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Authenticity of wines made with economically important grape varieties grown in Anatolia by their phenolic profiles

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ABSTRACT

The aim of this study was to characterize and compare the wines of main native and nonnative grape varieties grown in Turkey. The polyphenol compositions of red and white monovarietal wines of 11 grape varieties have been evaluated for four vintages (2006–2009). Discrimination of wines with respect to variety and vintage was studied by partial least square-discriminant analysis. Boğazkere red wines were the highest and Kalecik Karası red wines were the lowest in total phenol content. Syrah wines were the highest in anthocyanin compounds. (+)-catechin and (−)-epicatechin contents of Cabernet Sauvignon, Merlot and Syrah wines were the highest, whereas those compounds were detected in lower amounts in native wines. For the case of white wines, the discrimination among Emir, Muscat and Sultaniye wines was based on hydroxycinnamic acids, flavan-3-ols and total phenol content. The vintage based discrimination of red wines was affected at most from malvidin compounds. White wines could also be discriminated according to their vintage based on the presence of quercetin-3-*O*-galactoside, vanillic acid and o-coumaric acid. The phenolic descriptors of wines can be used in the authentication of wines with respect to variety and harvest year.

1. Introduction

Wine quality depends on several issues such as geographical origin, grape variety, climate, vintage and process conditions. To successfully compete in the market, this desired quality should be protected by characterizing the product in terms of varietal and geographical origin. The label should be accurate and not misleading the consumer. This geographical origin and variety based wine classification is important in terms of quality and economic reasons. Wines from different regions may differ in quality and price. Reliable assessment of grape variety is necessary to protect consumer from adulteration and false labeling (Jaitz et al., 2010). This is directly related to the labels of the origin such as Protected Designation of Origin (PDO) and Protected Geographical Indications (PGI) defined by the European Regulation (EEC) 2081/92. The labels in different countries may get different names like denominazione di origine controllata (DOC), Appellation d'Origine Contrôlée (AOC) and Denominación de Origen (DO) and they ensure the desired product quality (Gonzalez-Fernandez, Marcelo, Valenciano, & Rodriguez-Perez, 2012; Martinez-Carrasco, Brugarolas, & Martinez-Poveda, 2005; Perez-Magarino, Ortega-Heras, & Gonzalez-San Jose, 2002).

Phenolic compounds of wine determine not only its nutritional and sensory properties, but also the characterization of wine according to its geography and grape variety. The polyphenolic profile of a cultivar indicates its genetic potential due to the enzymatic reactions involved in the biosynthesis. The enzymatic activity depends on the environmental factors, i.e. sun exposure, water deficiency of the plant, degree of grape ripeness, berry size or vegetative vigour of the plant, varying at different geographical regions. Therefore, the polyphenol concentrations of wine samples even from the same cultivars may vary based on their geographic regions or vice versa. The aging and technological influences are other factors that could alter the polyphenol composition (Makris, Kallithraka, & Mamalos, 2006; Montealegre, Peces, Vozmediano, Gascuena, & Romero, 2006).

The wine polyphenols are monomeric, oligomeric and polymeric compounds which are mainly classified as flavonoids ($C_6C_3C_6$ skeleton) including anthocyanins, flavan-3-ols, flavonols and non-flavonoids, including hydroxybenzoic and cinnamic acids and stilbenes (Oliveira, Ferreira, Freitas, & Silva, 2011). They have been reported to have several biological activities such as cardioprotective, anti-inflammatory, anti-carcinogenic, anti-microbial and anti-aging, which rely on mainly their antioxidant and

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Table 1
The grape varieties, grape growing regions and sample numbers of commercial wine samples.

Grape variety	Number of sample	Regions
Boğazkere	8	Diyarbakır, Cappadocia, Tokat
Cabernet Sauvignon	7	İzmir, Cappadocia, Thrace, Tokat
Kalecik Karası	16	Ankara, Denizli, İzmir, Thrace
Merlot	10	Denizli, İzmir, Thrace
Öküzgözü	12	Elazığ, Cappadocia, Tokat
Syrah	12	Denizli, Manisa
Emir	10	Cappadocia
Muscat	9	Denizli, İzmir, Manisa
Narince	9	Denizli, Manisa, Tokat
Sultaniye	8	Denizli, İzmir, Manisa
Chardonnay	10	Denizli, İzmir, Cappadocia, Thrace

antiradical activities. They also play a critical role in the color formation and astringent or bitter taste of wines (Ivanova et al., 2011; Porgali & Buyuktuncel, 2012).

There are different phenological periods during the berry growth (Nicholas, Matthews, Lobell, Willits, & Field, 2011). It was reported that the accumulation of proanthocyanidins (procyanidin B_1, B_2, B_3 and B_4) mainly took place between late May and early August, before veraison. Similarly, the flavan-3-ols such as (+)-catechin, (−)-epicatechin and (−)-epicatechin gallate start accumulating with the growth of berry until the beginning of veraison. Quercetin being the main component, flavonoid synthesis starts before veraison and increases during berry ripening. Finally, the veraison period takes place generally between late July and till September, which is the ripening stage of the grape, at the same time. The changes such as accumulation of color (anthocyanins in red grapes), aroma compounds, tannins and minerals as well as color changes from green to red or yellow-green occur in veraison period. Essentially, the synthesis of anthocyanins starts two weeks before veraison, and then the anthocyanins reach a well-defined profile during veraison and are at their maximum at the ripening stage (Ivanova et al., 2011).

Turkey has a very suitable climate and soil characteristic for grape production with 500 thousands of hectares of vineyard area, which is the largest fifth in the world. Turkey locates between 35 and 42°00 north latitudes and 25–44°00 east longitudes. It is the first in raisin production and the third for fresh consumption (OIV, 2013a). There is a great potential for increasing production in the wine industry.

The aim of this study was to characterize monovarietal red and white wines from native and non-native grape varieties grown in Turkey using their polyphenol composition. It was also aimed to highlight the differences between wines of indigenous types (Boğazkere, Öküzgözü, Kalecik Karası, Emir, Narince, Sultaniye) and wines of widely cultivated types (Cabernet Sauvignon, Merlot, Syrah, Chardonnay, Muscat). Partial least squares-discriminant analysis (PLS-DA) was employed for the classification of wines with respect to grape variety and harvest year. To our knowledge, this is the most comprehensive study about the polyphenolic characterization of Turkish wines from economically important wine grapes.

2. Materials and methods

2.1. Wine samples

The commercial bottled wine samples were purchased from local market. The wines were chosen at similar price ranges. A total number of 111 wine samples were collected from the vintage years of 2006, 2007, 2008 and 2009. These mono-varietal wines were produced from 11 different grape varieties, five of which were Cabernet Sauvignon, Merlot, Syrah, Chardonnay and Muscat. The indigenous varieties were Boğazkere, Öküzgözü, Kalecik Karası as red, Emir, Narince and Sultaniye as white, which were commercially valuable grapes in Turkey (Table 1). Total of 65 red and 46 white wines were collected with ethanol contents ranging between 10 and 15% (v/v) according to the labels. The grape varieties were cultivated in 9 different wine growing regions of Turkey (Fig. 1). The geography and grape variety information in Table 1 were obtained from the labels on the wine bottles.

