A DIRECT RP-HPLC DETERMINATION OF PHENOLIC COMPOUNDS IN TURKISH RED WINES

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Abstract

Red wines from four different Turkish grape cultivars were analyzed in order to determine their phenolic contents. For the analyses, reversed phase-high performance liquid chromatography (RP-HPLC) coupled with diode array detection was used. The concentrations of eight phenolic acid standards (ferulic, *o*-coumaric, *p*-coumaric, caffeic, syringic, *trans*-cinnamic, chlorogenic and gallic acids) and five flavonoid standards ((+)-catechin, (-)-epicatechin, quercetin, vanillin and rutin) were used to determine characteristic differences among red wines from Kalecik karası, Öküzgözü, Boğazkere and Papazkarası cultivars grown in the viticultural region of Türkiye. In the wine samples, the most abundant phenolics (+)-catechin (17.82–33.59 mg L^{-1}) as flavonoid and gallic acid (13.25-16.39 mg L^{-1}) as phenolic acid while chlogenic acid was not detected in any samples. As a result, it was determined that types and concentrations of phenolics changed according to the wines from different cultivars.

Keywords: Turkish wines, phenolic compounds, RP-HPLC, direct injection

Türk Kırmızı Şaraplarında Fenolik Bileşiklerin Direkt RP-HPLC ile Belirlenmeleri

Özet

Dört farklı Türk üzüm çeşidinden yapılan kırmızı şaraplar, fenolik içeriklerini belirlemek amacıyla analiz edilmişlerdir. Analiz için diode array detectör eşliğinde ters fazlı-yüksek basınçlı sıvı kromotografisi (RP-HPLC) kullanılmıştır. Türkiye'de yetiştirilen Kalecik karası, Öküzgözü, Boğazkere ve Papazkarası üzüm çeşitlerinden elde edilen kırmızı şaraplar arasındaki karakteristik farkları belirlemek için, sekiz fenolik asit (ferulik, *o*-kumarik, *p*-kumarik, kaffeik, siyringik, *trans*-sinnamik, klorogenik ve gallik asitler) ile beş flavonoid ((+)-kateşin, (-)-epikateşin, kuersetin, vanillin ve rutin) standartı kullanılmıştır. Şarap örneklerinde en fazla bulunan fenolikler, flavonoidlerden (+)-kateşin (17,82–33,59 mg L^{-1}) ve fenolik asitlerden de gallik asit (13,25-16,39 mg L^{-1}) olurken, klorogenik asit hiçbir şarap örneğinde belirlenememiştir. Sonuç olarak, fenolik maddelerin tip ve konsantarsyonlarının farklı çeşitlerden elde edilen şaraplara göre değiştiği belirlenmiştir.

Anahtar kelimeler: Türk Şarapları, Fenolik Bileşikler, RP-HPLC, Direkt Enjeksiyon

1. Introduction

The phenolic compounds of red wines are substances which play an important role in several sensory properties such as colour, flavour, astringency and hardness (Rubichaud and Noble, 1990). Phenolics, a large and complex group of compounds of red wines, also serve as important oxygen reservoirs and substrates for browning reactions (Proestos et al., 2005). Furthermore its well known that wines are rich in phenolic compounds, which have been exhibited to be powerful antioxidants. Antioxidants play a crucial role in the prevention of many diseases such as cancer, inhibiting tumour initiation and heart disease (Briviba and Sies, 1994; Husain et al., 1987). These protective health effects derived from the consumption of wines have been attributed to their phenolic contents (Huang et al., 1992; Rice-Evans and Packer, 1998).

The types and concentrations of the phenolic compounds in wines depend on grape cultivars, ripening and climatic conditions (Goldberg et al., 1998). High performance liquid chromatography (HPLC) technique has been generally used in order to determinate the phenolic compounds in wine samples (Rosa, 1994; Revilla & Ryan, 2000; Rodriguez-Delgado et al., 2001; Lopez et al., 2001). In the determination of the wine phenolics, different extraction methods including solid-phase extraction with C18 or strong anionexchange anionic cartridges, liquid-liquid extraction with different organic solvents have been used. In

this study, wine samples injected directly to HPLC. So, the method permitted the determination of phenolic compounds in wines without any prior purification. Direct RP-HPLC injection techniques without sample preparation was used by Revilla and Ryan (2000), Lopez et al. (2001), Castellari et al. (2002), Ibern-Gomez et al. (2002), de Villiers et al. (2004), Suarez et al. (2005) before.

