INVESTIGATING THE EFFECT OF AGING ON THE PHENOLIC CONTENT, ANTIOXIDANT ACTIVITY AND ANTHOCYANINS IN TURKISH WINES

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ABSTRACT

Regular consumption of red wine in moderate amounts is proven to possess a positive effect on health because of the phenolic compounds and their ability to act as antioxidants. Aging is one the many factors that affects the phenolic composition of red wines. In this study, the effect of aging on total phenolics (TP), flavonoids (TF), anthocyanins (TA), antioxidant capacity (TAC) and individual phenolic compounds of red wines obtained from the two well-known Turkish wineries were investigated. Results showed, except for few samples, aging generally caused no significant difference in TP, TF and TAC ($P > 0.05$). On the other hand, for all samples, wine aging resulted in significant decrease in both the total (44–88%) and individual (53–99%) TA ($P < 0.05$). This study provided valuable insights into the biochemical composition of several commercial Turkish red wines and how this may change during aging.

PRACTICAL APPLICATIONS

Knowledge about the changes in antioxidant components in red wines as a result of aging may be applied in the production of wines with higher nutritional value.

INTRODUCTION

Epidemiological studies have suggested that regular consumption of red wine in moderate amounts may reduce the risk of cardiovascular diseases (Naissides *et al.* 2006) and in general, have a positive effect on health. The possible health-promoting properties of red wine are related in part to phenolic compounds and their ability to act as antioxidants (Porgali and Buyuktuncel 2012). Phenolic compounds in red wines can be divided into two main groups: flavonoids and nonflavonoids (Tarara *et al.* 2008). Flavonoids comprise flavonols (e.g., quercetin, myricetin), flavan-3-ols (e.g., catechin and epicatechin), as well as polymers of the latter, defined as procyanidins and anthocyanins (Minussi *et al.* 2003). Anthocyanins are responsible for the color of red wine and in general they occur as glycosides in the skin of the grapes. Flavan-3-ols and procyanidins are mostly located in the grape seeds and are responsible for the astringency and bitterness of wines. Nonflavonoids include hydroxybenzoates and hydroxycinnamates that are extracted mainly from the skin and pulp of the grapes and juice during the maceration/fermentation process (Borazan and Bozan 2013). The phenolic composition of wines depends on several factors such as the grape variety, environmental factors in the vineyard, the wine processing techniques, soil and atmospheric conditions during ripening and the ageing process (Granato *et al.* 2010).

Turkey ranks the fourth place in terms of viticultural area and fifth in total grape production (after Italy, France, Spain, U.S.A.) with 3,850,000 tons of annual produce (Kelebek *et al.* 2010). For wine production, grape varieties of both local and French origin are used in Turkey (Anli and Vural 2009). Kalecik karasi, Bogazkere and Okuzgozu are some examples of native Turkish grape varieties of *Vitis vinifera*, which are used to produce high-quality red wines in the industry.

So far, a great number of researches on the polyphenol composition of red wines have been carried out. Nevertheless, there have been few studies concerning the polyphenol composition of aged red wines and there is a lack of knowledge concerning the composition of antioxidants in Turkish wines having undergone aging. This is of prime importance considering that only a small number of red wines are consumed young, whereas the vast majority is aged for at least

12 months in oak barrels and 12 months in the bottle (Arnous *et al.* 2001). Moreover, wine aging implies a significant financial cost for the industry, so it is important to understand the timing, the factors and changes during bottle aging (Gambuti *et al.* 2012). Given the above, the aim of this study was to investigate the effect of aging on total phenolics, flavonoids, anthocyanins and antioxidant capacity as well as major phenolic compounds of several different wine samples obtained from the two well-known Turkish wineries.

MATERIALS AND METHODS

Wines

All commercial wine samples were supplied as triplicates by the two well-known Turkish wineries in spring of 2011. Samples covered some of the commercially available and widely consumed wines produced from Kalecik karasi, Shiraz, Bogazkere and Okuzgozu grape varieties of *V. vinifera*, as listed in Table 1. All samples examined were produced according to standard procedures with defined varietal composition. For both wineries, aging was carried in oak barrels. After all samples were transferred to the laboratory, they were stored at 4C in the dark and analyzed shortly after opening.

Chemicals

All chemicals and reagents used in this study were of analytical or high-performance liquid chromatography (HPLC) grade and purchased from Sigma-Aldrich Chemie GmbH and Co. KG (Steinheim, Germany) unless otherwise specified.

