Available online at www.sciencedirect.com
SCIENCE DIRECT
Food Chemistry 100 (2007) 518–525
Food Chemistry
www.elsevier.com/locate/foodchem

Determination of *flavan-3-ols* and *trans*-resveratrol in grapes and wine using HPLC with fluorescence detection

Ozan Gürbüz [a,*], Duygu Göçmen [a], Fatih Dağdelen [a], Murat Gürsoy [b], Sami Aydin [b], İsmet Şahin [a], Levent Büyükuysal [b], Mehmet Usta [c]

[a] *Uludag University, Faculty of Agriculture, Department of Food Engineering, Gorukle Campus, 16059 Bursa, Turkey*
[b] *Uludag University, Faculty of Medicine, Department of Pharmacology, 16059 Bursa, Turkey*
[c] *Department of Nephrology, 16059 Bursa, Turkey*

Received 25 July 2005; received in revised form 11 October 2005; accepted 19 October 2005

Abstract

Concentrations of *trans*-resveratrol, catechin and epicatechin were analyzed in musts and wines produced from seven red and four white grape cultivars from various wine growing regions of Turkey. Phenolics were quantified using an HPLC method optimized for the separation of wine phenolics. Wine samples contained higher phenolics levels than the corresponding musts. With the exception of Semillion, white wines and musts contained lower concentrations of phenolics than red wines and musts. However, the white cultivar Semillion had the highest concentrations of catechin and epicatechin among all wine and must samples. Semillion wine catechin and epicatechin were 13.7 and 11.8 mg/L, respectively. The highest level of *trans*-resveratrol among the white cultivars was found in Narince wine (1.93 mg/L). Within the red wine and must cultivars, Boğazkere, Öküzgozü, and Cabernet contained the highest concentrations of *flavan-3-ols* and *trans*-resveratrol. Catechin was the major phenolic in all wines and most musts. Epicatechin was the major phenolic in 6 of the 11 must samples, but none of the wine samples. *trans*-Resveratrol was generally found in lowest concentrations in both wines and musts.

Keywords: Catechin; Epicatechin; *trans*-Resveratrol; Wine

1. Introduction

The amount and types of phenolics present in wines and juice may play an important role in controlling oxidation in the human body. Phenolic compounds, primarily flavanols, have antioxidant properties which may be the source of putative health benefits derived from wine consumption. Wines contain a wide range of polyphenolic constituents that have reported anticancer, and anti-inflammatory effects in vitro, as well as the ability to block cellular events predisposing to atherosclerosis and coronary heart disease (CHD), (Aznar, Lopez, Cacho, & Ferreira, 2001; Faustino, Sobrattee, Edel, & Pierce, 2003; Franco et al., 2002; Goldberg, Karumanchiri, Soleas, & Tsang, 1999; Gonzalez-Paramas, Esteban-Ruano, Santos-Buelga, De Pascual-Teresa, & Rivas-Gonzalo, 2004; Guendez, Kallithraka, Makris, & Kefalas, 2005; Jeandet et al., 1997; Monagas, Bartolome, & Gomez-Cordoves, 2005; Teissedre & Landrault, 2000; Vitrac, Monti, Vercauteren, Deffieux, & Merillon, 2002). In France, CHD mortality is lower than in other industrialized countries, even though the dietary intake of saturated fat is higher. Regular consumption of red wine has been hypothesized to be the most likely cause for this phenomenon known as the "*French Paradox*" (Adrian et al., 2000a; Aznar et al., 2001; Burns et al., 2001; Faustino et al., 2003; Gambelli & Santaroni, 2004; Kolouchova-Hanzlikova, Melzoch, Filip, & Smidrkal, 2004; Landrault et al., 2001; Monagas et al., 2005; Saucier

* Corresponding author. Tel.: +90 224 442 89 70; fax: +90 224 442 80 77.
E-mail address: ozang@uludag.edu.tr (O. Gürbüz).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.10.008

& Waterhouse, 1999; Teissedre & Landrault, 2000; Vinas, Lopez-Erroz, Marin-Hernandez, & Hernandez-Cordoba, 2000).

Grapes contain a large amount of various phenolic compounds in the skins, pulp and seeds. During fermentaion and aging, wine compounds undergo structural transformations through oxidation and condensation reactions which have an impact influence on wine astringency and color (Gonzalez-Manzano, Rivas-Gonzalo, & Santos-Buelga, 2004; Monagas et al., 2005). The extension of maceration time (skin contact) and fermentation periods increases phenolic content, but also increases undesirable quality components such bitterness, astringency and off-flavors.

Concentrations of phenolic compounds increase during wine fermentation which employs commercial and endogenous enzymes (Vazquez, Perez-Coello, & Cabezudo, 2002). However, the occurrence of phenolic substances in wines is not only a consequence of their extraction from grapes during winemaking. These compounds are present in complex polymeric and glycosidic forms that are not easily degraded by digestive juices and so their absorption into the body is limited. During fermentation, these aggregates are broken down to monomeric forms. This beneficial effect is important from a nutritional point of view and so it would be useful to find specific chemical parameters that could be used to develop a "*nutritional card*" for wine (Gambelli & Santaroni, 2004).

Once grapes are crushed and before the beginning of alcohol fermentation, several condensation reactions take place which involve anthocyanins, catechins, and procyanidins, and result in the formation of new polymeric pigments (Revilla & Ryan, 2000). Therefore, the final composition of polyphenol compounds in wine depends on the polyphenols contained in the grapes, the extraction parameters, wine production methods as well as chemical reactions taking place during wine ageing (Czyzowska & Pogorzelski, 2004; Garciaviguera & Bridle, 1995; Sun, Spranger, Roque-do-Vale, Leandro, & Belchior, 2001).

