Chemistry of Natural Compounds, Vol. 42, No. 2, 2006

ORGANIC ACID, TOCOPHEROL, AND PHENOLIC COMPOSITIONS OF SOME TURKISH GRAPE CULTIVARS

Nilgun Gokturk Baydar

UDC 547.56.58

The organic acid, tocopherol, and phenolic compositions of three different grape cultivars, Emir, Kalecik karasi, and Narince were studied in order to evaluate their nutritive values and the contents of natural antioxidants. Organic acids, tocopherols, phenolic acids, flavonoids, and trans*-resveratrol contents were analyzed by high-performance liquid chromatography (HPLC). In addition, pH, soluble solid, titratable acidity, and total phenolic contents were also determined. It was determined that the contents of organic acids, tocopherols, and phenolic compounds were changed according to the cultivars.*

Key words: Grape cultivars, organic acid, tocopherol, phenolic compound.

In this study we determine the organic acid, tocopherol, and phenolic compositions of three different grape cultivars, Emir, Kalecik karasi, and Narince.

The organic acid compositions of three grape cultivars were determined by HPLC, and the data are given in Table 1. As shown in the table, tartaric and malic acids are the most abundant organic acids in grapes. Citric, oxalic, and fumaric acids were found to be in lower concentrations as compared to tartaric and malic acids. The results obtained are generally in agreement with the literature data [1–3].

The parameters of soluble solids, titratable acidity, and pH in grapes are also presented in Table 1. Soluble solid values of grapevine are found as 18.00% for Narince, 23.25% for Kalecik karasi, and 18.75% for Emir. Titratable acidity changed from 0.42 g/100 mL (Narince) to 0.63 g/100 mL (Emir). PH values also varied from 3.53 to 3.83.

The contents of individual tocopherols in the grapes were determined as μg/100 g berry (fresh weight, FW), and data are given Table 2. α-Tocopherol was the most abundant tocopherol in all the cultivars compared to γ and δ-tocopherols. Among the tocopherols present in foods, the α-homologue shows the highest vitamin E activity, thus making it the most important for human health and biological activity [4]. On the other hand, β-tocopherol was not found in the berries. The values of total tocopherol content varied from 61.28 (Emir) to 330.68 μg/100g (Kalecik karasi) in this study. To our knowledge, there is no information on tocopherol contents of grape berries. Tocopherols are present mostly in plant oils such as olive, sunflower, canola, palm etc. [5, 6]. So published studies on the tocopherol contents of grape are mostly focused on grape seed oil [7–9]. Regarding the tocopherol contents of grape seed oil, the study [7] determined that α-tocopherol was dominant in grape seed oil, with a value of 100.55 mg/kg oil.

The contents of *trans*-resveratrol, phenolic acids, and flavonoids in three different grape cultivars are given in Table 3. As regards grapes, the concentrations of these substances seem to vary considerably, since it depends on the cultivars.

trans-Resveratrol was found in the berries of Kalecik karasi, with 0.014 μg/g, while in the other cultivars it was not detected. *trans*-Resveratrol is a stilbene that is produced by plants in response to fungal infection or abiotic stresses such as heavy metal ions or UV light exposure. *trans*-Resveratrol also has a biological effect that provides health benefits such as protection against atherosclerosis, coronary hearth disease and cancer [10, 11]. The phenolic acids, including gallic, chlorogenic, ferulic, caffeic, *o*-coumaric and *p*-coumaric, syringic, and *trans*-cinnamic acids, showed differences according to the cultivars. Chlorogenic and caffeic acids were not detected in Kalecik karasi. However, syringic acid was found only in Kalecik karasi, 0.55 μg/g. The highest gallic acid value was found in Kalecik karasi.

University of Suleyman Demirel, Agricultural Faculty, Department of Horticulture, 32270, Isparta-Turkey, fax (90 246) 237 16 93, e-mail: nilgun@ziraat.sdu.edu.tr. Published in Khimiya Prirodnykh Soedinenii, No. 2, pp. 128-130, March-April, 2006. Original article submitted October 17, 2005.

