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Assessing wines based on total phenols, phenolic acids and chemometrics by multivariate analyses

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The aim of this study was to investigate the phenolic profile of some red wines produced from native Turkish grape varieties (*Vitis vinifera* Öküzgözü, *V. vinifera* Boğazkere and *V. vinifera* Shiraz) and some red fruit wines produced from pomegranate (*Punica granatum* L.), myrtle (*Myrtus communis* L.) and black mulberry (*Morus nigra* L.). Red grape wines and fruit wines were produced according to accepted conventional methods for wines and fruit wines regulations. At the end of productions all samples, were analyzed for the following parameters: total phenols (mg/L) and color parameters such as %D280, %D420, %D520, %D620, %DA, %IC,T, %IC^{I} ,%R, %Y and %B. By using HPLC method, the following parameters were determined: gallic acid, p-hydroxybenzoic acid, caffeic acid, syringic acid, p-coumaric acid and ferulic acids. The order of wines according to the total phenolic contents from the highest to the lowest values was determined to be: Boğazkere > Öküzgözü > mulberry > Shiraz > myrtle > pomegranate wines. The highest values for gallic acid, p-hydroxybenzoic acid, caffeic acid, syringic acid, p-coumaric acid and ferulic acid were determined in bilberry, pomegranate, Shiraz, mulberry, Boğazkere and Öküzgözü wines, respectively. Analyses of n-dimensional scale demonstrated the relationship between pomegranate wine and p-hydroxybenzoic/syringic acid; bilberry wine and gallic acid; Shiraz/Boğazkere and caffeic acid/p-coumaric acids; Öküzgözü and ferulic acid; total phenols and Shiraz/pomegranate/Boğazkere wines.

Key words: Phenols, colors, fruit wines, red wines.

INTRODUCTION

Phenolic compounds, especially anthocyanins, flavonols, catechins and other flavonoids, play a major role in wine quality since they contribute to the sensory characteristics of wines, particularly colour and astringency. Phenolic compounds have recently been demonstrated to have a wide range of biochemical and pharmacological effects, including anti-carcinogenic, anti-atherogenic, anti-inflammatory, antimicrobial and anti-oxidant activities (Frankel et al., 1993). The antioxidant activity of phenolics is mainly because of their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Landbo and Meyer, 2001). Phenolic compounds in wine, notably red wine, have been shown to inhibit *in vitro* oxidation of human low-density lipoprotein (LDL) (Frankel et al., 1993; Kanner et al., 1994; Teissedre et al., 1996). The ability of wine phenols to inhibit LDL oxidation has been suggested to be a possible mechanism explaining the 'French Paradox' (Frankel et al., 1993). Phenolic compounds act as antioxidants, scavenge free radicals that induce vascularrelaxation (Del Alamo et al., 2004) and they have been reported to have anti-inflammatory (Terra et al., 2007), anti-carcinogenic (Nichenametla et.al., 2006), anti-atherogenic, anti-thrombotic (Erlund et al., 2008), anti-mutagenic (Ferguson, 2001), antiviral (Kwon et al., 2010)

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and antibacterial (Tosi et al., 2007) properties. Okuzgozu grapes which is a native grape variety of *Vitis vinifera*, widely planted in the Denizli and Elazig region is used for the production of one of the highest quality red wines in Turkey. It is an important red grape variety for Turkey, which produces well-balanced and characteristics wines with fruity notes such as strawberry, cherry and blackberrylike odors (Kelebek et al., 2007).

Long-term health benefits associated with flavonols have increased the interest in the content of these flavonoids in various foods and beverages. Recent studies have shown that many berries are rich in flavonols (Hakkinen and Auriola, 1998a; Hakkinen et al., 1998b, 1999a, b). In contrast to grape wines, the phenolic profile of fruit wines is poorly known. Some studies have demonstrated that berry wines possess antioxidant activity on the oxidation of methyl linoleate, wines made from blackcurrant and crowberry or berry being slightly superior to red grape wines (Heinonen et al., 1998a).

