

Effect of maceration duration on physicochemical characteristics, organic acid, phenolic compounds and antioxidant activity of red wine from *Vitis vinifera* L. Karaoglan

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Abstract Effects of different maceration times (5, 10 and 15 days) on composition, phenolic compounds and antioxidant activities of red wines made from the *Vitis vinifera* L. Karaoglan grown in Malatya were investigated. Maceration duration changed some chemical constituents and color of Karaoglan red wines. A linear relationship was observed between antioxidant activity of wine and maceration duration. Major organic acid was tartaric acid which was at the highest concentration in wine macerated for 10 days. A total of 25 phenolic compounds was determined in wine samples. Within these phenolics; procyanidin B2, *trans*-caftaric acid, gallic acid, *trans*-caffeic acid, (+) catechin, (–) epicatechin and quercetin-3-*O*-glucoside were the most abundant phenolics regardless of maceration duration. In general, extended maceration duration resulted in increase in the concentration of phenolic compounds, reflecting the antioxidant activities of wine. In conclusion, the highest concentrations of total and individual phenolic compounds as well as antioxidant activities were found in wines macerated for 15 days.

Keywords Karaoglan red wine · Maceration time · Phenolic composition · Antioxidant activity · Chemical composition

Introduction

Grape (*Vitis vinifera* L.) contains a number of nutritional and functional compounds and it is rich in phenolic compounds. A number of phenolic compounds are present in seed and skin of grape. It was reported that the total extractable phenolics mainly distributed in skin (28–35 %) and seeds (60–70 %) in comparison to the pulp (10 %) in fresh grapes (Sagdic et al. 2011) and the phenolics in skin are transferred into the final product by the maceration process in red winemaking. In particular, red wine is rich in phenolic compounds, including flavonoids (anthocyanins, flavan-3-ols, proanthocyanidins or condensed tannins and flavanols) and non-flavonoids (hydroxybenzoic and hydroxycinnamic acids and their derivatives, stilbenes and phenol alcohols) based on grape variety, growing techniques (viticulture) and winemaking conditions (Budic-Leto et al. 2008; Ginjom et al. 2011). In this context, the qualitative and quantitative properties of phenolics in grape are affected by ripening stage of grape, climate, soil, growth area of grape and winemaking conditions including maceration duration, temperature, pressing intensity, inoculation level and type of yeast, the amount of sulfur dioxide, and other practices (Ivanova et al. 2011). The phenolic compounds (e.g., anthocyanins, stilbens, flavan-3-ols) are important for a high quality of red wine and these contribute to antioxidant activity which is associated with some health benefits (Lucena et al. 2010). It was reported that the moderate consumption of red wine has some biological activities such as cardioprotective effects, prevention of atherosclerosis, coronary heart disease, anti-inflammatory responses, prevention of low density lipoprotein oxidation, antihypertensive and carsinostatic properties, etc. (Lucena et al. 2010). Phenolic compounds also affect the sensory characteristics of wine, in particular

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color as a result of their interactions with colorless phenolics, polysaccharides, metals and anthocyanins. Colored (anthocyanins) and colorless phenolic compounds are extracted from grape skins into wine by means of maceration at a controlled temperature and time (Kelebek et al. 2006; Hernanz et al. 2007). Maceration process may cause considerable variations in wine quality depending on grape cultivar employed. It was reported that the prolongation of the maceration time greatly increases the levels of phenolic compounds and improves the color stability of wine (Kelebek et al. 2009).

Karaoglan is an autochthonous cultivar of *Vitis vinifera* L. grown in Arapgir, county of Malatya (Turkey). This aromatic variety has a round shaped grapes with a thick skin. It is traditionally used for table grape consumption; however, it has been recently used for red winemaking due to its dark color and high aroma potential. Karaoglan wines have a typical aroma characterized by fruit (raspberry, cherry, and strawberry) flavours. Although volatile and sensory characterization of Karaoglan red wines have been made in some extent (Yilmaztekin et al. 2015); however, chemical composition, phenolics and antioxidant characteristics has not yet been studied. The objective of the present study was to identify and quantify some physical properties, chemical composition and individual phenolic compounds of Karaoglan red wine and to investigate the effects of varying maceration duration on phenolic compounds and antioxidant activities of wine.