Spectrophotometric Methods

Total phenolic content (TP), total flavonoid content (TF), total anthocyanin content (TA) and total antioxidant capacity (TAC) assays were performed using a Shimadzu UV-1700 UV-Vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

The TP of samples was determined using Folin–Ciocalteu reagent according to the method adopted from Singleton and Rossi (1965). One hundred microliters of sample and 900 μL of distilled water were added to 5 mL of Folin–Ciocalteu reagent. Then, 4 mL of sodium carbonate solution was added to the mixture. After 2 h of incubation at room temperature, absorbance was measured at 765 nm. Results were expressed as mg of gallic acid equivalent (GAE) per 100 mL sample.

The TF was measured as described by Dewanto *et al.* (2002). 0.25 mL of sample and 1.25 mL of distilled water were mixed with 75 μL of 5% $NaNO_2$ solution. After 5 min, 150 μL of 10% $AlCl_3$ was added. Then after 6 min, 0.5 mL of 1 M NaOH was added to the mixture. Immediately, the volume was adjusted to 2.5 mL with distilled water and the absorbance was measured at 510 nm. Results were expressed as mg of catechin equivalent (CE) per 100 mL sample.

The TA was reported according to the pH differential method (AOAC 2006). Absorbance was measured at 520

TABLE 1. WINE SAMPLES EXAMINED IN THIS STUDY

Winery	Grape variety	Region	Aging period (years)	Abbreviation
Winery 1	Kalecik karasi	Ankara	1	W1 KK 1
			2	W1 KK 2
			3	W1 KK 3
			4	W1 KK 4
			5	W1 KK 5
	Shiraz	Denizli	2	W1 S 2
			3	W1 S 3
			4	W1 S 4
	Bogazkere–Okuzgozu	Denizli, Elazig	2	W1 BO 2
			3	W1 BO 3
			4	W1 BO 4
			5	W1 BO 5
Winery 2	Kalecik karasi	Ankara	3	W2 KK 3
			7	W2 KK 7
	Bogazkere	Diyarbakir	4	W2 B 4
			7	W2 B 7
	Okuzgozu	Elazig	3	W2 O 3
			7	W2 O 7

and 700 nm in buffers at pH 1.0 (KCl, 0.025 M) and pH 4.5 (CH_3CO_2Na, 0.4 M) and the results were calculated using the following equation:

$$TA = (A \times MW \times DF \times 1{,}000)/(\varepsilon \times l)$$

where $A = (A_{520nm}\text{-}A_{700nm})_{pH1.0} - (A_{520nm}\text{-}A_{700nm})_{pH4.5}$, MW = molecular weight of cyanidin-3-*O*-glucoside (C3G; 449.2 g/mol), DF = dilution factor, ε = molar extinction coefficient of C3G (26,900 L/[mol.cm]), and l = path length (cm). Results were expressed as mg of C3G per 100 mL sample.

The TAC was estimated using three different assays. The 2,2-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazil (DPPH) and cupric ion reducing antioxidant capacity (CUPRAC) assays were performed according to Miller and Rice-Evans (1997), Kumaran and Karunakaran (2006) and Apak *et al.* (2004), respectively. For ABTS assay, 100 µL of sample was mixed with 1 mL of ABTS-working solution and the absorbance was measured at 734 nm exactly 1 min after initial mixing. For DPPH assay, 100 µL of sample was mixed with 2 mL of 0.1 mM DPPH in methanol and after 30 min of incubation at room temperature, the absorbance was measured at 517 nm. For CUPRAC assay, 100 µL of sample was mixed with 1 mL of 10 mM $CuCl_2$, 7.5 mM neocuproine and 1 M NH_4Ac. Immediately, 1 mL of distilled water was added to the mixture to make the final volume 4.1 mL. After 60 min of incubation at room temperature, absorbance was read at 450 nm. In all assays, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was used as a standard and results were expressed as mg of Trolox equivalent (TE) per 100 mL sample.

For each spectrophotometric assay, samples were analyzed in triplicates and the average values were reported.

HPLC-Photodiode Array Analysis of Phenolic Compounds

Individual phenolic compounds were determined following the method of Kamiloglu and Capanoglu (2013). Samples were passed through a 0.45-µm-membrane filter and injected into HPLC system. The chromatographic separation in Waters 2695 HPLC (Waters Co., Milford, MA) took place in photodiode array (PDA) detector (Waters 2996) with a Supelcosil LC-18 25 cm × 4.60 mm, 5 µm column (Sigma-Aldrich) at 40C. The following solvents with a flow rate of 1 mL/min and 10 µL injection volumes were used for spectral measurement at 280, 312, 360 and 520 nm: as mobile phase solvent A, Milli-Q water with 0.1% (v/v) TFA and solvent B, acetonitrile with 0.1% (v/v) TFA. The linear gradient used is shown in Table 2. Quantification was done using external standards. All analyses were performed in triplicates.

