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Analysis of sugars, organic acids and vitamin C contents of blackberry genotypes from Turkey

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Abstract

The paper reports the composition of some quality characteristics of five blackberry varieties (“C. Thornless”, “Bursa 2”, “Navaho”, “Jumbo” and “Loch Ness”). Main soluble sugar and acid contents of experimental varieties were separated, identified and quantified using high-performance liquid chromatography with photo diode array spectrophotometric and refractive index detection, for organic acids, ascorbic acids and soluble sugars, respectively. According to the results, malic acid was detected as the main organic acid while citric acid was not detected in blackberry fruits. Ascorbic acid content was found very low quantity and also was not detected in all the cultivars. As for the sugars, fructose was found as the most abundant sugar and highly detected in “Navaho”. However, the highest total sugar/malic acid ratio was found in cv. C. Thornless.

Keywords: Blackberry; Quality; Sugars; Organic acids; HPLC

1. Introduction

Blackberries are one of the easiest to grow and are extremely tolerant of site and soil conditions. Several species of *Rubus* are called blackberries. Some are upright and require no support but others are trailing and require a trellis. The trailing varieties are thorny; the semi-trailing varieties are thornless; and the erect varieties may be thorny or thornless. As a rule, the erect varieties are more cold-hardy than the trailing or semi-trailing varieties. Erect varieties also fruit about one month earlier than other varieties (Darrow, 1967).

The blackberry fruits can be used in the industry for ice cream, juice, jam, marmalade, cake, etc., (Türemiş, Kafkas, Kafkas, & Onur, 2003) production. Flavonoids and phenolic compounds in the fruit are anticarcinogen (Ames, Shigena, & Hugen, 1993; Bilyk & Sapers, 1986). In addition, recent studies have demonstrated the strong antioxidant activities of anthocyanins such as cyanidin-3-glucoside (Tsuda et al., 1994) detected in blackberries. Furthermore, a novel zwitterionic anthocyanin was isolated from evergreen blackberry and structurally characterized as cyanidin-3-dioxalylglucoside (Stintzing, Stintzing, Carle, & Wrolstad, 2001). Therefore, blackberry fruits are also used in dietary supplements. Jiao and Wang (2000) studied antioxidant capacities and their relation with some important antioxidant enzymes which are responsible for reducing of the risk of some important health disorders. A similar study was performed by Gonzalez, de Ancos, and Cano (2000) describing partial peroxidase and polyphenol oxidase activities in blackberry fruits partially.

Consumption of fresh and frozen blackberries has increased in the past few years in Turkey. In search for alternative crops for farmers, blackberry appears as a potential crop of high market value. Turkey is one of the origins of blackberries and blackberry growing can be done in all parts of Turkey where irrigation is possible. Blackberry cultivation started in the Marmara region several

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decades ago and now has been introduced as a new crop in the Mediterranean region (Türemiş et al., 2003).

Limited data are available on the sugars and nonvolatile acids of blackberry cultivars (Gerald, Burgher, & Philips, 1985; Wang, Quian, & Finn, 2004). In our previous study (Türemiş et al., 2003), we characterized the yield and pomological fruit characteristics of some berries such as average fruit mass, total soluble solid (TSS) content, acidity, TSS/acidity rate, pH, color and harvesting periods of nine thornless blackberry cultivars grown in Adana province, in the Mediterranean region of Turkey and compared these cultivars according to their aroma profile. Blackberry may be a good alternative and a potential crop with high market value for farmers in this region. So far, we have little information about the blackberry fruit composition especially as regards sugars and acids. Our objective in this study was to detect sugars, organic acids and ascorbic acid among the suggested commercial varieties using high performance liquid chromatography technique.

2. Materials and methods

2.1. Materials and reagents

Blackberries were grown in the implementation area of the University of Cukurova, Faculty of Agriculture, Department of Horticulture in Turkey. Five varieties (Chester Thornless, Loch Ness, Navaho, Bursa 2 and Jumbo) used as plant materials. The experiment was designed as a complete randomized block with three replicate and 20 plants were used in each replicate. The fruits of experimental genotypes were harvested at ripe stage then immediately treated with liquid nitrogen and stored −80 °C until extraction.

Ultrapure water (18.2 MΩ cm) was prepared by using a Millipore system (Millipore Corp., Bedford, MA).

All the standards for the chromatography reagents and solvents were purchased from Sigma Chemical, Co. (St. Louise, MO).