2.2. Reagents

$NH_4H_2PO_4$ and H_3PO_4 (85%) were purchased from Merck (Darmstadt, Germany), HPLC grade acetonitrile and Folin Ciocalteu reagent were purchased from Sigma–Aldrich (Steinheim, Germany). Sodium carbonate was purchased from Riedel-de Häen (Honeywell, Seelze, Germany). HPLC grade pure standards were employed: (+)-catechin hydrate (Dlcatec), malvidin-3-O-glucoside (90%) (mal3G), quercetin (95%) (quer), quercetin-3-rutinoside

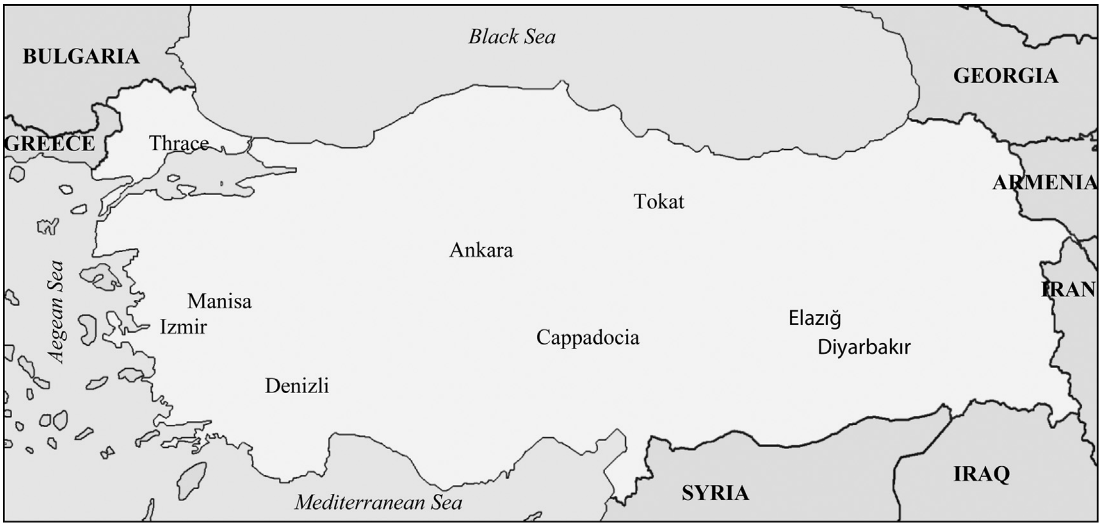

Fig. 1. Wine regions in Turkey.

(95%) (rutn), quercetin-3-*O*-glucoside (90%) (Q3glucosi), and quercetin-3-*O*-galactoside (97%) (Q3galact) were purchased from Sigma–Aldrich (Steinheim, Germany). (−)-epicatechin (95%) (−)-epicat, caffeic acid (95%) (caffe), ferulic acid (99%) (ferul), gallic acid (98%) (gallic), kaempferol (96%) (kaemp), myricetin (96%) (myric), o-coumaric acid (97%) (o-coum), p-coumaric acid (98%) (p-coum) and vanillic acid (97%) (vanill) were purchased from Fluka (Steinheim, Germany). Resveratrol (99%) (tresv), procyanidin B_1 (80%) (PB1) was purchased from Extrasynthese (Genay, France). The phenolic compounds were mentioned with their short names given in parenthesis in the text, Figures and Tables.

2.3. Instrumentation

2.3.1. Polyphenol analysis

The polyphenol content was determined by the HPLC method developed by Gomez-Alonso, Garcia-Romero, and Hermosin-Gutierrez (2007). The wine samples were filtered through 0.45-μm pore sized membrane filters (Sartorius, Goettingen, Germany),then they were directly injected into the HPLC. Chromatographic analyses were performed on an Agilent 1200 series HPLC with a diode array detector (Agilent Technologies, Santa Clara, CA, USA). A C18 (5 μm, 250 × 4.6 mm) column was used (AC Technologies, Aberdeen, Scotland). Column oven was set to 20 °C. Chromatograms were recorded at 280, 320, 360 and 520 nm. Identification and quantification of phenolic compounds were performed according to the retention times of pure standards and external standard method, respectively. Some anthocyanins and phenolic compounds were quantified by using the calibration curves of the most similar compounds (Table 2). These phenolics were identified from the chromatograms of wine samples studied elsewhere (Gomez-Alonso et al., 2007) as well as by the control of UV–VIS spectra. Duplicate measurements were performed. The calibration curves of the standard compounds were prepared each year from at least five concentration points ($R^2 \geq 0.990$). The chromatogram of Kalecik Karası red wine was shown in Fig. 2.

2.3.2. Total phenol content (TP)

Folin Ciocalteu method was employed with the micro scale protocol to reduce the assay volume (Arnous, Makris, & Kefalas, 2001). For white wines, 20 μL of sample and 3.16 ml water was mixed with 200 μL of 2 N Folin Ciocalteu reagent. For red wines, samples were diluted by one half with water. After 5 min, 600 μL of saturated sodium carbonate solution was added. The mixture was left for 2 h at 20 °C and the absorbance was read at 765 nm against blank (UV 2450; Shimadzu Inc., Kyoto, Japan). The measurements were repeated three times. The results were expressed in terms of gallic acid equivalent (mg GAE/L) by using a gallic acid calibration curve.

2.4. Statistical analyses and method validation

The detection limits were calculated according to the OIV method (OIV, 2013b) by using the graphical approach based on the background noise of a blank sample. The following formulas were employed: Limit of detection (LOD): $3 \times h_{max} \times$ RF, Limit of quantification (LOQ): $10 \times h_{max} \times$ RF. The response factor (RF) of the instrument is the quantity/signal ratio. The quantity is the concentration of the analyte and signal is the height of the analyte peak. h_{max} is the greatest variation in absorbance unit on the *y*-axis of chromatogram of between two points. The distance between two points is twenty times the width at mid-height of the analyte peak. Recovery values were based on the difference between the spiked and un-spiked sample and the ratio of this difference to the known value. Moreover, repeatability was checked by relative standard deviation of replicate analysis. The data were statistically evaluated

Table 2
Analytical conditions of polyphenols in wine samples (mg/L).