TABLE 2. THE GRADIENT ELUTION PROGRAM USED FOR THE DETERMINATION OF PHENOLIC COMPOUNDS IN WINE SAMPLES

Time (min)	Solvent A (%)	Solvent B (%)
0	95	5
45	65	35
47	25	75
54	95	5

Statistical Analysis

The data obtained from three independent experiments was reported as mean ± standard deviation (SD). Statistical analysis was carried out using SPSS software (version 20.0). Treatments were compared using one-way analysis of variance followed by Tukey post hoc test ($P < 0.05$). The correlation coefficients (R^2) were calculated using Microsoft Office Excel 2011 software (Microsoft Corporation, Redmond, WA).

RESULTS

TP

Results showed that samples from winery 1 were generally richer in phenolic material compared with samples from winery 2 (Table 3). Moreover, Bogazkere–Okuzgozu sample contained the highest TP among the samples from winery 1, while Bogazkere sample had the highest TP in case of winery 2 samples. Aging caused no significant difference in TP of all samples besides the Okuzgozu sample from winery 2, which was found to decrease significantly (22%) after 7 years of aging compared with 3 years of aging ($P < 0.05$).

TF

TF of all samples is expressed in Table 3. Unlike the results obtained for TP, in general, higher TF was achieved for samples from winery 2 instead of samples from winery 1. On the other hand, similar to the results obtained for TP, Bogazkere–Okuzgozu sample had the highest TF from winery 1, whereas Bogazkere sample contained the highest TF among the samples from winery 2. For samples from winery 2, no significant difference in TF was determined as a result of aging ($P > 0.05$). Nevertheless, TF of Kalecik karasi sample from winery 1 was found to increase significantly after 4 and 5 years of aging (27 and 38%, respectively; $P < 0.05$). Yet, also a significant decrease in TF (15%) was observed after 3 years of aging for Bogazkere–Okuzgozu sample from winery 1 ($P < 0.05$).

TA

Although the pattern of variation in TA was different than that observed in TP and TF, generally samples from winery

TABLE 3. TOTAL PHENOLIC (TP), TOTAL FLAVONOID (TF) AND TOTAL ANTHOCYANIN (TA) CONTENTS OF THE WINE SAMPLES

Group	Sample	TP (mg GAE/100 mL)	TF (mg CE/100 mL)	TA (mg C3G/100 mL)
1	W1 KK 1	207 ± 22a	433 ± 32b	11,001 ± 276a
	W1 KK 2	229 ± 34a	508 ± 44ab	8,878 ± 147b
	W1 KK 3	220 ± 44a	514 ± 59ab	5,229 ± 103c
	W1 KK 4	242 ± 49a	551 ± 28a	2,989 ± 630d
	W1 KK 5	230 ± 32a	596 ± 17a	1,364 ± 125e
2	W1 S 2	329 ± 45a	597 ± 97a	21,942 ± 421a
	W1 S 3	331 ± 21a	677 ± 59a	12,879 ± 852b
	W1 S 4	349 ± 12a	686 ± 11a	6,201 ± 194c
3	W1 BO 2	423 ± 21a	932 ± 28a	17,615 ± 425a
	W1 BO 3	369 ± 28a	794 ± 35b	11,476 ± 929b
	W1 BO 4	388 ± 39a	850 ± 14ab	6,028 ± 209c
	W1 BO 5	413 ± 53a	920 ± 61a	2,937 ± 170d
4	W2 KK 3	130 ± 15a	607 ± 64a	5,713 ± 430a
	W2 KK 7	141 ± 10a	699 ± 24a	1,338 ± 143b
5	W2 B 4	267 ± 24a	1,150 ± 42a	5,394 ± 897a
	W2 B 7	231 ± 19a	1,178 ± 53a	3,039 ± 383b
6	W2 O 3	184 ± 6a	764 ± 95a	7,849 ± 969a
	W2 O 7	143 ± 9b	718 ± 88a	3,002 ± 575b

The data presented in this table consist of average values ± SD of three independent samples. Different letters in the columns within each group (1 through 6) represent statistically significant differences ($P < 0.05$). Abbreviations used for samples are expressed in Table 1.

