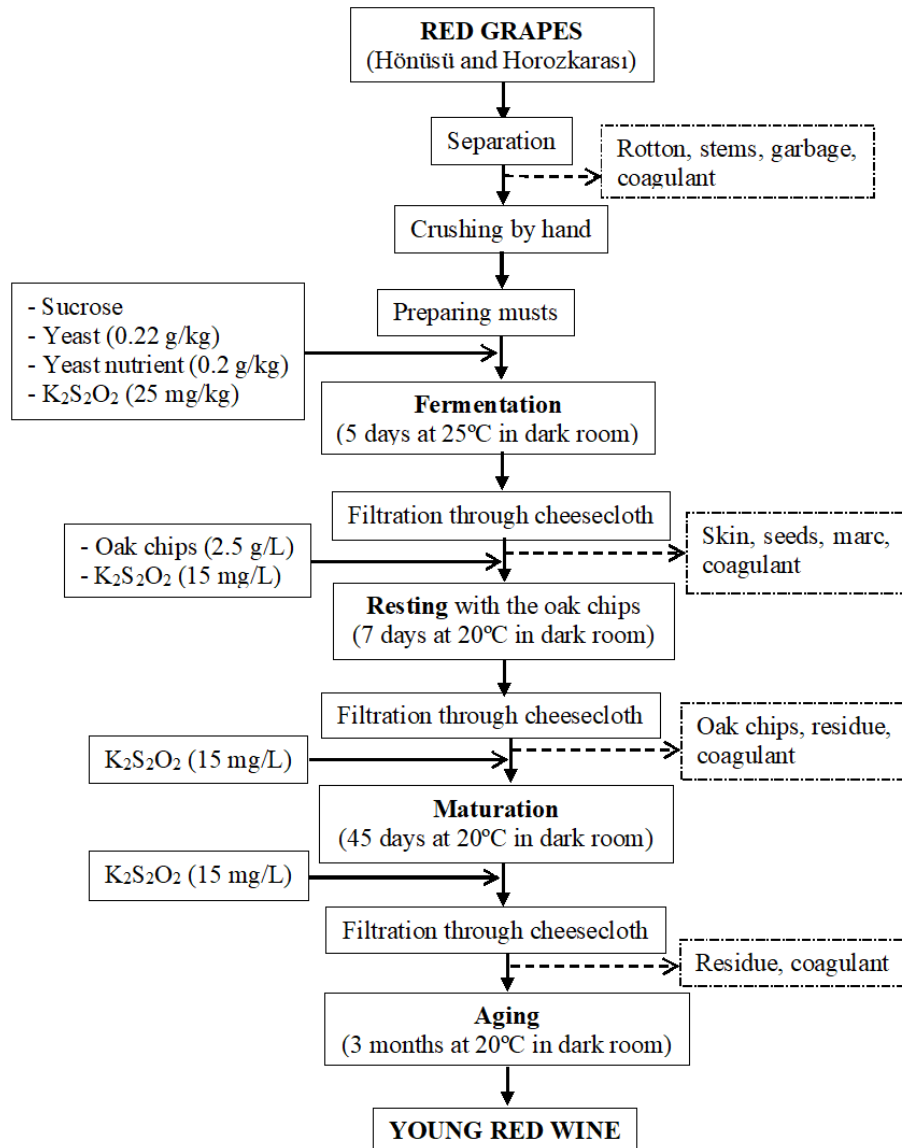
10. Anli R, Vural N, Yılmaz S, Vural I. The determination of biogenic amines in Turkish red wines. *J Food Comp Analysis*. 2004;17(1):53-62. [https://doi.org/10.1016/S0889-1575\(03\)00104-2](https://doi.org/10.1016/S0889-1575(03)00104-2)
11. TFC. Communique on food additives other than colorants and sweeteners. Turkish Food Codex (TFC), Notification No: 2008/22, Ankara: Ministry of Agriculture and Rural Affairs, 2008.
12. Li L, Li Z, Wei Z, Yu W, Cui Y. Effect of tannin addition on chromatic characteristics, sensory qualities and antioxidant activities of red wines. *RSC Adv*. 2020;10:7108-7117.
13. Gomez-Plaza E, Gil-Munoz R, Lopez-Roca JM, Martinez-Cutillas A, Fernandez-Fernandez JI. Maintenance of colour composition of a red wine during storage. Influence of prefermentative practices, maceration time and storage. *LWT - Food Sci Technol*. 2002;5:46-53. <https://doi.org/10.1006/fstl.2001.0809>
14. Jiang B, Zhang Z-W. Comparison on phenolic compounds and antioxidant properties of Cabernet Sauvignon and Merlot wines from four wine grape-growing regions in China. *Molecules*. 2012;17:8804-8821. <https://doi.org/10.3390/molecules17088804>
15. Kelebek H, Canbas A, Selli S, Saucier C, Jourdes M, Glories Y. Influence of different maceration times on the anthocyanin composition of wines made from *Vitis vinifera* L. cvs. Boğazkere and Öküzgözü. *J. Food Eng*. 2006;77(4):1012-1017.
16. Kocabey N. Determination of Phenol Compounds and Aroma Substances of Wines Obtained from Karaoğlan and Aşık White Grapes grown in Arapgir. MSc Thesis, İnönü University, Institute of Science and Technology, Malatya, Turkey, 2013.
17. Markoski MM, Garavaglia J, Oliveira A, Olivaes J, Marcadenti A. Molecular properties of red wine compounds and cardiometabolic benefits. *Nutr Metaboli Ins*. 2016;2(9):51-57. <https://doi.org/10.4137/NMIS32909>