 0009-3130/06/4202-0156

TABLE 1. Organic Acids, pH, Titratable Acidity and Soluble Solid Values of Grape Cultivars

Cultivars	Organic acids					pH	Titratable acidity, g/100 mL	Soluble solids, %
	tartaric, mg/g	malic, mg/g	citric, μg/g	oxalic, μg/g	fumaric, μg/g			
Emir	3.35±0.10	1.28±0.09	42.76±1.10	24.15±2.19	8.14±0.43	3.53±0.07	0.63±0.02	18.75±0.16
Kalecik karasi	2.96±0.11	1.70±0.08	54.13±2.64	24.42±1.00	13.23±0.14	3.83±0.01	0.51±0.07	23.25±0.50
Narince	4.83±0.18	2.10±0.32	62.14±0.14	18.16±3.16	11.00±0.20	3.69±0.02	0.42±0.03	18.00±0.50

TABLE 2. Tocopherol Contents of Grape Cultivars

Cultivars	Tocopherols, μg/100 g FW				
	α-tocopherol	β-tocopherol	γ-tocopherol	δ-tocopherol	Total tocopherol
Emir	53.43±3.22	N.d.	7.46±0.89	0.39±0.02	61.28±4.16
Kalecik karasi	286.67±11.12	N.d.	43.52±2.16	0.49±0.02	330.68±14.22
Narince	84.73±4.46	N.d.	20.27±1.17	4.97±0.18	109.97±6.86

N.d.:not detected.

TABLE 3. The Contents of *trans*-Resveratrol, Phenolic Acids, and Flavonoids in Grape Cultivars

Phenolic compounds	Cultivars		
	Emir	Kalecik karasi	Narince
trans-Resveratrol, μg/g	N.d.	0.014±0.00	N.d.*
Phenolic acids, μg/g:			
Gallic	0.62±0.02	5.20±0.82	1.30±
Chlorogenic	0.22±0.01	N.d.	2.26±0.04
Caffeic	0.18±0.00	N.d.	0.33±0.02
Syringic	N.d.	0.55±0.00	N.d.
p-Coumaric	0.13±0.00	0.15±0.00	0.46±0.04
Ferulic	0.20±0.02	0.21±0.02	N.d.
o-Coumaric	0.30±0.01	0.46±0.02	1.22±0.08
trans-Cinnamic	0.02±0.00	0.05±0.00	0.08±0.00
Flavonoids, μg/g:			
(+)-Catechin	30.71±2.13	66.20±5.46	89.25±7.16
Vanillin	0.03±0.00	N.d.	0.08±0.00
(-)-Epicatechin	1.49±0.05	1.00±0.06	1.79±0.01
Rutin	0.28±0.03	18.95±1.86	1.08±0.04
Quercetin	0.35±0.00	0.87±0.04	0.60±0.02
Total phenolic content, mg_{GAE}/100 g	73.78±2.82	137.48±3.45	142.79±6.14

N.d.: not detected.

On the other hand, Narince had the highest chlorogenic, caffeic, *p*-coumaric, *o*-coumaric, and *trans*-cinnamic acid contents as compared to the other cultivars.

The most abundant flavonoid was (+)-catechin in the berries of all cultivars. The highest (+)-catechin content was found in Narince followed by Kalecik karasi and Emir, respectively. (–)-Epicatechin values changed between 1.00 and 1.79 μg/g. Rutin concentration was rather high in Kalecik karasi as compared to the other cultivars. Quercetin concentrations were relatively low

in the berries. Vanillin was not found in Kalecik karasi, while Narince and Emir had vanillin in small quantities, 0.03 and 0.08 μg/g, respectively.

The total phenolic contents of cultivars determined with the colorimetric method and data are presented in Table 3. Comparing the phenolic contents in the berries, it is seen that there are some differences among the cultivars. Narince showed the highest phenolic content followed by Kalecik karasi with very close value.

When the results were evaluated, the levels of phenolic compounds in berries changed according to the grape cultivar. These results were in agreement with the findings of Lee and Jaworski [12], and Oszmianski and Lee [13].

In recent years, much attention has been devoted to phenolic compounds. In particular, flavonoids and related phenolics attract increasing attention due to their antioxidant properties, which may help to explain the protective effect of vegetable-fruit rich diets on coronary hearth disease [14].