Flavan-3-ols or flavanols are found in the solid parts of the berry (seed, skin and stem) in monomeric, oligomeric, or polymeric forms; the latter two forms are also know as proanthocyanidins or condensed tannins (Monagas et al., 2005). Flavanols, the other phenolic components of wine, also have antioxidant properties (Guendez et al., 2005; Vinas et al., 2000). It has also been found that alcohol and SO_2 remarkably enhanced antioxidant properties of flavanols (Saucier & Waterhouse, 1999). The neutral phenolics are composed of *flavan-3-ols*, with catechin and epicatechin as the two major compounds (Auw, Blanco, Okeefe, & Sims, 1996; Blanco, Auw, Sims, & O'Keefe, 1998). Catechin and procyanidins have been shown to be powerful in vitro inhibitors of LDL oxidation and platelet aggregation (Careri, Corradini, Elviri, Nicoletti, & Zagnoni, 2003; Gambuti, Strollo, Ugliano, Lecce, & Moio, 2004; Gonzalez-Paramas et al., 2004; Padilla et al., 2005; Teissedre & Landrault, 2000). Minussi et al. (2003) & Mitjans et al. (2004) reported that the antioxidant and immunomodulatory activity of wine has a positive correlation with catechin and epicatechin concentration. Catechins have many other beneficial health effects such as antitumorgenic, anti-mutagenic, anti-pathogenic and anti-oxidative properties (Buettner, 2004).

Phytoalexins occur naturally in various families of plants, but grapes and related products are considered the most important dietary source of these substances (de Lima et al., 1999). They are stilbenes which include resveratrol (*trans*-3,5,4′-tri-hydroxystilbene), pterostilbene, piceid and viniferins (Adrian et al., 2000a; Adrian, Jeandet, Douillet-Breuil, Tesson, & Bessis, 2000b). Stilbenes concentrations can vary depending on factors such as grape cultivar, mechanical injury, fungal infection mainly by *Botrytis cinerea* (Careri et al., 2003; Jeandet et al., 1997; Kolouchova-Hanzlikova et al., 2004), vinification procedures (de Lima et al., 1999; Dixon, 2001), environmental conditions (temperature, humidity, latitude, height above sea level and geochemical characteristics) (Gambelli & Santaroni, 2004; Gambuti et al., 2004) and abiotic stresses such as ultraviolet (UV) (Adrian et al., 2000a; Careri et al., 2003).

In recent years, it has been discovered that resveratrol has several biological effects, including anticancer activity for certain cancer types, cardio protection activity (Adrian et al., 2000a; Aznar et al., 2001; Blanco et al., 1998; Careri et al., 2003; Franco et al., 2002; Jeandet et al., 1997; Mitjans et al., 2004; Padilla et al., 2005), antioxidant activity and inhibition of platelet aggregation, as well as anti-inflammatory activity (Gambuti et al., 2004; Padilla et al., 2005). There is increasing interest in resveratrol research owing to its pharmacological activity (Dourtoglou, Makris, Bois-Dounas, & Zonas, 1999; Zhou et al., 2004).

Because of their similar chemical characteristics, flavanols have been difficult to separate and quantify. However, HPLC can provide different retention times to allow the identification of these compounds (Garciaviguera & Bridle, 1995) and fluorescence detections should provide the necessary selectivity for accurate quantification.

The objective of this study was to determine the relative concentrations of phenols such as *trans*-resveratrol, catechin and epicatechin in both wines and musts from various red and white grape cultivars grown in Turkey.

2. Materials and methods

2.1. Chemicals and standards

Acetonitrile and acetic acid of HPLC-grade were obtained from Merck (Darmstadt, Germany). HPLC-grade methanol was purchased from Carlo Erba Reagent (Milan, Italy). Standards of (+)-Catechin, (−)-Epicatechin and *trans*-resveratrol were supplied from Sigma (St. Louis, Missouri, USA). Pectinex 2000 L and Pectinex AR were obtained from Novo Ferment (Switzerland). Potassium meta-bisulfide was purchased from Merck (Darmstadt,

Germany). *Saccharomyces bayanus* (67 J INRA Narbonne) was used in wine fermentation as yeast starter and it was supplied from Fermicamp (France). Stock solutions (1 mg/mL) were prepared by dissolving 2.5 mg of the commercial product in 2.5 mL of methanol. They were kept in dark bottles at 4 °C. Working solutions of each standard were prepared by dilution with distilled water 1/10 (v/v) just prior to analyzing.

2.2. Must and wine preparation

Eleven grape cultivars used in this study were supplied from five different vineyard regions in Turkey and harvested in 2003. Seven red wines and four white wines were produced from these cultivars. Each grape cultivar was obtained from regions where growing conditions were most favorable for that cultivar.

Each cultivar was harvested at the optimal stage of ripening for commercial use from selected locations then transferred and processed in the pilot scale winery at our department. Pectinex 2000 L (0.05%) and Pectinex AR (0.05%) were added to the pulp after fruit disintegration. The maceration and pectinolysis process lasted 4 h. After maceration, some parts of the must samples were analyzed prior to fermentation. Potassium meta-bisulfide was added (50 mg $S0_2$ kg^{-1}) to the mash and pressed in a vertical press. The must was inoculated with 2% yeast starter (6×10^8 CFU/mL). Vinification procedures were completed for each cultivar must by dividing it into three 50 L flasks and storing them at 17 ± 2 °C until the sugar was consumed. Other steps followed were the same as classic wine making practices; wines were racked and bottled following filtration and kept in cold stabilization (−4 °C) for two weeks. They were then stored in cellar conditions for 3 months prior to analyzing.

