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Evaluation of Phenolic Compounds in Different Parts of a Native Red-Fleshed Apple (*Malus baccata*) in Northern Iran

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ABSTRACT

In this study, the phenolic compound content including (+)–catechin, quercetin 3-galactoside, phloridzin, chlorogenic acid, cyaniding 3-galactoside (anthocyanin) from the peel, pulp and seeds of a wild red-fleshed apple genotype growing in Northern Iran were investigated, using high performance liquid chromatography (HPLC). There were significance differences in phenolic composition among pulp, peel and seeds in fruits. Chlorogenic acid was the predominant phenolic compound in the pulp (0.62 mg/g FW). Seeds had the highest content of phloridzin (2.84 mg/g FW) and pulp showed the highest catechin content (0.53 mg/g FW). Quercetin 3-galactoside content in peel (0.46 mg/g FW) was higher than in other parts. According to the results, the seeds (0.52 mg/g FW) had the highest content of cyanidin 3-galactoside while there was no significant difference between peel and pulp (0.33 and 0.17 mg/g FW, respectively). Besides light, other factors are involved in anthocyanin synthesis. Anthocyanin accumulated in the pulp might be synthesized in the leaves and then transferred to the fruit, although this requires further study. Red-fleshed apple fruit is good material for studying factors involved in anthocyanin biosynthesis and could be a good resource of valuable antioxidants for the human diet.

Keywords: anthocyanin, catechin, chlorogenic acid, phloridzin, quercetin

INTRODUCTION

Phenolic compounds are a class of low molecular weight secondary metabolites found in most land plants including vegetables and fruits (Boyer and Liu 2004; Carbone *et al*. 2011). 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). Plant polyphenolics are presented in human diet, and being of great interest as they seem to present potential anticarcinogenic properties and reduce the incidence of cardiovascular disease, asthma and diabetes, due to their antioxidant activity and their function as free radical scavengers (Alonsa-Salces *et al*. 2001; Boyer and Liu 2004; Stevenson and Lowe 2009).

Apple is an excellent source of several phenolic compounds and also possesses high total antioxidant capacity (Khanizadeh *et al*. 2008). Less than 0.4% of the antioxidant activity (AOA) of apples is attributed to ascorbic acid content, indicating that other factors, such as phenolics, are the main contributors (Vieira *et al*. 2011). The major flavonoids classes occurring in apple fruit are flavonols such as quercetin 3-glycosides, monomeric and oligomeric flavan 3-ols such as catechin, epicatechin and procyanidins, dihydrochalcones such as phloridzin, and in red-colored cultivars, anthocyanins such as cyaniding 3-glycosides. Apple fruit also contain considerable amounts of hydroxycinnamic acid derivatives which are mainly represented by chlorogenic acid (Awad *et al*. 2000; Shoji *et al*. 2004; Ceymann *et al*. 2012).

Different research results show that the phenolic content of apple extracts are determined by genetic, environmental and post-harvest factors, including fruit season, fruit maturity, light exposure, storage and processing (Awad *et al*. 2000; Van der Sluis 2001; Awad and de Jager 2002; Chinici *et al*. 2004). Also, distribution of these metabolites differs within a fruit (Awad *et al*. 2000; Carbone *et al*. 2011). Vieira *et al*. (2011) and Drogoudi *et al*. (2008), with their investigation and comparison of phenolic compounds and antioxidant activity between peel and pulp of apple fruits, indicated that the concentration of phenolic compounds and antioxidant activity in the peel is higher than in the pulp. Bakhshi (2006) reported that the accumulation of different phenolics was different in various parts of an individual fruit of 'Fuji' apple.