Materials and methods

Winemaking

Red grapes of *Vitis vinifera* L. Karaoglan were manually harvested at optimal ripening stage, which was recommended by an Oenologue, from the vineyards in Arapgir (Malatya, Turkey) and used in red winemaking in a local winery (Yeni Dogus, Yazili village, Arapgir, Malatya). Must from Karaoglan grape have a titratable acidity (as tartaric acid) of 6.4 g/L, pH 3.3 and reducing sugar 218 g/L. After harvest, the grapes were subjected to three different maceration duration (5, 10 and 15 days) in duplicate to examine the effect of maceration time. The grapes were destemmed and crushed on a grape destemmer-crusher, transferred into 500-L stainless-steel tanks, and then treated with sulfur dioxide (35 mg/L). Alcoholic fermentation was started by 20 g/100 L commercial wine yeast *Saccharomyces cerevisiae* “Laffort RX 60” (Enologica Vason S.r.l., Verona, Italy) at 15 °C; the temperature was allowed to rise during fermentation and maintained at 24–25 °C. The extractability of polyphenolic and aroma compounds during the maceration increased by punching down the

mixture twice per day. The punching down was carried out by crushing the grape mash and stirring the mixture for 5 min. No additional oxygenation was performed during the alcoholic fermentation. Following the completion of each maceration time, the mash was pressed gently in a horizontal press. Free-run and press wines were combined and transferred into new 500-L stainless-steel tanks. Fermentation was controlled daily by recording the optical density and temperature. A commercial lactic acid bacteria culture (*Oenococcus oeni*; Preac 450 Laffort; Viniflora Oenos; Chr. Hansen, Denmark) was inoculated into the wines for malolactic fermentation at 20 °C. Malolactic fermentation stopped in 2–3 week with confirmation by TLC (thin layer chromatography) and the wines were then racked. The wines were racked again after 1 mo, treated with sulfur dioxide (30 mg/L), and filtered through 3-mm membranes. The resultant wines were bottled into 750-mL bottles, stopped using natural cork stoppers, and stored in a room at a temperature of 16–18 °C for 3 mo prior to analysis.

Chemical and physical analysis

Total acidity (by the alkali titration method using 0.1 N NaOH), pH (using a pH meter, WTW Inolab, Germany), total solids (by oven drying method), density (using a pycnometer, Isolab, Istanbul, Turkey), total alcohol (using an ebulliometer, Dujardin—Salleron, Paris, France), volatile acids (by distillation method), total SO₂ (by titration method) and ash (using a furnace at 550 °C) were determined by the methods described in Ough and Amerine (1988). Color intensity and tint of the wine were determined by measuring absorbance of the wines at 420, 520 and 620 nm using a spectrophotometer (Shimadzu UV-1800 model, Kyoto, Japan) as described in Kelebek et al. (2010a). Color intensities, which represent the contents and structure of anthocyanins, were calculated by the total absorbance values of wines measured at 420, 520 and 620 nm, while the tint of wine was calculated as the ratio of the absorbance at 420–520 nm.

Total phenolic compounds were determined by using the Folin–Ciocalteu’s method and expressed as mg gallic acid equivalents per liter of wine (mg GAE/L) as described in Singleton et al. (1999). Total anthocyanin contents in wine samples were determined by the methods given by Mazza et al. (1999). The absorbance of the samples was read at 520 nm and the results were expressed as mg of malvidin-3-*O*-glucoside per L of wine (mg Malv/L).

Antioxidant capacity by ABTS⁺ and DPPH[•] methods

The ABTS⁺ [2,2-azino-di-(3-ethylbenzothiazoline-sulphonic acid)] was assayed by the method described in Re

et al. (1999). The absorbance of the samples were measured at 734 nm and compared with the Trolox standard solutions, giving the results as mg Trolox/L of wine. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was measured at 517 nm as described in Lucena et al. (2010) with some modifications. The DPPH[•] solution was prepared by dissolving 2.5 mg of DPPH[•] radical in 100 mL of methanol. A 100 μ L of wine and 100 μ L of Trolox standard solutions (concentration ranging from 5 to 100 mg/L) were diluted with 3.9 mL of DPPH[•] solution. The mixture was vortexed and rest for 45 min at dark. The absorbance of the resultant solution was measured at 517 nm using the same spectrophotometer against methanol and the results were expressed as mg Trolox/L of wine.