Therefore, the aim of this study was to determine and evaluate the total phenolic content, phenolic acid (gallic, p-hydroxybenzoic, caffeic acid, syringic, p-coumaric, ferulic acids) and colour values (A280, A420, A520, A620, IC, T, IC', %dA, %R, %Y, %B) of wines produced from grapes of *V. vinifera* L. cv. origin var: Öküzgözü, Boğazkere, Shiraz and fruits such as pomegranate (*Punica granatum* L.), myrtle (*Myrtus communis* L.) and black mulberry (*Morus nigra* L.).

MATERIALS AND METHODS

Materials used were grapes of *V. vinifera* L. cv. origin var: Öküzgözü, Boğazkere and Shiraz. The other fruits used were pomegranate (*P. granatum* L.), myrtle (*M. communis* L.) and black mulberry (*M. nigra* L.). Fruits were processed in Food Engineering Department, Ege University (Izmir, Turkey) within 24 h of harvest.

Wine processing

Grapes were crushed, destemmed and prepared for skin fermentation treatments. The crushed grapes were sulfited (50 mg/L SO_2), inoculated with 'Fermirouge' (20 g/hL *Saccharomyces cerevisiae* 7000 INRA; Gist-Brocades Co.), supplied with pectolytic enzyme 'Rapidase-ex-color' (4 g/hL) 100 units; (Barny, 1987) and left for skin fermentation lasting for 5 days at 25°C. The pomace was stirred and pushed down twice daily. After alcohol fermentation, wines were allowed to dry in glass vessels. Fining agent used was gelatine (250 g/hL) (Merck / Darmstadt / Germany). After filtration ('Seitz, D.6800 / Mannheim, Germany, plate filter) and bottling, wines were stored at 15°C. The process of production of fruit wines other than grapes was performed according to the accepted procedures for fruit wines.

Total phenols analysis

Total phenolic content was determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965) by carrying out the following modification to reduce the assay volume. To 3.90 ml of H_2O, 0.1 ml sample was added followed by 0.5 ml (25 ml Folin Ciocalteu reagent in 75 ml H_2O) Folin-Ciocalteu reagent (Merck, Darmstadt, Germany) and was mixed. After 3 to 6 min, 0.5 ml saturated sodium carbonate (20 g Na_2CO_3 in 100 ml H_2O) (Merck) was added. After vigorous mixing with a vortex and waiting for 30 min, the reading was performed at 725 nm (Pharmacia LKB Novaspec II spectrophotometer, Uppsala, Sweden). The results were expressed as gallic acid equivalents (GAE) using a calibration curve against a gallic acid (Merck) Standard (100 mg/L).

High-performance liquid chromatography analysis

Gallic acid, p-hydroxybenzoic acid, syringic acid, ferulic acid, p-coumaric acid and caffeic acid were obtained as standards from Sigma Chemical Company Frankfurt, Germany). Solvents used for chromatography were methanol and phosphoric acid (of high-performance liquid chromatography ultragradient grade) supplied by J. T. Baker (New Jersey/USA) and Riedel-de Haen AG (Darmstadt/Germany), respectively. Membranes (0.45 µm pore size) used for filtration of the samples were obtained from Sartorius AG (Darmstadt/Germany) (16555 Minisart). The liquid chromatographic system (HP 1100 series) supplied by SEM Company (Izmir, Turkey) was equipped with an electochemical detector (HP1049A Programmable Electrochemical Detector), a pump (HP 1100 series G1310A isocratic pump), a manual injector (HP 1100 series G1328A Rheodyne 7725I) with 20 µl loop and a chromatographic data processing software (HP Chem Station LC Rev. A.06. 03.509). Separation was performed by (C18) column (Hichrom 5 C18, 7.75 x 300 mm, 5 µm particle size).