In general, the genus *Malus* is comprised of about 30–35 species of small deciduous trees or shrubs in the family Rosaceae (Mulabagal *et al*. 2007). The domesticated orchard or table apple, *Malus* × *domestica* Borkh., is considered to be a complex interspecific hybrid. The main ancestor of commercial apple is thought to be *M. sieversii* (Harris *et al*. 2002) along with other ancestors, those being *M. sylvestris*, *M. pumila*, and *M. dasyphylla* (Mulabagal *et al*. 2007). Most of the *Malus* species are generally known as "wild apples", "crab apples", or "crabs", names derived from their typically small and tart fruit (Robinson *et al*. 2001; Wolf *et al*. 2003). The Central Asian wild apple is closely related to a group of apples that have different-sized fruits. One of these is *Malus baccata* which has small and red fruits (Harris *et al*. 2002). Whilst most apples have white or cream pulp, a small number of varieties have red or pink pulp. According to the citations, the red-fleshed wild apples growing in northern Iran must belong to *M. baccata* species. In this study, phenolic compounds variations in different parts of a native crabapple with red pulp grown in Ramsar region located in Mazandaran Province, Iran were investigated. The fruits are small with astringent and tart taste and non-juicy and oxidized easily, thus do not have commercial uses and only native people use them in traditional foods and jams. However, there is no published data on the botanical and physiological aspects of this variety.

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Original Research Paper

MATERIALS AND METHODS

Plant material

The fruits of a native wild red-fleshed apple genotype were collected from Javaher Deh region with geographical coordinates as 36° 51' 21" North, 50° 27' 47" East situated at an altitude of 1800 meters above sea-level, near Ramsar City in Mazandaran Province, north of Iran.

Chemicals

Cyanidin chloride, quercetin 3-galactosid (hyperoside), (+)-catechin were obtained from Extrasynthese (Genay Cedex, France). Phloridzin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Chlorogenic acid was obtained from Cayman Chemical Co. (Michigan, USA).

Phenolic extraction

The pulp, peel and seeds were obtained from five randomly selected apples. These specimens of fruit peel, pulp and seeds were frozen in liquid nitrogen and then kept at -80°C until use. Phenolic extraction was carried out using a mixture of 15% acetic acid in methanol which was added to 2 g of fine powder and left at 4°C overnight and then centrifuged in 10,000 rpm for 10 min (Bakhshi and Arakawa 2006a). The supernatant of the centrifuged samples was filtered through a disposable syringe filter (0.45 µm; Jet-Biofil, Canada) and stored at -20°C until analysis.

Determination of phenolic compounds by high performance liquid chromatography (HPLC)

Identification of phenolics including chlorogenic acid, catechin, phloridzin, quercetin 3-galactoside and cyanidin 3-galactoside was carried out with a high performance liquid chromatography system (Waters, MA, USA) coupled to a dual λ absorbance detector (Waters Dual λ Absorbance 2487). Separation of phenolic compounds was performed in a column Symentery C18 (4.6 × 150 mm with 5 µm pore size; Waters, Dublin Ireland) using two solvents: A 95: 5 (v/v) water: methanol (HPLC grade) and B 5: 95 (v/v) water: methanol. 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. The flow was 1 ml min^{-1}. Fifty µl of extracts was injected onto the C18 column which was maintained at room temperature. Identification of the compounds was carried out by comparing their retention times with those of mentioned phenolic compounds standards. All samples were analysed in triplicate.

Statistical analysis

The experiment was conducted in a completely randomized design. Data were subjected to an analysis of variance (ANOVA) using the General linear Model (GLM) procedure of SAS (v. 9.1). Means were compared with Tukey's test at $P \leq 0.01$.

RESULTS AND DISCUSSION

As the other studies results shows (Ayala-Zavala *et al*. 2010), there were significant differences regarding all measured phenolic compounds among peel, pulp and seeds of fruits. This indicated that the synthesis and accumulation of phenolic compounds is tissue specific (Awad *et al*. 2000; Carbone *et al.* 2011). Moreover, it is well established that genetic plays a major role in controlling polyphenol composition of apples (Khanizadeh *et al*. 2008; Vieira *et al*. 2011), and the genetic activity of the various parts of the apple fruit might be different as it is a pome fruit (not a true fruit). The seeds originate from the ovary and the pulpy part from the floral tube, the fused bases of sepals, petals and stamens (Awad *et al*. 2000).