Despite the wealth of information on wines in general, there appears to be little information on phenolic compounds of Turkish wines obtained from different grape cultivars. Therefore the aim of the study is to characterize the phenolic compounds of red Turkish wines by (RP) HPLC with DAD detector using the direct quantative determination and to compare the major differences among the red wines obtained from different cultivars.

2. Materials and Methods

2.1. Wine Samples

Three bottles of each different brands (Yazgan, Kavaklıdere and Doluca for Kalecik karası and Öküzgözü; Yazgan and Kavaklıdere for Boğazkere and Yazgan and Sevilen for Papaz karası) were purchased from a hypermarket in Isparta, Turkey. Before analyses, different brands of each wine were combined in equal volumes. The HPLC analysis was performed without any particular treatment except filtration. Each determination was carried out in triplicate.

2.2. Standards

Gallic acid, caffeic acid, *p*-coumaric acid, *o*-coumaric acid, ferulic acid, syringic acid, (−)-epicatechin, rutin and quercetin were purchased from Sigma-Aldrich (Steinheim, Germany). T*rans*-cinnamic acid and vanillin were obtained from Acros (Geel, Belgium) while (+)-catechin and chlorogenic acid were supplied from Fluka (Buchs, Switzerland). Stock solutions of all the standards were prepared in methanol.

2.3. HPLC Analysis

Phenolic compounds were evaluated by reversed phase - high performance liquid chromatography (RP-HPLC) with direct injection. Detection and quantification was carried out with a SCL-10Avp System controller, a SIL–10AD vp Autosampler, a LC-10AD vp pump, a DGU-14a degasser, a CTO-10 A vp column heater and a diode array detector with wavelengths set at 278 nm. The 250 x 4,6 mm i.d., 5μm column used was filled with Luna Prodigy. The flow rate was 1 ml min^{-1}, injection volume was 10 μl and the column temperature was set at 30 °C. Gradient elution of two solvents was used: Solvent A consisted of: acetic–water (2:98 v/v), solvent B: methanol and the gradient programme used is given Table 1. The data were integrated and analyzed using the Shimadzu Class-VP Chromatography Laboratory Automated Software system. The wine samples, standard solutions and mobile phases were filtered by a 0.45-μm pour size membrane filter. The amount of phenolic compounds in the extracts was calculated as μg/l wine using external calibration curves, which were obtained for each phenolic standard. Phenolic compositions of wines were determined by the modified method of Capanio et al. (1999).

Table 1. Solvent gradient conditions with linear gradient

Final Time	A%	B%
(Initial)	100	0
3	95	5
18	80	20
20	80	20
30	75	25
40	70	30
50	60	40
55	50	50
65	0	100

2.4. Statistical analysis

Statistical analysis was performed using SPSS 10.01 software for windows.

3. Results and Discussion

Reversed phase high-performance liquid chromatography (RP-HPLC) with direct injection was used for separation of phenolic compounds. Although normal-phase chromatography has been used for the separation of phenolic compounds, it is now generally agreed that reversed-phase HPLC is the method of choice for the separation of a wide variety of phenolic compounds. The separation of standards of phenolic compounds is shown in Figure 1. As can be seen separation was achivied for 13 components including phenolic acids and flavonoids. Generally, such separations are rapid and provide high resolution and sensitivity (Lopez et al., 2001). Phenolic contents of four Turkish grape wines (mg L^{-1}) were shown in Table 2. Levels of phenolic compounds were significantly influenced by the wine grape cultivars (P<0.05). The major differences were observed in wine samples obtained from different cultivars. The most abundant phenolic substances detected were (+)-catechin as a flavonoid and gallic acid as a phenolic acid. The values ranged from 17.82 to 33.59 mg L^{-1} for +(-) catechin and from 13.25 to 16.39 mg L^{-1} for gallic acid in the