The operating conditions were carried out at 21 ± 4°C. The separation of phenolic compounds was performed with a flow rate of 1 ml/min for 80 min. Amperometric detection was carried at +/0.65 V (versus Ag/AgCl, 0.5 mA full scale) in the electrochemical cell. The solvents used and their proportions were as follows: methanol/0.01 N phosphoric acid (30/70 v/v). Both solvents were degassed (ELMA LC 30/H ultrasonic bath/Darmstadt/Germany) for 15 min before use. Each compound was tentatively identified by retention time under the same conditions. Quantitative determinations were carried out by the external standard method based on peak areas (Yildirim et al., 2005).

Colour measurements

Spectrophotometric measurements were made using a 1-mm cell path length at A420, A520, A620 nm. Colour density (IC) was calculated as the sum of A420 and A520 nm (Yıldırım et al., 2006).

(IC) Colour density = $\left(A_{420}+A_{520}\right)$

Tint (T) was calculated as the ratio of A420 to A520 nm.

(T) Tint value = $\left(A_{420}/A_{520}\right)$

Colour intensity (IC') was determined as the sum of A420, A520 and A620 nm.

(IC) Colour intensity = $\left(A_{420}+A_{520}+A_{620}\right)$

The Da (%) value was calculated as follows:

(Da %) Proportion of red colour produced by flavylium cations $= \left(\frac{A_{520} - \frac{A_{420} - A_{620}}{2}}{A_{520}} \times 100 \right)$