2.3. Chromatographic procedure

trans-Resveratrol, catechin and epicatechin levels in wine and must samples were measured by a HPLC system (HP 1100 series, Hewlett–Packard, Palo Alto, CA, USA) after small modifications of a previously reported method (Vinas et al., 2000). This system was combined with a quaternary pump (HP, G1311A), a fluorometric detector (HP, G1321 A) and an autosampler (HP, G1329 A). *trans*-Resveratrol, catechin and epicatechin were separated on C18 Hypersil H5 ODS column (Phenomenex, Aschaffenburg, Germany) (250×4.6 mm i.d.) with gradient elution of three mobile phases (flow rate 1 mL min^{-1}). The mobile phases consisted of (A) 9% acetonitrile, 91% (5%: aqueous acetic acid) (v/v); (B) 25% acetonitrile, 75% (5%: aqueous acetic acid) (v/v); (C) 70% acetonitrile, 30% (5%: aqueous acetic acid) (v/v), respectively. The step gradient started with 100% mobile phase A for 10 min at a wavelength ($\lambda_{Ex}/\lambda_{Em}$) 280/315 nm (for catechin and epicatechin), step gradient to 100% mobile phase B in 1 min and held for 11 min at a wavelength ($\lambda_{Ex}/\lambda_{Em}$) 324/370 nm (for *trans*-resveratrol). To prepare the column for the next run, mobile phase C and then mobile phase A were run for 5 min and 15 min, respectively, after each injection.

Wine and must samples (0.5 mL) were centrifuged at 10,000*g* for 5 min and were placed in a thermostat controlled autosampler (+4 °C). Hundred micro liters of samples and standards (0,10,100 and 1000 ng/mL) was injected directly into the column. All measurements were performed in triplicate ($n = 3$), values were averaged and the standard deviation calculated.

trans-Resveratrol, catechin and epicatechin peaks were identified by the comparison of retention times and UV spectrums with commercial standards of phenolic compounds. To evaluate the efficiency of the HPLC procedure, each sample was also spiked with standards (1000 ng for must and 100 ng for wine samples). Chromatograms were analyzed with the HP Chemstation software, (Revision A.08.03.847) and quantification was done using linear regression analysis (Pharmacological Calculation System, Version 4.0).

2.4. Statistical analysis

The concentration of *flavan-3-ols* and *trans*-resveratrol were measured and analyzed using one-way ANOVA. Values ($n = 3$) were reported as mean concentration ± standard deviation. The SD was calculated by analysis of variance using the Minitab statistical package (Minitab Inc., PA). Furthermore, Duncan's multiple range test was used to determine the differences ($p < 0.01$) between variances by using the MSTAT statistical package (N. Drinkwater, Madison, WI).

3. Results and discussion

During the process of wine preparation significant changes take place in the composition and content of phenolic compounds resulting from the process of fruit disintegration as well as wine fermentation and ageing (Lopez-Velez, Martinez-Martinez, & Del Valle-Ribes, 2003). The total polyphenol content is highest for wines made using an enzymatic treatment (Czyzowska & Pogorzelski, 2004). Low molecular weight catechins could significantly participate in the reducing or antioxidant power of red wine and could directly correlated with its concentration (Katalinic, Milos, Modun, Music, & Boban, 2004). Although the final composition of wine phenolic compounds can be altered by must extraction and wine production methods the major factor appears to be the amount of polyphenols contained in the initial grape cultivar.

Enzymatic maceration and yeast fermentation were used in this study as a processing method during wine production. This method was chosen because more phenolic compounds are extracted from the grapes and undesirable effects in the wine quality, such as secondary metabolites

Table 1
Mean levels of catechin, epicatechin and resveratrol in the analyzed must samples

Cultivars	Color[B]	Location	Region	Concentration of phenols (mg/L)[A]		
				Catechin	Epicatechin	Resveratrol
Kalecikkarası	R	Ankara	MA	0.044 ± 0.003^{cdC}	0.160 ± 0.0057^{c}	0.0056 ± 0.0014^{c}
Çalkarası	R	Denizli	A	0.812 ± 0.01^{b}	0.335 ± 0.0086^{bc}	0.0007 ± 0.0001^{c}
Boğazkere	R	Diyarbakır	EA	0.262 ± 0.01^{c}	0.224 ± 0.0084^{bc}	0.0246 ± 0.013^{bc}
Öküzgözü	R	Elazığ	EA	1.014 ± 0.031^{b}	0.652 ± 0.013^{b}	0.102 ± 0.033^{b}
Cabernet sauvignon	R	Tekirdağ	T	0.055 ± 0.002^{cd}	0.214 ± 0.0025^{c}	0.0004 ± 0.0003^{c}
Cinsaut	R	Tekirdağ	T	0.113 ± 0.004^{cd}	0.098 ± 0.0046^{c}	0.001 ± 0.00003^{c}
Merlot	R	Tekirdağ	T	0.019 ± 0.0005^{d}	0.099 ± 0.0006^{c}	0.0008 ± 0.000006^{c}
Emir	W	Ankara	MA	0.036 ± 0.0023^{cd}	0.04 ± 0.0006^{c}	0.291 ± 0.0008^{a}
Narince	W	Tokat	BS	0.054 ± 0.015^{cd}	0.110 ± 0.0011^{c}	0.023 ± 0.0017^{bc}
Clairette	W	Tekirdağ	T	0.067 ± 0.0072^{cd}	0.157 ± 0.004^{c}	0.02 ± 0.00003^{c}
Semillion	W	Tekirdağ	T	6.829 ± 0.144^{a}	4.527 ± 0.194^{a}	0.0082 ± 0.0004^{c}

[A] Mean values and ±S.D. ($n = 3$) expressed in mg/L.
[B] R: red; W: white; MA: middle anatolia; A: aegean; EA; east anatolia; T: thrace; BS: black sea.
[C] Different superscripts within the same column for each value mean significant difference $p < 0.01$.

and bacterial fermentation, do not occur. Table 1 shows the grape sample originations from five different vineyard regions.