2.2. Extraction of sugars and acids

For sugars; approximately 500 g of each frozen sample was used and each replicate was prepared separately from this homogenized material 1 g of sample was weighted and powdered with liquid nitrogen in a mortar and then transferred to a screw cap Eppendorf tube with 20 ml of aqueous ethanol (80%, v/v). Reaction mixture was placed in an ultrasonic bath and sonicated for 15 min at 80 °C then filtered and the extraction procedure was repeated three times more. All the filtered extracts were combined and evaporated to dryness on the boiling water bath. The residue was dissolved with 2 ml of distilled water and filtered before HPLC analysis (Miron & Schaffer, 1991).

From the same homogenate described above, 1 g of frozen sample was weighted and powdered with liquid nitrogen in a mortar and mixed with 20 mL of aqueous *meta*-phosphoric acid (3%) at room temperature for 30 min using a shaker for the carboxylic acids and vitamin C detections. Acidic extract was filtered and made up to 25 mL with the same solvent, then used for HPLC analysis (Bozan, Tunalıer, Koşar, Altıntaş, & Başer, 1997).

2.3. HPLC conditions for acids and sugars

For the carboxylic acids and vitamin C; the liquid chromatographic apparatus (Shimadzu LC 10A*vp*) consisted of an in-line degasser, pump and controller coupled to a photo diode array detector (Shimadzu SPD 10A*vp*) equipped with an automatic injector (20 μL injection volume) interfaced to a PC running Class VP chromatography manager software (Shimadzu, Japan). Separations were performed on a 250 × 4.6 mm i.d., 5 μm, reverse-phase Ultrasphere ODS analytical column (Beckman) operating at room temperature with a flow rate of 1 mL min^{-1}. Detection was carried out with a sensitivity of 0.1 a.u. fs between the wavelengths of 200 and 360 nm. Elution was effected using an isocratic elution of the solvent, 0.5% aqueous *meta*-phosphoric acid. Components were identified by comparison of their retention times to those of authentic standards under analysis conditions and UV spectra with our in-house PDA-library and quantified by external standard method. A 10 min equilibrium time was allowed between injections. Three replicate were done for each genotype and each injection also.

As for the sugars, the liquid chromatographic apparatus (Shimadzu LC-10A*vp*) consisted of an in-line degasser, pump and controller coupled to a refractive index detector equipped with an automatic injector (20 μL injection volume) interfaced to a PC running Class VP chromatography manager software (Shimadzu, Japan). Separations were performed on a 150 × 4.6 mm i.d., 5 μm, reverse-phase Nucleosil NH_2 analytical column (Shimadzu, Japan) operating at room temperature with a flow rate of 1 mL min^{-1}. Elution was effected using an isocratic elution of 75% aqueous acetonitrile as a solvent. Components were identified by comparison of their retention times with those of authentic standards under analysis conditions and quantified by external standard method. A 10 min equilibrium time was allowed between injections. The reproducibility of the chromatographic separation of the components was determined by making five injections of the standard solutions and blackberry sample.

2.4. Quantitative and statistical analyses

All the samples were directly injected to the reverse phase chromatography column. For the stock solution of the organic acid standards, L-ascorbic acid, malic acid, citric acid, were dissolved in methanol at a concentration of 1 mg mL^{-1} and the sugar standards, glucose, fructose, sucrose, were dissolved in water at a concentration of 30 mg mL^{-1}. All the samples and standards were injected three times each and mean values were used (Table 1).

Table 1
Concentration ranges and calibration equations of reference compounds used for calibration of the HPLC analysis[a]

	Organic acids and ascorbic acid			Sugars		
	Citric acid	Malic acid	Ascorbic acid	Fructose	Glucose	Sucrose
Concentration range [mg mL^{-1}]	0.066–0.660	0.069–0.690	0.087–0.696	3–30	3–30	3–30
Calibration equation	$y = 53840254x + 54147.6$	$y = 563007.49x + 3430.82$	$y = 804200.76x - 5560.65$	$y = 58125x + 2348.7$	$y = 92538x - 25576$	$y = 76014x - 12010$

[a] All calibration coefficients >0.99.

The results were statistically evaluated by one way analysis of variance (ANOVA). Statistical differences with *p*-values under 0.05 were considered significant and means were compared by Tukey's test, using SPSS program version 10.01 (SPSS Inc., Chicago, IL).

3. Results and discussion

Fruit characteristics of blackberry cultivars like other fruits play an important role for their marketability. The ratio of sugars to organic acids has been related to flavor quality for a variety of fruits and specifically to berry flavor (Shaw, 1988) and determine the optimum time for harvesting, because it is considered as an index of quality (Cordenunsi, Nascimento, Genovese, & Llajalo, 2002).