Phenolic compounds

Phenolic compounds in wine samples were determined by the method of Porgali and Buyuktuncel (2012). Shimadzu LC-20AD Prominence HPLC system (Shimadzu Corp., Kyoto, Japan) consisted of diode array detector model SPD-M20A equipped with a pump system with an auto sampler model SIL-20A HT, CTO-20A column heater and DGU-20A5 degasser units. The column was C18 ODS-3 (250 \times 4.6 mm I.D.) with a 5 μ m packing (GL Sciences, Kyoto, Japan) for separation. The chromatographic conditions were arranged according to a previously described method with slight modification (Perez-Magarino et al. 2008) to determinate phenolic compounds. Analytical standards (min 99 % purity) [protocatechuic acid, 4-hydroxy benzoic acid, vanillic acid, syringic acid, *trans*-caftaric acid, 2,5-dihydroxy benzoic acid, chlorogenic acid, *trans*-caffeic acid, *p*-coumaric acid, ferulic acid and sinapic acids, catechin, procyanidin-B2, (–) epicatechin, 4-methyl catechol, epicatechin 3-*O*-gallate and *trans*-resveratrol] were purchased from Sigma-Aldrich (St. Louis, USA) while gallic acid was supplied from Merck (Darmstadt, Germany). The phenolic compounds were quantified by comparison with peak areas of each standard. All analyses were made in triplicate.

Organic acids

Organic acids were analyzed with the method described by Sturm et al. (2003). Before analysis by high pressure liquid chromatograph (HPLC) coupled to photodiode array detector (PDA), wine sample was filtered through 0.45 μ m syringe filters (Lubitech, nylon filter, Shanghai, China). The analysis was performed using a Shimadzu 20A series HPLC (Shimadzu Cooperation). Separation was achieved on an organic acid column (Rezex ROA; 300 \times 7.8 mm, Phenomenex Co, Torrance, Calif., USA) reverse phase C18

column (5 μ m, 250 \times 4.6 mm i.d) thermostatted at 50 °C. The elution was performed with sulphuric acid 0.005 N using a flow rate of 0.5 mL/min. Detection was carried out in a PDA, using 210 nm as preferred wavelengths. The organic acids found were quantified by comparison of the area of their peaks recorded at 210 nm with calibration curves obtained from analytical standards of each compound.

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) using the SPSS 9.0 statistical software program (SPSS Inc., Chicago, IL, USA) in order to evaluate the effect of maceration duration. All analyses were performed at least three times and the results were given as mean \pm standard deviation (SD). To compare the significant differences of the mean values at $P < 0.05$, Duncan's multiple-range tests were used.

Results and discussion

Chemical composition and physical properties of wine

Different maceration duration (5, 10 or 15 days) significantly influenced the chemical composition of the wine samples (Table 1). The levels of ethanol determined in all samples were in accordance with the regulations for the Turkish Food Codex (Anonymous 2009) for red wine (9–15 %, v/v). Ethanol concentration was higher in K10 (13.11 %, v/v) wine than K5 (12.04 %) and K15 (11.68 %) wines and these differences were found to be significant ($P < 0.05$). It was reported that almost the same concentrations of ethanol were determined for Boğazkere (Kelebek et al. 2006), Öküzgözü (Kelebek et al. 2010a), Mencia red (Ortega-Heras et al. 2012) wines. The reducing sugar, volatile acid and total acid contents were higher in K5 than those of K10 and K15. The values of volatile acid in K5 sample (0.35 g/L) was significantly higher than the other wine samples of K10 (0.25 g/L) or K15 (0.30 g/L). Total acidity in wine samples was observed between 4.13 and 4.59 g/L (as tartaric acid) and these values were in accordance with regulations for red wines in Turkey. Density and color values in the wine samples were proportionally changed by maceration time. Similar relative density values were found by Kelebek et al. (2010a) for Öküzgözü and Budic-Leto et al. (2008) for Plavac Mali red wines. The highest color intensity was observed for K5 wine due to high absorbance measured at 420, 520 and 620 nm of absorbance. These were highly correlated

Table 1 Chemical composition of Karaoglan wine as affected different maceration duration (5, 10 and 15 days)

Parameters	Wines ¹		
	K5	K10	K15
Ethanol (v/v, %)	12.04 ± 0.16ab	13.11 ± 0.40b	11.68 ± 0.5a
Reducing sugar (g/L)	2.10 ± 0.14a	1.45 ± 0.07b	1.55 ± 0.07b
pH	3.66 ± 0.00a	3.65 ± 0.10a	3.72 ± 0.05a
Volatile acid (g acetic acid/L)	0.35 ± 0.01a	0.25 ± 0.01b	0.30 ± 0.02ab
Total acid (g tartaric acid/L)	4.59 ± 0.05a	4.44 ± 0.06a	4.13 ± 0.00b
Total SO ₂ (mg/L)	66.00 ± 25.46a	53.50 ± 2.12a	75.50 ± 14.85a
Free SO ₂ (mg/L)	25.60 ± 8.15a	22.70 ± 0.42a	26.88 ± 2.72a
Total solid (mg/L)	32.00 ± 0.4a	30.00 ± 0.4b	35.00 ± 0.2c
Ash (g/100 g)	0.22 ± 0.00a	0.19 ± 0.01a	0.21 ± 0.00a
Density (20/20, °C)	0.9925 ± 0.00a	0.9932 ± 0.00ab	0.9940 ± 0.00b
Color intensity	0.936 ± 0.001a	0.814 ± 0.005b	0.756 ± 0.007c
Tint	0.925 ± 0.001a	0.936 ± 0.000b	0.974 ± 0.002c