18. Meng JF, Ning PF, Xu TF, Zhang ZW. Effect of rain-shelter cultivation of *Vitis vinifera* cv. Cabernet Gernischt on the phenolic profile of berry skins and the incidence of grape diseases. *Molecules*. 2013;18(11):381-397. <https://doi.org/10.3390/molecules18010381>
19. Kelebek H, Canbas A, Jourdes M, Teissedre PL. Characterization of colored and colorless phenolic compounds in Öküzgözü wines from Denizli and Elazığ regions using HPLC-DAD-MS. *Ind Crops Prod*. 2010;31:499-508. <https://doi.org/10.1016/j.indcrop.2010.01.012>
20. Issa-Issa H, Guclu G, Noguera-Artiaga L, López-Lluch D, Poveda R, Selli S, Carbonell-Barrachina AA. Aroma-active compounds, sensory profile, and phenolic composition of Fondillón. *Food Chem*. 2020;316:e-126353.
21. Mendoza L, Matsuhiro B, Aguirre MJ, Isaacs M, Sotés G, Cotoras M, Melo R. Characterization of phenolic acids profile from Chilean red wines by high-performance liquid chromatography. *J Chilean Chem Soc*. 2011;56(2):688-691. <http://dx.doi.org/10.4067/S0717-97072011000200014>
22. Gurbuz O, Gocmen D, Dagdelen F, Gursoy M, Aydin S, Sahin I, Buyukuysal L, Usta M. Determination of flavan-3-ols and trans-resveratrol in grapes and wine using HPLC with fluorescence detection. *Food Chem*. 2007;100(5):18-525. <https://doi.org/10.1016/j.foodchem.2005.10.008>
23. Gris EF, Mattivi F, Ferreira EA, Vrhovsek U, Filho DW, Pedrosa CR, Bordignon-Luiz MT. Stilbenes and tyrosol compounds in the assessment of antioxidant and hypolipidemic activity of *Vitis vinifera* L. red wines from Southern Brazil. *J Agr Food Chem*. 2011(59):795-796. <https://doi.org/10.1021/jf2008056>
24. Tuck KL, Freeman MP, Hayball PJ, Stretch GL, Stupans I. The in vivo fate of hydroxytyrosol and tyrosol, antioxidant phenolic constituents of olive oil, after

intravenous and oral dosing of labeled compounds to rats. *J Nutr.* 2001;131:1993-1996.  
<https://doi.org/10.1093/jn/131.7.1993>