Phenolic name	Acronym	LOD	LOQ	Recovery of white wines (%)	Recovery of red wines (%)
Malvidin-3-*O*-glucoside	mal3G	0.0270	0.0900	81	83 ± 7
Rutin	rutn	0.0289	0.0963	97 ± 2	87 ± 10
Kaempferol	kaemp	0.0279	0.0932	98 ± 6	95 ± 10
Quercetin	quer	0.0196	0.0653	87 ± 7	95 ± 11
Myricetin	myric	0.0192	0.0639	100 ± 14	86 ± 4
Resveratrol	tresv	0.0011	0.0037	81 ± 5	93 ± 1
p-Coumaric acid	p-coum	0.0109	0.0363	95 ± 11	80 ± 1
Ferulic acid	ferul	0.0149	0.0498	84 ± 2	88 ± 9
Caffeic acid	caffe	0.0194	0.0647	80	82 ± 6
Gallic acid	gallic	2.1351	7.1170	101 ± 1	86 ± 8
(+)-Catechin	Dlcatec	1.3166	4.3888	94 ± 6	97 ± 1
Vanillic acid	vanill	0.0352	0.1172	86 ± 3	85 ± 5
(−)-Epicatechin	(−)-epicat	0.2774	0.9247	100 ± 6	86 ± 5
o-Coumaric acid	o-coum	0.0525	0.1751	80 ± 4	84 ± 3
Quercetin-3-*O*-glucoside	Q3glucosi	0.0245	0.0815	84 ± 5	93 ± 8
Quercetin-3-*O*-galactoside	Q3galact	0.0612	0.2039	97 ± 1	89 ± 1
Procyanidin B_1	PB1	0.2113	0.7043	85	88
Peonidin-3-*O*-glucoside[a]	peo3G	–	–	–	–
Petunidin-3-*O*-glucoside[a]	pet3G	–	–	–	–
Delphinidin-3-*O*-glucoside[a]	del3G	–	–	–	–
Vitisin-A[a]	vitA	–	–	–	–
Delphinidin-3-*O*-glucoside acetate[a]	del3Ga	–	–	–	–
Petunidin-3-*O*-glucoside acetate[a]	pet3Ga	–	–	–	–
Peonidin-3-*O*-glucoside acetate[a]	peo3Ga	–	–	–	–
Malvidin-3-*O*-glucoside acetate[a]	mal3Ga	–	–	–	–
Delphinidin-3-*O*-glucoside coumarate[a]	del3Gc	–	–	–	–
Pinotin-A[a]	pinA	–	–	–	–
Malvidin-3-*O*-glucoside coumarate[a]	mal3Gc	–	–	–	–
Quercetin-3-*O*-glucuronide[b]	Q3glucuron	–	–	–	–
Myricetin-3-*O*-glucoside[b]	myric3G	–	–	–	–

[a] The compounds were quantified using the malvidin-3-*O*-glucoside standard curve.
[b] The compounds were quantified using the quercetin-3-*O*-glucoside standard curve.

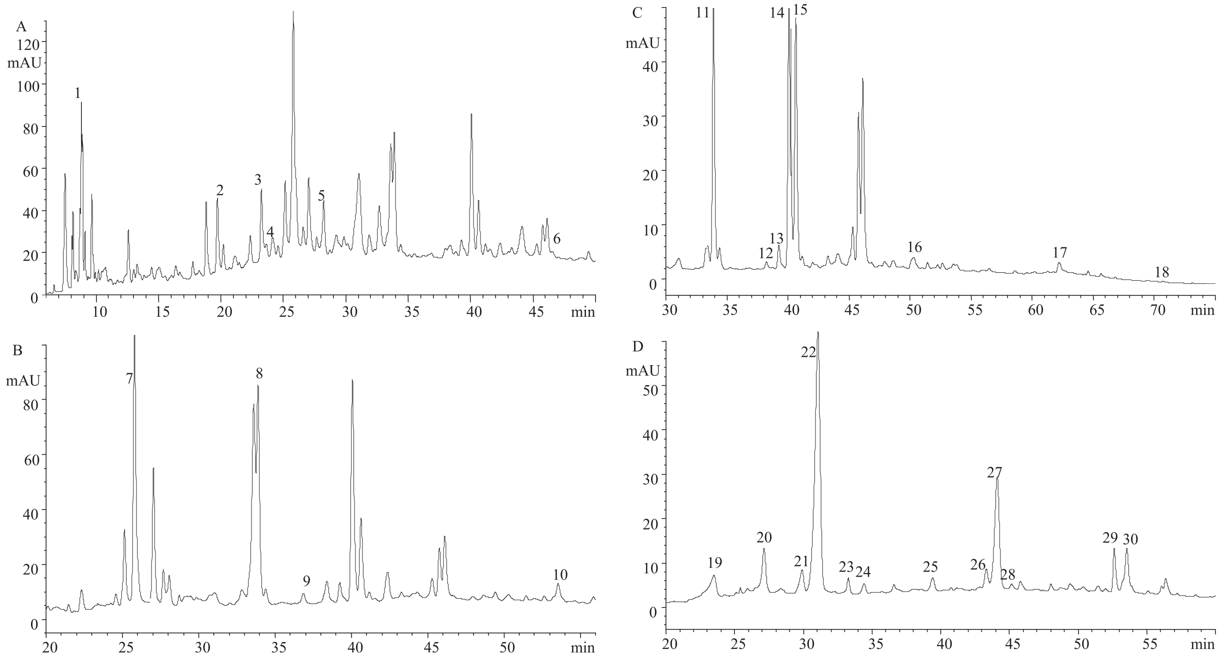

Fig. 2. Chromatograms of a Kalecik Karası red wine sample at 280 nm (A), 320 nm (B), 360 nm (C) and 520 nm (D). Peak Assignment: 1 gallic, 2 PB1, 3 Dlcatec, 4 vanill, 5 (−)-epicat, 6 o-coum, 7 caffe, 8 p-coum, 9 ferul, 10 tresv, 11 myric3G, 12 rutn, 13 Q3galact, 14 Q3glucosi, 15 Q3glucuron, 16 myric, 17 quer, 18 kaemp, 19 del3G, 20 pet3G, 21 peo3G, 22 mal3G, 23 vitA, 24 del3Ga, 25 pet3Ga, 26 peo3Ga, 27 mal3Ga, 28 del3Gc, 29 pinA, 30 mal3Gc.

with Simca-P software (v. 10.5; Umetrics Inc., Umea, Sweden). Partial least square-discriminant analysis (PLS-DA) was used to evaluate the effect of grape variety and vintage on wine chemical properties. Following the standardization, transformation to normality (if necessary) was employed on the variables to minimize skewness. Approximately 80% of the data set was chosen for model development and the remaining 20% constituted the validation set of PLS-DA models. The performance of the developed PLS-DA model to predict the wine samples in validation set was tested by the probability of the sample belonging to the model with a value greater than 10% (Simca-P). The significant variables affecting the models were determined with the variable importance in the projection (VIP) plots of PLS-DA models created by Simca software. VIP parameter greater than 1.0 indicated that the variable had a significant influence on the model (Eriksson, Johanson, Wold, & Wold, 2001, p. 504).

$$VIP_{ak} = \sqrt{\frac{n \times \sum_{l=1}^{k} w*_{al}^{2} \times SS_l(Y)}{\sum_{l=1}^{k} SS_l(Y)}}$$

where $w*_{al}^{2}$ is weighted sum of squares of the PLS weights, n is the number of terms in the model and SS (Y) is the sum of squares of that PLS dimension. The multivariate models were defined with their number of principal components, R^2 value and leave-one-out cross validation coefficient (R^2_{pred}).

Table 3
Anthocyanin concentrations of red wines (mg/L).