Sugar accumulation, especially the concentration of high level of fructose, is a very important physiological process that determines the dessert fruit quality. The amounts of fructose, glucose, sucrose and total sugars in the genotypes are given in Table 2. The concentrations of mean fructose, glucose, sucrose and total sugars among the genotypes were found statistically significant ($p < 0.05$). As shown in Table 2, fructose was found to be dominant compared with other sugars in all experimental cultivars. The fructose concentration was varied between 21.1 and 33.8 $mg/g_{extract}$ and the values were obtained from Bursa 2 and Navaho cultivars, respectively. Glucose content was followed by that of fructose and it changed from 15.8 in the cultivar Bursa 2 to 26.1 $mg/g_{extract}$ in the cultivar Navaho. Sucrose was detected in lower concentrations compared to other sugars in all the genotypes due to the fact that it may be converted to invert sugars during the ripening process. When the experimental cultivars were evaluated according to their total sugar contents; the highest value was obtained from Navaho cultivars. Similar results were obtained from Wang et al. (2004). The authors characterized the contents of sugar and acid of 17 representative blackberry varieties planted in the Pacific Northwest using high performance liquid chromatography. The authors reported the glucose level ranged from 22.6 to 45.8 $mg/g_{extract}$ with a mean of 35.3 $mg/g_{extract}$ while the fructose level ranged from 22.6 to 40.3 $mg/g_{extract}$ with a mean of 33.0 $mg/g_{extract}$. In our study, both glucose (15.8–26.1 $mg/g_{extract}$) and fructose (21.1–33.8 $mg/g_{extract}$) concentration was found lower than Wang et al. (2004) reports. The results reflect that there must be genetic variation among the blackberry varieties according to their individual sugars. In addition, the different climatic conditions may also be a reason to affect the sugar composition. Sucrose was found the lowest level in all experimental genotypes among the detected sugars. It varied from 1.2 to 2.6 $mg/g_{extract}$ in Bursa 2 and Navaho, respectively. Moore and Clark (1989), detected some of the pomological traits of Navaho, Cheyenne and Shawnee cultivars and the authors reported that cultivar Navaho was found more aromatic and sweeter than other cultivars.

The malic, citric and ascorbic acid concentrations are given in Table 3. As shown in this table, citric acid was not detected in any of the experimental cultivars, whereas malic acid was detected in all the cultivars as the main organic acid. The amounts of malic acid changed from 0.6 (C. Thornless) to 11.0 $mg/g_{extract}$ (Loch Ness), but these values were not found statistically significant. Ascorbic acid was detected in C. Thornless (2.5 $mg/g_{extract}$), Bursa 2 (4.6 $mg/g_{extract}$) and Loch Ness (14.9 $mg/g_{extract}$) and not detected in Navaho and Jumbo cultivars.

The ratio of sugar/acid plays an important role for evaluating the fruit quality. The relationship between total sugar/total acids and total sugar/malic acid ratio are shown in Fig. 1. As shown Fig. 1, although a high level

Table 2
HPLC qualitative and quantitative data of sugars in blackberry genotypes ($mg/g_{extract}$)

Genotypes	Fructose	Glucose	Sucrose	Total sugars
Navaho	33.8 ± 0.3^a	26.1 ± 0.1^a	2.6 ± 0.1^a	62.5 ± 0.6^a
C. Thornless	25.1 ± 1.2^c	16.9 ± 0.4^c	1.5 ± 0.2^c	43.6 ± 1.9^c
Jumbo	25.5 ± 0.5^c	16.6 ± 0.6^d	1.4 ± 0.2^{cd}	43.5 ± 1.4^c
Bursa 2	21.1 ± 0.8^d	15.8 ± 0.8^e	1.2 ± 0.1^d	38.2 ± 1.1^c
Loch Ness	30.2 ± 1.8^b	20.3 ± 1.3^b	2.0 ± 0.6^b	52.5 ± 0.3^b

Values (mg/g) are expressed as means ± standard error.
[a, b, c, d, e] Statistical differences ($p < 0.05$) ($n = 3$).