25. Garaguso I, Nardini M. Polyphenols content, phenolics profile and antioxidant activity of organic red wines produced without sulfur dioxide/sulfites addition in comparison to conventional red wines. *Food Chem.* 2015;179:336-342.  
<https://doi.org/10.1016/j.foodchem.2015.01.144>
26. McRae JM, Day MP, Bindon KA, Kassara S, Schmid SA, Schulkin A, Kolouchova R, Smith PA. Effect of early oxygen exposure on red wine colour and tannins. *Tetrahedron.* 2015;71:3131-3137. <https://doi.org/10.1016/j.tet.2014.08.059>

UNDER PEER REVIEW



**Fig. 1. Young red wine production scheme**

**Table 1. pH, Brix, alcohol and free SO<sub>2</sub> values of red wines\***

Time (day)	pH			Brix (%)			Alcohol (%)			Free SO <sub>2</sub> (mg/L)		
	1	2	3	1	2	3	1	2	3	1	2	3
Must	3.52±0.02 <sup>aA</sup>	3.47±0.02 <sup>aB</sup>	3.42±0.03 <sup>aC</sup>	22.78±0.21 <sup>aA</sup>	22.73±0.39 <sup>aA</sup>	22.57±0.45 <sup>aB</sup>	-	-	-	-	-	-
1	3.63±0.02 <sup>bA</sup>	3.59±0.01 <sup>cB</sup>	3.62±0.01 <sup>cC</sup>	17.57±0.47 <sup>bA</sup>	15.53±0.48 <sup>bB</sup>	15.58±0.11 <sup>bB</sup>	0.01±0.01 <sup>aA</sup>	0.25±0.32 <sup>aA</sup>	0.05±0.03 <sup>aA</sup>	-	-	-
3	3.43±0.01 <sup>cA</sup>	3.45±0.02 <sup>aA</sup>	3.51±0.02 <sup>bA</sup>	8.79±0.18 <sup>cA</sup>	7.70±0.03 <sup>cB</sup>	7.78±0.10 <sup>cB</sup>	9.38±0.30 <sup>bA</sup>	10.24±0.09 <sup>bB</sup>	10.52±0.14 <sup>bB</sup>	-	-	-
5	3.47±0.02 <sup>dA</sup>	3.57±0.02 <sup>bB</sup>	3.56±0.03 <sup>cC</sup>	7.58±0.35 <sup>dA</sup>	6.77±0.02 <sup>dB</sup>	7.47±0.03 <sup>dB</sup>	13.56±0.11 <sup>cA</sup>	13.46±0.09 <sup>cA</sup>	13.26±0.22 <sup>cA</sup>	20.56±1.53 <sup>aA</sup>	19.27±0.58 <sup>aA</sup>	19.58±0.58 <sup>aA</sup>
12	3.35±0.01 <sup>eA</sup>	3.43±0.01 <sup>aA</sup>	3.40±0.01 <sup>aB</sup>	7.76±0.24 <sup>dA</sup>	6.64±0.40 <sup>deB</sup>	6.44±0.05 <sup>eB</sup>	13.20±0.46 <sup>cdA</sup>	13.40±0.02 <sup>cA</sup>	13.09±0.09 <sup>cdA</sup>	23.00±2.00 <sup>bA</sup>	24.00±1.00 <sup>bAB</sup>	19.67±1.53 <sup>bC</sup>
57	3.32±0.01 <sup>eA</sup>	3.42±0.02 <sup>dB</sup>	3.35±0.02 <sup>dC</sup>	7.74±0.24 <sup>dA</sup>	6.52±0.08 <sup>deB</sup>	6.38±0.05 <sup>dB</sup>	12.91±0.33 <sup>deA</sup>	13.10±0.14 <sup>dA</sup>	12.85±0.11 <sup>dA</sup>	23.67±1.53 <sup>cB</sup>	25.33±1.53 <sup>cAB</sup>	21.00±1.00 <sup>cA</sup>
147	3.64±0.01 <sup>fA</sup>	3.61±0.01 <sup>dB</sup>	3.58±0.01 <sup>cC</sup>	6.94±0.12 <sup>eA</sup>	6.23±0.05 <sup>eB</sup>	6.10±0.02 <sup>gB</sup>	12.66±0.24 <sup>eA</sup>	12.59±0.20 <sup>eA</sup>	12.58±0.28 <sup>eA</sup>	22.00±1.00 <sup>cA</sup>	23.00±1.00 <sup>dA</sup>	19.67±0.58 <sup>eB</sup>

