

Effects of Cultural Practices on Total Phenolics and Vitamin C Content of Organic Table Grapes

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The total phenolic and vitamin C contents of organic table grapes were determined by spectrophotometry and HPLC, respectively, after several quality-increasing treatments were applied at véraison for two growing seasons. Cane girdling and cluster-berry thinning resulted in different responses in the cultivars. ‘Red Globe’ generally was found to produce the lowest quantity of total phenolics in the berries, while ‘Trakya İlkeren’ was the cultivar that accumulated the highest phenolics. Vitamin C accumulation in the berries was also affected by the treatments. The effect of the treatments was inconclusive in ‘Buca Razakısı’. The application of both girdling and thinning caused more accumulation of vitamin C in the ‘Alphonse L.’ and ‘Trakya İlkeren’ berries.

INTRODUCTION

There is increased awareness in the world about consuming organic food products. A vast number of published studies have dealt with comparisons of the nutritive value of agricultural products derived from both conventional and organic systems. Although there is a general consensus that organic foods contain a high dry matter and lower nitrate content (Huber *et al.*, 2011), regarding their content of vitamins and phenolic compounds some studies have indicated that they are higher (Carbonaro *et al.*, 2002; Wang *et al.*, 2008) and others that they are at similar or lower levels (Lombardi-Boccia *et al.*, 2004).

Grape berries, especially the coloured ones, contain phenolic compounds in their skins and seeds (Poudel *et al.*, 2008). They contribute not only to colour but also to sensory properties such as the bitterness and astringency of the juice and wine. Phenolic compounds are also regarded as preservatives against microbes and the oxidation of food (Heaton, 2001; Benbrook *et al.*, 2008). In this respect, vitamin C is another very important compound in the protection against oxidative stress in plants (Rock *et al.*, 1996). It also is a co-factor of several enzymes that participate in the synthesis of anthocyanidins (Barata-Soares *et al.*, 2004).

Table grape production usually involves treatments such as adjusting crop load by cultivar thinning and shoot topping, as well as increasing crop quality by girdling, topping or tipping, and using growth regulators, mainly gibberellic acid. The effects of these treatments on yield and quality have been studied intensely, with varying results depending on the level, time, cultivar and site.

However, there is little information on the impact of various cultural practices on the production of phenolic metabolites in organically produced table grape cultivars. This study focused on the determination of the effects of crop thinning and cane girdling on the total phenolic compounds and vitamin C contents of organic table grapes.

MATERIALS AND METHODS

Three coloured table grape cultivars (*Vitis vinifera* L.) (Alphonse Lavallée, Red Globe and Trakya İlkeren) and one white table grape cultivar (Buca Razakısı) were grown organically at the experimental vineyard (38°27'32"N, 27°13'21"E) of the Department of Horticulture, Ege University, İzmir, Turkey. ‘Trakya İlkeren’ is a blue-black cultivar with round and large berries. Its cluster is very large and it ripens seven to eight days before ‘Cardinal’. ‘Buca Razakısı’ is a white-coloured, midseason cultivar with winged conical clusters carrying 3 to 4 g berries. The vines grafted onto 41B rootstock were trained to a Guyot on a Y system with 2.5 x 3 m spacing. The vines were seven years old at the time of the experiment. Cultivation practices and the control of pests and diseases were employed according to organic production regulations (European Council Regulation No: 834/2007). Soil conditions were as follows: sand 40%, silt 40%, clay 20%, low salinity, pH 7.8, organic matter 2.6%.

Cluster-berry thinning (CBT) was performed at véraison, on 1 August for ‘Red Globe’ and ‘Alphonse L.’ and on 15 June for ‘Trakya İlkeren’ and ‘Buca Razakısı’. ‘Red Globe’ and

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Trakya Ilkeren' were thinned to 25 clusters per vine, while 20 clusters were retained on the other two cultivars. Clusters left on the vine were tipped to weigh approximately 500 g. Cane girdling (CG) was also performed at véraison on the fruiting canes just below the first basal cluster from the bottom. A 0.4 cm wide ring of bark was completely removed with a double-bladed knife. Control vines of each cultivar were neither thinned nor girdled. When the clusters on the control vines reached approximately 16°Brix, the grapes were harvested at technological maturity and analyses were carried out. Trials were carried out during the growing seasons of 2012 and 2013.