Except for Semillion, red grape cultivars generally had higher levels of catechin, epicatechin and *trans*-resveratrol compared to white cultivars. White Semillion grapes had the highest concentration of catechin (6.829 mg/L) and epicatechin (4.527 mg/L), however, Emir had the greatest *trans*-resveratrol levels (0.291 mg/L). Semillion white grapes had contained the highest *flavan-3-ols* concentrations but the lowest *trans*-resveratrol. We observed the same results in Semillion wines. This pattern was not repeated between the red grapes and its wines. Therefore, it can be said that each grape cultivar did not give the same response to the same wine making practices. Within the group of red grape cultivars, Ökügözü grapes contained the highest levels of all three phenolics. The phenolic profile of wine is not the same as that of fresh grapes because significant changes in phenolic composition occur during the wine-making process, both very early at the grape-crushing stage and during wine fermentation and aging (Lopez-Velez et al., 2003).

Tables 1 and 2 show the phenolic compound levels in the must and wine samples. All of the wine samples contained higher levels of flavanols than the corresponding must samples. Faustino et al. (2003) also reported that grape cultivars have a lower amount of catechin, epicatechin and *trans*-resveratrol than found in wines. The biological activity of *trans*-resveratrol and it's derivatives, that may be relatively abundant in grapes and wines, has been reported (Burns et al., 2001; Kolouchova-Hanzlikova et al., 2004; Revilla & Ryan, 2000; Sato, 1997; Wang, Catana, Yang, Roderick, & van Breemen, 2002).

Fig. 1 shows the HPLC profiles corresponding to the must (a), wine (b) and three phenolics standards (c) using fluorometric detection. The elution order and the retention characteristics were: (1) catechin ($t_r = 5.7$); (2) epicatechin ($t_r = 8.9$); (3) *trans*-resveratrol ($t_r = 19.6$).

Table 2
Mean levels of catechin, epicatechin and resveratrol in the analyzed wine samples

Cultivars	Concentration of phenols (mg/L)[A]		
	Catechin	Epicatechin	Resveratrol
Kalecikkarası	14.226 ± 0.21^{aB}	2.792 ± 0.070^{bc}	0.467 ± 0.007^{e}
Çalkarası	9.822 ± 0.642^{d}	1.028 ± 0.027^{e}	0.323 ± 0.013^{ef}
Boğazkere	14.357 ± 0.725^{a}	0.873 ± 0.007^{e}	1.495 ± 0.020^{c}
Öküzgözü	5.823 ± 0.186^{e}	1.772 ± 0.030^{d}	4.403 ± 0.084^{a}
Cabernet sauvignon	11.439 ± 0.491^{bcd}	3.232 ± 0.024^{b}	0.311 ± 0.006^{ef}
Cinsaut	10.489 ± 0.166^{cd}	1.716 ± 0.015^{d}	1.245 ± 0.048^{d}
Merlot	9.776 ± 0.261^{d}	2.665 ± 0.077^{c}	0.176 ± 0.008^{fg}
Emir	3.029 ± 0.150^{f}	1.075 ± 0.043^{e}	1.243 ± 0.013^{d}
Narince	12.343 ± 0.027^{abc}	1.100 ± 0.022^{e}	1.931 ± 0.022^{b}
Clairette	5.832 ± 0.295^{e}	0.545 ± 0.025^{f}	0.273 ± 0.013^{fg}
Semillion	13.748 ± 0.601^{ab}	11.775 ± 0.124^{a}	0.116 ± 0.00002^{g}

[A] Mean values and ±S.D. ($n = 3$) expressed in mg/L.
[B] Different superscripts within the same column for each value mean significant difference $p < 0.01$.

3.1. Flavan-3-ols concentrations

The largest concentration of the three phenolic compounds examined in the musts and wines (Fig. 2) was catechin. Of our 11 wines studied, Boğazkere red and Semillion white wines had the highest levels. Catechin and *trans*-resveratrol, had much higher average levels in the red wines. However, epicatechin average levels were higher in the white wines. These results show great similarity to those previously reported by Minussi et al. (2003). Catechin concentrations in the white wines also were similar to a number of studies (Darias-Martin, Rodriguez, Diaz, & Lamuela-Raventos, 2000; Landrault et al., 2001; Teissedre & Landrault, 2000). As shown in Table 3 the average catechin concentrations in must samples (0.85 mg/L) were lower compared to the wine samples (10.08 mg/L). These differences were found to be