Phenol	Boğazkere			Cabernet Sauvignon			Kalecik Karası			Merlot			Öküzgözü			Syrah		
	[a]min	[b]max	[c]med	[a]min	[b]max	[c]med	[a]min	[b]max	[c]med	[a]min	[b]max	[c]med	[a]min	[b]max	[c]med	[a]min	[b]max	[c]med
mal3G	5.84	69.0	20.5	14.2	32.1	24.8	7.01	48.2	19.0	2.63	56.1	29.8	4.50	47.1	30.3	10.6	72.0	34.1
peo3G	0.49	8.57	1.38	0.56	2.04	1.00	0.43	2.64	0.98	0.20	6.75	2.76	0.34	7.79	1.93	1.10	10.9	2.11
pet3G	0.98	15.1	3.82	0.86	4.00	1.79	0.50	4.28	1.47	0.32	7.88	4.91	0.93	12.3	5.23	1.83	13.4	3.41
del3G	0.94	13.0	3.50	0.82	3.13	1.41	0.51	2.79	1.14	0.56	6.88	3.57	0.79	10.8	4.05	1.27	10.4	2.42
vitA	0.97	4.61	1.38	1.23	4.05	1.50	0.33	1.10	0.60	0.70	4.66	1.31	0.78	4.71	1.26	1.23	4.84	1.79
del3Ga	0.03	5.64	0.16	0.23	0.85	0.33	0.10	0.78	0.25	0.04	6.03	0.88	0.07	6.66	0.39	0.31	6.25	0.50
pet3Ga	0.13	6.17	0.43	0.32	1.07	0.54	0.16	1.07	0.40	0.06	5.93	1.21	0.14	5.88	0.69	0.42	6.13	0.84
peo3Ga	0.28	5.97	0.67	0.54	1.16	0.68	0.37	1.70	0.70	0.17	5.82	1.36	0.00	5.50	0.70	0.64	8.21	1.19
mal3Ga	0.61	15.5	3.03	4.98	13.1	9.77	1.68	16.5	5.48	0.48	19.7	10.5	0.35	11.0	4.37	3.28	28.4	10.1
del3Gc	[d]n.d.	6.36	0.62	[d]n.d.	0.38	0.21	0.07	0.87	0.24	0.05	4.82	0.52	[d]n.d.	5.30	0.63	0.16	5.70	0.38
pinA	0.51	5.00	0.95	0.51	1.23	0.78	0.44	2.89	1.26	0.19	5.19	0.63	0.27	5.15	0.99	0.31	5.37	1.17
mal3Gc	0.96	16.8	3.59	1.07	3.32	2.09	0.73	11.4	2.54	0.22	7.88	3.83	0.36	10.6	3.99	0.90	12.6	2.91
vitA/pinA	0.78	2.67	1.23	1.00	6.50	1.92	0.11	1.68	0.56	0.90	7.08	1.45	0.60	6.86	1.36	0.43	5.15	1.13
Tace	1.19	32.8	4.13	6.26	16.1	11.3	2.34	19.7	6.81	0.75	32.5	15.4	0.56	28.5	6.36	4.71	48.7	12.7
Tcoum	0.96	23.1	4.16	1.28	3.65	2.24	0.80	12.2	2.79	0.27	12.7	4.40	0.40	15.9	4.53	1.17	18.3	3.28
Tace/Tcoum	0.84	2.59	1.17	3.73	7.08	4.88	1.39	4.12	2.95	2.31	5.47	2.66	0.85	2.57	1.57	2.36	4.46	3.54

[a] Min: minimum.
[b] Max: maximum.
[c] Med: median.
[d] n.d: not detected.

3. Results and discussion

The limit of detection (LOD), limit of quantification (LOQ) and recovery values of polyphenol analysis were reported in Table 2. The most abundant anthocyanin was mal3G in red wine samples due to its high stability (Saavedra et al., 2011). Other main anthocyanin-glucosides were peo3G, pet3G and del3G (Table 3). The malvidin values were consistent with the data in the literature (Garcia-Falcon, Perez-Lamela, Martinez-Carballo, & Simal-Gandara, 2007; Gomez-Alonso et al., 2007). The malvidin-3-glucoside concentrations of Syrah, Cabernet Sauvignon and Merlot wines were in good agreement with the same cultivars of southern France (Landrault et al., 2001). There was no statistical significance among wines in terms of total coumaroylated (Tcoum) and acetylated (Tace) anthocyanin derivatives and their ratio, however the following observations could be made: Total amount of coumaroylated anthocyanins was the highest in Öküzgözü, Boğazkere and Merlot wines, the lowest in Cabernet Sauvignon and Kalecik Karası wines. The acetylated anthocyanin derivatives were higher for Syrah, Merlot and Cabernet Sauvignon wines. Relatively, the ratio of acetates to coumarates was the lowest in Boğazkere and Öküzgözü wines and the highest in Cabernet Sauvignon wines. Similarly, the same property was observed for Cencibel variety wines in Spain, as opposed to those from Merlot and Cabernet Sauvignon (Gomez-Alonso et al., 2007). VitA and pinA, the so-called pyranoanthocyanins are formed by the interaction of mal3G with pyruvic acid and caffeic acid through yeast metabolism. Due to the slow pathway of pinA, this pigment can be used as an aging indicator in red wines. VitA results were similar to the data observed in the literature (Morata, Calderon, Gonzalez, Gomez Cordoves, & Suarez, 2007). It was reported that young wines contained the maximum concentrations of vitA and trace amounts of pinA (Rentzsch, Schwarz, Winterhalter, Blanco-Vega, & Hermosin-Gutierrez, 2010). In our study, median value of the ratio of vitA to pinA (vitA/pinA) of red wine samples was greater than 1.0 for all red wine varieties except Kalecik Karası wines (0.56). The Cabernet Sauvignon and Merlot wines had significantly high vitA/pinA. The Dlcatec, gallic and (−)-epicat values of Kalecik Karası wines were lower than those of Cabernet Sauvignon and Merlot wines and similar to the study of Anli and Vural (2009). Gallic and Dlcatec were the dominant phenolic compounds in red wines in accordance with the results of Porgali and Buyuktuncel (2012). The detected flavonol compounds were rutn, quer, myric, kaemp and their glucosides (Table 4). Syrah red wines were the richest of all red wines in flavonol content. The results were in agreement with the data reported elsewhere (Anli, Vural, Demiray, & Ozkan, 2006; Garcia-Falcon et al., 2007). In white wine samples, no anthocyanin compounds were detected (Table 5). Q3galact was the most abundant flavonol in white wines. Muscat white wines could be distinguished from the other white wines with higher hydroxycinnamic acid contents (caffe, ferul and p-coum acids). On the other hand, Narince white wines were rich in flavan-3-ol content [(−)-epicat and Dlcatec] and Emir wines were rich in tresv The flavan-3-ol contents of the Narince and Emir wines were higher than those reported elsewhere (Gurbuz et al., 2007). The tresv contents of our wines were lower than those reported in the same reference. Sultaniye white wines were the poorest of all white wines in terms of polyphenol contents.

The red and white wines were statistically evaluated separately due to colorimetric and chromatographic differences. The statistical analyses for some samples having values below the limit of detection values were performed by assigning the corresponding limit of detection value.

Table 4
Polyphenol concentrations of red wines (mg/L).