\*Values are the mean±SD (n = 3). In the columns, different small letters represent significant differences and in the rows, different capitalized letters represent.

**Table 2. Flavonoid contents of musts and red wines (mg/L)\***

Wine	(+)-Catechin	Procyanidin B2	Myricetin	Quercetin	Rutin
Must					
1	60.98±0.64 <sup>aA</sup>	45.42±1.80 <sup>aA</sup>	0.54±0.01 <sup>aA</sup>	0.11±0.01 <sup>aA</sup>	0.12±0.02 <sup>aA</sup>
2	61.23±0.92 <sup>aA</sup>	50.23±1.17 <sup>aB</sup>	0.67±0.02 <sup>aB</sup>	0.23±0.01 <sup>aB</sup>	0.24±0.01 <sup>aB</sup>
3	61.49±1.05 <sup>aA</sup>	51.16±2.31 <sup>aB</sup>	1.11±0.02 <sup>aC</sup>	0.28±0.02 <sup>aC</sup>	0.28±0.02 <sup>aC</sup>
Fermentation (5 days)					
1	63.08±0.07 <sup>bA</sup>	274.42±10.63 <sup>bA</sup>	4.80±0.02 <sup>bA</sup>	3.40±0.03 <sup>bA</sup>	1.60±0.02 <sup>bA</sup>
2	64.00±0.85 <sup>bA</sup>	247.43±10.98 <sup>bB</sup>	5.43±0.03 <sup>bB</sup>	7.57±0.06 <sup>bB</sup>	2.00±0.01 <sup>bB</sup>
3	70.26±0.76 <sup>bB</sup>	278.53±13.32 <sup>bA</sup>	5.62±0.03 <sup>bC</sup>	8.52±0.04 <sup>bC</sup>	2.21±0.0 <sup>bB</sup>
Resting of wines with oak chips (7 days)					
1	66.62±1.29 <sup>cA</sup>	423.60±11.47 <sup>cA</sup>	4.78±0.01 <sup>cA</sup>	3.26±0.01 <sup>cA</sup>	1.51±0.02 <sup>cA</sup>
2	68.10±0.72 <sup>cB</sup>	480.69±21.52 <sup>cB</sup>	5.42±0.02 <sup>cB</sup>	3.94±0.02 <sup>cB</sup>	1.83±0.03 <sup>cB</sup>
3	78.77±0.99 <sup>cC</sup>	643.67±10.39 <sup>cC</sup>	5.53±0.02 <sup>cC</sup>	6.27±0.02 <sup>cC</sup>	2.11±0.02 <sup>bC</sup>
Maturation of wines (45 days)					
1	72.00±0.48 <sup>dA</sup>	531.37±11.82 <sup>dA</sup>	4.56±0.04 <sup>dA</sup>	3.23±0.03 <sup>dA</sup>	1.50±0.01 <sup>dA</sup>
2	73.24±0.54 <sup>dA</sup>	561.31±27.16 <sup>dB</sup>	4.90±0.03 <sup>dB</sup>	3.11±0.02 <sup>dB</sup>	1.77±0.03 <sup>dB</sup>
3	87.53±0.65 <sup>dA</sup>	732.34±22.34 <sup>dC</sup>	4.94±0.02 <sup>dC</sup>	6.03±0.07 <sup>dC</sup>	2.10±0.02 <sup>cC</sup>
Aged red wines (3 months)					
1	62.12±1.61 <sup>eA</sup>	298.30±9.59 <sup>eA</sup>	1.12±0.01 <sup>eA</sup>	2.86±0.02 <sup>eA</sup>	1.38±0.01 <sup>dA</sup>
2	65.18±1.69 <sup>eB</sup>	273.27±11.51 <sup>dA</sup>	1.26±0.01 <sup>eB</sup>	3.89±0.02 <sup>dB</sup>	1.58±0.02 <sup>dB</sup>
3	71.71±1.38 <sup>eB</sup>	396.50±10.78 <sup>eB</sup>	1.68±0.02 <sup>eC</sup>	4.73±0.02 <sup>eC</sup>	1.61±0.02 <sup>dC</sup>

