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Total Phenolic and Flavonoid Contents, Phenolic Compositions and Color Properties of Fresh Grape Leaves

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Abstract

Grape leaves have been consumed as fresh and canned for years. Stuffed grape leaves (dolma) is a traditional delicacy for Turkish, Balkan and Middle East nations. In this study; grape leaves from Sultani Çekirdeksiz (SÇ), Sultan1 (S1), Sultan7 (S7) Saruhanbey (SB) and Narince (N) grape cultivars were assayed for their total phenolics, total flavonoids, some phenolic compounds and color properties. Total phenolic and total flavonoid contents of the samples were determined by Folin Ciocalteu and colorimetric aluminum chloride methods respectively. Results were expressed as Gallic acid and (+)-catechin equivalent mgg^{-1}. The phenolic compositions of the samples were separated by HPLC. *L*, a*, b** values of the samples were measured by Minolta Colorimeter and a/b values were calculated.

Total phenolic contents varied from 9.72 to 14.22 mg g-1 fresh leaves and total flavonoid contents from 5.08 to 7.22 mg g-1 fresh leaves. *L** values of samples were measured between 37.9–45.0 and *a** values -8.3– -3.9 and *b** values 8.6 – 15.0. a/b values were calculated between -0.57– -0.45. (+)-catechin. (-) epicatechin, gallic acid, caffeic acid and vanillic acid were detected in all grape leaf samples.

Keywords: Grape leaves, Phenolics, Flavonoids, Color properties

Introduction

Grape is an important fruit crop grown all over the world. Turkey has the most suitable climate conditions for vine growing in the world and is the origin of the vine genes. Grape and grape derived products have been produced since ancient times. Grape leaves are also one of the grape products and have been consumed as fresh and canned for years. Stuffed grape leaves (dolma) are a traditional delicacy for Turkish. Balkan and Middle East nations. Colors of grape leaves are green, yellow-green, dark green and red. They have sugars, organic acids, amino acids, phenolic compounds and vitamins (Ribereau ve Reynold. 1971).

Grape is a major source of phenolic compounds (Maxcheix et al., 1990) with good nutraceutical potential (Doshi et al., 2006). Phenolic compounds have attracted an increased attention in the field of nutrition, health and medicinelargely because of their anticarcinogenic /antimutagenic, antiulceric, antiallergic, antiatherogenic, anti-inflammatory, antiarthritic, antimicrobial properties and, very importantly, antioxidant activity (Amellai et al., 1985; Mangiapane et al., 1992; Liviero & Puglisi, 1994; Teissedre et al., 1996; Bagchi et al., 1998; Siato et al., 1998; Catterall et al., 2000, Doshi et al., 2006). The antioxidant activity of grapes is positively correlated with the concentration of phenolic compounds and further to their composition, anthocyanins, flavonols, flavan-3-ols, hydroxybenzoates, etc. (Landbo & Meyer, 2001, Doshi et al., 2006). It was reported that grapevine parts and berries had high phenolic compounds (Doshi et al., 2006). In this study; grape leaves from Sultani Çekirdeksiz (SÇ), Sultan1 (S1), Sultan7 (S7), Saruhanbey (SB) and Narince (N) grape cultivars were assayed.

Material and Methods

SÇ, S1, S7, SB and N leaf samples were obtained from Manisa Viticulture Research Station vineyards. Thirty leaves samples were randomly collected from every grape variety. Leaves were washed and cleaned with deionized water and ethanolic solution respectively. L*, a*, b* values of the samples were measured by Minolta Colorimeter and a/b values were calculated. Chlorophyll values were measured by SPAD-502plus chlorophyll meter.

Total phenolic contents (TP) of the leaf samples were determined spectrophotometrically

using a Multiskan FC Microplate Spectrophotometer (Thermo scientific. Finland) according to the Folin-Ciocalteu colorimetric method (Singleton & Rossi 1965). The concentration of total phenolics was expressed as gallic acid equivalent (mg GAE g-1). Total flavonoids (TF) were determined using the colorimetric assay with aluminum chloride and (+)-catechin as standard for calibration according to Zhishen et al. (1995). 5 g fresh samples were crushed and homogenized using by IKA Ultra Turrax at 9000 rpm for 5 min. Then the mixtures were centrifuged at 7000 rpm for 10 min. The supernatants were utilized for analysis of total phenolic and total flavonoid content.