¹ K5, K10 and K15 wines macerated for 5, 10 15 days, respectively. Mean ± SD values with different letter within row differed significantly ($P < 0.05$)

Table 2 Antioxidant activity and total phenolic compounds in Karaoglan wine as affected different maceration duration (5, 10 and 15 days)

Parameters	Wines ¹		
	K5	K10	K15
ABTS (mg Trolox/L)	59.59 ± 2.68a	68.72 ± 2.44b	72.86 ± 2.49b
DPPH (mg Trolox/L)	152.90 ± 0.82a	153.83 ± 0.24a	135.92 ± 0.72b
Total Phenolics (mg GAE/L)	3131.67 ± 53.33a	2955.42 ± 32.03b	3866.04 ± 77.19c
Total Anthocyanin (mg Malv/L)	347.13 ± 3.25a	272.41 ± 17.88b	242.53 ± 1.63b

GAE gallic acid equivalent, Malv malvidin-3-*O*-glucoside

¹ K5, K10 and K15 wines macerated for 5, 10 15 days, respectively. Mean ± SD values with different letter within row differed significantly ($P < 0.05$)

with total anthocyanin contents of wines (Table 2). Similar results were also reported by Budic-Leto et al. (2008), that color intensity was decreased with increase in maceration time, which was correlated with a decrease of anthocyanins during maceration. Tint values increased by increasing of maceration time and the highest values were observed for wines macerated for 15 days (K15). Increase in tint values by maceration duration may be linked to the lowering of absorbance at 520 nm. These results are in agreement with the findings from other workers for red wines (Gomez-Plaza et al. 2001; Budic-Leto et al. 2008). However, Kelebek et al. (2006, 2009) pointed out that the color intensity increased from days 3 to 6 and then decreased after days 10 or 12 of maceration time. Different maceration time did not influence the pH, total SO₂, free SO₂ and ash contents of the wines ($P > 0.05$). The pH values of these wines were in accordance with those reports by Kelebek et al. (2006, 2009) and Budic-Leto et al. (2008) for red wines.

Antioxidant activity, total phenolic and anthocyanin contents of wine

Antioxidant activity in wine was determined by ABTS and DPPH assays and significant differences ($P < 0.05$) were found between the wine samples (Table 2). The lowest ABTS values were observed after 5 days of maceration (59.59 mg Trolox/L) and these values increased steadily with increase in maceration duration. Wine K15 contained the highest level of ABTS value (72.86 mg Trolox/L) with similar values for ABTS observed in K10 wine (Table 2). The changes in polyphenolic content during the vinification process, including maceration have some effect on the antioxidant capacity of the wine (Ortega-Heras et al. 2012), as antioxidant activity in wine was dependent on the concentration of some the flavonoids, including hydroxybenzoic and hydroxycinnamic acids, flavan-3-ols, flavonols, flavanones and stilbens. These compounds play a role as antioxidants by their concentration and the free radical scavenging properties of their constituent hydroxyl groups,

allowing them to act as reducing agents, hydrogen- or electron-donating agents or singlet oxygen scavengers (Paganda et al. 1999). This may be explained by the levels of total phenolic compounds in K15 wine. Indeed, the highest level of total phenolic compounds were observed in K15 wine (3866 mg GAE/L). The same linear relationship between ABTS assay and total phenolic compound were also observed in Turkish (Porgali and Buyuktuncel 2012), Croatian (Vrcek et al. 2011) and Slovakian and Austrian (Stasko et al. 2008) wines.

