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SCIENCE DIRECT
Food Chemistry 97 (2006) 732–736
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

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

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Received 21 April 2005; received in revised form 15 August 2005; accepted 25 September 2005

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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doi:10.1016/j.foodchem.2005.09.023

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).