Extraction of phenolics for liquid chromatography was done according to Taware et al. (2010) and Rusjan and Korosec-Koruza (2007). The frozen leaves were crushed and extraction was carried out with 1 g sample in 10 ml acidified Methanolic water (Methanol with 1% Formic acid: water, 1:1 v/v) using by IKA Ultra Turrax at 9000 rpm for 5 min. The extracts were centrifuged for 10 min (7000 rpm), filtered through 0.45 µm syringe filter and stored -20 ºC until injection.

Agilent 1260 Infinity HPLC system and diode array detector was used. Analyses were conducted at 30 ºC using Agilent C18 column (4.6 x 250 mm, 5 µm). The compounds were detected at 280 nm. The Mobile phase consisted of A (water-formic acid, 98:2 v/v) and B (acetonitrile-formic acid, 98:2 v/v). The gradient program was used based upon the methods of Breksa et al. (2010) and J. Meng et al. (2011) with some modifications and was as follows: a 3 min isocratic elution step with 5% B, followed by linear gradient from 5% to %9 B, 7 min linear gradient to 13.5% B, 4 min linear gradient to 18.5% B, 4 min linear gradient to %5 B and 15 min isocratic elution with 15% B. The flow rate was 1.0 ml/min and injection volume 5 µl.
Statistical analyses of the data were done by using SPSS packet program and Duncan's multiple range tests were used to compare means.

Results

L, a*, b** and a/b values of grape leaf samples were presented in Table1. Chlorophyll values also were indicated in Table1 and expressed as spad units. Statistically differences were found in terms of all examined color parameters among leaf samples ($p \leq 0.05$). The highest L* values were observed in SB and N samples, a* values in S1, SÇ and S7 samples, b* values in SB and N and a/b values in S1 and N samples. Chlorophyll values were determined between 27.83 and 32.23 spad. S7, SÇ and S1 showed highest chlorophyll values.
Total phenolic and flavonoid content of leaves differed significantly in five varieties *($p \leq 0.05$).* Total Phenolics were determined between 9.72 and 14.25 mg g^{-1} and flavonoids between 5.08 and 6.22 mg g^{-1}. N leaves showed the lowest TF values and S1 leaves TP values.

Table 1. Color and chlorophyll properties of leaves

Variety	L^*	a^*	b^*	a/b	*SPAD*
SB	44.91^{a}	-8.26^{b}	15.03^{a}	-0.55^{c}	29.13^{bc}
S7	40.31^{b}	-4.68^{a}	8.56^{b}	-0.53^{bc}	31.10^{ab}
S1	40.26^{b}	-3.86^{a}	8.96^{b}	-0.43^{a}	32.83^{a}
N	45.00^{a}	-6.62^{ab}	14.93^{a}	-0.45^{ab}	27.83^{c}
SÇ	37.92^{b}	-4.18^{a}	7.34^{b}	-0.56^{c}	32.23^{a}

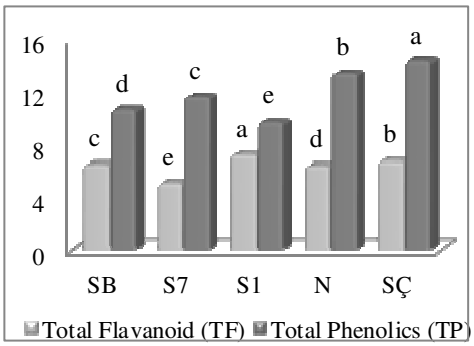
*Values with the different letters within each column are significantly different for *p≤0.05*

Table 2. Phenolic compounds of leaves (mg kg^{-1})

Variety	*(+)-Catechin*	*Epicatechin*	*Vanilic Acid*	*Gallic Acid*	*Caffeic Acid*
SB	112.33^{ab}	20.47^{c}	66.07^{c}	6.67	52.93^{b}
S7	101.03^{b}	34.03^{b}	60.00^{d}	6.15	51.20^{b}
S1	103.34^{b}	34.60^{b}	64.20^{cd}	6.26	45.10^{c}
N	91.20^{b}	20.07^{c}	73.40^{b}	6.03	70.14^{a}
SÇ	131.73^{a}	55.50^{a}	78.92^{a}	6.02	51.23^{b}

*Values with the different letters within each column are significantly different for *p≤0.05*

Phenolic content of leaf samples were presented in Table 2. Figure 2 and 3 showed HPLC chromatograms of phenolic compounds for standards and samples. Statistically differences were observed in terms of (+)-catechin, epicatechin, vanilic acid and caffeic acid values among leaf samples *(p≤0.05)*.