The antioxidant activity of the wines was also measured by DPPH radical-scavenging assay and the wine samples of K15 (135.92 mg Trolox/L) exhibited significantly lower DPPH value than those of K5 (152.90 mg Trolox/L) and K10 (153.83 mg Trolox/L) wines (Table 2). The data from DPPH assay were not consistent with the ABTS results, which may be due to the different reaction mechanisms of these two analyses (ABTS or DPPH). It was reported that a negative correlation was found between the level of total phenolic compound and DPPH radicals-scavenging assay by Sagdic et al. (2011) in grape pomace extracts. These authors pointed out that some phenolic compounds react with the Folin–Ciocalteu's reagent as antioxidants; however, these compounds may not react with the DPPH free radicals. Also, DPPH free radical may scavenge phenolic compounds at free form, while Folin–Ciocalteu's reagent assays the phenolics both at free or bound form (Singleton et al. 1999). In summary for antioxidant assays, ABTS assay was more useful than DPPH assay; the former was proportionally changed with maceration duration. Similarly, Floegel et al. (2011), who suggested that the ABTS may be more useful assay than DPPH for determination of antioxidant status of many foods containing hydrophilic, lipophilic or high-pigmented antioxidant compounds.

Total phenolic compounds in wine samples were significantly influenced by maceration duration (Table 2). Although a small depletion in total phenolic compound contents was observed after 10 days maceration (from 3131.67 to 2955.42 mg GAE/L), a sharp increase was evident after 15 days of maceration (3866.04 mg GAE/L). In general, total phenolic contents increased with increased maceration duration and similar levels of phenolic compounds were also reported by Kelebek et al. (2006, 2010b), Budic-Leto et al. (2008), Ivanova et al. (2011) and Hernanz et al. (2007).

The concentration of anthocyanin in Karaoglan wines decreased with maceration duration and the highest amounts of anthocyanin was observed in K5 wines (Table 2). Decrease in the anthocyanin contents of the wine may be linked to the degradation of these compounds and condensation with tannin (Ribéreau-Gayon and Glories

1986). The decrease in anthocyanin level could be due to the reactions of anthocyanins with proanthocyanins that form copolymerization products or anthocyanin adsorption by yeast lees (Budic-Leto et al. 2008). It was previously reported that the total concentration of anthocyanin was at the highest level in 3 or 6 days-macerated red *Vitis rotundifolia* wine (Sims and Bates 1994), 6 days-macerated Öküzgözü and Boğazkere (Kelebek et al. 2006) and Kalecik Karasi (Kelebek et al. 2009) red wines. Budic-Leto et al. (2008) also reported that anthocyanin content in young wines obtained by skin contact of 17 days was significantly lower ($P < 0.001$) than 'Plavac mali' wines which were macerated 5 and 8 days.

Individual phenolic contents of wine

Phenolic compounds in wine samples macerated for 5, 10 or 15 days are listed in Table 3 and shown Fig. 1. A total of 25 phenolic compounds was determined in the samples and these phenolics can be grouped as hydroxybenzoic acid (7) and hydroxycinnamic acid (6) derivatives, flavanols (5), stilbene (1) and flavonols (6). In general, flavanols and hydroxybenzoic and hydroxycinnamic acids derivatives were the predominant phenolic groups and procyanidin B2 (492.75–713.48 mg/L), *trans*-caftaric acid (247.17–302.74 mg/L) and *trans*-caffeic acid (109.11–114.87 mg/L) were the most abundant phenolic compounds. Procyanidin B2, gallic acid, *trans*-caftaric acid, *trans*-caffeic acid, (+) catechin, (–) epicatechin and quercetin-3-*O*-glucoside were predominant phenolics for wine samples and their concentrations (except for *trans*-caffeic acid) increased with longer maceration duration. From these phenolic compounds (e.g., gallic acid, (+) catechin, (–) epicatechin, procyanidin B3 and *trans*-caftaric acid) were also determined in Mandilaria and Voidomatis wines produced in Greece (Anastasiadi et al. 2010). Kelebek et al. (2010b) reported that *trans*-caftaric acid, catechin, *trans*-coutaric acid and procyanidin B1 were the most abundant phenolic compounds in Öküzgözü red wines. Effects of maceration duration on total phenolic compounds in Karaoglan red wines were significant ($P < 0.05$) and the total concentrations of phenolic compounds increased from 1198.03 mg/L (5 days macerated wine) to 1700.50 mg/L (15 days macerated wine). The concentrations of phenolic acids (hydroxybenzoic and hydroxycinnamic acid derivatives) increased with maceration duration ($P < 0.05$). Gallic acid, 4-hydroxybenzoic acid, vanillic acid, syringic acid, epicatechin 3-*O*-gallate, hesperidin, *trans*-caftaric acid, chlorogenic acid and *p*-coumaric acid were higher in K15 wines in comparison to K5 and K10 wines. It can be concluded in this instance, that the longer maceration time produce high levels of phenolic acids in red wine. Gallic acid was also found at higher levels in comparison to other