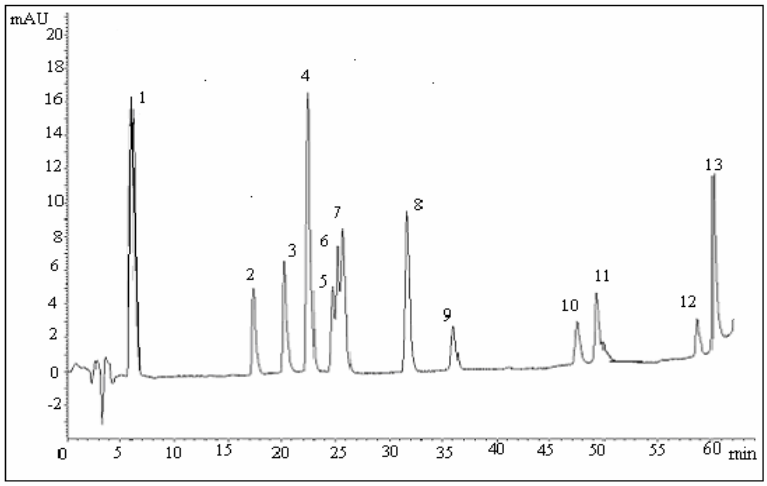

Figure 1. A Chromatogram of Phenolic Standards at 278 nm: 1-Gallic acid, 2-(+)- Catechin, 3-Chlorogenic acid, 4-Caffeic acid, 5-Syringic acid, 6-(-)-Epicatechin, 7-Vanillin, 8- *p*-Coumaric acid, 9-Ferulic acid, 10- *o*-Coumaric acid, 11-Rutin, 12- *Trans*-Cinnamic acid, 13- Quercetin.

Table 2. Phenolic contents of four Turkish wines (mg L^{-1}).

Phenolic compounds	Retention Time (min)	Wine making cultivars			
		Öküzgözü	Boğazkere	Papaz karası	Kalecik karası
Gallic acid	6.17	14.93±1.23b*	13.25±1.24d	16.39±1.23a	14.69±1.23c
(+)-Catechin	17.59	22.26±0.68c	17.82±0.68d	31.70±0.70b	33.59±0.71a
Chlorogenic acid	nd	nd	nd	nd	Nd
Caffeic acid	22.59	11.55±1.38	9.05±0.13	23.58±1.35	5.92±0.12
Syringic acid	24.72	1.55±0.03c	1.68±0.03b	1.86±0.03a	Nd
(-)-Epicatechin	25.22	9.46±0.09b	5.62±0.09d	12.65±0.10a	8.99±0.09c
Vanillin	25.90	1.03±0.01c	1.40±0.01a	1.33±0.01b	1.01±0.10d
p-Coumaric acid	31.83	2.11±0.20a	2.03±0.20c	7.38±0.00a	0.54±0.01d
Ferulic acid	36.21	Nd	0.14±0.00b	0.23±0.00a	Nd
o-Coumaric acid	47.64	0.64±0.01a	nd	0.56±0.01b	0.27±0.01c
Rutin	49.47	Nd	4.67±0.36b	nd	14.92±0.39a
Trans-Cinnamic acid	58.78	0.30±0.01c	0.42±0.00a	0.38±0.01b	Nd
Quercetin	60.11	2.20±0.13d	2.37±0.12c	3.87±0.10a	3.38±0.10b

* : Differences in same rows were statistically important (Duncan test, p<0.05)
nd: Not detected

all wine samples. Proestos et al. (2005) were also found the most abundant phenolic compounds in Greek wines as (+)-catechin (11.80-40.00 mg L^{-1}). On the other hand in this study, chlorogenic acid was not detected in any wine samples.