Chlorogenic acid, a phenolic acid, was the predominant phenolic in pulp (**Fig. 1**). Bakhshi (2006) reported that chlorogenic acid content increase from peel to the core of

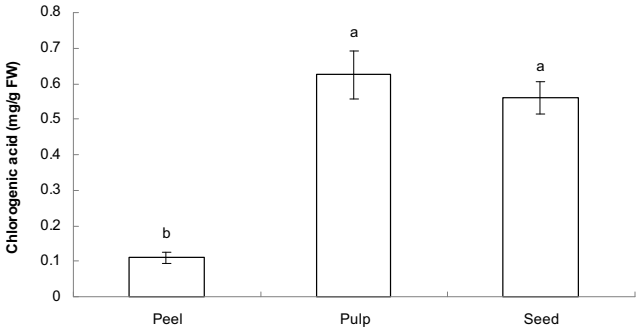

Fig. 1 Chlorogenic acid content of different parts of red-fleshed apple fruit.

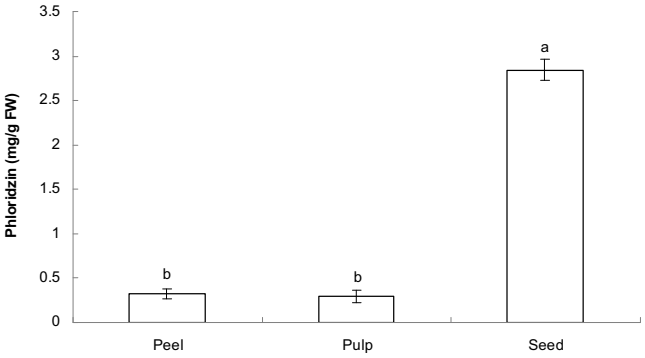

Fig. 2 Phloridzin content of different parts of red-fleshed apple fruit.

'Fuji' apple. Awad *et al* (2000) showed that there is interaction between tissue and cultivar. In 'Jonagold' the chlorogenic acid concentration was maximal in the core area; whereas it was highest in the seeds of 'Elstar'. As the results show here, in the studied genotypes the pulp had the highest content of chlorogenic acid, followed by seed and peel, respectively (**Fig. 1**). However, the seeds did not show significant differences with the pulp. Therefore, the higher levels of chlorogenic acid in deeper tissue zones may indicate that the genes controlling its synthesis are not light dependent.

The highest content of phloridzin was observed in the seeds (**Fig. 2**). Awad *et al.* (2000) deduced that phloridzin is the principle flavonoid in the seeds, where it contributed 98% of total flavonoids, and except for the flesh, the level of phloridzin increased from the peel to the core (Awad *et al.* 2000; Bakhshi 2006). In the red-fleshed apple of Iran, the phloridzin concentration was maximal in the seed showing that the expression of genes controlling phloridzin synthesis is not light dependent and is affected by genotype. Moreover, the phloridzin contributes more than 90% of the soluble phenolic compounds in apple leaves (Gosch *et al*. 2009). It is possible that phloridzin is transmitted from leaves to the all fruit parts and this note that seeds had the highest content of phloridzin indicating that the seeds are more vigorous sinks for this flavonoids rather than peel and pulp.

Catechin in pulp was higher than in other parts of fruits; and it was lowest in seeds (**Fig. 3**). It has been already reported that the peel catechin content is higher than pulp (Escarpa and Gonzales 1998; Awad *et al*. 2000), which was unlike the results here.

Quercetin 3-galactoside was the major peak in peel, followed by seeds and pulp, respectively (**Fig. 4**). Quercetin 3-galactoside (0.46 mg/g FW) was mainly located in the peel, which this result is in agreement with other authors (5.29-12.14 mg/g DW) (Awad *et al*. 2000; Jakopic *et al*. 2009). Thus, light have an important role in quercetin synthesis. The fruits in outside part of tree canopy have more content of quercetin 3-galactoside rather than those placed inside of tree canopy (Awad *et al*. 2001; Jakopic *et al*. 2009). On other hand, Solovchenko and Eiberger (2003) referred to

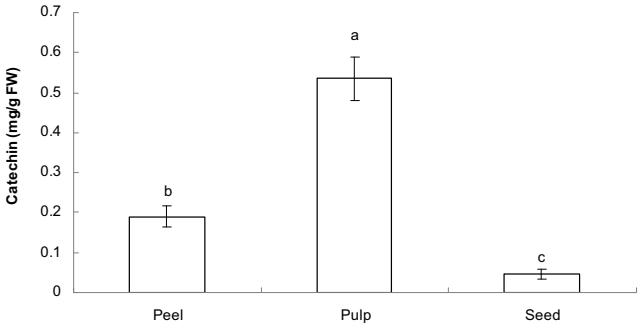

Fig. 3 Catechin content of different parts of red-fleshed apple fruit.

