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Effect of Harvesting Time on Flavonoid Content and Antioxidant Activity of Raspberries

Mahtab Moradi Digehsara • Davood Bakhshi* • Ensieh Ghorbani

Department of Horticultural Science, Faculty of Agriculture, University of Guilan, Rasht, Iran

Corresponding author: * **bakhshi-d@guilan.ac.ir**

ABSTRACT

In this investigation, major phenolic compounds of raspberry fruits including cyanidin 3-galactoside (anthocyanin) and quercetin 3-galactoside, and the extract antioxidant activity were investigated. Results revealed that early season fruits (in July) had the highest (5.22 mg/g FW) content of quercetin 3-galactoside. Highest (23.43 mg/g FW) and lowest (1.62 mg/g FW) content of anthocyanin found in the fruits in September and October, respectively. Among different harvesting times, fruits in July showed the highest percentage of antioxidant activity (75.84%). This study pointed out that phenolic content and antioxidant potential of raspberry fruit is strongly influenced by ripening time. Based on the results, it could be concluded that early season fruits are richer in antioxidants.

Keywords: Antioxidant activity, Cyanidin 3-galactoside, Quercetin 3-galactoside, *Rubus idaeus* L.
Abbreviations: HPLC, high performance liquid chromatography; **DPPH**, 2,2-diphenyl-1-picrylhydrazyl

INTRODUCTION

In recent decades, fruit and vegetable consumption has attracted growing interest because several studies have demonstrated the correlation between consumption of fresh fruits and vegetables with the prevention, delay or onset of chronic degenerative diseases including cancer (Çekiç and Özgen 2010). The protection that fruits and vegetables provide against these maladies has been attributed to the presence of several antioxidants, especially to antioxidative vitamins, including ascorbic acid (vitamin C), α-tocopherol (vitamin E) and β-carotene (provitamin A). Nevertheless, recent studies seem to indicate that (poly) phenolic substances are the main phytochemicals with antioxidant properties found in higher plants (Liazid *et al*. 2007; Magalhães *et al*. 2009; Rodríguez-Medina *et al*. 2009). In addition, phenolic compounds have an important role in the nutritional, organoleptic and commercial properties of agricultural foodstuffs, since they enhance sensory properties such as color, astringency, bitterness and flavor (Boyer and Liu 2004). Thus, the determination of phenolic compounds in fruits, vegetables, and other foods has been of increasing interest in recent years (Palma *et al*. 2002).

Among fruits, raspberries (*Rubus idaeus* L.) have a high free radical scavenging capacity and are rich in both vitamin C and total phenolics (de Ancos *et al*. 2000). The major phenolic compounds in berries are hydrolysable tannins (galloand ellagitannins) and anthocyanins, hydroxycinnamic acids, flavonols, flavan-3-ols, including proanthocyanidins being present in lower amounts (Häkkinen 1999; Siriwoharn and Wrolstad 2004; Bobinaite *et al*. 2012). Recently, it has been shown that anthocyanins are important antioxidants in raspberry (Mullen *et al*. 2002). Major anthocyanins in red raspberry have been identified as cyanidin and pelargonidin glycosylated with rutinose and sophorose (Torre and Barritt 1977; Spanos and Wrolstad 1987). However, in humans the bioavailability of dietary anthocyanins is low (Mazza *et al*. 2002; Wu *et al*. 2002).

Numerous factors other than variety may affect on the polyphenol content in plants. The content of phenolic compounds in berry fruits is determined by many factors too, such as the species, variety, cultivation, region, weather conditions, ripeness, harvesting time, storage time and conditions (Kondakova *et al*. 2009; Pincemail *et al*. 2012). On other hand, raspberries ripen at different times. Most varieties keep producing fruit from 1-2 months. Therefore, in this study, two main flavonoids, including quercetin 3-galactoside and cyanidin 3-galactoside, and antioxidant activity of one raspberry genotype fruits in four different harvesting times were compared.

MATERIALS AND METHODS

Plant materials

In this study the raspberry fruits growing in Rasht, Guilan province, Iran, were investigated in 2009. The fruits were harvested at full ripe stage in different harvesting times including July, August, September and October and analyzed separately. For each sample, 10 fruits were blended and then 2 g of this specimen was used for analysis.

Chemicals

Cyanidin chloride and quercetin 3-galactoside (hyperoside) were obtained from Extrasynthese (Genay, France). DPPH was obtained from Sigma-Aldrich Co. (St. Louis, USA).

Preparation of extracts

Phenolic extraction was carried out using 15% acetic acid in methanol added to fine powder of two grams of specimen prepared and kept in 4°C overnight and centrifuged at 10,000 × *g* for 10 min (MERMLE: Z233M-2). The supernatant of the centrifuged samples was filtered through a disposable syringe filter (0.45 µm) and stored at -20°C until analysis (Bakhshi and Arakawa 2006).

Identification of flavonoids with HPLC

The content of cyaniding 3-galactoside and quercetin 3-galactoside were determined using high-performance liquid chromatography (HPLC, Waters, MA, USA) coupled to a dual λ absorbance detector (Waters Dual λ Absorbance 2487). The column was a Waters Symmetry C18 5 µm 4.6 × 150 mm (Waters, Dublin, Ire-

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land). Solvent used for elution was (A) 95:5 H_2O/methanol (HPLC grade) and (B) 5:95 H_2O/methanol, where the flow was 1 ml min^{-1}. The analysis pH was 3. The solvent program started at an initial composition of 90% A and 10% B, increasing to 55% A and 45% B at 20 min, and then 45% A and 55% B at 30 min. Fifty µl of extracts was injected onto the column which was maintained at room temperature. Identification of the compounds was carried out by comparing their retention times with those of standards.