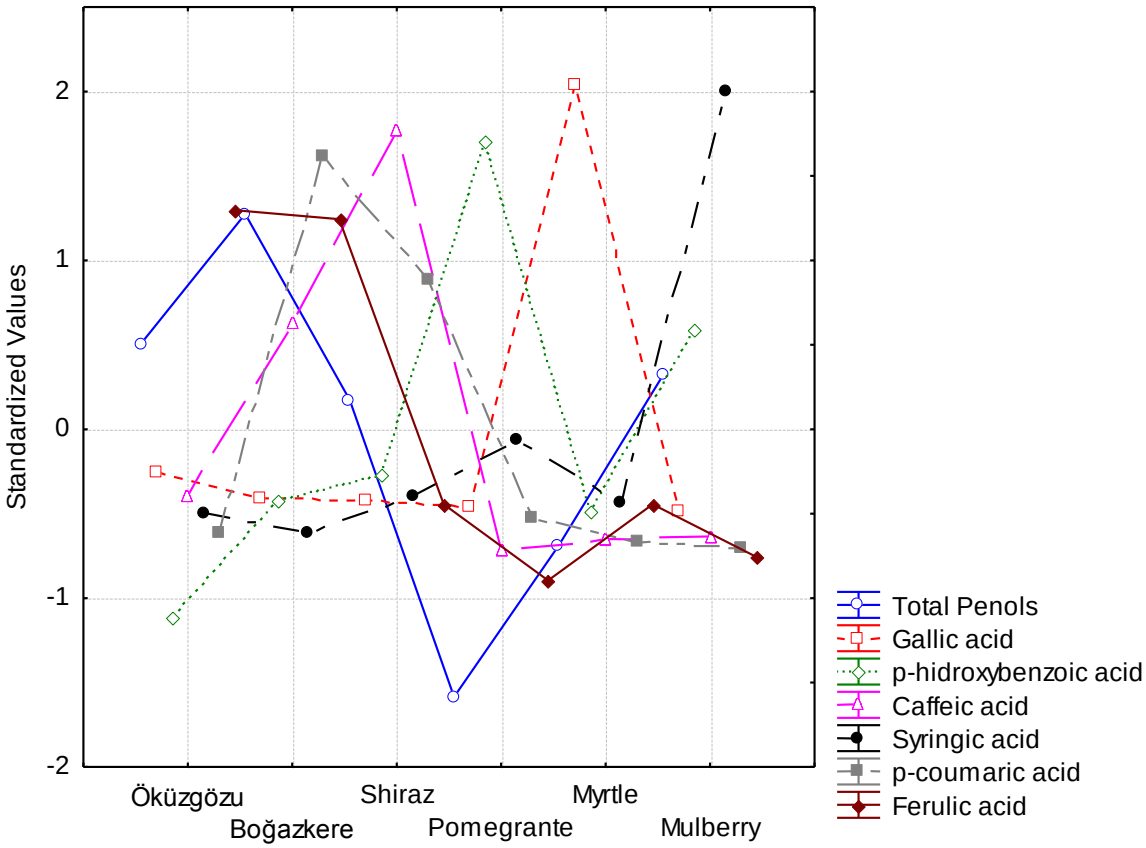

Figure 1. The phenolic acid (gallic, p-hydroxybenzoic, caffeic acid, syringic, p-coumaric and ferulic acids) profiles of the different wines.

Proportions of red (%R), yellow (%Y) and blue (%B) were determined as given below (Singleton and Rossi, 1965):

$$\text{(\%Y) Proportion of yellow colour} = \left(\frac{A_{420}}{CI}\times 100\right)$$

$$\text{(\%R) Proportion of red colour} = \left(\frac{A_{520}}{CI}\times 100\right)$$

$$\text{(\%B) Proportion of blue colour} = \left(\frac{A_{620}}{CI}\times 100\right)$$

Statistical evaluation

Significant differences between averages were obtained at a 95% significance level. Using multivariate exploratory techniques, principal component analysis was performed. Principal component analysis permits visualization of the original arrangement of wines in an n-dimensional space, by identifying the directions in which most of the information is retained. The cluster analysis was performed as a joining type (tree cluster) by using raw data with complete linkage and the Euclidean distance. The scale plot was demonstrated as link/d_{max}/100. In order to be comparable, all values were standardized.

RESULTS AND DISCUSSION

The standardized values of the studied phenolic acids (gallic, p-hydroxybenzoic, caffeic acid, syringic, p-coumaric and ferulic acids) and total phenols found in different wines produced from grapes (*V. vinifera* L. cv. origin var:. Öküzgözü, Boğazkere, Shiraz.) and fruits such as pomegranate (*P. granatum L.*), myrtle (*M. communis L.*) and black mulberry (*Morus nigra L.*) are given in Figure 1. As could be seen, the highest value of total phenols was determined in Boğazkere wine, followed by Öküzgözü, Shiraz and mulberry wines.

The highest concentrations of polyphenols were detected in Buzbağı wine produced from Bogazkere–Öküzgözü grapes (Porgali and Büyüktuncel, 2012). In other studies, the total phenolic content of fruit wines made from apples, blackcurrants, bilberries, cowberries, crowberries, cherries, strawberries, and in artic bramble and cherry liqueurs, were determined to be between 18 and 132 mg/l GAE. Wines made of cherries (1080 mg/l GAE), red raspberries and blackcurrants (1050 mg/l GAE), blackcurrants and bilberries (average of 1040 mg/l GAE), blackcurrants and crowberries (1020 mg/l GAE), blackcurrants and redcurrants (average 890 mg/l GAE)