Trials were carried out on a split-plot design in which the cultivars and the treatments were in the main and sub-parcels respectively. All cultivars were in the same block and replicates were planted randomly. Every treatment consisted of three replicates of four vines. At harvest, 10 clusters from each replicate of a treatment were picked and sampled for total phenolics and vitamin C contents. Analyses were repeated three times.

Extractions were performed by the method of Ojeda *et al.* (2002). For each replication, skins from 10 berries from the major class were separated manually, weighed and ground to a powder with liquid nitrogen using a porcelain mortar and pestle. Phenolic compounds were extracted into 70% acetone in water (v/v) to give an average powder concentration of 2.5% (w/v) in 2 mL Eppendorf tubes. The tubes were agitated mechanically for 15 min at room temperature, sonicated (Branson 220; Roucaire, Courtaboeuf, France) for 30 min, and centrifuged for 10 min at 6 500 g. The supernatant was recovered and stored at -20°C. Six extractions were carried out for each sample.

The total phenol content (mg/g per fresh weight) was determined by the Folin-Ciocalteu method (Singleton & Rossi, 1965) and expressed as gallic acid equivalents (GAE) by determining the absorbance at 765 nm. Folin-Ciocalteu reagents were purchased from Sigma Aldrich (F-9252).

The vitamin C content (mg per 100 mg fresh weight) was determined by the HPLC method described by Cemeroglu (2007), with some modifications. Whole berries were crushed and ground with a hand blender and prepared into a mesh. Three g was weighed and transferred to 50 ml falcon tubes, to which 6 mL of 2.5% metaphosphoric acid solution was added. The mixture was centrifuged at 4°C and 6 500 g for 10 min. Clear supernatant (2 mL) was filtered through a 0.45 µm Teflon filter. A C₁₈ column (Phenomenex Luna C₁₈, 250 x 4.60 mm, 5 µ) was used and the column temperature was set to 25°C. The mobile phase was ultrapure water adjusted to pH 2.2 with H₂SO₄ at 1 mL per min flow rate. Readings were performed in a diode array detector at 254 nm wavelength using L-ascorbic acid (Sigma A5960) at varying concentrations (50, 100, 500, 1 000, 2 000 ppm). Each analysis of total phenolics and vitamin C was repeated three times.

Because there were no significant effects of the growing seasons, the data obtained from the analyses over two growing seasons were pooled before the statistical analysis was carried out. The descriptive statistics for the studied variables (characteristics) were presented as mean and standard error. ANOVA was employed to compare for

means of practices (treatments and cultivars) for the studied variables. Duncan's multiple comparison test was carried out for determination of statistically significant differences in the means. The statistical significance level considered was 5%. The SPSS (SPSS Inc., IL., USA, ver. 13) statistical program was used for all computations.

RESULTS AND DISCUSSION

The statistical analysis showed that both the total phenolic and vitamin C contents of the cultivars were influenced by the treatments. 'Trakya Ilkeren', the earliest ripening cultivar, always had the highest amount of total phenolics (2.09 mg/g GAE) compared to the other cultivars. The effect of a treatment on the phenolic contents changed within the cultivars. The treatment that caused a cultivar to accumulate the most phenolics compared to the others differed, such as CG+CBT in 'Trakya Ilkeren' (2.20 mg/g GAE) and 'Buca Razakısı' (1.82 mg/g GAE), CG in 'Alphonse L.' (2.00 mg/g GAE), and CBT in 'Red Globe' (0.84 mg/g GAE).