Phenol	Boğazkere			Cabernet Sauvignon			Kalecik Karası			Merlot			Öküzgözü			Syrah		
	[a]min	[b]max	[c]med	[a]min	[b]max	[c]med	[a]min	[b]max	[c]med	[a]min	[b]max	[c]med	[a]min	[b]max	[c]med	[a]min	[b]max	[c]med
rutn	1.73	3.99	3.18	2.10	5.31	3.40	1.46	3.92	2.28	0.87	6.03	2.52	2.57	5.87	3.90	1.96	5.31	3.20
quer	1.35	7.71	3.59	2.89	11.4	5.46	0.48	10.1	3.93	1.79	7.55	5.78	1.20	6.73	3.64	3.15	15.8	8.40
myric	1.57	7.00	2.64	3.15	6.81	4.46	<loq	6.08	1.87	1.83	5.48	3.58	1.04	5.73	2.18	2.55	16.6	4.94
kaemp	0.54	1.35	0.68	0.19	1.07	0.67	<loq	2.73	0.82	0.40	1.18	0.62	0.53	1.32	0.69	0.56	1.75	0.90
Q3glucosi	0.98	11.3	6.27	0.18	33.9	9.37	1.94	45.9	12.9	0.43	29.0	16.9	3.19	6.56	4.67	2.19	47.0	28.9
Q3galact	0.11	4.77	2.46	2.12	8.35	5.05	1.42	11.2	7.23	0.20	9.24	5.06	1.56	12.5	2.34	2.17	12.5	8.70
Q3glucuron	5.42	11.4	9.02	0.69	19.2	9.46	4.33	17.9	9.15	3.16	26.4	13.1	3.24	12.9	9.52	9.92	20.0	14.1
myric3G	0.87	19.0	11.8	0.00	32.0	13.5	2.58	39.6	12.1	0.00	27.7	15.6	5.49	12.2	9.86	10.8	40.4	30.3
caffe	13.3	38.3	20.4	4.19	31.6	10.4	8.62	37.8	20.5	4.20	36.3	11.7	8.19	44.1	13.0	4.61	25.6	16.3
p-coum	3.48	9.66	6.26	4.74	11.1	7.87	3.15	11.1	5.87	2.68	10.4	7.05	3.19	16.5	4.89	2.17	11.8	7.61
ferul	0.97	3.02	1.68	0.63	2.93	1.21	0.73	1.71	1.16	0.56	1.96	1.01	0.49	1.82	1.23	0.31	1.45	1.03
tresv	0.19	1.02	0.24	0.19	0.60	0.39	0.13	0.56	0.38	0.15	0.78	0.40	0.21	1.14	0.48	0.17	0.75	0.50
gallic	17.0	83.4	51.8	38.6	120	47.0	13.7	45.5	27.2	23.5	79.0	47.0	45.6	110	61.5	18.6	68.7	36.9
Dlcatec	27.6	41.0	35.5	35.2	99.6	44.0	26.3	48.5	38.5	34.3	153	59.7	28.5	90.9	51.3	30.7	70.9	37.1
vanill	5.56	9.74	6.38	4.48	11.0	6.24	5.12	10.8	6.76	4.08	9.97	6.61	4.64	8.55	5.92	4.15	8.95	7.00
(−)-epicat	7.16	15.0	10.9	17.6	53.2	25.7	9.03	28.4	17.2	18.1	81.9	33.1	7.20	26.9	14.4	18.6	42.0	23.0
o-coum	0.46	3.82	2.97	0.93	2.01	1.43	0.57	1.47	1.15	0.23	2.11	1.36	0.25	3.71	2.12	0.59	1.26	1.01
PB1	21.1	39.3	33.1	23.7	75.4	42.8	8.16	50.9	42.2	30.9	99.2	43.3	27.1	70.6	44.0	24.2	62.4	30.4
TP	2370	3904	2996	2284	3355	2427	1522	2487	1858	1646	3364	2447	926	2849	1988	1962	3150	2193

[a] Min: minimum.
[b] Max: maximum.
[c] Med: median.

Table 5
Polyphenol concentrations of white wines (mg/L).

Phenol	Emir			Chardonnay			Narince			Muscat			Sultaniye		
	^{a}min	^{b}max	^{c}med	^{a}min	^{b}max	^{c}med	^{a}min	^{b}max	^{c}med	^{a}min	^{b}max	^{c}med	^{a}min	^{b}max	^{c}med
rutn	^{d}<loq	1.26	0.07	^{d}<loq	0.79	0.12	^{d}<loq	0.27	0.11	^{d}<loq	0.22	0.11	^{d}<loq	0.25	0.06
quer	^{d}<loq	2.81	1.17	^{d}<loq	3.01	0.33	0.57	6.47	1.65	^{d}<loq	8.07	0.87	^{d}<loq	0.99	0.29
myric	^{d}<loq	2.56	0.02	^{d}<loq	1.21	0.02	^{d}<loq	0.58	0.02	^{d}<loq	0.02	0.02	^{d}<loq	0.38	0.02
kaemp	^{d}<loq	0.92	0.03	^{d}<loq	1.04	0.03	^{d}<loq	0.94	0.03	^{d}<loq	0.93	0.03	^{d}<loq	0.10	0.03
Q3glucosi	^{d}<loq	1.39	0.10	^{d}<loq	2.17	0.13	^{d}<loq	3.43	0.04	^{d}<loq	1.25	0.02	^{d}<loq	2.18	0.02
Q3galact	^{d}<loq	12.1	2.18	^{d}<loq	17.1	1.54	^{d}<loq	15.1	0.80	^{d}<loq	14.1	2.24	^{d}<loq	1.72	0.06
Q3glucuron	0.11	4.97	0.52	^{e}n.d.	2.53	0.10	^{e}n.d.	3.05	0.90	^{e}n.d.	1.26	0.04	^{e}n.d.	0.61	0.00
myric3G	^{e}n.d.	0.21	0.00	^{e}n.d.	0.30	0.00	^{e}n.d.	0.18	0.00	^{e}n.d.	0.22	0.00	^{e}n.d.	0.11	0.00
caffe	1.54	8.32	4.49	1.10	5.96	2.94	2.65	21.0	4.94	1.90	22.4	15.1	0.37	1.99	1.38
p-coum	0.87	4.65	2.03	0.43	3.06	1.52	1.05	6.32	1.48	2.00	13.6	9.85	0.08	1.19	0.60
ferul	0.36	1.12	0.72	0.33	0.93	0.68	0.50	1.44	0.75	0.63	1.43	0.97	0.06	0.70	0.47
tresv	0.07	0.71	0.21	0.04	0.14	0.07	0.03	0.29	0.13	^{d}<loq	0.22	0.12	^{d}<loq	0.08	0.03
gallic	^{d}<loq	15.1	9.48	^{d}<loq	17.2	10.0	^{d}<loq	33.1	12.4	^{d}<loq	12.1	7.21	^{d}<loq	17.2	10.7
Dlcatec	^{d}<loq	9.63	6.02	^{d}<loq	12.8	5.04	^{d}<loq	13.1	10.0	^{d}<loq	9.26	5.22	^{d}<loq	4.46	2.72
vanill	0.91	1.96	1.32	0.48	1.69	0.94	0.70	2.47	1.30	^{d}<loq	1.31	0.67	0.37	1.06	0.62
(−)-epicat	1.67	4.90	2.37	^{d}<loq	6.09	2.23	1.40	4.37	2.71	^{d}<loq	3.11	1.83	^{d}<loq	2.22	1.35
o-coum	^{d}<loq	2.88	1.68	^{d}<loq	0.99	0.19	^{d}<loq	1.84	0.83	^{d}<loq	1.33	0.32	0.24	0.62	0.40
PB1	1.81	6.46	4.19	0.81	7.98	3.84	1.38	6.63	4.26	1.03	4.12	1.73	1.50	5.29	2.40
TP	240	527	310	194	414	288	236	416	345	264	369	316	178	317	212

[a] Min: minimum.
[b] Max: maximum.
[c] Med: median.
[d] loq: limit of quantification.
[e] n.d: not detected.