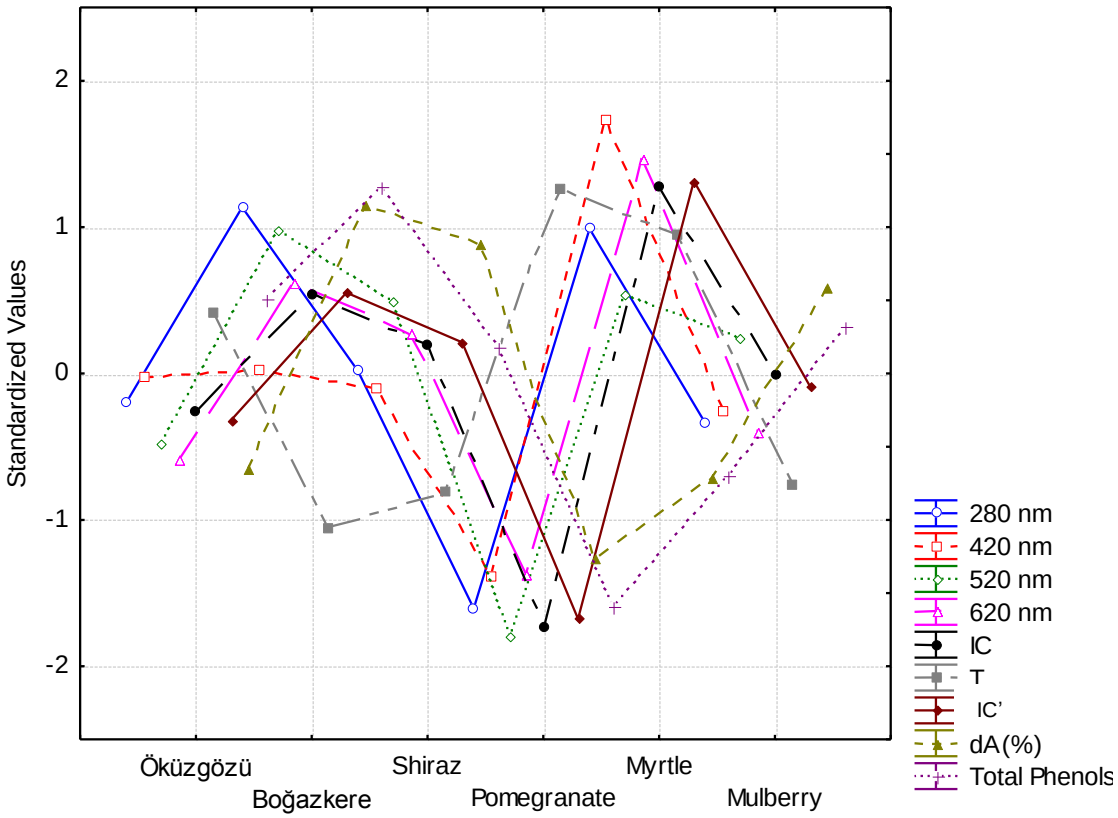

Figure 2. The colour profiles (A280, A420, 520, 620, IC, T, IC', dA%) of different wines.

and blackcurrants (average of 870 mg/l GAE) were found to contain the highest amounts of phenolic compounds (Heinonen et al., 1998a).

Evaluating the gallic acid content in samples, the order from the highest to the lowest values was determined as: myrtle wine > Öküzgözü wine > Boğazkere wine > Shiraz > pomegranate wine = mulberry wine. The order of wines concerning the p-hydroxybenzoic acid content was found to be: pomegranate wine > mulberry wine > Shiraz wine > Boğazkere wine > myrtle > Öküzgözü wine. The highest value of caffeic acid was determined in Shiraz wine, followed by Boğazkere, Öküzgözü, mulberry, myrtle and pomegranate wines. The order of wines ranged according to their syringic acid contents as: mulberry wine > pomegranate wine > Shiraz wine > myrtle wine > Öküzgözü wine > Boğazkere wine. The wine order related to the values of p-coumaric acid was determined as Boğazkere wine > Shiraz wine > Öküzgözü wine > pomegranate wine > myrtle wine > mulberry wine. According to the contents of the last analyzed phenolic acid (ferulic acids), wines were ranged in the order of: Öküzgözü wine > Boğazkere wine > myrtle wine = Shiraz wine > pomegranate wine > mulberry wine. It was determined that Okuzgözü wines from Elazig region contained higher level of phenolic acids than wines obtained from Denizli region in different vintage. Concentration of trans-caftaric and transcoutaric acid in Denizli wines ranged from 42.50 to 68.9 mgL^{-1} and from 20.15 to 33.10 mgL^{-1}, whereas Elazig wines contained 51.56 to 78.40, and 20.77 to 45.40 mgL^{-1} of these compounds, respectively. Some authors considered that the trans-coutaric acid/trans-caftaric acid ratio may characterize wines according to their varietals origin. Wine may also contain their corresponding p-hydroxycinnamic acids (p-coumaric and caffeic acid) due to the hydrolysis of the tartaric moiety in wine (Gomez-Alonso et al., 2007).