The vitamin C content of the cultivars showed limited variations. Mean values of the vitamin C accumulated in the cultivars ranged from 12.76 ('Red Globe') to 16.34 ('Alphonse L.') (Table 1). Interestingly, the control groups of both 'Buca Razakısı' and 'Alphonse L.' contained similar amounts of vitamin C. While its level in 'Buca Razakısı' did not change significantly with treatments, it showed significant variations in the 'Alphonse L.'. 'Red Globe' did not change its vitamin C content with the treatments. However, the 'Red Globe' vines that received both girdling and thinning accumulated less vitamin C in their berries. 'Alphonse L.' had a significantly lower amount of vitamin C in its control and thinned vines. The combined effects of girdling and thinning resulted in the highest vitamin C levels. Phenolic compounds and vitamin C are important in the production of colour and taste and in plant defence. Their content has been investigated mostly in wine grape cultivars and their wines. However, their fresh consumption in the human diet is important and also encouraged. It therefore is important to investigate these compounds in table grapes that are exposed to different treatments for increasing quantity and quality.

Varietal differences in response to the girdling and/or thinning activities in terms of total phenolic and vitamin C accumulation were observed in this study. Similar results have also been shown in several other studies. Kanner *et al.* (1994) found that three seedless table grape cultivars ('Thompson Seedless', 'Flame Seedless' and 'Black Seedless') contained 260, 850 and 920 mg per kg of total phenolics respectively. Cantos *et al.* (2002) reported varietal differences in the polyphenol profiles of seven table grape cultivars, ranging between 114.9 (cv. 'Dominga') and 361.2 mg per kg fresh weight ('Flame Seedless'). Lachman *et al.* (2004) reported the highest phenolics in the 'Royal' table grape compared to the other must varieties studied. Proteggente *et al.* (2002) showed that green grape cultivars had 80 mg total phenolics and 2 mg vitamin C per 100 g fresh weight (FW).

Studies involving cultural practices such as girdling and generative organ thinning in table grapes are abundant. However, there are few regarding organically grown grapes. Girdling and cluster/berry thinning are two of the cultural practices that can cause an imbalance in vine nutrition and

TABLE 1
Effects of various treatments of contents on total phenolics and vitamin C of the organic table grape cultivars (\pm SEM).

Treatments	Total phenolics (mg GAE/g FW)			
	Cultivars			
	Alphonse L.	Red Globe	Trakya Ilkeren	Buca Razakısı
Control	1.71 \pm 0.01 ^{Bd}	0.78 \pm 0.01 ^{Db}	2.05 \pm 0.01 ^{Ac}	1.31 \pm 0.01 ^{Cb}
Girdling	2.00 \pm 0.01 ^{Aa}	0.71 \pm 0.01 ^{Bc}	2.00 \pm 0.01 ^{Ad}	0.60 \pm 0.01 ^{Cd}
Cluster thinning	1.77 \pm 0.01 ^{Bb}	0.84 \pm 0.01 ^{Da}	2.11 \pm 0.01 ^{Ab}	0.94 \pm 0.04 ^{Cc}
Girdling+Cluster thinning	1.72 \pm 0.01 ^{Cc}	0.72 \pm 0.01 ^{Cc}	2.20 \pm 0+01 ^{Aa}	1.82 \pm 0.02 ^{Ba}
Mean	1.80	0.76	2.09	1.17

Treatments	Vitamin C (mg/100 g FW)			
	Cultivars			
	Alphonse L.	Red Globe	Trakya Ilkeren	Buca Razakısı
Control	15.49 \pm 0.24 ^{Ac}	13.67 \pm 0.04 ^{Ba}	14.18 \pm 0.26 ^{Bb}	15.55 \pm 0.53 ^{Aa}
Girdling	15.64 \pm 0.01 ^{Ac}	14.00 \pm 0.07 ^{Ba}	13.94 \pm 0.31 ^{Bb}	15.59 \pm 0.00 ^{Aa}
Cluster thinning	16.51 \pm 0.38 ^{Ab}	12.39 \pm 0.86 ^{Bab}	15.42 \pm 0.31 ^{Aa}	16.72 \pm 0.22 ^{Aa}
Girdling+Cluster thinning	17.70 \pm 0.04 ^{Aa}	10.99 \pm 0.05 ^{Bb}	16.21 \pm 0.19 ^{Aa}	16.21 \pm 0.91 ^{Aa}
Mean	16.34	12.76	14.94	16.02