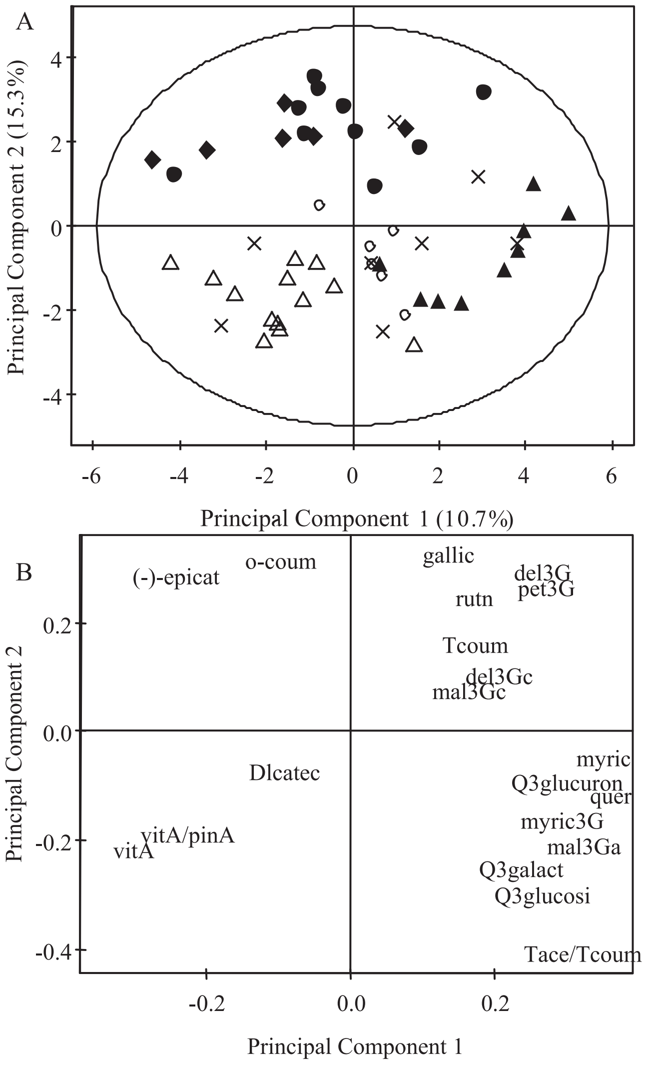

Fig. 3. The PLS-DA scores (A) and loadings (B) plots of varietal discrimination of red wines: ◆:Boğazkere, ○: Cabernet Sauvignon, Δ: Kalecik Karası, ×: Merlot, ●: Öküzgözü, ▲: Syrah.

3.1. Varietal discrimination

The phenolic variables used in building multivariate models were determined with the VIP statistics of the SIMCA software, which gave significantly effective variables in the classification. The PLS-DA model of red wines was developed with 20 polyphenol variables (all flavonol compounds except kaemp and all flavan-3-ol compounds, gallic, o-coum, and vitA, all malvidin compounds except their acetate derivatives, mal3G and peo3G). The model with three principal components produced a regression coefficient of Y matrix ($R^2{}_Y$) of 0.364 and a leave-one-out cross validation coefficient ($R^2{}_{pred}$) of 0.274. The classes were established for Boğazkere (6 observations), Cabernet Sauvignon (6 observations), Kalecik Karası (13 observations), Merlot (8 observations), Öküzgözü (10 observations) and Syrah (9 observations). The scores plot yielded clusters of all varieties except the Merlot wines (Fig. 3A). The first principal component (PC) was responsible for the discrimination of Kalecik Karası and Syrah wines and was influenced by quer, myric, (−)-epicat and vitA (Fig. 3B). The Syrah wines were richer in flavonol content and vitA. Cabernet Sauvignon wines together with Syrah wines were clustered close to each other based on their high ratio of total acetylated to total coumaroylated compounds (Tace/Tcoum). Boğazkere and Öküzgözü wines could be discriminated from the remaining varieties via second PC with higher values of malvidins and coumaroylated derivatives (pet3G, del3G, mal3Gc and del3Gc), gallic, o-coum and lower values of (−)-epicat and Tace/Tcoum ratio. The observations in the validation set (13 samples) were correctly predicted with membership probability values between 0.21 and 0.98.

Boğazkere and Öküzgözü are the two native grape varieties widely grown in the eastern Anatolia (Diyarbakır and Elazığ) regions, which are used for high quality wine production in Turkey (Kelebek et al., 2006). The majority of Boğazkere and Öküzgözü wines in this study were from the eastern wine growing regions (Diyarbakır and Elazığ). There were only four wines from central Anatolia (Cappadocia and Tokat). Their cluster indicated that these two varieties were similar based on their high gallic, o-coum, del3G contents and their low Tace/Tcoum, Q3glucosi, Q3galact and (−)-epicat contents. The statistical analysis showed the discrimination of these two native varieties from the remaining wines; it can be concluded that Boğazkere and Öküzgözü wines define the characteristics of red wines from eastern Anatolia (Diyarbakır and Elazığ). The Syrah wines collected from the western Anatolia (Denizli and Manisa) were also rich in anthocyanins. Moreover Syrah wines were rich in flavonol-glucosides unlike Boğazkere and Öküzgözü wines. Kalecik Karası wines of another important native grape variety have been collected from different regions (Denizli, İzmir, Thrace, Ankara). These wine samples could be discriminated from other wines of İzmir, Denizli and Manisa (Syrah and Cabernet Sauvignon varieties) based on their low contents of polyphenols, vitA/pinA, and malvidin compounds. These findings indicate that the grape variety has more influence on the phenolic character of wines than the geographical origin.