\*Values are the mean±SD (n = 3). In the columns, different small letters represent significant differences and in the rows, different capitalized letters represent.

**Table 3. Phenolic acids, stilbene and phenolic alcohol contents of musts and red wines (mg/L)\***

	Gallic acid	<i>p</i> -Coumaric acid	Chlorogenic acid	Resveratrol	Tyrosol
Must					
<b>1</b>	2.80±0.13 <sup>aA</sup>	0.05±0.02 <sup>aA</sup>	8.42±0.04 <sup>aA</sup>	0.05±0.01 <sup>aA</sup>	1.05±0.03 <sup>aA</sup>
<b>2</b>	4.59±0.05 <sup>aB</sup>	0.06±0.02 <sup>aA</sup>	8.86±0.10 <sup>aB</sup>	0.07±0.01 <sup>aB</sup>	2.57±0.12 <sup>aB</sup>
<b>3</b>	6.53±0.06 <sup>aC</sup>	0.10±0.02 <sup>aB</sup>	9.20±0.02 <sup>aC</sup>	0.08±0.01 <sup>aB</sup>	3.54±0.05 <sup>aC</sup>
Fermentation (5 days)					
<b>1</b>	32.77±0.54 <sup>bA</sup>	11.27±0.69 <sup>bA</sup>	9.71±0.07 <sup>bA</sup>	2.03±0.05 <sup>bA</sup>	13.75±0.09 <sup>bA</sup>
<b>2</b>	23.94±1.49 <sup>bB</sup>	11.37±0.80 <sup>bA</sup>	10.90±0.07 <sup>bB</sup>	2.14±0.03 <sup>bB</sup>	19.74±0.10 <sup>bB</sup>
<b>3</b>	40.54±1.01 <sup>bC</sup>	12.16±0.11 <sup>bA</sup>	11.20±0.06 <sup>bC</sup>	2.26±0.02 <sup>bC</sup>	23.83±0.22 <sup>bC</sup>
Resting of wines with oak chips (7 days)					
<b>1</b>	40.39±1.57 <sup>cA</sup>	10.21±0.41 <sup>cA</sup>	10.50±0.07 <sup>cA</sup>	2.63±0.05 <sup>cA</sup>	12.86±0.10 <sup>cA</sup>
<b>2</b>	39.59±1.11 <sup>cB</sup>	10.31±0.43 <sup>bB</sup>	11.71±0.07 <sup>cB</sup>	2.74±0.10 <sup>cA</sup>	19.02±0.30 <sup>cB</sup>
<b>3</b>	46.67±1.74 <sup>cC</sup>	10.92±0.53 <sup>cB</sup>	12.59±0.15 <sup>cB</sup>	2.91±0.04 <sup>cB</sup>	22.15±0.09 <sup>cC</sup>
Maturation of wines (45 days)					
<b>1</b>	21.60±1.07 <sup>dA</sup>	7.38±0.05 <sup>dA</sup>	7.23±0.03 <sup>dA</sup>	2.55±0.03 <sup>dA</sup>	10.56±0.09 <sup>dA</sup>
<b>2</b>	17.64±0.14 <sup>dB</sup>	7.21±0.05 <sup>cB</sup>	7.27±0.02 <sup>dAB</sup>	2.73±0.07 <sup>cA</sup>	11.96±0.12 <sup>dB</sup>
<b>3</b>	36.47±1.27 <sup>dC</sup>	7.30±0.05 <sup>dA</sup>	7.33±0.06 <sup>dB</sup>	2.89±0.04 <sup>cB</sup>	18.35±0.10 <sup>dC</sup>
Aged red wines (3 months)					
<b>1</b>	11.49±1.49 <sup>eA</sup>	7.35±0.06 <sup>dA</sup>	7.22±0.09 <sup>eA</sup>	2.39±0.10 <sup>cA</sup>	6.57±0.04 <sup>eA</sup>
<b>2</b>	15.51±1.14 <sup>eA</sup>	7.15±0.06 <sup>cB</sup>	7.24±0.04 <sup>eA</sup>	2.69±0.01 <sup>cB</sup>	11.05±0.10 <sup>eB</sup>
<b>3</b>	24.78±1.96 <sup>eB</sup>	7.33±0.02 <sup>dA</sup>	7.32±0.09 <sup>dA</sup>	2.87±0.03 <sup>cC</sup>	11.49±0.05 <sup>eC</sup>