*ANOVA to compare data ($p = 0.05$): Different upper cases in each row represent different cultivar means (A, B, C, D) Different lower cases in each column represent different treatment means (a, b, c, d). SEM: Standard error of mean

source-sink relationships. Girdling and thinning applications alone or in combination seemed to have provided more photosynthesis products to the remaining upper organs or clusters, leading to more accumulation of phenolics. Cluster thinning is reported to increase phenolic composition by changing the leaf-area-to-fruit ratio, or by hastening maturity (Prajitna *et al.*, 2007) and to increase the anthocyanins (Guidoni *et al.*, 2002). The coloured varieties, 'Alphonse L.', 'Red Globe' and 'Trakya Ilkeren', contained more phenolics in their berries, as expected, compared to 'Buca Razakısı', the white cultivar. Coloured grape cultivars accumulate more anthocyanins in the skin and has the highest total polyphenols (Ivanova *et al.*, 2011). One interesting result was that 'Red Globe' had less total phenolic compounds than 'Buca Razakısı' in all the treatments, except for the girdling alone. This is a result that conflicts with previous studies, which have reported that white varieties generally have fewer phenolic compounds than coloured varieties (Cantos *et al.*, 2002; Lachman *et al.*, 2004; Ivanova *et al.*, 2011). This might have been the result of the 'Red Globe' vines being harvested before they had the chance to reach their "phenological maturity".

Organic fruit growing is considered a practice that increases the fruit's quality and composition in favour of human health. There are studies on grapes showing either a decrease or an increase in the phenolic and vitamin contents. For instance, a higher total phenolic content of organically grown wine grapes (Malusà *et al.*, 2004) and Labrusca varieties (Dani *et al.*, 2007) has been recorded. Dani *et al.* (2007) found that the juice of purple and white organic grapes contained statistically higher amounts of vitamin C. Mulero *et al.* (2010), in contrast, reported no changes in Monastrel grapes. However, the total phenolic contents of the cultivars studied here showed comparable results to previous studies,

such as that of Kanner *et al.* (1994), who studied the seedless table grapes, and Revilla *et al.* (1995), who studied white and red grapes.

CONCLUSIONS

The response of the cultivars to the girdling and/or cluster thinning differed. Girdling and cluster thinning applied together caused white grape cultivars to accumulate more total phenolics. The quantity of vitamin C was more in all the cultivars except for 'Red Globe'. The empirical data, although with contradictory results, suggest that cultural practices used on grapes might influence the phenolic contents of the grapes. As consumer preferences for organic grapes increases, a better understanding and more detailed information on the effects of agricultural practices on beneficial nutrients will be required.

LITERATURE CITED

- Barata-Soares, A.D., Gomez, M.L.P.A., De Mesquita, C. & Lajolo, F.M., 2004. Ascorbic acid biosynthesis: A precursor study on plants. *Braz. J. Plant Physiol.* 16, 147-154.
- Benbrook, C., Zhao, X., Yanez, J., Davies, N. & Andrews, P., 2008. New evidence confirms the nutritional superiority of plant-based organic foods. *State Sci. Rev.* Available: http://www.organiccenter.org/reportfiles/Nutrient_Content_SSR_Executive_Summary_Final.pdf (Accessed April 2012).
- Cantos, E., Espin, J.C. & Tomas-Barberan, F.A., 2002. Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC-DAD-MS-MS. *J. Agric. Food Chem.* 50, 5691-5696.
- Carbonaro, M., Mattera, M., Nicoli, S., Bergamo, P. & Cappelloni, M., 2002. Modulation of antioxidant compounds in organic vs. conventional fruit (peach, *Prunus persica* L., and pear, *Pyrus communis* L.). *J. Agric. Food Chem.* 50, 5458-5462.