1 were found to contain higher TA compared with samples from winery 2 (Table 3) as observed with TP. Furthermore, in contrast to the results obtained for TP and TF, Shiraz sample had the highest TA from winery 1, whereas Okuzgozu sample contained the highest TA among the samples from winery 2. Wine aging resulted in significant decrease in TA for all samples (44–88%; $P < 0.05$).

TAC

TAC was measured using three different assays (ABTS, DPPH, CUPRAC), which showed different trends (Table 4). In agreement with the findings of TF, majority of the samples of winery 2 demonstrated higher TAC in comparison to samples from winery 1, with DPPH assay giving the

TABLE 4. TOTAL ANTIOXIDANT CAPACITY (TAC) OF THE WINE SAMPLES

Group	Sample	ABTS (mg TE/100 mL)	DPPH (mg TE/100 mL)	CUPRAC (mg TE/100 mL)
1	W1 KK 1	221 ± 51b	2,803 ± 139a	1,000 ± 156a
	W1 KK 2	226 ± 20b	2,957 ± 335a	1,182 ± 165a
	W1 KK 3	352 ± 39a	3,142 ± 1,001a	1,093 ± 140a
	W1 KK 4	396 ± 12a	3,441 ± 763a	1,196 ± 111a
	W1 KK 5	319 ± 27a	3,387 ± 858a	1,236 ± 99a
2	W1 S 2	410 ± 121a	4,616 ± 925a	1,471 ± 253a
	W1 S 3	522 ± 82a	4,964 ± 991a	1,513 ± 275a
	W1 S 4	461 ± 101a	4,902 ± 970a	1,526 ± 11a
3	W1 BO 2	718 ± 70a	6,266 ± 1,619a	2,039 ± 122a
	W1 BO 3	513 ± 112a	5,467 ± 1,326a	1,626 ± 37b
	W1 BO 4	567 ± 124a	5,872 ± 1,307a	1,926 ± 159ab
	W1 BO 5	575 ± 101a	5,940 ± 1,739a	1,976 ± 106a
4	W2 KK 3	466 ± 76a	4,245 ± 349a	1,574 ± 63a
	W2 KK 7	532 ± 110a	4,341 ± 654a	1,593 ± 48a
5	W2 B 4	767 ± 148a	6,511 ± 781a	2,538 ± 13a
	W2 B 7	749 ± 166a	7,052 ± 851a	2,477 ± 233a
6	W2 O 3	548 ± 29a	5,559 ± 990a	1,893 ± 49a
	W2 O 7	483 ± 24b	4,720 ± 676a	1,714 ± 109a

The data presented in this table consist of average values ± SD of three independent samples. Different letters in the columns within each group (1 through 6) represent statistically significant differences ($P < 0.05$). Abbreviations used for samples are expressed in Table 1.

