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A STUDY ON TOTAL PHENOLICS AND VITAMIN C CONTENTS OF KALECIK KARASI (*Vitis vinifera* L.) CLONES

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In this study total phenolic and vitamin C contents of the fully ripe berries of 23 clones of Kalecik Karasi which is one of the leading Turkish local red-wine grape cultivar originally grown in Kizilirmak valley near Kalecik/Ankara region were examined under the clone selection project supported by TÜBİTAK (Project Nr: 107 O 731). High-pressure liquid chromatography (HPLC) was used for vitamin C and spectrophotometer for total phenolics estimation. One way ANOVA was used to compare means of clone for their total phenolic and vitamin C contents. In addition to this univariate method, hierarchical cluster analysis was performed to identify similarity levels among the clones by considering total phenolics and vitamin C content together. Differences among the clones were found statistically significant for both characteristics. Total phenolic contents of the clones varied from 3.310 mg (clone 21) to 3.389 mg (clone 6) as GAE g fw. Vitamin C content ranged from 14.010 mg (clone 6) to 16.500 mg (clone 19) in 100g fw. Furthermore, similarity level for all clones was 83.1% that means variation rate is about 17% among the clones. As a summary of whole data, the first three performing clones are 6 (3.389 mg), 10 (3.374 mg) and 1 (3.365 mg) for total phenolics, and 19 (16.500 mg), 9 (16.020 mg), and 21 (16.015 mg) for vitamin C contents of the berries.
Keywords: Grapes clones, cluster analysis, Kalecik Karasi, phenolics, vitamin C

INTRODUCTION

Polyphenolics (flavonoids, anthocyanins and stilbenes "resveratrol") are structurally a diverse class of compounds known as secondary metabolites and naturally present in grapes and wines. Two main substances in this group are anthocyanins and tannins. Anthocyanins are the pigments and responsible for red or purple-black color of grapes and wines. Tannins are quite complex compounds and yellow, brown or red colored large molecules as well as astringent and bitter taste. Polyphenolics contribute to sensory attributes such as color, bitterness and astringency of grapes and wines. They also play a key role in aging and maturation of wines. Many polyphenolics have beneficial biological effects on human health such as antioxidant, antimicrobial, antiviral, anticarcinogenic antienflamatory, antihypertensive and antiulcer (Agarwal *et al*., 2000; Downey *et al*., 2003; Baydar *et al.,* 2004; Peng *et al*., 2005; Kennedy, 2008; Ali *et al*., 2010; Mitic *et al*., 2010; Çetin, 2010; Liang *et al*., 2011; Çelik *et al*., 2012). Procyanidolic oligomers (PCOs) also known as leukocyanidins or pycnogenols which belongs to flavonoid family, are very powerful antioxidant activity 30-50 times stronger than those of vitamin C and vitamin E, β-Carotene and Selenium.

Although external factors such as viticultural practices and weather conditions are likely to effect the levels of phenolic substances, genetic diversity for varieties or clones are much more decisive (Bavaresco and Fregoni, 2001; Bayhan, 2004; Anli *et al*., 2006; Ivanova *et al*., 2010; Oberholster *et al*., 2010; Burin *et al.,* 2011; Bunea *et al*., 2012; Du *et al*., 2012; Çelik *et al*., 2012).

Vitamin C is also an important antioxidant (radical scavenger), helps to protect against cancers, heart disease and stress. As a part of cellular biochemistry, it provides energy, also essential for sperm production, and for composing the collagen protein involved in building and health of cartilage, joint, skin and blood vessels; helps in maintaining a healthy immune system, aids in neutralizing pollutants, is needed for antibody production, acts to increase the absorption of nutrients in the gut, and thins the blood (*http://www.naturalhub.com* Natural Food-Fruit Vitamin C Content). Ascorbic acid is also a biosynthetic precursor to both tartaric acid and oxalic acid revealed by specific radiolabel studies (De Bolt, 2008; Melino, 2009).

Grapes with colored or green-yellow skins are considered to good source of vitamin C with its concentration between 10-15 mg/100 g fw which is 17-24% of the recommended daily amount. However, it should be emphasized that the significant interaction between grape seed and vitamin C treatments for effects on blood pressure (BP) this means that treatment with the combination of vitamin C and polyphenols increases systolic blood pressure (Ward *et al*., 2005).

The aim of this study, corresponding to the clone selection project supported by TÜBİTAK, was to determine the variations for the contents of total phenolic compounds and vitamin C in 23 clones of Kalecik Karasi grown in Kalecik (Ankara) where is the original territory of the variety, during the years of 2008 and 2009.

MATERIALS AND METHODS

Fully ripe berries (24-26 °B) were collected in the limited-drip irrigated (Çelik *et al*., 2005) clone selection vineyard established in 1999 at the Viticulture Research Station of Faculty of Agriculture in Kalecik where is 70 km northeast of Ankara. Clones were grafted on 41B clone 172, with 2x3 m planting density, trained as bilateral Guyot on quite high trunk (80 cm) in 2008 and 2009 vintage seasons.