Table 3 Phenolic compounds in Karaoglan wine as affected different maceration durations (5, 10 and 15 days)

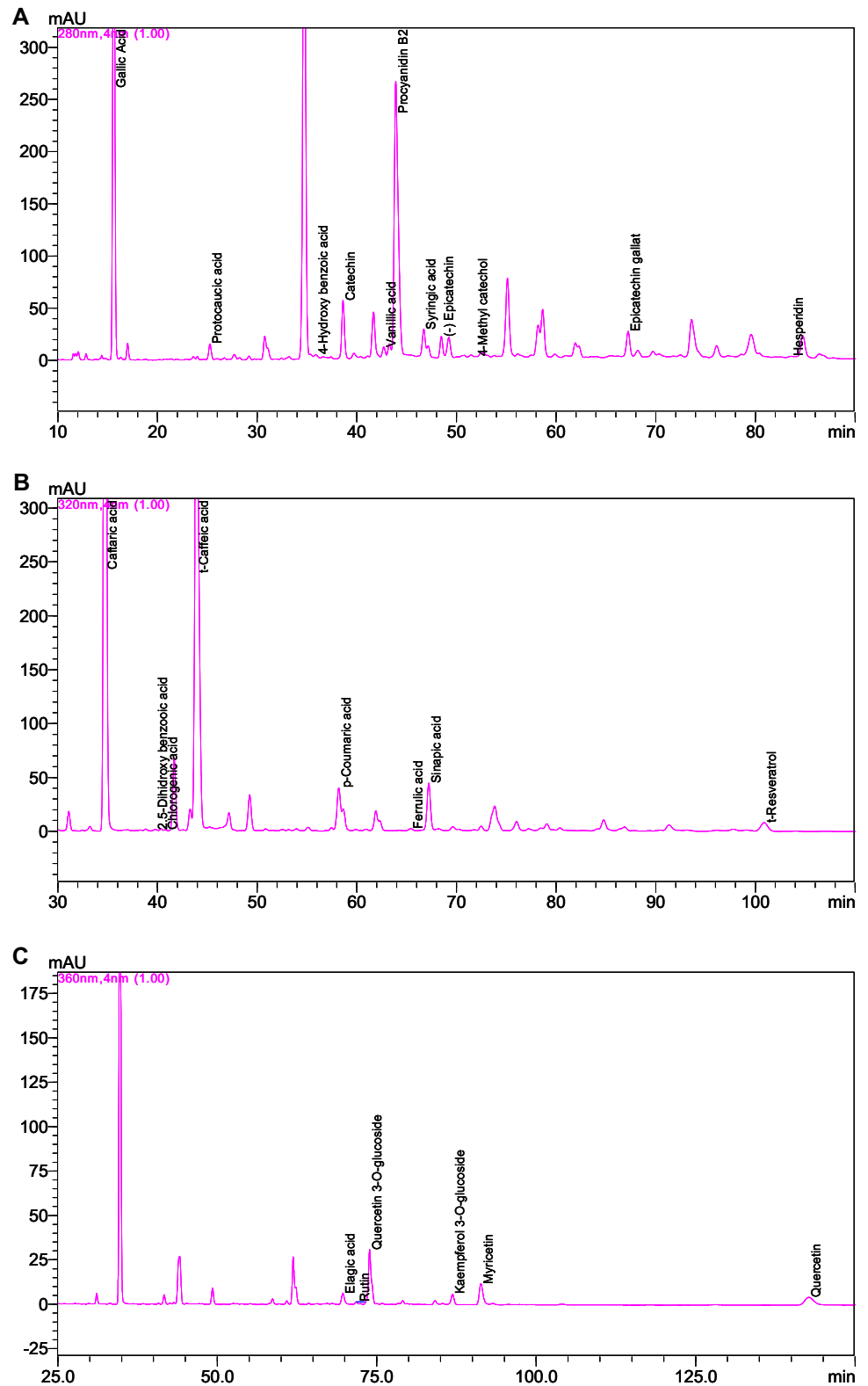
Phenolic compounds (mg/L)	Wines ¹		
	K5	K10	K15
Gallic acid	129.37 ± 1.90a	134.91 ± 9.81a	154.03 ± 31.42b
Protocatechuic acid	8.39 ± 0.12a	9.54 ± 0.25b	9.30 ± 1.76b
4-Hydroxy benzoic acid	8.38 ± 3.85a	4.39 ± 0.40b	14.80 ± 2.25c
2,5-Dihydroxy benzoic acid	2.38 ± 0.00a	2.06 ± 0.07b	1.99 ± 0.00b
Vanillic acid	2.54 ± 1.03a	5.51 ± 0.88a	29.85 ± 1.16b
Syringic acid	7.30 ± 0.06a	11.61 ± 1.83ab	14.04 ± 3.13b
4-Methyl catechol	12.85 ± 0.15a	9.44 ± 2.88a	16.39 ± 0.45b
Epicatechin 3- <i>O</i> -gallate	6.34 ± 0.56a	6.11 ± 2.22a	12.94 ± 2.06b
Hesperidin	12.53 ± 0.21a	8.71 ± 3.02ab	16.24 ± 1.83b
Procyanidin B2	492.75 ± 66.96a	705.67 ± 21.43b	713.48 ± 32.21b
(+) Catechin	55.02 ± 18.48a	55.77 ± 17.00a	114.51 ± 24.70b
(−) Epicatechin	43.00 ± 17.89a	53.50 ± 8.45a	97.27 ± 21.84b
<i>t</i> -Caffaric acid	247.17 ± 6.23a	258.44 ± 33.08a	302.74 ± 13.18b
Chlorogenic acid	1.46 ± 0.05a	1.54 ± 0.00ab	1.67 ± 0.06b
<i>t</i> -Caffeic acid	109.11 ± 1.91a	107.90 ± 7.73a	114.87 ± 2.24a
<i>p</i> -Coumaric acid	3.67 ± 0.60a	4.01 ± 0.21a	7.15 ± 2.13b
Ferulic acid	1.42 ± 0.11a	1.57 ± 0.09a	1.62 ± 0.11a
Sinapic acid	4.31 ± 0.06a	6.86 ± 2.48a	7.50 ± 0.82a
<i>t</i> -Resveratrol	2.55 ± 0.03a	2.19 ± 0.26a	2.68 ± 0.16a
Ellagic acid	5.94 ± 0.52a	6.77 ± 0.62a	7.23 ± 0.70a
Rutin	1.25 ± 0.00a	0.70 ± 0.10b	0.69 ± 0.04b
Quercetin-3- <i>O</i> -glucoside	28.42 ± 0.51a	22.66 ± 0.94b	40.20 ± 2.49c
Kaempferol-3- <i>O</i> -glucoside	3.72 ± 0.05a	3.88 ± 0.70a	5.61 ± 1.26b
Myricetin	4.80 ± 2.70a	7.39 ± 0.55b	8.20 ± 0.83b
Quercetin	3.34 ± 2.08a	4.70 ± 0.26a	5.53 ± 0.73a
Total phenolic compounds	1198.03	1437.85	1700.50