TABLE 5. PHENOLIC ACIDS, FLAVONOLS AND FLAVAN-3-OLS PRESENT IN WINE SAMPLES

		Phenolic acids		Flavonols		Flavan-3-ols	
Group	Sample	Gallic acid (mg/100 mL)	*p*-coumaric acid (mg/100 mL)	Quercetin (mg/100 mL)	Kaempferol (mg/100 mL)	(+)-catechin (mg/100 mL)	(–)-epicatechin (mg/100 mL)
1	W1 KK 1	1.6 ± 0.1cd	0.64 ± 0.02a	0.8 ± 0.1b	0.087 ± 0.004bc	4.1 ± 0.2ab	2.7 ± 0.1a
	W1 KK 2	1.99 ± 0.01b	0.45 ± 0.01b	0.7 ± 0.1b	0.07 ± 0.01c	4.3 ± 0.3a	2.8 ± 0.1a
	W1 KK 3	1.9 ± 0.1bc	0.624 ± 0.004a	1.26 ± 0.01a	0.19 ± 0.02a	3.9 ± 0.5ab	2.11 ± 0.02b
	W1 KK 4	1.5 ± 0.1d	0.46 ± 0.02b	0.5 ± 0.2b	0.10 ± 0.02bc	2.33 ± 0.03c	1.4 ± 0.2c
	W1 KK 5	2.7 ± 0.2a	0.380 ± 0.001c	0.6 ± 0.1b	0.14 ± 0.02ab	3.1 ± 0.3bc	2.94 ± 0.01a
2	W1 S 2	3.5 ± 0.1a	0.297 ± 0.002c	1.51 ± 0.01a	0.101 ± 0.001a	3.1 ± 0.3a	5.32 ± 0.02a
	W1 S 3	3.7 ± 0.1a	0.76 ± 0.02a	1.47 ± 0.04a	0.095 ± 0.002b	3.1 ± 0.5a	4.8 ± 0.1b
	W1 S 4	2.8 ± 0.1b	0.609 ± 0.001b	0.685 ± 0.004b	0.060 ± 0.001c	2.6 ± 0.7a	3.3 ± 0.1c
3	W1 BO 2	4.2 ± 0.2b	0.297 ± 0.003d	0.59 ± 0.01a	0.02 ± 0.01bc	2.82 ± 0.01a	3.86 ± 0.01c
	W1 BO 3	3.5 ± 0.1b	0.33 ± 0.01c	0.12 ± 0.01c	0.011 ± 0.001c	2.59 ± 0.01a	2.59 ± 0.01d
	W1 BO 4	4.32 ± 0.01b	0.618 ± 0.003b	0.30 ± 0.01b	0.022 ± 0.001ab	3.0 ± 0.6a	5.02 ± 0.02b
	W1 BO 5	6.2 ± 0.4a	0.71 ± 0.01a	0.5 ± 0.1a	0.029 ± 0.001a	2.9 ± 0.2a	6.16 ± 0.01a
4	W2 KK 3	2.9 ± 0.1a	0.58 ± 0.01b	1.41 ± 0.02b	0.17 ± 0.02b	2.7 ± 0.2a	3.7 ± 0.2b
	W2 KK 7	3.1 ± 0.3a	0.9 ± 0.1a	1.673 ± 0.004a	0.278 ± 0.001a	2.8 ± 0.4a	5.3 ± 0.4a
5	W2 B 4	3.1 ± 0.1b	0.39 ± 0.02b	0.24 ± 0.04b	0.014 ± 0.001b	0.8 ± 0.1b	3.32 ± 0.03b
	W2 B 7	5.3 ± 0.1a	1.19 ± 0.04a	0.45 ± 0.03a	0.029 ± 0.001a	1.4 ± 0.2a	5.7 ± 0.3a
6	W2 O 3	5.52 ± 0.01b	0.5 ± 0.1b	0.5 ± 0.1a	0.032 ± 0.001a	3.43 ± 0.01a	5.8 ± 0.3a
	W2 O 7	5.81 ± 0.01a	0.65 ± 0.02a	0.36 ± 0.02a	0.027 ± 0.002a	2.60 ± 0.03b	5.2 ± 0.3a

The data presented in this table consist of average values ± SD (determined using HPLC-PDA at 280, 312 and 360 nm) of three independent samples. Different letters in the columns within each group (1 through 6) represent statistically significant differences ($P < 0.05$). Abbreviations used for samples are expressed in Table 1.

highest values. According to all three assays carried out, the highest TAC was determined for Bogazkere–Okuzgozu sample among the samples of winery 1, while the Bogazkere sample possessed the highest TAC from samples of winery 2. On the basis of the DPPH assay, for all samples, aging ended up with no significant difference in TAC ($P > 0.05$). On the other hand, according to ABTS assay, TAC of Kalecik karasi sample from winery 1 was found to increase significantly after 3, 4 and 5 years of aging (59, 79 and 44%, respectively) ($P < 0.05$). In contrast, again according to ABTS assay, TAC decreased significantly in Okuzgozu sample of winery 2 after 4 years of aging (12%; $P < 0.05$). Additionally, as judged by CUPRAC assay, after 3 years of aging, TAC of Bogazkere–Okuzgozu sample of winery 1 were also found to decrease significantly compared with 2 years of aging (20%; $P < 0.05$).

Phenolic Acids

From the group of phenolic acids, gallic acid and *p*-coumaric acid were detected in wine samples, with gallic acid being the major compound in all samples (Table 5). Generally, both for the gallic acid and *p*-coumaric acid contents, samples from wineries 1 and 2 were found to be quite close to each other. On the other hand, the gallic acid contents of Bogazkere–Okuzgozu sample from winery 1 and Okuzgozu sample from winery 2 were determined to be the highest ones. The effect of aging on gallic acid content of wines varied, showing mostly significant increases (5–69%) but also a significant decrease (20% for Shiraz sample of winery 1; $P < 0.05$) or no change (Kalecik karasi sample of winery 2; $P > 0.05$). Nevertheless, aging the wines resulted in significant increase in *p*-coumaric acid content in all samples (30–205%) except for the Kalecik karasi sample from winery 1, which was found to decrease significantly (41%) after 5 years of aging ($P < 0.05$).