***Determination of total phenolics*:** Total phenolic contents of the clones were determined using Folin-Ciocalteou's method proposed by Singleton and Rossi (1965). Quantification of the samples was performed by spectrophotometer in 765 nm and results were expressed as mg gallic acid equivalent (GAE) in g fresh weight.

***Determination of vitamin C*:** Vitamin C contents of the clones were determined with some modification of the method proposed by Cemeroğlu (2007) and expressed as mg 100 g fresh weight. After crushing of the grapes by hand blender, they were scaled as 5 g and transferred into 50 mL test tubes. Then, 5 mL of 2.5% M- phosphoric acid solution was added. The mixture was centrifuged (6500 rpm) at 4°C for 10 min. By taking 0.5 ml solution from clear part of the centrifuge tube, it was supplemented with 2.5% M-phosphoric acid solution to obtain a 10 mL final mixture. This mixture was filtered through a 45 µm Teflon filter and then injected into HPLC (High-pressure liquid chromatography) device. C18 column (Phenomenex Luna C18, 250 x 4.60 mm, 5µ) was employed to analyze vitamin C. Column oven temperature was fixed as 250ºC. Ultra distilled water fixed at pH 2.2 with H_2SO_4 in 1 ml/min. flow rate was used for mobile phase in the system. Quantification for vitamin C was performed with DAD detector in 254 nm. Various concentrations (50, 100, 500, 1000, 2000 ppm) of L–ascorbic acid (Sigma A5960) were employed to identify amount and peak value of vitamin C.

***Statistical analysis*:** Descriptive statistics for studied variables (characteristics) were presented as mean and standard error of the mean. One way ANOVA was used to compare clones for their total phenolic and vitamin C contents, and then Duncan multiple comparison test was carried out for determination of different means. In addition to this univariate method, hierarchical cluster analysis which is multivariate method was performed to identify similarity levels among the clones by considering total phenolics and vitamin C contents together. Statistically significance level was considered to 5% and SPSS (ver. 13) statistical program was used for all statistical computations.

RESULTS AND DISCUSSION

Descriptive statistics and comparative results for total phenolic (mg GAE / g fw) and vitamin C (mg / 100 g fw) contents of Kalecik Karasi clones are presented as the averages of 2008 and 2009 in Table 1.

Table 1. Total phenolic and vitamin C contents of the berries of Kalecik Karasi clones

Clones	Total phenolics (mg GAE/ g fw)	Vitamin C (mg/ 100 g fw)
	Mean ± SE Mean	Mean ± SE Mean
1	3.365 ± 0.0005 c*	14.935 ± 0.0350 b-f
2	3.345 ± 0.0045 e-h	15.500 ± 0.2400 a-d
3	3.348 ± 0.0030 def	15.695 ± 0.1950 abc
4	3.338 ± 0.0020 hij	15.485 ± 1.0750 a-d
5	3.346 ± 0.0010 efg	15.270 ± 0.0800 a-f
6	3.389 ± 0.0005 a	14.010 ± 0.0400 f
7	3.314 ± 0.0001 m	15.325 ± 0.1250 a-f
8	3.347 ± 0.0025 def	15.410 ± 0.0700 a-e
9	3.332 ± 0.0025 jk	16.020 ± 0.2300 ab
10	3.374 ± 0.0010 b	15.140 ± 0.1600 a-f
11	3.345 ± 0.0050 e-h	15.020 ± 0.5700 b-f
12	3.338 ± 0.0020 hij	14.225 ± 0.3750 def
13	3.340 ± 0.0005 f-i	15.400 ± 1.1400 a-f
14	3.351 ± 0.0015 de	15.635 ± 0.5750 abc
15	3.337 ± 0.0020 ij	14.060 ± 0.1700 ef
16	3.325 ± 0.0050 kl	15.450 ± 0.1500 a-e
17	3.323 ± 0.0035 l	14.960 ± 0.0900 b-f
18	3.327 ± 0.0025 kl	15.330 ± 0.2300 a-f
19	3.326 ± 0.0015 kl	16.500 ± 0.1200 a
20	3.326 ± 0.0015 kl	15.530 ± 0.4100 a-d
21	3.310 ± 0.0005 m	16.015 ± 0.0350 ab
22	3.354 ± 0.0010 d	14.070 ± 0.1100 ef
23	3.339 ± 0.0005 g-j	14.585 ± 0.1350 b-f
Mean	3.341 ± 0.00269	15.198 ± 0.1129

*Different lower cases represent significant differences between the clones' means ($p<0.05$)

***Total phenolics*:** Among the clones, the highest total phenolics were obtained from clone 6 (3.389 mg) followed by clone 10 (3.374 mg) and clone 1 (3.365 mg), however the lowest values were recorded in clone 21 (3.310 mg) and followed by clone 7 (3.314 mg) and clone 17 (3.323 mg). Although total phenolic contents of the berries ranged from 3.310 mg (clone 21) to 3.389 mg (clone 6) and differences were statistically significant, the variation between these two extreme levels is only 2.4 mg.