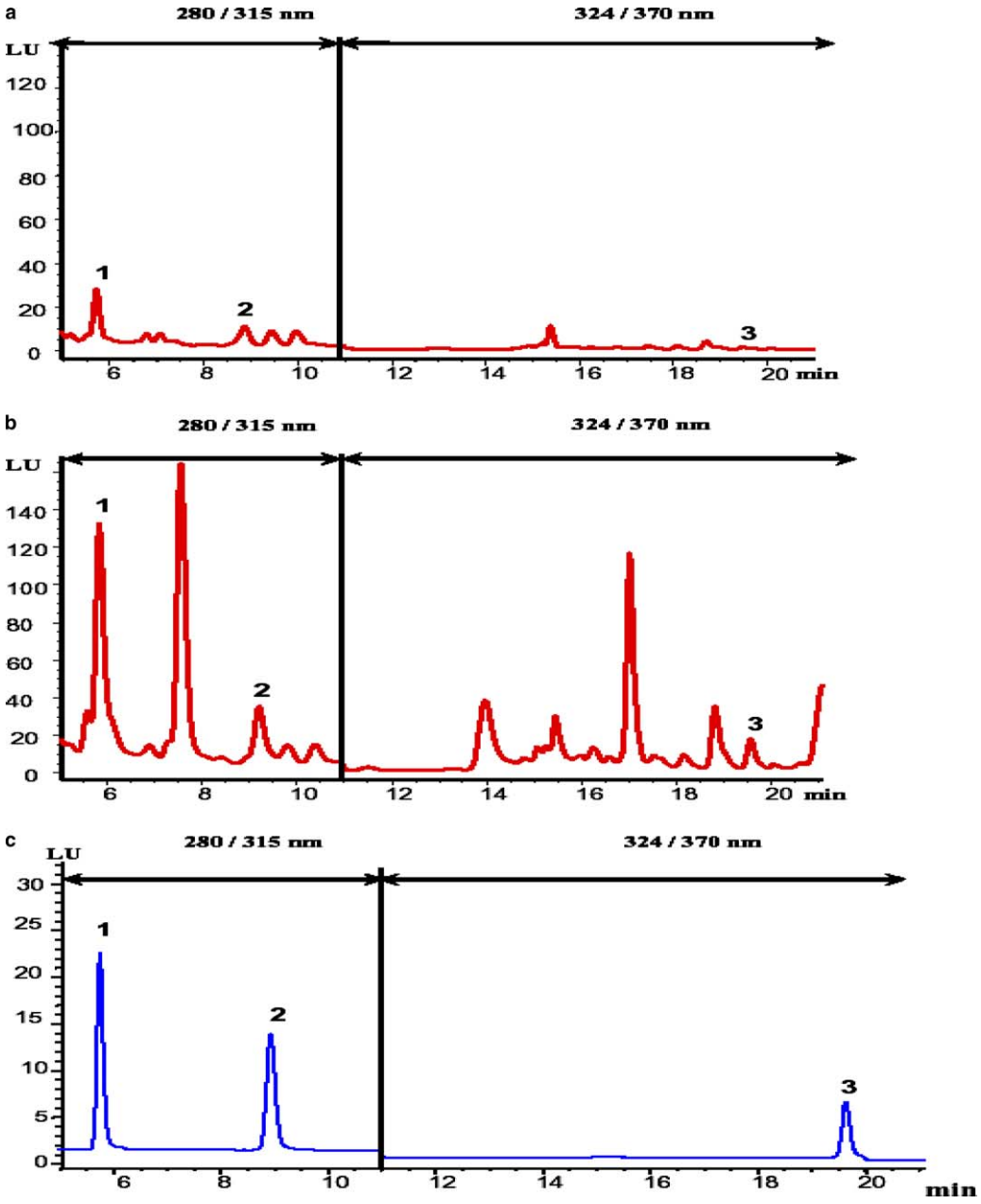

Fig. 1. Chromatographic profiles of must (a), wine (b) and the standards (c). The peaks correspond to: (1) catechin (1 μl/mL); (2) epicatechin (1 μl/mL); (3) *trans*-resveratrol (1 μl/mL). The wavelength changes are shown at the top of the figure.

significantly different ($p < 0.01$). The phenolic compound accumulation in wine may come from the enzymatic preparation, yeast fermentation, and oxidative polymerization reactions. Research by Czyzowska & Pogorzelski (2004) also shows that the total content of polyphenols is highest for wines treated with an enzymatic preparation. The levels of catechin and epicatechin in all wine samples showed a great similarity to that reported by Minussi et al. (2003) and Garciaviguera & Bridle (1995). However, these levels were at lower concentrations than reported in other studies (Bonilla, Mayen, Merida, & Medina, 1999).

French Merlot and Cabernet wines had remarkably high levels of catechin and epicatechin compared to our observations in Merlot and Cabernet wines (Landrault et al., 2001; Teissedre & Landrault, 2000). And in Spanish red wines, (Gambuti et al., 2004) levels of catechin (65–195.4 mg/L) and epicatechin (42.4–46.6 mg/L) were found. However, young red wines were found to have levels of (catechin) 4.96–7.14 mg/L and (epicatechin) 2.02–3.02 mg/L which were very similar to that found in the red wines of this study (Gomez-Plaza, Gil-Munoz, Lopez-Roca, & Martinez, 2000). Also Blanco et al. (1998) reported comparable levels of catechin 6.8 mg/L and epicatechin 1.5 mg/L in red wines.

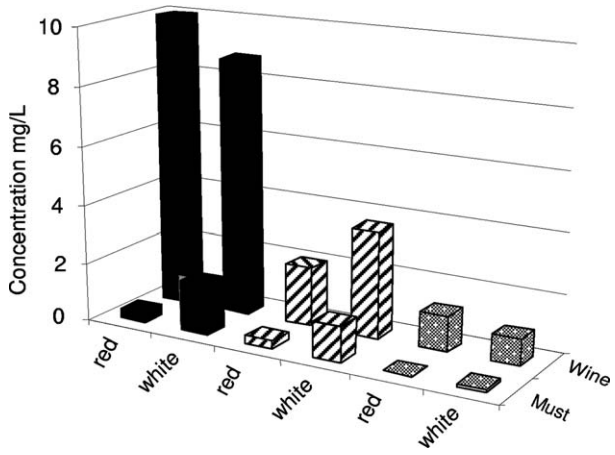

Fig. 2. Comparative diagram shows mean values of catechin, epicatechin and resveratrol in red and white wines. Black bars: (+)-catechin; Striped bars: (−)-epicatechin; Dotted bars: *trans*-resveratrol.

Table 3
Average levels of catechin, epicatechin and resveratrol in must and wine

	Average values of phenols (mg/L)[A]		
	Catechin	Epicatechin	Resveratrol
Must	0.846 ± 0.339^{bB}	0.601 ± 0.222^{b}	0.043 ± 0.015^{b}
Wine	10.080 ± 0.643^{a}	2.597 ± 0.534^{a}	1.089 ± 0.002^{a}

[A] Mean values and ±S.D. ($n = 3$) expressed in mg/L.
[B] Different superscripts within the same column for each value mean significant difference $p < 0.01$.

Among our must samples, the highest catechin level was obtained in the Semillion must (6.829 mg/L). In the wine samples, this flavanol was found to be the highest in red Boğazkere wine (14.357 mg/L).