This study provides basic information on the organic acid, tocopherol, and phenolic composition of grapes. It is known that grapes are one of the most important fruits in human health and its importance arises from not only the nutritive value but also its natural antioxidant compounds.

EXPERIMENTAL

Materials. Grapes of some popular cultivars grown in Turkey, Emir (white), Kalecik karasi (red), and Narince (white) were collected at optimal maturity from the experimental vineyard of the Agricultural Faculty of Ankara University (Ankara, Turkey).

Determination of Soluble Solids, pH, and Titratable Acidity. The soluble solid content of grapes was determined as Brix using a refractometer; the pH of berries was determined with a pH meter (WTW pH 526), and the titratable acidity by titrating 10 mL sample with 0.1 N NaOH to pH 8.1. Titratable acidity was expressed as g tartaric acid/100 mL.

Determination of Organic Acids. Five grams of berries was mixed with 5 mL of methanol and the mixtures were homogenized using an ultratorax at 24.000 rpm and centrifuged at 3500 rpm for 10 min at ambient temperature. Then, 0.2 mL of mixture was diluted with 1.8 mL of 0.05 M phosphoric acid [H_3PO_4 (pH:2.2)]. The final mixture was filtered through a 0.45 μm membrane filter before 20 μL injections. HPLC analysis of organic acids was performed by HPLC on a Shimadzu Class LC VP HPLC system with class LC-VP software equipped with a UV-VIS detector (SPD-10AV vp) and a pump (LC-6AD); 0.05 M H_3PO_4 prepared in water and adjusted to pH 2.2 with NaOH was used as the mobile phase. The elution was conducted at room temperature using a YMC Pack-ODS-AM (250 × 4.6 mm i.d., 5 μm) column at a flow rate of 0.8 mL/min. The UV detector was set at 210 nm. Initial identity assignment of organic acids was based on comparison retention data obtained with a UV detector for standard compounds and sample components. Quantization was achieved by using peak areas from external calibration with standard [tartaric, malic, citric, oxalic, fumaric acids (Sigma)] solutions. All determinations were done three times by using three different samples.

Determination of Tocopherols. Undamaged and disease-free berries were snipped from clusters. After seeds were manually separated from whole berries, berries were dried at room temperature. Dried grape samples were crushed in a grinder for 2 min. Powdered 2 g of samples of the grapes were weighed and materials were extracted by triple-extraction with 10 mL hexane at room temperature. Then hexane was removed by rotary evaporation under vacuum at 35°C. The extract was diluted in a mixture of heptane:tetrahydrofuran (THF) (95:5) (v/v), filtered (0.5 pm Millipore) and placed in non-actinic vials. They were overlayered with nitrogen and stored for up to 24 h at +4°C.

Tocopherols were analyzed by HPLC. In the tocopherol analyses, the HPLC method of [15] was modified. The HPLC system (Shimadzu) was equipped with an autosampler (SIL-10AD vp), system controller (SCL-10Avp), pump (LC-10Advp), degasser (DGU- 14A), and column oven (CTO-10Avp), and the column temperature was 30°C. The detector used was fluoresans detector with wavelengths set at 295 nm for extinction and 330 nm for emission. Tocopherols were separated on a normal phase column (Luna, 150 × 4.6 mm i.d., 5 μm particle size) with mobile phase flow rate 1.2 mL/min. The mobile phase was a mixture of heptane:THF (95:5) (v/v). The data were integrated and analyzed using the Shimadzu Class-VP Chromatography Laboratory Automated Software system. Standard samples of α, β, γ, and δ isomers of tocopherol (Sigma Chemical Co., St. Louis, Mo., USA) were dissolved in hexane and used for identification and quantification of peaks. The amount of tocopherols in the extracts was calculated as μg tocopherols in 100 g berry (fresh weight, FW) using external calibration curves obtained for each tocopherol standard.

Determination of Total Phenolic Content. For total phenolic content, extraction was done by the method of Ojeda et al. [16]. The concentration of total phenolics in the berries was determined by the Folin-Ciocalteu colorimetric method [17]. Estimations were carried out in triplicate and calculated from a calibration curve obtained with gallic acid. Total phenolic contents were expressed as gallic acid equivalents (mg_{GAE}/100g).