Table 3
HPLC qualitative and quantitative data of carboxylic and ascorbic acids in blackberry genotypes ($mg/g_{extract}$)

Genotypes	Malic acid	Ascorbic acid	Citric acid	Total acids
Navaho	1.3 ± 0.1	nd	nd	1.3 ± 0.1
C. Thornless	0.6 ± 0.7	2.5 ± 0.3	nd	3.1 ± 0.5
Jumbo	1.5 ± 0.1	nd	nd	1.5 ± 0.1
Bursa 2	5.5 ± 0.4	4.6 ± 0.4	nd	10.1 ± 0.4
Loch Ness	11.0 ± 2.7	14.9 ± 2.7	nd	25.9 ± 2.5

Values (mg/g) are expressed as means ± standard error.
nd, not detected ($n = 3$).

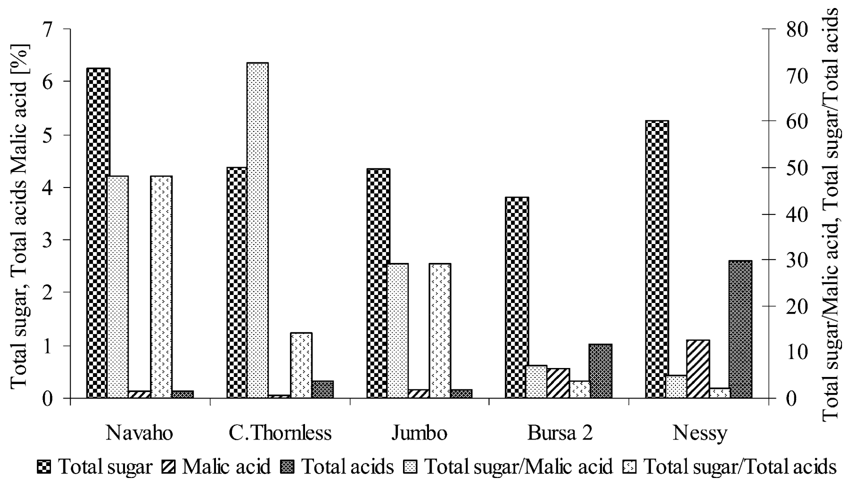

Fig. 1. Relationship between total sugar and total acids in blackberry cultivars.

of total sugar/total acids ratio was obtained in the Navaho cultivar. The total sugar/malic acid ratio in C. Thornless was detected higher than Navaho. The amounts of fructose, glucose and sucrose in Navaho genotype were found the highest while Loch Ness was the richest genotype regarding malic and ascorbic acids.

Türemiş et al. (2003) compared fruit characteristics of nine thornless blackberry genotypes which were grown in Adana province, Mediterranean region of Turkey. The fruits of five blackberry cultivars (Chester, Jumbo, Loch Ness, Navaho and Dircksen) and four selections (Bursa-1, Bursa-2, Bursa-3 and Bartin) were characterized by interms of some fruit quality characteristics. The berries were compared as to their percentages of total soluble solid (TSS) content, fruit acidity, TSS/acidity ratio. According to those authors results, the cultivar Navaho had the best TSS content (11.3%) and TSS/acidity rate (9.6), whereas the cultivar Jumbo had the lowest TSS content (9.0%). The cultivar Loch Ness had the lowest TSS/acidity rate. In that study, the sugar content of berries were found higher than our results. The reason may be due to using two different measuring methods. The authors had measured total soluble solids by hand refractometer, while we used HPLC method which is more reliable and accurate. However, the highest sugar content was detected in the cultivar Navaho in both experiments. Like sugars, the malic acid content of the berries were found to be higher (from 0.9% to 1.3%) in the previous study compared with our results (from 0.06% to 1.10%). On the other hand, the highest malic acid or titratable acid was obtained from the cultivar Loch Ness in both experiments. The differences between the results may be due to the using different measuring methods.

Taking everything into consideration, the fruits of blackberry genotypes differed in both individual sugars and acid contents. In addition, fructose and glucose concentrations were found dramatically higher than sucrose, respectively. As regards carboxylic acids: malic acid was detected in all experimental genotypes whereas citric acid was not detected. Ascorbic acid level also varied from genotype to genotype and not detected in all genotypes.

4. Conclusion

This study yielded information about sugars, some carboxylic acids and ascorbic acid contents of five blackberry cultivars grown in Mediterranean climatic conditions. Among the detected sugars, fructose was found to be the most abundant in all the genotypes. As for the detected acids, malic acid was found as a major one. Citric acid which is a major acid in other berries especially in strawberries was not detected in any of the experimental cultivars. Similarly, ascorbic acid was not detected in all the varieties studied. Among the experimental genotypes even Navaho seemed to have a high in percentage of total sugars, the ratio of total sugar/malic acid concentration was found less than in C. Thornless but the ratio of total sugar/total acid of Navaho was higher than C. Thornless.

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