Kelebek et al. (2010), investigated and determined the phenolic acid contents of Öküzgözü wines produced from grapes supplied from two regions in Turkey (Denizli and Elazig) during two years. According to their results, the grape location and production year could cause significant differences in values.

In Figure 2, color values of different wines are given. The highest values of absorbance at 280 and 520 nm were found in Boğazkere and at 420 and 620 nm were determined in myrtle wines. The IC value was found to be in greatest amount in myrtle (*M. communis* L.) wine. The maximum value for Tint (T) was obtained in pomegranate wine. IC' value was found to be in highest amount in myrtle (*M. communis* L.) wine. The maximum dA(%) value was obtained in Boğazkere wine. The proportion

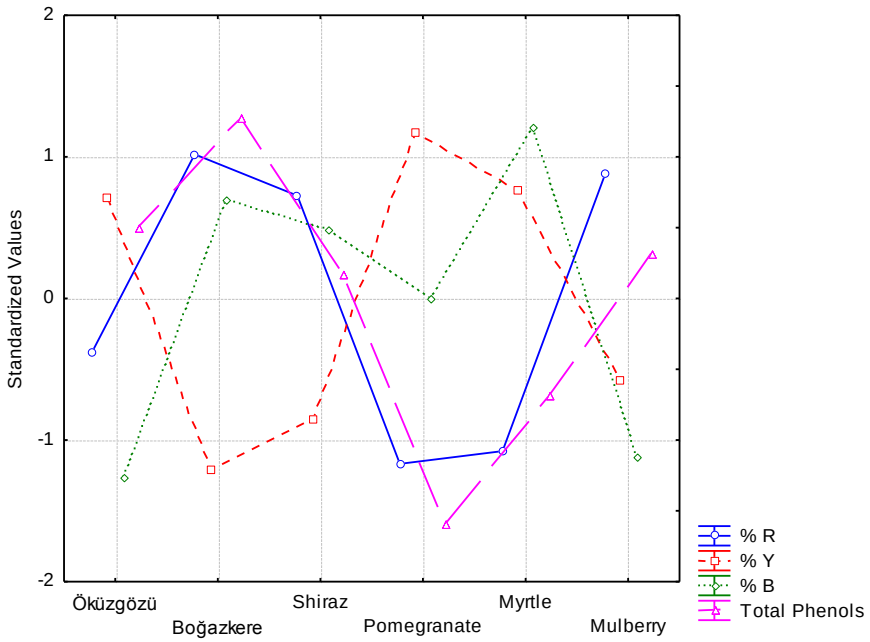

Figure 3. The colour profiles (%R, %Y, %B) of different wines.