Flavonols

Of the flavonols, quercetin and kaempferol were determined in the analyzed wine samples and for all samples, quercetin was determined to be the main flavonol (Table 5). Similar to the results obtained for phenolic acid contents, flavonol contents of samples from wineries 1 and 2 were found to be pretty close to each other. On the other hand, within the samples of winery 1, Shiraz sample contained the highest amount of quercetin, whereas Kalecik karasi had the highest kaempferol content. Nevertheless, for both flavonols, Kalecik karasi was found to contain the highest amounts among the samples of winery 2. After aging, both quercetin and kaempferol contents showed varying results; generally significant increases (19–107% for Kalecik karasi and Bogazkere samples from winery 2; $P < 0.05$) or no change (Kalecik karasi sample from winery 1 and Okuzgozu sample from winery 2; $P > 0.05$) but also a significant decrease

TABLE 6. ANTHOCYANINS PRESENT IN WINE SAMPLES

Group	Sample	Malvidin-3-*O*-glucoside (mg/100 mL)	Cyanidin-3-*O*-glucoside (mg/100 mL)	Peonidin-3-*O*-glucoside (mg/100 mL)
1	W1 KK 1	5.8 ± 0.6a	0.27 ± 0.01a	0.20 ± 0.03a
	W1 KK 2	4.6 ± 0.6a	0.20 ± 0.03a	0.16 ± 0.01a
	W1 KK 3	2.1 ± 0.7b	0.11 ± 0.02b	0.07 ± 0.03b
	W1 KK 4	0.7 ± 0.1b	0.05 ± 0.02bc	0.04 ± 0.02b
	W1 KK 5	0.22 ± 0.01b	0.006 ± 0.001c	0.02 ± 0.01b
2	W1 S 2	10.6 ± 0.6a	0.7 ± 0.2a	0.6 ± 0.1a
	W1 S 3	5.3 ± 0.6b	0.3 ± 0.1ab	0.3 ± 0.1b
	W1 S 4	1.4 ± 0.4c	0.10 ± 0.04b	0.07 ± 0.01b
3	W1 BO 2	7.39 ± 0.01a	0.80 ± 0.01a	0.312 ± 0.001a
	W1 BO 3	3.6 ± 0.1b	0.36 ± 0.01b	0.157 ± 0.001b
	W1 BO 4	0.8 ± 0.3c	0.08 ± 0.02c	0.041 ± 0.002c
	W1 BO 5	0.3 ± 0.1c	0.06 ± 0.01c	0.040 ± 0.003c
4	W2 KK 3	1.8 ± 0.1a	0.10 ± 0.01a	0.065 ± 0.002a
	W2 KK 7	0.15 ± 0.01b	0.007 ± 0.001b	0.026 ± 0.001b
5	W2 B 4	0.4 ± 0.1a	0.041 ± 0.004a	0.06 ± 0.01a
	W2 B 7	0.19 ± 0.01b	0.011 ± 0.004b	0.022 ± 0.001b
6	W2 O 3	1.8 ± 0.2a	0.19 ± 0.01a	0.06 ± 0.01a
	W2 O 7	0.3 ± 0.1b	0.04 ± 0.01b	0.035 ± 0.001b

The data presented in this table consist of average values ± SD (determined using HPLC-PDA at 520 nm) of three independent samples. Different letters in the columns within each group (1 through 6) represent statistically significant differences ($P < 0.05$). Abbreviations used for samples are expressed in Table 1.

(in Shiraz sample from winery 1: 55 and 41% for quercetin and kaempferol contents, respectively; $P < 0.05$).

Flavan-3-ols

Both (+)-catechin and (−)-epicatechin from the group of flavan-3-ols were detected in wines (Table 5). For all samples except for Kalecik karasi sample of winery 1, the amount of (−)-epicatechin was found to be higher than (+)-catechin. Similar to phenolic acids and flavonols, the flavan-3-ol content of samples from winery 1 and winery 2 were determined to be rather close to each other. Aging mostly caused no significant difference in (+)-catechin content of samples ($P > 0.05$); however, (+)-catechin content in Kalecik karasi sample from winery 1 and Okuzgozu sample from winery 2 were found the decrease significantly (76% after 4 years of aging and 24% after 7 years of aging, respectively), whereas Bogazkere sample aged for 7 years was determined to contain significantly higher amount of (+)-catechin (75%) compared with samples aged for 4 years ($P < 0.05$). In case of (−)-epicatechin content, aging the wines generally resulted in significant increases (60% for Bogazkere–Okuzgozu sample from winery 1, 43% for Kalecik karasi sample from winery 2 and 68% for Bogazkere sample from winery 2), but also insignificant changes (Kalecik karasi sample from winery 1 and Okuzgozu sample from winery 2) and a significant decrease (61% for Shiraz sample from winery 1) was observed ($P < 0.05$).