Determination of *trans*-Resveratrol, Phenolic Acids, and Flavonoids by HPLC. The phenolic compounds were extracted using the method described by Dragovic-Uzelac et al. [18]. Separation of phenolics was performed by the modified method of Caponio et al. [19]. Reversed phase (RP)-HPLC analysis was done using 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 × 4.6 mm i.d. 5 μm column used was filled with Luna Prodigy. The flow rate was 1 mL/min, the injection volume was 10 μL, and the column temperature was set at 30°C. For gradient elution, mobile phase A contained 2% acetic acid in water; solvent B contained methanol. The following gradient was used: 0–3 min, from 100% A to 95% A, 5% B; 3–20 min, from 95% A, 5% B to 80% A, 20% B; 20–30 min, from 80% A, 20% B to 75% A, 25% B; 30–40 min, from 75% A, 25% B to 70% A, 30% B; 40–50 min 70% A, 30% B to 60% A, 40% B; 50–55 min, 60% A, 40% B to 50% A, 50% B; 55–65 min, 50% A, 50% B to 100% B. The data were integrated and analyzed using the Shimadzu Class-VP Chromatography Laboratory Automated Software system. The grape samples, standard solutions, and mobile phases were filtered by a 0.45 μm pore size membrane filter. The amount of phenolic compounds in the extracts was calculated as μg/g berry using external calibration curves obtained for each phenolic standard.

REFERENCES

1. J. J. Hunter, J. H. Visser, and O. T. De Villiers, *Am. J. Enol. Vitic*., **42** (3), 237 (1991).
2. A. K. Kanellis and K. A. Roubelakis-Angelakis, In: *Biochemistry of Fruit Ripen*, G. B. Seymour, J. E. Taylor & G. A. Tucker (Eds.), London: Chapman & Hall, 1993, p. 189.
3. Y. Soyer, N. Koca, and F. Karadeniz, *J. Food Com. Anal*., **16**, 629 (2003).
4. N. Guthrie and E. M. Kurowska, In: *Nutraceuticals and Functional Foods*, Wildman CRC Press, Boca Raton, Florida, 2001, pp. 269-280.
5. W. Degreyt, *PhD Thesis*, Universite des Sciences Appliques de Gent, Belgique (1998).
6. M. P. Aguilera, G. Beltran, D. Ortega, A. Fernandez, A. Jimenez, and M. Uceda, *Food Chem.*, **89**, 387 (2005).
7. A. Gliszcynska-Swiglo and E. Sikorska, *J. Chromatogr. A*, 1048, 195 (2004).
8. N. Gokturk Baydar and M. Akkurt, *Turk. J. Agric. For.*, **25**, 163 (2001).
9. B. H. Oomah, J. Liang , D. Godfrey, and G. J. Mazza, *J. Agric. Food Chem*., **46**, 4017 (1998).
10. E. N. Frankel, A. L. Waterhouse, and J. E. Kinsella, *Lancet*, **341**, 1103 (1993).
11. S. Ulrich, F. Wolter, and J. M. Stein, *Mol. Nutr. Food Res.*, **49**, 452 (2005).
12. C. Y. Lee and A. Jaworski, *Am. J. Enol. Vitic*., **41**, 87 (1990).
13. J. Oszmianski, C. Y. Lee, *Am. J. Enol. Vitic*., **41**, 204 (1990).
14. O. I. Aruoma, A. Murcia , J. Butler, and B. Halliwell, *J. Agric. Food Chem.*, **41**, 1880 (1993).
15. A. M. Lampi, L. Kataja, A. Kamal-Eldin, and V. Piironen, *J. Amer. Oil Chem. S*oc., **76**, 749 (1999).
16. H. Ojeda, C. Andary, E. Kraeva, A. Carbonneou, and A. Deloire, *Am. J. Enol. Vitic*., **53**, 261 (2002).
17. V. L. Singleton and J. R. Rossi, *Am. J. Enol. Vitic*., **16**, 144 (1965).
18. V. Dragovic-Uzelac, J. Pospisil, B. Levaj, and K. Delonga, *Anal. Nutr. Clin. Meth*., **91**, 373 (2005).
19. F. Caponio, V. Alloggio, and T. Gomes, *Food Chem*., **64**, 203 (1999).