*TF: Catechin equivalent (mg g^{-1})
*TP: Gallic acid equivalent (mg g^{-1})

Figure 1. Total phenolic and flavonoids of leaves

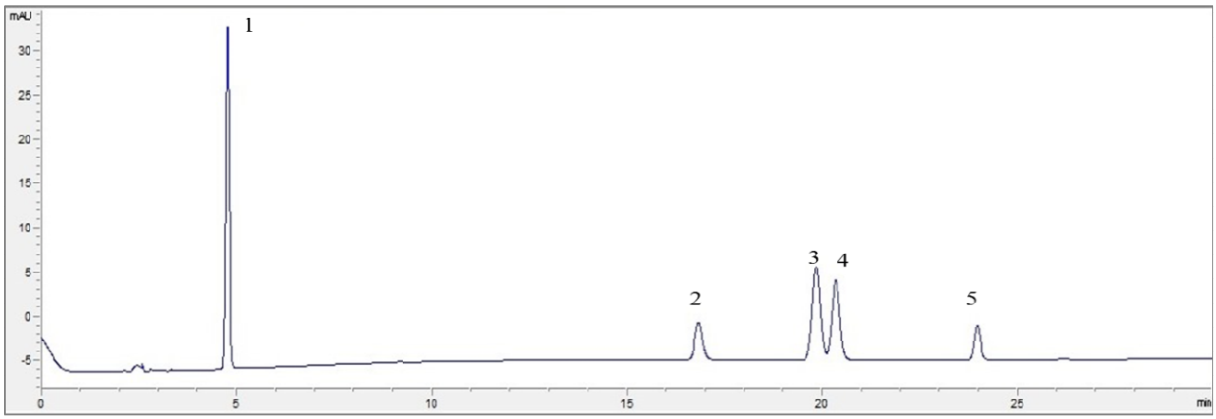


Figure 2. HPLC chromatograms obtained for standards (1, gallic acid; 2, (+)-catechin; 3, vanilic acid; 4, caffeic acid; 5, epicatechin)

Discussion

Color is one of the most important parameters for fresh grape leaves. These parameter directly affects the consumer choice. Especially; L* and a* values are important parameters for fresh consumed grape leaves. Gülcü and Demirci (2011) studied on quality properties of some pickled grape leaves and Doshi et al., (2006) were investigated the phenolic composition and antioxidant activity in grapevine parts and berries (Vitis vinifera L.) cv. Kishmish Chornyi (Sharad Seedless) during maturation. They have reported that the highest flavonoid values were determined in leaf samples at first harvest times (29.15 mg g-1 (+)-catechin equivalent). They found that total phenolic contents of Thompson seedless leaves were between 1.5 and 2.5 mg g^{-1} GAE (Taware et al., 2010). Total flavonoid content of leaf samples in our study were low and total phenolic content were high compared to literature.

It was reported by different researches that grapevine leaves are rich source of phenolic compounds (Doshi et al., 2006, Taware et al., 2010, Bonilla et al., 2003). Bonilla et al. (2003) expressed that that first time studied on the phenolic content and antioxidant capacity of muscadine leaves. Major phenolics in muscadine leaves were investigated color properties. They have reported that L* values were between 28.84 and 35.99, a* between -1.44 and -2.69 and b* values between 16.23 and 21.70. Although our results of color parameters were generally similar with this research, some except for minor differences. Our L* values were higher and a* and b* values lower than the findings of this research.

myricetin, ellagic acid, kaempferol, quercetin, and gallic acid, with average concentrations of 157.6, 66.7, 8.9, 9.8, and 8.6 mg per 100 g fresh weight respectively. Average total phenolic contents of leaves were 351.9 mg GAE per 100 g fresh weight (Bonilla et al., 2003). Some phenolic compounds in Thomson seedless leaves were gallic acid, vanilic acid, caffeic acid, rutin hydrate, quercetin hydrate, quercetin, catechin, epicatechin and average concentrations of these phenolic were 362.00, 4.72, 4.25, 239.80, 3.34, 2.64, 0.58, mg kg^{-1} respectively (Taware et al., 2010). Our phenolic compound results were showed differences with the other two studies. These differences have been estimated as a result of location, soil, climate, variety, sampling time, etc.