The significant differences ($p < 0.01$) of the average values between musts (0.601 mg/L) and wines (2.597 mg/L) were determined for epicatechin levels. The highest epicatechin levels again were seen in Semillion must (4.527 mg/L) and the Semillion wine also had the highest concentration of epicatechin (11.775 mg/L). In comparison, Goldberg et al. (1996) reported epicatechin levels between 16.7 and 64.4 mg/L in commercial wines. Padilla et al. (2005) also found higher concentrations of epicatechin in Spanish wines between 5.3 and 46 mg/L and Vitrac et al. (2002) reported average epicatechin levels of 32.9 mg/L in red wines.

3.2. *trans*-Resveratrol concentrations

trans-Resveratrol occurs in grape skin in a few $\mu g\ g^{-1}$ fresh weights, mainly in its free form the corresponding glucoside, piceid. *cis*-Resveratrol is not a natural constituent of grapes and there is no evidence about factors that could facilitate such conversion during winemaking.

trans-Resveratrol concentration in grape skins is also effected by their sanitary state. In this study the must average level of *trans*-resveratrol (Table 3) was low (0.043 mg/L). This fact may be easily explained by the grape stock's response to mold infections and physiological stresses which produce higher levels of *trans*-resveratrol and other stilbenes. If those phenomena do not appear, the levels in grapes and wines may be low (Revilla & Ryan, 2000). These differences can also be explained by the intensity of gray mold infection at harvest time (Adrian et al., 2000b). In addition to the previous reasons, some grape varieties may be genetically richer in this compound (Burns et al., 2001; Kallithraka, Arvanitoyannis, El-Zajouli, & Kefalas, 2001).

Wine flavanols, which come from grape skin, are extracted at the beginning of alcohol fermentation and they are a source of *trans*-resveratrol after degradation due to the β-glucosidase activity (Bavaresco, Fregoni, Cantu, & Trevisan, 1999).

trans-Resveratrol appeared in lower concentrations than the other phenols in this study. These results confirm previous research made by Vinas et al. (2000). Table 3 shows that the average *trans*-resveratrol contents of wines (1.089 mg/L) were higher than in must samples (0.043 mg/L). There were statistical differences between concentrations found in the musts and those found in the wines ($p < 0.01$). The concentrations of *trans*-resveratrol in musts and wines are profoundly influenced by some practices, such as the use of enzymes. As regards enological practices, vinification conditions seem to exert substantial influence on *trans*-resveratrol and isomers concentrated in wine (Dourtoglou et al., 1999). *trans*-Resveratrol levels in the red grapes ranged between 0.0004 and 0.102 mg/L and the white grapes varied between 0.008 and 0.291 mg/L. Emir must (white cultivar) contained the highest *trans*-resveratrol concentration of all the musts (0.291 mg/L). Ranges for the red wines were 0.176–4.403 mg/L and white wines 0.116–1.931 mg/L. In wines, the highest *trans*-resveratrol level was measured in the red wine, Öküzgözü (4.403 mg/L).

The *trans*-resveratrol levels in the red wines studied were lower than previously reported (Adrian et al., 2000a; Kolouchova-Hanzlikova et al., 2004). However, Baptista, Tavares, & Carvalho (2001)found similar *trans*-resveratrol amounts in Portuguese red wines (0.63–5.21 mg/L). And in white wines, Darias-Martin et al. (2000) reported *trans*-resveratrol levels similar to this study. Also Vitrac et al. (2002) reported averages within the range of the wines in this study (red wines 2.3 mg/L and white wines 0.06 mg/L).

In Italian red wines, the levels of *trans*-resveratrol have been reported between 0.56 and 2.86 mg/L (Careri et al., 2003). Goldberg et al. (1996) reported *trans*-resveratrol levels ranging from 0.30 to 4.68 mg/L in commercial wines. Padilla et al. (2005) found concentrations of *trans*-resveratrol in Spanish wines between 0.06 and 4 mg/L. Also, Gambuti et al. (2004) found levels of *trans*-resveratrol between 2.1 and 2.5 mg/L in Spanish wines.

4. Conclusion

The HPLC procedures provided excellent separation and enabled quantitation of these three major compounds present in must and wine. Our data has demonstrated the differences of phenolic content were influenced by enzymatic preparation and controlled fermentation conditions. In the research conducted by Czyzowska & Pogorzelski (2004), the data shows that considerable modification of flavanol composition takes place during enzymatic maceration in the process of wine production. However, soil type and fungal pressure are additional factors reported to influence phenolic concentrations (Baptista et al., 2001).

We determined that the concentrations of *trans*-resveratrol, catechin and epicatechin increased from the original must as a result of wine fermentation. Observed levels of *flavan-3-ols* and *trans*-resveratrol were similar to that reported by other researchers (Darias-Martin et al., 2000; Landrault et al., 2001; Teissedre & Landrault, 2000). On average, the largest increases from must to wine were observed for catechin in both red and white cultivars followed by epicatechin and *trans*-resveratrol (Fig. 2). Since the phenolic content of wine, particularly catechins and proanthocyanidins, have been of interest due to their potential health benefits (Gonzalez-Paramas et al., 2004) this study should be of value to those wine makers and consumers seeking high levels of these compounds.

Acknowledgments

The authors gratefully acknowledge University of Uludag, Bursa, Turkey for financial support of this research project (Project no: 2002/12).

References

Adrian, M., Jeandet, P., Breuil, A. C., Levite, D., Debord, S., & Bessis, R. (2000a). Assay of resveratrol and derivative stilbenes in wines by direct injection high performance liquid chromatography. *American Journal of Enology and Viticulture, 51*(1), 37–41.

Adrian, M., Jeandet, P., Douillet-Breuil, A. C., Tesson, L., & Bessis, R. (2000b). Stilbene content of mature vitis vinifera berries in response to uv-c elicitation. *Journal of Agricultural and Food Chemistry, 48*(12), 6103–6105.