The PLS-DA model of white wines was developed with 9 polyphenol variables (TP, all flavan-3-ols, all phenolic acids excluding gallic and o-coum). The model with three principal components produced an R^2_Y of 0.383 and R^2_{pred} of 0.272. The classes were established for Emir (8 observations), Chardonnay (8 observations), Narince (8 observations), Muscat (8 observations) and Sultaniye (6 observations). According to the first PC, Emir and Narince wines overlapped each other; on the other hand the discrimination of this group from the Muscat and Sultaniye wines was clear (Fig. 4A). Chardonnay and Sultaniye wines could be discriminated from the remaining varieties based on the lower concentrations of caffe, p-coum, and ferul. The second PC discriminated the Muscat wines from the remaining varieties with the higher concentrations of p-coum, ferul, caffe and lower concentrations of PB1 and gallic of Muscat wines. With the first and second PCs, the clusters of Emir and Narince wines could be explained with their higher concentrations of PB1 and vanill, than the other wines (Fig. 4A and C). On the other hand, the scores plot between the first and third PC showed the discrimination between the Emir and Narince wines based on higher tresv content of Emir wines and higher Dlcatec content of Narince wines (Fig. 4B and D). The membership probability values of white wine samples (9 samples) in the validation set were between 0.16 and 0.84 indicating correct predictions.

Flavonols exerted no impact on the varietal discrimination of the white wines. Emir and Narince are the two native varieties of *Vitis vinifera* grown in Cappadocia and Tokat (central Anatolia), respectively, whereas Sultaniye is widely grown in the western Anatolia (Denizli and İzmir). In this study, Emir wines of Cappadocia and Narince wines of Tokat regions were discriminated from Muscat and Sultaniye wines of western Anatolia. This discrimination can be explained by high vanill and PB1 contents of Emir and Narince wines. On the other hand, Muscat and Sultaniye white wines could be clearly discriminated from each other even though they all originated from the western regions (Denizli-Manisa-İzmir) due to the highest amount of hydroxycinnamic acids of Muscat wines among other white wines.

3.2. Vintage discrimination

For the discrimination of red wines with respect to harvest year, four classes were built with 14, 17, 15 and 6 observations for the 2006, 2007, 2008 and 2009 vintages, respectively. The model with four principal components produced an R^2_Y of 0.580 and R^2_{pred} of 0.434 (Fig. 5A). 2009 harvest year wines could be discriminated from the remaining wines with higher vitA, pinA and malvidin levels (Fig. 5B). 2006 and 2007 vintage wine's discrimination from

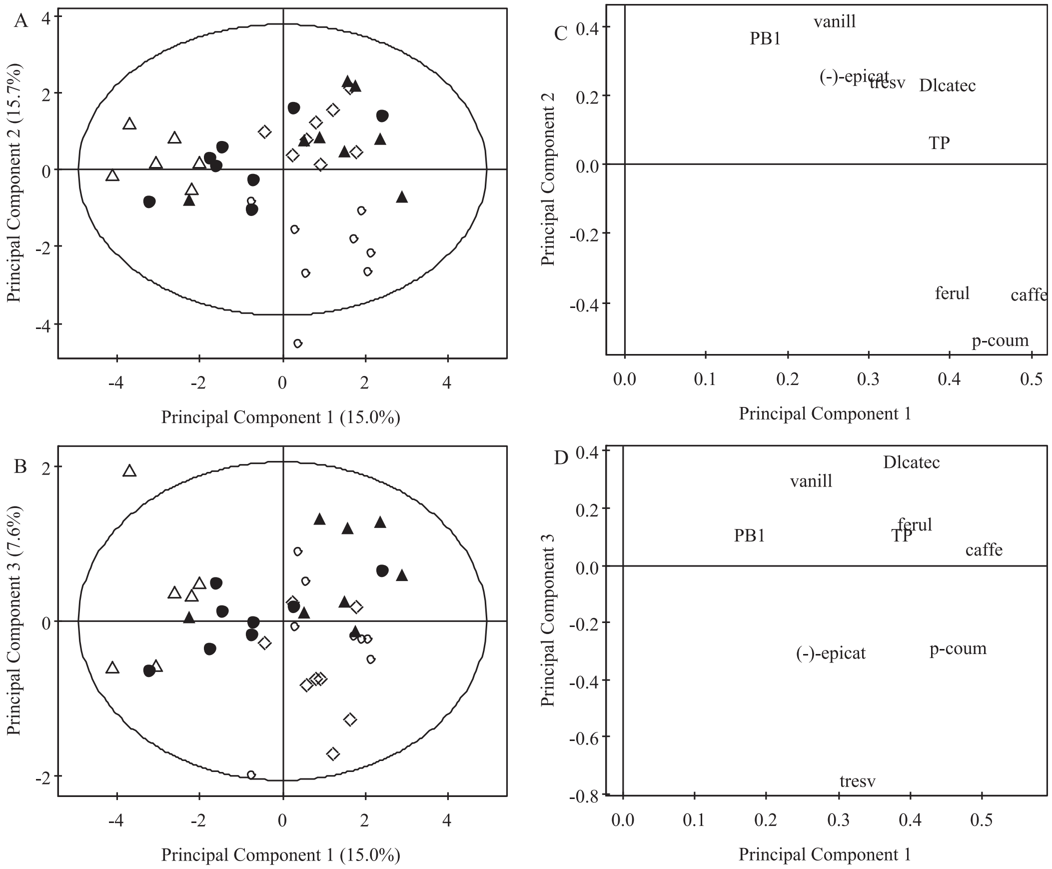

Fig. 4. The PLS-DA scores (A, B) and loadings (C, D) plots of varietal discrimination of white wines: (A, C) PC1 vs PC2, (B, D) PC2 vs PC3. ◊:Emir, ●: Chardonnay, ▲: Narince, ○: Muscat, Δ: Sultaniye.

the 2008 was enhanced by the second PC based on the greater values of tresv and lower values of p-coum of the 2006–2007 vintage wines than the 2008 wines.

The model for the discrimination of white wines included again four classes with 9, 11, 9 and 8 observations for the 2006, 2007, 2008 and 2009 vintages, respectively. The model with three principal components produced an R^{2}_{Y} of 0.524 and R^{2}_{pred} of 0.348 (Fig. 6A). 2006 and 2007 vintage wines could be discriminated from the 2008 and 2009 with the first PC based on the higher values of Q3galact and Dlcatec of 2008 and 2009 vintage wines (Fig. 6B). 2008 discriminated itself from the 2006 and 2009 vintage wines according to the second PC depending on the higher values of ferul, vanill and o-coum of 2008 vintage wines.

The meteorological data have been collected from Meteorological Service of Turkish State for the four harvest years to investigate the effect of climate conditions of wine growing regions on the polyphenol synthesis of berry at different growth periods (http://www.dmi.gov.tr/index.aspx). According to the climatic data, the average temperature values (°C) for the veraison periods (August) of four harvest years were the highest for the western and eastern regions: Denizli (29.4), İzmir (28.8), Manisa (29.2), Diyarbakır (31.1) and Elazığ (27.4). It was the lowest for Cappadocia (23.4). The average sunshine values (h/day) of August in Cappadocia was 11.7, as the highest of all regions. The tresv content was significantly high in Emir wines of Cappadocia region and low in the Muscat, Chardonnay and Sultaniye wines of Denizli and İzmir regions. According to a study by Cassidy, Hanley, and Lamuela-Raventos (2000), the factors affecting the tresv amounts in wine include grape variety, climatic conditions and UV light exposure. The high insolation and low temperature characteristics of Cappadocia region and Emir grape variety might have influenced the high concentrations of tresv in Emir wines of Cappadocia region. The high tresv concentrations of Emir wines in comparison to other white wines were also reported elsewhere (Gurbuz et al., 2007).