¹ K5, K10 and K15 wines macerated for 5, 10 15 days, respectively. Mean ± SD values with different letter within row differed significantly ($P < 0.05$)

phenolic acids and its concentrations ranged 129.37–154.03 mg/L in wines. It has previously been reported that gallic acid was predominant phenolic acid in red wines (Porgali and Buyuktuncel 2012). The concentrations of *trans*-caffeic, ferulic and sinapic acids and *trans*-resveratrol were not influenced by maceration duration ($P > 0.05$); however, other compounds, including *t*-caftaric, chlorogenic and *p*-coumaric acids were influenced significantly ($P < 0.05$). Phenolic acids such as *trans*-caffeic, ferulic and sinapic acids were also determined in white wine (Hernanz et al. 2007), Öküzgözü (Kelebek et al. 2010b) and Queensland (Ginjom et al. 2011) red wines. Three flavanols, including procyanidin B2, (+) catechin and (−) epicatechin were determined in Karaoglan red wine samples and their concentrations except rutin increased by maceration duration ($P < 0.05$). The concentrations of (+) catechin and (−) epicatechin increased twofold in 15 days when compared to 5 days of maceration. The concentrations of (+) catechin and (−)

epicatechin in Karaoglan red wines were almost the same as for French (Carando et al. 1999) and Öküzgözü (Kelebek et al. 2010b) wines; however, procyanidin B2 was higher in Karaoglan red wine than those of other wines. Only 1 stilbene (*trans*-resveratrol) was determined in wine samples and its concentration (2.19–2.68 mg/L) was not influenced by maceration duration ($P > 0.05$). It was reported that *trans*-resveratrol concentration was 0–2.0 mg/L for Queensland red wine (Ginjom et al. 2011), 1.207 mg/L (as mean values) for 7 different brand of Turkish red wines (Gürbüz et al. 2007), 2.1–2.5 mg/L for Italian wines (Gambutì et al. 2004), 0.88–1.48 mg/L for two Greek red wines (Anastasiadi et al. 2010) and 0.56–2.86 mg/L for Italian red wines (Careri et al. 2003). The authors also recommended the fluorescence detector for the determination of (+) catechin and (−) epicatechin in red wines. A total of five flavonols, including rutin, quercetin-3-*O*-glucoside, kaempferol-3-*O*-glucoside, myricetin and quercetin, were determined in Karaoglan red