Auw, J. M., Blanco, V., Okeefe, S. F., & Sims, C. A. (1996). Effect of processing on the phenolics and color of cabernet sauvignon, chambourcin, and noble wines and juices. *American Journal of Enology and Viticulture, 47*(3), 279–286.

Aznar, M., Lopez, R., Cacho, J. F., & Ferreira, V. (2001). Identification and quantification of impact odorants of aged red wines from rioja. Gc-olfactometry, quantitative gc-ms, and odor evaluation of HPLC fractions. *Journal of Agricultural and Food Chemistry, 49*(6), 2924–2929.

Baptista, J. A. B., Tavares, J. F. d. P., & Carvalho, R. C. B. (2001). Comparison of polyphenols and aroma in red wines from portuguese mainland versus azores islands. *Food Research International, 34*(4), 345–355.

Bavaresco, L., Fregoni, C., Cantu, E., & Trevisan, M. (1999). Stilbene compounds: from the grapevine to wine. *Drugs under Experimental and Clinical Research, 25*(2–3), 57–63.

Blanco, V. Z., Auw, J. M., Sims, C. A., & O'Keefe, S. F. (1998). Effect of processing on phenolics of wines. *Process-induced chemical changes in food* (Vol. 434, pp. 327–340). New York: Plenum Press.

Bonilla, F., Mayen, M., Merida, J., & Medina, M. (1999). Extraction of phenolic compounds from red grape marc for use as food lipid antioxidants. *Food Chemistry, 66*(2), 209–215.

Buettner, A. (2004). Investigation of potent odorants and after-odor development in two chardonnay wines using the buccal odor screening system (boss). *Journal of Agricultural and Food Chemistry, 52*(8), 2339–2346.

Burns, J., Gardner, P. T., Matthews, D., Duthie, G. G., Lean, M. E. J., & Crozier, A. (2001). Extraction of phenolics and changes in antioxidant activity of red wines during vinification. *Journal of Agricultural and Food Chemistry, 49*(12), 5797–5808.

Careri, M., Corradini, C., Elviri, L., Nicoletti, I., & Zagnoni, I. (2003). Direct HPLC analysis of quercetin and *trans*-resveratrol in red wine, grape, and winemaking byproducts. *Journal of Agricultural and Food Chemistry, 51*, 5226–5231.

Czyzowska, A., & Pogorzelski, E. (2004). Changes to polyphenols in the process of production of must and wines from blackcurrants and cherries. Part II. Anthocyanins and flavanols. *European Food Research and Technology, 218*(4), 355–359.

Darias-Martin, J. J., Rodriguez, O., Diaz, E., & Lamuela-Raventos, R. M. (2000). Effect of skin contact on the antioxidant phenolics in white wine. *Food Chemistry, 71*(4), 483–487.

de Lima, M. T. R., Waffo-Teguo, P., Teissedre, P. L., Pujolas, A., Vercauteren, J., Cabanis, J. C., et al. (1999). Determination of stilbenes (*trans*-astringin, *cis*- and *trans*-piceid, and *cis*- and *trans*-resveratrol) in portuguese wines. *Journal of Agricultural and Food Chemistry, 47*(7), 2666–2670.

Dixon, R. A. (2001). Natural products and plant disease resistance. *Nature, 411*(6839), 843–847.

Dourtoglou, V. G., Makris, D. P., Bois-Dounas, F., & Zonas, C. (1999). *trans*-Resveratrol concentration in wines produced in greece. *Journal of Food Composition and Analysis, 12*(3), 227–233.

Faustino, R. S., Sobrattee, S., Edel, A. L., & Pierce, G. N. (2003). Comparative analysis of the phenolic content of selected chilean, canadian and american merlot red wines. *Molecular and Cellular Biochemistry, 249*(1–2), 11–19.

Franco, M. A., Coloru, G. C., Del Caro, A., Emonti, G., Farris, G. A., Manca, G., et al. (2002). Variability of resveratrol (3,5,4′-trihydroxystilbene) content in relation to the fermentation processes by saccharomyces cerevisiae strains. *European Food Research and Technology, 214*(3), 221–225.

Gambelli, L., & Santaroni, G. P. (2004). Polyphenols content in some italian red wines of different geographical origins. *Journal of Food Composition and Analysis, 17*(5), 613–618.

Gambuti, A., Strollo, D., Ugliano, M., Lecce, L., & Moio, L. (2004). *trans*-Resveratrol, quercetin, (+)-catechin, and (−)-epicatechin content in south italian monovarietal wines: relationship with maceration time and marc pressing during winemaking. *Journal of Agricultural and Food Chemistry, 52*(18), 5747–5751.

Garciaviguera, C., & Bridle, P. (1995). Analysis of non-colored phenolic-compounds in red wines – a comparison of high-performance liquid-chromatography and capillary zone electrophoresis. *Food Chemistry, 54*(4), 349–352.

Goldberg, D. M., Karumanchiri, A., Soleas, G. J., & Tsang, E. (1999). Concentrations of selected polyphenols in white commercial wines. *American Journal of Enology and Viticulture, 50*(2), 185–193.

Goldberg, D. M., Tsang, E., Karumanchiri, A., Diamandis, E., Soleas, G., & Ng, E. (1996). Method to assay the concentrations of phenolic constituents of biological interest in wines. *Analytical chemistry FIELD Publication Date: 1996 May 15*, 68 (10): 1688-94. FIELD Reference Number: FIELD Journal Code: 0370536 FIELD Call Number.

Gomez-Plaza, E., Gil-Munoz, R., Lopez-Roca, J. M., & Martinez, A. (2000). Color and phenolic compounds of a young red wine. Influence of wine-making techniques, storage temperature, and length of storage time. *Journal of Agricultural and Food Chemistry, 48*, 736–741.