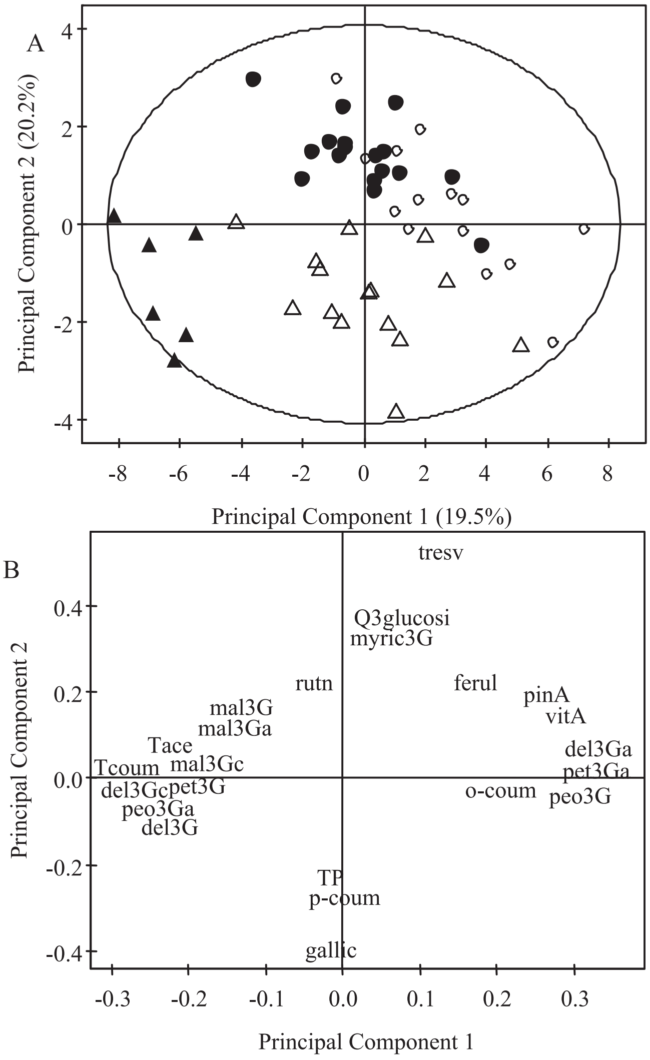

Fig. 5. The PLS-DA scores (A) and loadings (B) plots of harvest year discrimination of red wines. ○: 2006, ●: 2007, Δ: 2008, ▲: 2009.

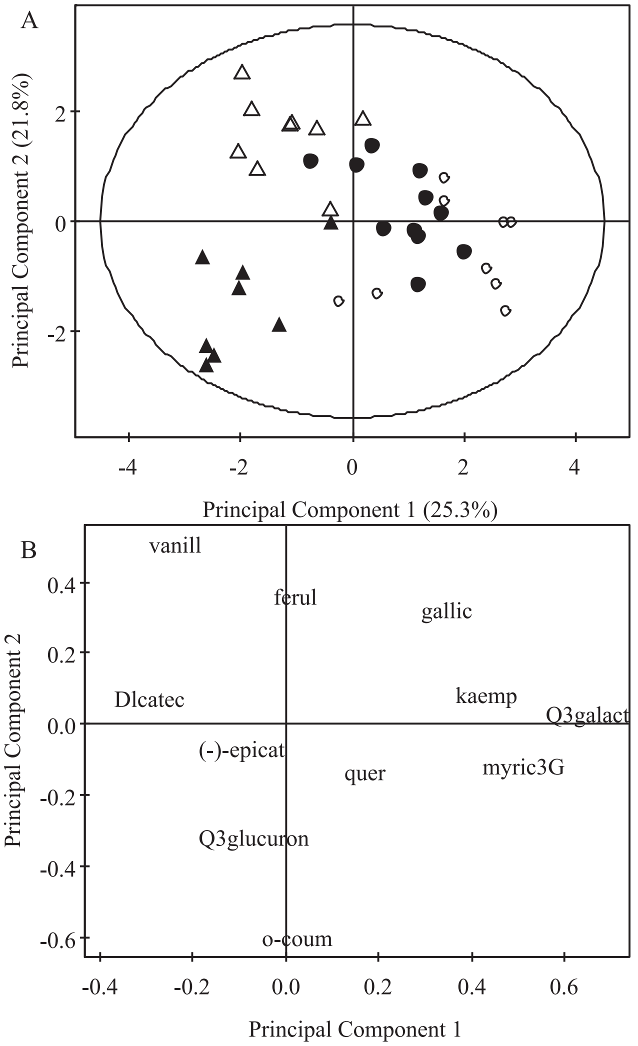

Fig. 6. The PLS-DA scores (A) and loadings (B) plots of harvest year discrimination of white wines. ○: 2006, ●: 2007, Δ: 2008, ▲: 2009.

The vintage classification of red wines in this study indicated that malvidin compounds had the most significant impact on the model. The maximum anthocyanin concentrations were observed in 2009 wine samples. The average total rainfall (mm) for 2009 was minimum (1.5) in the veraison period, whereas average total rainfall amounts for the 2006, 2007 and 2008 vintages at the same period were 6.4, 6.6 and 10.9, respectively (It should be noted that, the highest average precipitation (mm) at flowering season, June and July, occurred in 2009 as 22.3, as opposed to the lowest rainfall statistics in August 2009. For the previous years, on the other hand, the precipitation in june and july was between 10.8 and 17.2 mm). The increase in the anthocyanin contents of 2009 vintage red wines might be based on the increased biosynthesis of anthocyanins at water deficiency during veraison period (August). It was reported that the anthocyanins along with the flavonols were synthesized via the phenylpropanoid pathway during the veraison period. The vine water stress significantly affected the berry development and composition (Van Leeuwen et al., 2004). Anthocyanin content of Syrah grapes increased after the beginning of veraison period and strong water deficiency between the veraison and harvest periods increased the biosynthesis of anthocyanins as reported elsewhere (Ojeda, Andary, Kraeva, Carbonneau, & Deloire, 2002). In the same study it was also found that strong levels of early water deficit before the veraison had adverse effects on the anthocyanin and flavonol synthesis in the berries. In this study, the 2009 vintage white wines were recognized with higher flavonol-glucoside levels. This result may be linked to the high rainfall before veraison.

4. Conclusion

Authentication and labeling has been gaining importance for wines. Phenolic profiles can serve as a fingerprint of wines of certain grape varieties or regions. The monovarietal wines produced from native and nonnative grapes grown in Turkey were characterized based on their polyphenol contents. Multivariate statistical technique, PLS-DA was useful to show differences and similarities of wines based on grape cultivar and vintage. These main phenolic characters of wines can be used for their authenticity. Boğazkere wines had the highest total phenol content. On the other hand, Cabernet Sauvignon, Merlot and Syrah had higher amounts of epicathecin and cathechin than native varieties. Kalecik Karası red wines and Sultaniye white wines were found to be poorest in total phenolic content. Muscat wines had the highest content of hydroxycinnamic acids. The wines of 2009 harvest year were shown to have the highest amount of anthocyanins and flavonol contents.

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