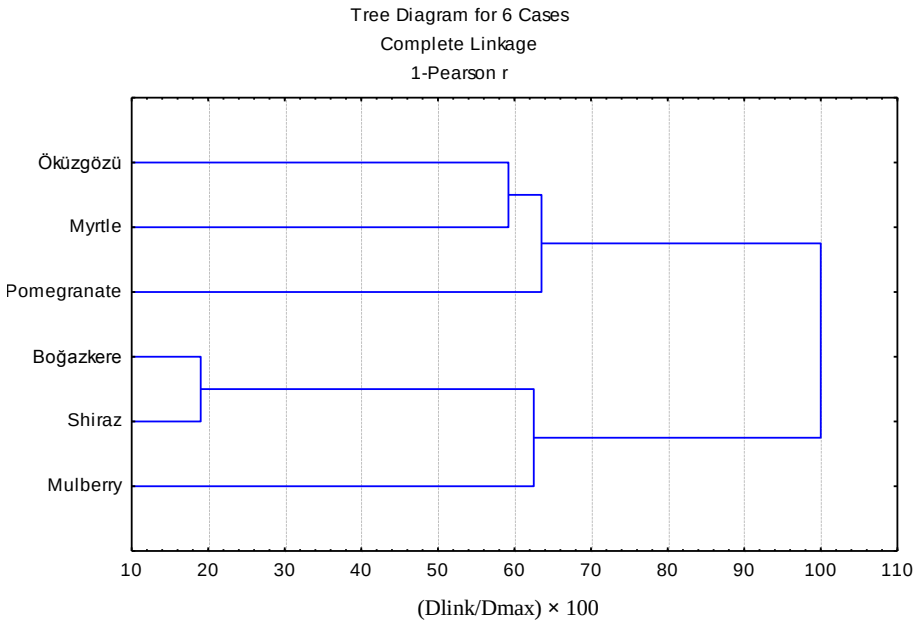

Figure 4. Cluster diagram of analyzed wines.

values of red, yellow and blue are given in Figure 3. The highest value of %R, %Y and %B were determined in Boğazkere, pomegranate and myrtle wines, respectively. It was determined that berries with a strong purple colour, such as myrtle had higher phenolic contents including anthosyanins (Heinonen et al., 1998a, b). These compounds are known for their influences on red wine coloration by copigmentation (Boulton, 2001), they also seem to contribute to bitterness of wine (Jackson, 2002) and also display some antioxidant activity (Majo et al., 2008).

Figure 4 visualizes the relationship among different wines based on all analysed parameters. As can be seen from the Figure, two main clusters were formed. In the