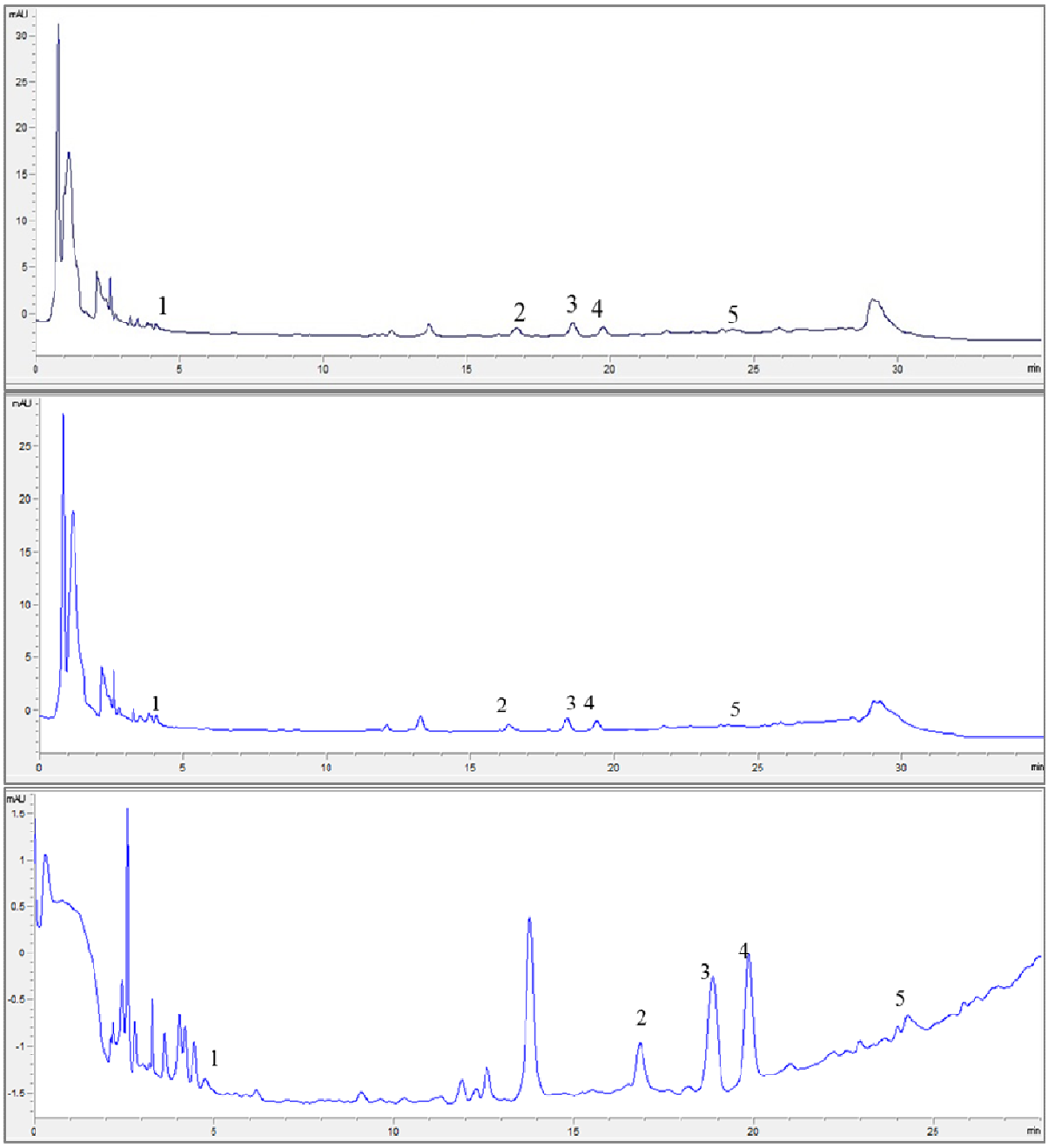


Figure 3. HPLC chromatograms obtained for three different grape leaves; SÇ, SB and N (1, gallic acid; 2, (+)-catechin; 3, vanilic acid; 4, caffeic acid; 5, epicatechin)

Conclusion

Grapevine leaves have been consumed for years. Five different grapevine leaves which commonly consumed were investigated in this study. Some phenolic compounds and physical characteristics of leaves were determined. It was found that leaf samples had high phenolic content such as grape berry and phenolic content and color properties changed among the varieties.