Antioxidant activity of raspberry extracts

The antioxidant activity was measured by the scavenging of DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) free radical. Briefly, 50 µl of diluted extracts were added to 950 µl of a 0.1 N solution of DPPH in methanol. After, the reaction was allowed to take place in the dark for 30 min, the absorbance was recorded using JENWAY–6405 UV/Vis, Belgium spectrophotometer at 715 nm. The antioxidant activity of the extracts is expressed in the form of the percentage of free radical scavenging.

Statistical analysis

This experiment was conducted in a completely randomized design. Data were analyzed using SAS procedures and software (SAS, 9.1). Analysis of variance was constructed using the PROC GLM procedure, and Mean separation was performed using Duncan's Multiple Range Test, at $P \leq 0.05$.

RESULTS AND DISCUSSION

Identification of flavonoids

There was considerable variation among different harvest times regarding quercetin 3-galactoside, cyaniding 3-galactoside and antioxidant activity. The studied genotype produces fruit in summer and autumn. Full ripe fruits were harvested 3 times in during summer and once in autumn. According to the obtained results, raspberry phenolic compounds changes during plant growth and different times of fruit set. Early season fruits (in July) had the highest content of quercetin 3-galactoside which declined through other harvesting times. In October, quercetin 3-galactoside slightly increased (**Fig. 1**). Anthocyanin in first harvesting time was low, but in August and September increased, and then declined in October (**Fig. 2**). Pincemail *et al.* (2012) reported that phenolic compounds and antioxidant activity of strawberries was affected by the harvesting time of the season. They reported that many factors determined fruits phenolic content, but the harvesting time (at the same ripening stage) appeared to be very important, even more important than genotype. Findings of the current study are in coincidence with their report. Milivojevic *et al.* (2011) also studied total phenolic content and antioxidant activity in 5 raspberry cultivars at different harvesting times and expressed that these compounds differed at different times. This difference among harvesting times can be related to plant physiological status and environmental conditions specifically temperature, light quantity and quality during plant growth and various stages of fruit growth and development.

Antioxidant activity

Among different harvest times, early season fruits (in July) showed highest percentage of antioxidant activity, followed by fruits in October, September and August (**Fig. 3**). However, in Milivojevic *et al.*'s (2011) study, fruits harvested in autumn had more antioxidant activity than those harvested in summer. This study pointed out that the nutritional value of raspberry fruit is strongly influenced by harvesting time. Raspberry fruits in first harvesting time had more antioxidants. Total antioxidant activity percentage affected by bioactive compounds such as phenolic compounds including phenolic acids and flavonoids. According to the results here, it could be concluded that early season fruits are the richest regarding antioxidants than other times.

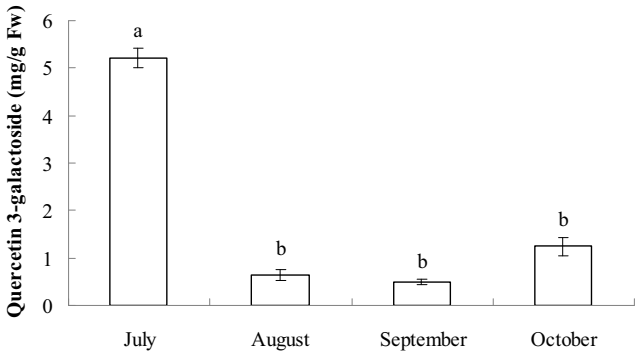

Fig. 1 Quercetin 3-galactoside content of raspberry fruits (mg/g Fw) at various harvesting times. Different letters indicate significant differences according to Duncan's Multiple Range Test ($P < 0.05$). Values represent mean ± Standard Error (SE). n = 3

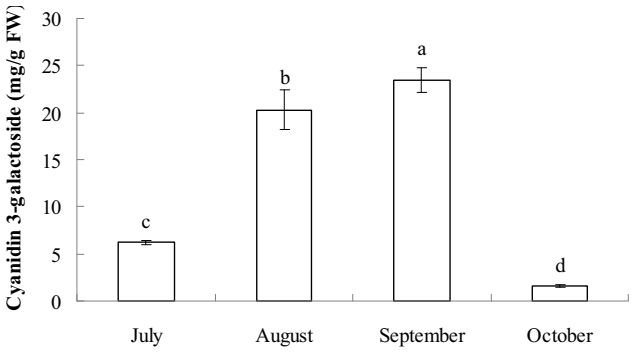

Fig. 2 Cyanidin 3-galactoside content of raspberry fruits (mg/g Fw) at various harvesting times. Different letters indicate significant differences according to Duncan's Multiple Range Test ($P < 0.05$). Values represent mean ± Standard Error (SE). n = 3

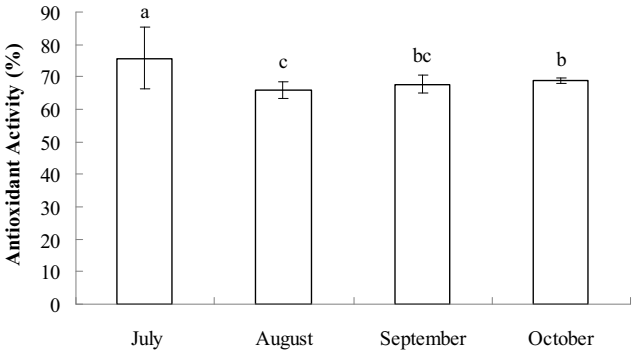

Fig. 3 Antioxidant activity of raspberry fruits (mg/g Fw) at various harvesting times. Different letters indicate significant differences according to Duncan's Multiple Range Test ($P < 0.05$). Values represent mean ± Standard Error (SE). n = 3

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