Anthocyanins

Malvidin-3-*O*-glucoside (M3G), C3G and peonidin-3-*O*-glucoside (P3G) were identified from the group of anthocyanins, with M3G being the major anthocyanin in all samples (Table 6). Samples from winery 1 were found to be richer in terms of anthocyanin content compared with the samples from winery 2, with Shiraz (for M3G and P3G) and Bogazkere–Okuzgozu (for C3G) samples being the richest ones. Confirming the results obtained for total anthocyanins, aging the wine samples also resulted in significant decreases in all individual anthocyanins (53–99%; $P < 0.05$).

Correlation between Assays

TP, TA and TAC showed a low correlation ($R^2 < 0.2$), whereas high correlations were obtained between TF and TAC, with CUPRAC being the highest ($R^2 = 0.9131$). Total and individual anthocyanins of wines also showed a linear relationship with high correlation coefficients ranging from $R^2 = 0.8927$ to $R^2 = 9{,}213$. The correlation data between the analyzed compounds and antioxidant activities revealed that gallic acid and catechin are the major contributors to the antioxidant capacity of the analyzed wine samples ($R^2 = 0.3166$–0.4372 and $R^2 = 0.3373$–0.5460). Overall, these results imply that flavonoids are the major contributors to the antioxidant capacity.

DISCUSSION

High-quality red wines generally require a period of aging, after which they are ready to be consumed. During this time, modifications of the polyphenol composition of the wine occur, mainly not only because of the formation of polymeric compounds but also because of oxidation, hydrolysis and other transformations that may take place in native grape phenolics. In the literature, there are some reports on the effect of aging on the polyphenol composition of red wines (Arnous *et al.* 2001; Zafrilla *et al.* 2003; Perez-Magarino and Gonzalez-San Jose 2004; Monagas *et al.* 2006); nevertheless, the present study represents the first published data describing the changes in polyphenols and antioxidant capacity of Turkish wines as a result of aging for long periods.

According to the results, aging caused no significant difference in TP of all samples besides the Okuzgozu sample from winery 2 ($P > 0.05$). Changes observed in TP of this sample during ageing may be possibly due to the transformation of phenolic compounds into condensed forms that possess slightly different chemical properties and reactivity toward the Folin–Ciocalteu reagent. Changes determined in TP could also be due to the enzymatic activity from residual microorganisms present in the wine (Monagas *et al.* 2006). Folin–Ciocalteu reagent is nonspecific to phenolic compounds as it reacts with phenolic compounds only under basic conditions and also many nonphenolic compounds can reduce it. Even with the undefined chemical nature of it, determination of TP using Folin–Ciocalteu reagent is convenient, simple and reproducible, and it is commonly employed in the wine industry, where gallic acid is usually selected as a standard (Paixao *et al.* 2007; Porgali and Buyuktuncel 2012). Gallic acid that was determined to be the predominant phenolic acid in the Bogazkere–Okuzgozu sample from winery 1 and Okuzgozu sample from winery 2 was found to increase significantly after aging ($P < 0.05$). The main sources of gallic acid in wine are grape seeds and oak barrels (Anli and Vural 2009). Thus, a possible explanation of the increased gallic acid content after aging might be the extraction of this compound from the oak barrels into the wine during the aging period.

In the analyzed samples, the most prominent anthocyanin was M3G, whereas C3G was the least abundant anthocyanin. Previously, similar anthocyanin profiles have been reported for different red wine varieties (Arnous *et al.* 2001; Kallithraka *et al.* 2005). For all samples, wine aging resulted in significant decrease in both the total and individual anthocyanins ($P < 0.05$). In this sense, the results of our study were compatible with other studies in which aged wines are resulted in anthocyanin degradation (Monagas *et al.* 2006; Ivanova *et al.* 2012). It has been reported that anthocyanins could be affected by storage and processing methods (Howard *et al.* 2014). In red wine grapes, anthocyanins exist exclusively as monomers. However, after few months of ageing, monomeric anthocyanins are transformed into polymeric pigments, which lead to an increase in the color stability against both bisulphite bleaching and pH changes (Gutierrez *et al.* 2005). Three mechanisms have been proposed to be possibly involved in the formation of polymeric pigments during red wine aging. The first two mechanisms involve direct reaction between proanthocyanidin and anthocyanin, producing colorless compounds or orange xanthylium salts. The third mechanism includes acetaldehyde-mediated condensation of anthocyanin and proanthocyanidin, providing purple pigments with an ethyl bridge (Wang *et al.* 2003). The assay used to determine TA is based on the pH structural-dependent property of anthocyanins. Considering the fact that polymeric pigments are more resistant to pH changes compared with monomeric anthocyanins, it can be assumed that TA determination mostly includes free monomeric or simple anthocyanins (Monagas *et al.* 2006). Additionally, anthocyanins can precipitate, leading to decrease of their concentration during the wine aging (Gomez-Plaza *et al.* 2000).