Gonzalez-Manzano, S., Rivas-Gonzalo, J. C., & Santos-Buelga, C. (2004). Extraction of *flavan-3-ols* from grape seed and skin into wine using simulated maceration. *Analytica Chimica Acta, 513*(1), 283–289.

Gonzalez-Paramas, A. M., Esteban-Ruano, S., Santos-Buelga, C., De Pascual-Teresa, S., & Rivas-Gonzalo, J. C. (2004). Flavanol content and antioxidant activity in winery byproducts. *Journal of Agricultural and Food Chemistry, 52*(2), 234–238.

Guendez, R., Kallithraka, S., Makris, D. P., & Kefalas, P. (2005). Determination of low molecular weight polyphenolic constituents in grape (vitis vinifera sp.) seed extracts: correlation with antiradical activity. *Food Chemistry, 89*(1), 1–9.

Jeandet, P., Breuil, A. C., Adrian, M., Weston, L. A., Debord, S., Meunier, P., et al. (1997). HPLC analysis of grapevine phytoalexins coupling photodiode array detection and fluorometry. *Analytical Chemistry, 69*(24), 5172–5177.

Kallithraka, S., Arvanitoyannis, I., El-Zajouli, A., & Kefalas, P. (2001). The application of an improved method for *trans*-resveratrol to determine the origin of greek red wines. *Food Chemistry, 75*(3), 355–363.

Katalinic, V., Milos, M., Modun, D., Music, I., & Boban, M. (2004). Antioxidant effectiveness of selected wines in comparison with (+)-catechin. *Food Chemistry, 86*(4), 593–600.

Kolouchova-Hanzlikova, I., Melzoch, K., Filip, V., & Smidrkal, J. (2004). Rapid method for resveratrol determination by HPLC with electrochemical and uv detections in wines. *Food Chemistry, 87*(1), 151–158.

Landrault, N., Poucheret, P., Ravel, P., Gasc, F., Cros, G., & Teissedre, P. L. (2001). Antioxidant capacities and phenolics levels of french wines from different varieties and vintages. *Journal of Agricultural and Food Chemistry, 49*(7), 3341–3348.

Lopez-Velez, M., Martinez-Martinez, F., & Del Valle-Ribes, C. (2003). The study of phenolic compounds as natural antioxidants in wine. *Critical Reviews in Food Science and Nutrition, 43*(3), 233–244.

Minussi, R. C., Rossi, M., Bologna, L., Cordi, L., Rotilio, D., Pastore, G. M., et al. (2003). Phenolic compounds and total antioxidant potential of commercial wines. *Food Chemistry, 82*(3), 409–416.

Mitjans, M., del Campo, J., Abajo, C., Martinez, V., Selga, A., Lozano, C., et al. (2004). Immunomodulatory activity of a new family of antioxidants obtained from grape polyphenols. *Journal of Agricultural and Food Chemistry, 52*(24), 7297–7299.

Monagas, M., Bartolome, B., & Gomez-Cordoves, C. (2005). Updated knowledge about the presence of phenolic compounds in wine. *Critical Reviews in Food Science and Nutrition, 45*(2), 85–118.

Padilla, E., Ruiz, E., Redondo, S., Gordillo-Moscoso, A., Slowing, K., & Tejerina, T. (2005). Relationship between vasodilation capacity and phenolic content of spanish wines. *European Journal of Pharmacology, 517*(1–2), 84–91.

Revilla, E., & Ryan, J. M. (2000). Analysis of several phenolic compounds with potential antioxidant properties in grape extracts and wines by high-performance liquid-chromatography-photodiode array detection without sample preparation. *Journal of Chromatography A, 881*(1–2), 461–469.

Sato, M. (1997). Beneficial effects of moderate alcohol consumption to health. A review of recent results on wines. *Book of Abstracts, 213th ACS National Meeting, San Francisco, April 13–17*: AGFD-151.

Saucier, C. T., & Waterhouse, A. L. (1999). Synergetic activity of catechin and other antioxidants. *Journal of Agricultural and Food Chemistry, 47*(11), 4491–4494.

Sun, B. H., Spranger, I., Roque-do-Vale, F., Leandro, C., & Belchior, P. (2001). Effect of different winemaking technologies on phenolic composition in tinta miuda red wines. *Journal of Agricultural and Food Chemistry, 49*(12), 5809–5816.

Teissedre, P. L., & Landrault, N. (2000). Wine phenolics: contribution to dietary intake and bioavailability. *Food Research International, 33*(6), 461–467.

Vazquez, L. C., Perez-Coello, M. S., & Cabezudo, M. D. (2002). Effects of enzyme treatment and skin extraction on varietal volatiles in spanish wines made from chardonnay, muscat, airen, and macabeo grapes. *Analytica Chimica Acta, 458*(1), 39–44.

Vinas, P., Lopez-Erroz, C., Marin-Hernandez, J. J., & Hernandez-Cordoba, M. (2000). Determination of phenols in wines by liquid-chromatography with photodiode array and fluorescence detection. *Journal of Chromatography A, 871*, 85–93.

Vitrac, X., Monti, J. P., Vercauteren, J., Deffieux, G., & Merillon, J. M. (2002). Direct liquid chromatographic analysis of resveratrol derivatives and flavanonols in wines with absorbance and fluorescence detection. *Analytica Chimica Acta, 458*(1), 103–110.

Wang, Y., Catana, F., Yang, Y. N., Roderick, R., & van Breemen, R. B. (2002). An lc–ms method for analyzing total resveratrol in grape juice, cranberry juice, and in wine. *Journal of Agricultural and Food Chemistry, 50*(3), 431–435.

Zhou, H., Cui, H., Wan, G. H., Xu, H., Pang, Y. Q., & Duan, C. F. (2004). Direct analysis of *trans*-resveratrol in red wine by high performance liquid chromatography with chemiluminescent detection. *Food Chemistry, 88*(4), 613–620.