Fig. 1 A representative chromatograms from phenolic compounds of Karaoglan (K10) red wine. Phenolic compounds monitored at 280 (a), 320 (b) and 360 (c) nm



wines and their concentrations except rutin increased with increase in maceration duration (Table 3). It was observed that the highest concentrations of flavonols was observed for in 15 days of maceration. The major flavonol was

quercetin-3-*O*-glucoside; however, it was reported that different flavonols including myricetin-3-*O*-glucoside (Kelebek et al. 2010b), quercetin (Ginjom et al. 2011), myricetin (Porgali and Buyuktuncel 2012) were

predominant in different red wines. Myricetin was the second most abundant flavonol and its concentration increased linearly with maceration duration. The concentrations of ellagic acid increased slightly with increase in maceration duration. Ellagic acid was the hydrolysis product of hydrolyzable tannins, which mainly come from oak tannins in oak-contacted wines (Ginjom et al. 2011). Quercetin, which was one of the most potent antioxidant of the phenolic compounds, ranged from 3.34 (K5) to 5.53 (K15) mg/L in wine samples and its concentration was not significantly changed by the maceration duration ($P > 0.05$).

Organic acid contents of wine

Four organic acids, including citric, tartaric, malic and succinic acids were determined in Karaoglan red wine samples using HPLC–DAD. Their concentration were observed to change significantly as a function of maceration time (Table 4). As expected, tartaric acid (ranged 3.34–4.23 g/L) was the principal organic acid in all samples with regardless of maceration time. It was emphasized that this acid has a crucial role for optimal taste, color and stability during fermentation (Lamikanra 1997). Citric acid was second most abundant organic acid in K5 and K10 while together tartaric and citric acid accounted for over 75 % of total organic acid in wine samples; however, citric acid was not identified in K15 wine. The highest concentrations of each organic acids were observed at 10 days of maceration (total organic acid was 6.64 g/L), then decreases were observed in all acids at 15 days. Furthermore, citric acid disappeared after 15 days of maceration time. It was previously reported that tartaric and malic acids were the most abundant organic acids in wines (Lamikanra 1997; Güven 2008) and total amount of tartaric and malic acid

accounted for 90 % of total organic acid in muscadine wine (Lamikanra 1997).

Conclusion

The effect of the maceration duration on some physico-chemical properties (e.g., color intensity, density, pH or chemical composition), phenolic composition and antioxidant activity of Karaoglan red wines was studied. In summary, the results obtained in this study showed that the length of the maceration duration influenced the composition of wine. Color intensity and tint values were increased with extended maceration duration. The total and individual phenolic compounds of Karaoglan red wines were enhanced by increasing maceration duration (from 5 to 15 days). The antioxidant activity of wines was positively influenced by phenolic compounds transferred from grapes into the wine during maceration. Prolonged maceration (15) days resulted in a significant increase in the concentrations of total and individual phenolic compounds and antioxidant activity. The phenolic contents of Karaoglan wine and their behavior during winemaking clarify the role of different winemaking practices on the antioxidant compounds.

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Compliance with ethical standards

Conflict of interest None.

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Table 4 Organic acids in Karaoglan wine as affected by different maceration durations (5, 10 and 15 days)

Organic acids (g/L)	Wines ¹		
	K5	K10	K15
Citric acid	1.39 ± 0.08a	0.77 ± 0.02b	ND ^b
Tartaric acid	3.62 ± 0.43a	4.23 ± 0.02b	3.34 ± 0.04a
Malic acid	0.39 ± 0.05a	0.69 ± 0.01b	0.44 ± 0.02a
Succinic acid	0.57 ± 0.04a	0.95 ± 0.07b	0.58 ± 0.23a
Total organic acid	5.97	6.64	4.36

¹ K5, K10 and K15 wines macerated for 5, 10 15 days, respectively. Mean ± SD values with different letter within row differed significantly ($P < 0.05$)

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