- Cemeroğlu, B., 2007. Gıda Analizleri. Gıda Teknolojisi Derneği Yayınları, pp. 168 – 171. No: 34, Ankara, Turkey [in Turkish].
- Dani, C., Oliboni, L.S., Vanderlinde, R., Bonatto, D., Salvador, M. & Henriques, J.A.P., 2007. Phenolic content and antioxidant activities of white and purple juices manufactured with organically or conventionally-produced grapes. *Food Chem. Toxicol.* 45, 2574-2580.
- Guidoni, S., Pierpaolo, A. & Schubert, A., 2002. Effect of cluster thinning on berry skin anthocyanin composition of *Vitis vinifera* cv. Nebbiolo. *Am. J. Enol. Vitic.* 53, 224-226.
- Heaton, S. 2001. Organic farming, food quality and human health. Soil Association Report, Bristol, UK.
- Huber, M., Rembikowska, E., Średnicka, D., Bugel, S. & Van De Vijver, L.P.L., 2011. Organic food and impact on human health: Assessing the status quo and prospects of research. *NJAS - Wageningen J. Life Sci.* 58(3-4), 103-109.
- Ivanova, V., Stefova, M., Vojnoski, B., Dornyei, Á., Márk, L., Dimovska, V., Stafilov, T. & Kilar, F., 2011. Identification of polyphenolic compounds in red and white grape varieties grown in R. Macedonia and changes of their content during ripening. *Food Res. Int.* 44, 2851-2860.
- Kanner, J., Frankel, J.E., Granitt, R., German, B. & Kinsella, J.E., 1994. Natural antioxidants in grapes and wines. *J. Agric. Food Chem.* 1004(42), 64-69.
- Lachman, J., Šulc, M., Hejtmánková, A., Pivec, V. & Orsák, M., 2004. Content of polyphenolic antioxidants and trans-resveratrol in grapes of different varieties of grapevine (*Vitis vinifera* L.). *Hort. Sci. (Prague)* 31(2), 63-69.
- Lombardi-Boccia, G., Lucarini, M., Lanzi, S., Aguzzi, A. & Cappelloni, M., 2004. Nutrients and antioxidant molecules in yellow plums (*Prunus domestica* L.) from conventional and organic productions: a comparative study. *J. Agric. Food Chem.* 52, 90-94.
- Malusà, E., Laurenti, E., Ghibaudi, E. & Rolle, L., 2004. Influence of organic and conventional management on yield and composition of grape cv. 'Grignolino'. *Acta Hort. (ISHS)* 640, 135-141.
- Mulero, J., Pardo, F. & Zafrilla, P., 2010. Antioxidant activity and phenolic compounds in conventional and organic red grapes (var. Monastrell). *CyTA - J. Food* 8(3), 185-191.
- Ojeda, H., Andary, C., Kraeva, E., Carbonneau, A. & Deloire, A., 2002. Influence of pre and postverasion water deficit on anthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. *Am. J. Enol. Vitic.* 53(4), 261-267.
- Poudel, P.R., Tamura, H., Kataoka, I. & Mochioka, R., 2008. Phenolic compounds and antioxidant activities of skins and seeds of five wild grapes and two hybrids native to Japan. *J. Food Comp. Anal.* 21, 622-625.
- Prajitna, A., Dami, I.E., Steiner, T.E., Ferree, D.C., Scheerens, J.C. & Schwartz, S.J., 2007. Influence of cluster thinning on phenolic composition, resveratrol, and antioxidant capacity in Chambourcin wines. *Am. J. Enol. Vitic.* 58, 346-350.
- Proteggente, A.R., Pannala, A.S., Paganga, G., Buren, L.V., Wagner, E., Wiseman, S., Put, F.V.D., Dacombe, C. & Rice-Evans, C.A., 2002. The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radicals Res.* 36(2), 217-233.
- Revilla, J.M.E.E., Alonso, E. & Kovac, V., 1995. The phenolic composition of table grapes. In: Charalambous, G. (ed.). *Proc. 8th Internat. Flavor Conf.*, July 1994, Cos, Greece. pp. 1579–1596.
- Rock, C.L., Jacob, R. & Bowen, P.E. 1996. Update on the biological characteristics of the antioxidant micronutrients: Vitamin C, vitamin E, and the carotenoids. *J. Am. Dietetic Assoc.* 96(7), 693-702.
- Singleton, V.L. & Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am. J. Enol. Vitic.* 16, 144-158.
- Wang, S.Y., Chen, C.T., Sciarappa, W., Wang, C.Y. & Camp, M.J., 2008. Fruit quality, antioxidant capacity, and flavonoid content of organically and conventionally grown blueberries. *J. Agric. Food Chem.* 56, 5788-5794.