In Boğazkere wine, eleven compounds were separated (Figure 2). While, only *o*-coumaric acid and chlorogenic acid were not found, main compounds of it were (+)-catechin, gallic acid, caffeic acid, (-)-epicatechin, rutin, quercetin and *p*-coumaric acid, respectively. Papaz karası wine had all phenolic compounds identified in this study except rutin and chlorogenic acid. In wine from Öküzgözü, ten of phenolic compounds were identified as (+)-Catechin (22.26 mg L^{-1}), gallic acid (14.93 mg L^{-1}), caffeic acid (11.55 mg L^{-1}), (-)-epicatechin (9.46 mg L^{-1}), quercetin (2.20 mg L^{-1}), *p*-coumaric acid (2.11 mg L^{-1}), syringic acid (1.55 mg L^{-1}), vanillin (1.03 mg L^{-1}), *o*-coumaric acid (0.64 mg L^{-1}), *trans*-cinnamic acid (0.30 mg L^{-1}). Ferulic acid, chlorogenic acid and rutin were no in this wine sample. While syringic, ferulic, chlorogenic acid and *trans*-cinnamic acids in Kalecik karası wine was not found, rutin (14.92 mg L^{-1}) was the second most abundant compound after (+)-catechin (33.59 mg L^{-1}). Gallic acid, (-)-epicatechin, caffeic acid and quercetin followed them, respectively. Vanillin and *o*-coumaric acid were determined lower amount in it. These differences may be explained by the differences among the cultivars which may affect phenolic compositions. The findings of Oszmianski and Lee (1990), Andrade et al. (2001), Lopez et al. (2001), Lopez-Velez et al. (2003), Proestos et al. (2005) studying phenolic compounds of wines making from different grape cultivars are in agreement with our results. Similarly, Kılınç and Kalkan (2003) determined that the evaluation of phenolic contents in white and red wines emphasized the importance of variety characteristics of wines. They found phenolic acids including gallic acid, p-hydroxybenzoic acid, syringic acid, 2,3-dihydroxybenzoic acid, ferrulic acid, p-coumaric acid and vanillic acid) in 13 different wines. Bayhan (2004) studying on the phenolic compositions of wines produced 16 different Kalecik karası clones found that catechin, epicatechin, quercetin and *trans*-resveratrol levels of wines ranged from 2.2 mg $L^{-1}$to 9.27 mg L^{-1}, 4.36 mg L^{-1} to 8.83 mg L^{-1}, 4.52 mg $L^{-1}$to 15.2 mg L^{-1} and 0 to 4.18 mg L^{-1}, respectively.

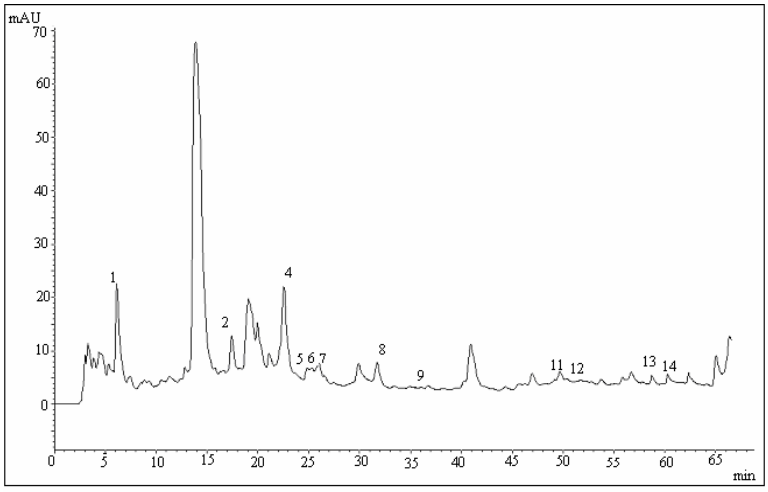

Figure 2. A Chromatogram recorded at 278 nm of the Boğazkere wine sample: 1-Gallic acid, 2-(+)-Catechin, 4-Caffeic acid, 5-Syringic acid, 6-(-)-Epicatechin, 7-Vanillin, 8- *p*-Coumaric acid, 9- Ferulic acid, 11-Rutin, 12- *Trans*-Cinnamic acid, 13- Quercetin.

Data presented in a manner to accomplish a fundamental objective is to compare the concentration of each phenolic compounds in wines from the different grape cultivars. Researches conducted on the compositions of phenolics in wines, are

generally focused on the concentrations of resveratrol and anthocyanidins (Kallithraka et al., 2001; Tsanova-Savova et al., 2002; Gambelli and Santaroni, 2004; Kolouchova et al., 2004; Zhou et al, 2004; de Villiers et al., 2004; Villano et al., 2005; Abril et al., 2005).

According to our knowledge, there are no detailed data regarding the composition of phenolic compounds in Turkish wine obtained several grape cultivars, so this preliminary study contributes new knowledge of the composition of the wines of different grapes. Further studies, with a larger number of samples, are necessary to confirm the differences observed.

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