\*Values are the mean±SD (n = 3). In the columns, different small letters represent significant differences and in the rows, different capitalized letters represent.

**Table 4. The content of anthocyanins in must and red wines (mg/L)\***

	Delphinidin-3-glucoside	Petunidin-3-glucoside	Malvidin-3-glucoside	Peonidin-3-glucoside	Malvidin-3,5-diglucoside	Peonidin-3-acetylglucoside	Cyanidin-3-glucoside	Peonidin-3,5-diglucoside
Must								
<b>1</b>	0	0	18.66±1.25 <sup>aA</sup>	18.12±1.04 <sup>aB</sup>	0	0.78±0.02 <sup>cC</sup>	2.43±0.37 <sup>aC</sup>	0
<b>2</b>	0	0	23.37±2.42 <sup>aA</sup>	22.39±1.42 <sup>aB</sup>	0	0.94±0.04 <sup>cC</sup>	4.41±0.28 <sup>aD</sup>	0
<b>3</b>	0	0	32.83±2.58 <sup>b</sup>	29.85±1.19 <sup>b</sup>	0	1.12±0.07 <sup>c</sup>	6.72±0.54 <sup>b</sup>	0
Aged red wines (3 months)								
<b>1</b>	28.69±1.06 <sup>aA</sup>	34.51±1.27 <sup>aA</sup>	28.62±1.56 <sup>cB</sup>	7.56±0.06 <sup>cB</sup>	17.48±0.90 <sup>aC</sup>	0	3.96±0.51 <sup>aD</sup>	4.61±0.65 <sup>aD</sup>
<b>2</b>	32.97±0.91 <sup>aA</sup>	40.85±1.26 <sup>bA</sup>	35.18±3.47 <sup>cB</sup>	9.37±0.08 <sup>cC</sup>	28.64±0.88 <sup>cD</sup>	0	4.89±0.67 <sup>aD</sup>	12.42±0.34 <sup>bE</sup>
<b>3</b>	40.45±1.51 <sup>bA</sup>	52.74±1.69 <sup>cA</sup>	46.50±2.58 <sup>dB</sup>	15.89±0.04 <sup>dC</sup>	39.13±0.75 <sup>bD</sup>	0.7±0.01 <sup>eE</sup>	8.37±0.83 <sup>bF</sup>	22.95±0.70 <sup>cC</sup>

\*Values are the mean±SD (n = 3). In the columns, different small letters represent significant differences and in the rows, different capitalized letters represent.