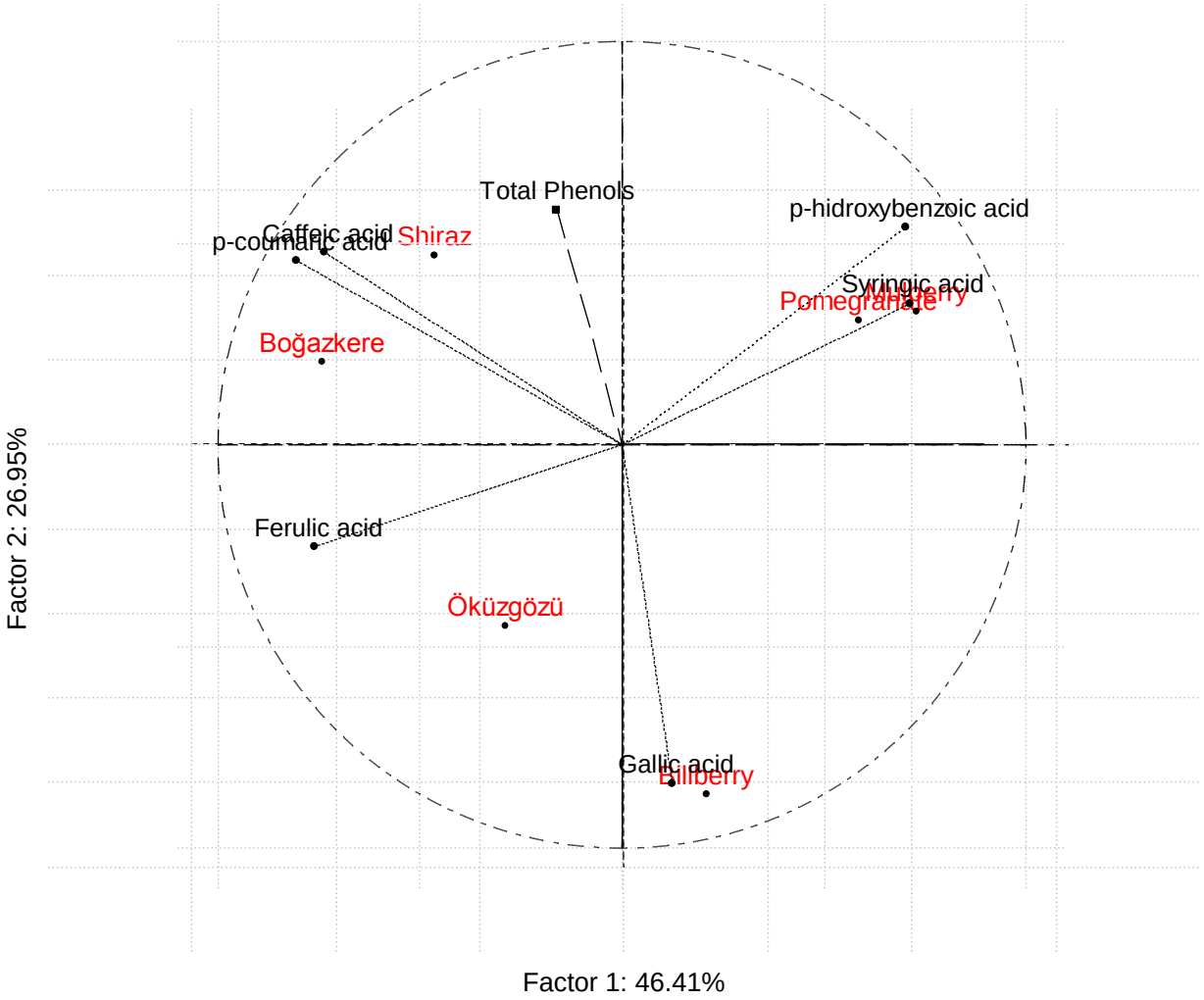

Figure 5. Evaluation of the relationship among phenolic acids, total phenols and analyzed wines by principal components analysis (PCA).

first one, was included Öküzgözü, myrtle and pomegranate wines. In the second one, was found Boğazkere, Shiraz and mulberry wines. Direct relationships were determined between Öküzgözü and myrtle wine; and between Boğazkere and Shiraz wines.

Principal component analysis was run on the set of data to examine the relationship among all parameters and wines. The projection of the variables (phenolic acids, total phenols, different wines) on the factor plane of the first two factors (46.41% x 26.95%) can be seen in Figure 5. In the first group was pomegranate, mulberry wines, p-hydroxybenzoic and syringic acids. In the second group, were found myrtle, Öküzgözü wines and gallic acid. In the third one was located Boğazkere, Shiraz wines, caffeic acid, and p-coumaric acids. The location of wines and analyzed parameters demonstrated the close relationship among compounds found in the same group. The results obtained by HPLC analysis and evaluations done by PCA, demonstrated the following close relations: bilberry wines and gallic acid; pomegranate wine and p-hydroxybenzoic acid; mulberry wine and syringic acid; Boğazkere, Shiraz wines and p-coumaric acid, caffeic acid; Öküzgözü wine and ferulic acid.

Conclusions

The colour analyses results demonstrate the importance of Boğazkere and bilberry wines. According to the cluster analyses, there are close relationship between Öküzgözü and myrtle (*M. communis L.*) wine; Boğazkere, Shiraz wines and mulberry wines.

The results of grouping of analyzed parameters in n-dimensional space with different wines revealed the importance of myrtle (*M. communis L.*) wines for gallic

acid content; pomegranate wine for p-ydroxybenzoic acid content; mulberry wine for syringic acid content; Boğazkere, Shiraz wines for p-coumaric acid, caffeic acid contents and Öküzgözü wine for ferulic acid amount found in wine.

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