References

Amellai, M., Bronner, C., Braincon, F., Haag, M., Anton, R. & Landry, Y. (1985). Inhibition of mast cells histamine release by flavonoids and bioflavonoids. Planta Medica, Feb (1), 16–20.

Bagchi, D., Garg, A., Krohn, R.L. et al. (1998). Protective effect of grape seed proanthocyanidins and selected antioxidants against TPA-induced hepatic and brain lipid peroxidation and DNA fragmentation and peritoneal macrophage activation in mice. General Pharmacology, 30, 771–776.

Bonillai, E. P., Akoh, C.C., Sellappan S., Krewer, G. 2003. Phenolic Content and Antioxidant Capacity of Muscadine Grapes. J. Agric. Food Chem. 2003, 51, 5497-5503 5497

Catterall, S., Souquet, J.M., Cheynier, V., Clifford, M.N. & Ioannides, C. (2000). Modulation of the mutagenecity of food carcinogens by oligomeric and polymeric procyanidins isolated from grape seeds: synergistic genotoxicity with nitrosopyrrolidin. Journal of the Science of Food and Agriculture, 80, 91–101.

Doshi, P., Adsule, P., Banarjee,K., 2006. Phenolic composition and antioxidant activity in grapevine partsand berries (Vitis vinifera L.) cv. Kishmish Chornyi (Sharad Seedless) during maturation. International Journal of Food Science and Technology 2006, 41 (Supplement 1), 1–9

Gülcü, M., Demirci, A.Ş., 2011. Salamuraya İşlenen Bazı Asma Yapraklarının Kalite Özellikleri Üzerine Bir Araştırma. Tekirdağ Ziraat Fakültesi Dergisi. 2011 8 (3)

Landbo, A.K. & Meyer, A.S. (2001). Ascorbic acid improves the antioxidant activity of Europeans grape juices by improving the juice's ability to inhibit lipid peroxidation of human LDL in vitro. International Journal of Food Science and Technology, 36, 727–735.

Liviero, L. & Puglisi, P.P. (1994). Antimutagenic activity of procyanidins from Vitis vinifera. Fitoterapia, LXV, 203–209.

Mangiapane, H., Thompson, J., Salter, A., Brown, S., Bell, G.D. & White, D. (1992). The inhibition of the oxidation of low-density lipoprotein by (+) catechin, a natural occurring flavonoid. Biochemical Pharmacology, 43, 445–450.

Meng, J., Fang, Y., Zhang, A., Chen, S., Xu, T., Ren, Z., Han, G., Liu, J., Zhang, Z., Wang, H, 2011. Phenolic content and antioxidant capacity of Chinese raisins produced in Xinjiang Province. Food Research International 44 (2011) 2830-2836.

Ribereau, G.J. and E, Reynold. 1971. Science et Techniques de la Vigne. Tome 1., Biologie de la Vigne. Sols de Vignobles.Ed. Dunod. Paris.

Rusjan, D., Korosec-Koruza, Z., 2007. A comparison of extraction methods for selected phenolic compounds from grape berry skins using liquid chromatography and spectrometry. Acta. Chim. Slov., 54:114-118 (2007).

Siato, M., Hosoyama, H., Ariga, T., Kataoka, S. & Yamaji, N. (1998). Antiulcer activity of grape seed extracts and procyanidins. Journal of Agricultural and Food Chemistry, 46, 1460–1464.

Singleton, V.L., Rossi, J. R., 1965. Colorimetry of total phenolics with Phospho molybdic phosphotungstic acid. American Journal of Enology and Viticulture 16: 144-158

Taware, P. B., Dhumal, K. N., Oulkar D.P., Patil S.H., Banarjee K. 2010. Phenolic Alterations in Grape Leavesi Berries and Wines Due to Foliar and Cluster Powdery Müldew Infections. International Journal of Pharma and Bio Sciences. V1(1) 2010.

Teissedre, P.L., Frankel, E.N., Waterhouse, A.L., Peleg, H. &German, J.B. (1996). Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wines. Journal of the Science of Food and Agriculture, 70, 55–61.

Zhishen, J., Mengchneg, T., Jianming, W., 1999. The determination of flavonoids contents in mulberry and their scavenging effects on superoxide radicals. Food Chem.. 64. 555–559 (1999).