Wine aging caused different effects on TAC of the examined red wines. Besides the grape variety, this could be mainly due to the difference in environmental and growing factors of the fields. Ultraviolet-B radiation, water stress and abscisic acid are known to affect grape berry (*V. vinifera* L.) growth, maturation and their quality for winemaking (Quiroga *et al.* 2012; Berli and Bottini 2013). On the other hand, the maceration techniques may also play a role in the antioxidant capacity of red wines. According to a research, the antioxidant activity of red wines produced with long maceration was found to have a higher capacity and to be responsible for 60% of the relative inhibition of LDL oxidation in the human body (Goldberg *et al.* 1996). Variations were also observed between different TAC measurement assays in terms of determining the effect of aging on the examined red wines. It has been confirmed in the literature that the capacity of antioxidants assessed by various methods often gives inconsistent and conflicting results (Niki 2011). The measurement of TAC cannot be interpreted satisfactorily by a single method because of the complex nature of phytochemicals (Jagtap and Bapat 2015). The principles of the methods alter greatly depending on the radical that is generated or the time of the reaction. Even ABTS and DPPH assays, which are based on the same principle, can show several important differences in their response to antioxidants (Ozcelik *et al.* 2003; Karadag *et al.* 2009). Therefore, it is recommended to apply several tests in order to obtain a reliable TAC evaluation, as was done in this study.

HPLC analysis revealed that quercetin and kaempferol are the major flavonol aglycones in the investigated red wines, which is in agreement with the data reported in the literature (McDonald *et al.* 1998; Tsanova-Savova and Ribarova 2002). Wine aging showed varying results in terms of flavonol content, generally significant increases or no change but also a significant decrease in case of Shiraz sample from winery 1 ($P < 0.05$). The reduction in this sample as a result of aging could be due to the combination of sugar and flavonol aglycones to form the flavonol glycosides. While more and more flavonol glycosides had been formed during wine aging, the flavonol glycosides themselves were hydrolyzed into flavonol aglycones in the oak barrels, which could explain why the amount of flavonols varied with aging (Fang *et al.* 2007). Furthermore, flavan-3-ol content of the aged wines also varied. This diversity in the amount of flavan-3-ols can be attributed to different reasons: on one hand, the flavan-3-ol concentration tends to decrease since they are involved in oxidation reactions, as well as in tannin–tannin and anthocyanin–tannin polymerization reactions; on the other hand, an increase in the concentration of flavan-3-ols is also expected because they can be released from their galloylated precursors (Gutierrez *et al.* 2005).

Aging of red wines in oak barrels is an expensive practice, and thus, it implies a significant financial cost. Lately, alternatives to oak barrel aging are being searched to carry out the wine-ageing process. In order to try to create conditions similar to those provided by oak barrels, oak chips are added to wine to give a woody aroma and taste to the wine. In addition to oak chips, another method called micro-oxygenation, which is the controlled introduction of oxygen into wine to simulate the oxygen uptake occurring during barrel aging, has also been proposed (Garde-Cerdan and Ancin-Azpilicueta 2006; Gambuti *et al.* 2012). In further studies, it might be interesting to employ these new technologies for aging of Turkish wines and compare the effects on polyphenol composition and antioxidant activity with results of the current study.

CONCLUSION

As far as we know, this is the first study reporting the effect of aging for long periods on polyphenol content of Turkish red wines. During red wine aging, when polymerization reaction occurs, decrease in polyphenol content with intensive decrease of anthocyanins was observed in the analyzed red wines, while generally, no significant change in TP, TF and TAC were noticed. Overall, the results demonstrated the complexity of the biochemical composition of Turkish red wines and how this is modified upon aging and the potential relevance of these changes to red wine quality.

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