Singleton (1966) pointed out that total phenolics in berry were recorded 3770 mg/kg as Gallic acid in 12 wine grape varieties. The author also stated that total phenolics have been likely to vary according to species and varieties. Similarly, Lee and Jaworski (1987) noted that there are considerable differences among the 19 varieties of *Vitis vinifera* in terms of phenolics. Furthermore, Roggero *et al*. (1986) conducted a study with clones of Syrah grapes and indicated that total phenolics can be varied even clones of the same varieties. According to their findings, statistically significant differences between the clones indicated that same genotypes have been likely to show considerable differences under the various stress factors. In addition, according to Bessis *et al*. (1998) the variation in the total phenolics of the grapes can be related with structure of soil, drought stress and different cultural practices. In fact, Mateus *et al*. (2001) stated that altitude of vineyard and some climatic factors, especially temperature and humidity play an important role in the accumulation of total phenolics of grapes.

Since phenolic contents in the skins and seeds of the berries of red wine grape varieties is the main source of wine phenolics which are very valuable compounds for human health with their highly beneficial biological activities, anthocyanin density of the berry skin (mg/kg) and resveratrol contents of wines (mg/L) were considered as selection criteria with same proportional value of 5% for each attribute in the long-term clone selection project on Kalecik Karasi which covers this study (Çelik *et al*., 2012). Therefore it can be suggested that this study may be helpful in clone selection programs on red wine grape varieties with some modifications.

***Vitamin C*:** Vitamin C content of the berries varied from 14.010 mg (clone 6) to 16.500 mg (clone 19), unlike to the phenolic contents, variation rate in vitamin C (17.8%) is considerable higher. Leading clones for their vitamin C performances are clone 19 (16.500 mg), clone 9 (16.020 mg), and clone 21 (16.015 mg), contrarily, clone 6 (14.010 mg), clone 15 (14.060 mg), and clone 22 (14.070 mg) showed the lowest performances.

The studies about determination of the vitamin C content in grape have been rare in the literature. According to Iqbal *et al*. (2006) average vitamin C content of the Pakistani grapes has been 1.64 mg/100 g fw. Similarly, Nikniaz *et al.* (2009) reported that average vitamin C content of Iranian grapes has been 16.83 mg/100 g fw for red varieties and 14.85 mg/100 g fw for white varieties. Likewise, Szeto *et al*. (2002) noted that vitamin C content of Chinese varieties was found 20 mg/100 g fw in white varieties and lower than 10 mg/100 g fw.

Although vitamin C content of grapevine berries is considerably important in human diet, this highly antioxidant compound mostly losses its activity during the vinification. Because of this inactivation, it is unlikely to be worthy to use this attribute as a selection criteria in clone selection programs for wine grape varieties, however it may be highly recommended for table grapes.

***Cluster analysis*:** Results of cluster analysis were presented in Table 2. The highest similarity (99.430%) was obtained for clone 8 and clone 13, followed by clone 7 and clone 18 (99.349%), clone 2 and clone 4 (99.217%), clone 15 and clone 22 (99.079%), clone 9 and clone 21 (98.946%). However, with the exceptions of clones 6, 9, 12, 15, 19, 21, 22, and 23; largest cluster was formed by the other 15 clones at about 94% similarity level. When both attributes are considered together, similarity level among all Kalecik Karası clones is 83.135%.

Table 2. Summarized results of cluster analysis

Step	Cluster	Similarity level	Cluster joined		New cluster	No. of obs. in cluster
1	22	99.430	8	13	8	2
2	21	99.349	7	18	7	2
3	20	99.217	2	4	2	2
4	19	99.079	15	22	15	2
5	18	98.946	9	21	9	2
6	17	98.341	2	20	2	3
7	16	98.256	2	16	2	4
8	15	97.856	2	8	2	6
9	14	97.717	1	17	1	2
10	13	97.193	3	14	3	2
11	12	97.067	5	7	5	3
12	11	97.023	1	11	1	3
13	10	96.744	6	15	6	3
14	9	96.675	2	5	2	9
15	8	94.959	2	3	2	11
16	7	94.234	1	10	1	4
17	6	93.921	9	19	9	3
18	5	93.789	1	2	1	15
19	4	92.722	6	12	6	4
20	3	84.951	1	9	1	18
21	2	83.607	1	23	1	19
22	1	83.185	1	6	1	23

In order to improve current cultivars as well as to breed new varieties in *vitis vinifera*, considerable variation is necessary for the cultivars or clones. In our study, this variation was observed among the clones in both traits. Roggero *et al*. (1986) , Bavaresco and Fregoni (2001), Bayhan (2004), Burin *et al*. (2011), and Çelik *et al.* (2012) also indicated similar variations in total phenolic contents of grapevine clones for the same variety.

***Conclusion*:** In this study similarities or phenotypic variations among twenty-three Kalecik Karasi clones were evaluated in terms of total phenolics and vitamin C. This variation is likely to be useful for selecting of high–value varieties in both vitamin C and phenolics. In addition as a result of this study, clone 10 and clone 1 can be suggested in the selection of future generations. Therefore, it is expected that this study will be useful for the researchers in the future. However, it should be noted that further researches are needed to understand the associations between clones and some quality attributes.

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