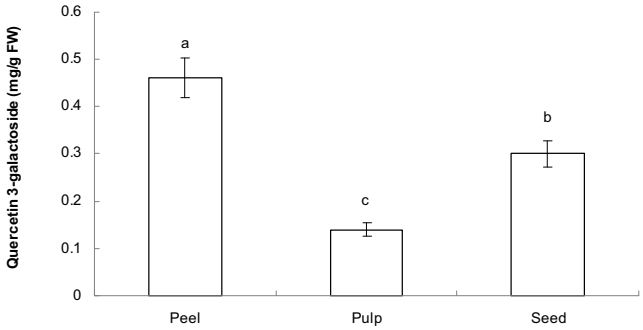

Fig. 4 Quercetin 3-galactoside in different parts of red-fleshed apple fruit.

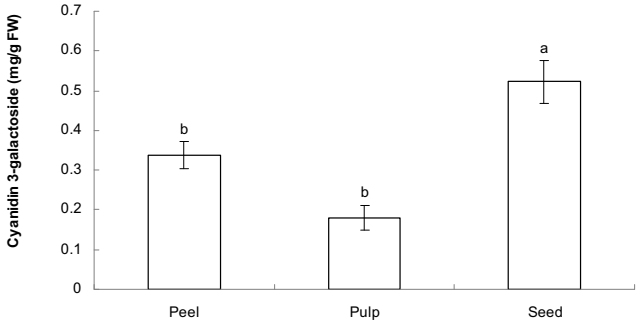

Fig. 5 Cyanidin 3-galactoside content of different parts of red-fleshed apple fruit.

quercetins role in UV absorption. Manach *et al*. (2004) also expressed that phenolic compounds accumulate in the outer and aerial tissue (skin and leaves), because their biosynthesis is stimulated by light.

As the results showed (**Fig. 5**), the seeds had the highest content of cyanidin 3-galactoside. The results of mean comparison did not show a significant variation between peel and pulp, but in peel was higher than in pulp. Red-skinned apples accumulate anthocyanin, while yellow-green ones do not and most of the commercial cultivars with red skin accumulate anthocyanin in the skin but not in the pulp (Bakhshi and Arakawa 2006b). But one interesting and considerable property of this genotype of apple was the anthocyanin synthesis in its pulp. Although, the biosynthesis mechanisms of anthocyanin and other phenolics have been widely studied in the skin, limited information about the pulp is available (Bakhshi and Arakawa 2006b). Bakhshi and Arakawa (2006b) investigated the phenolic compounds biosynthesis with light irradiation in the pulp and found that the pulp of apples had the potential for accumulating anthocyanin as well as many other polyphenolics.

Cyanidin 3-galactoside mainly locate in the peel (D'Abrosca *et al*. 2007), and light and UV-B irradiation stimulate the anthocyanin synthesis in peels (Awad *et al*. 2001; Bakhshi and Arakawa 2006b), but the HPLC analysis of this genotype indicated that the cyaniding 3-galactoside content of seed is higher than peel and pulp. These results may indicate that except of light, other factors are also involved in anthocyanin synthesis. Therefore, this wild apple is a valuable genetic resource for studying anthocyanin synthesis, and for breeding programs to producing red color in pulp of apples.

CONCLUSION

Phenolic content varied significantly in different parts of individual fruit. This indicated that the synthesis and accumulation of phenolic compounds is tissue specific. Moreover, seeds have the highest anthocyanin content, however, it has been reported that anthocyanin synthesis is light dependent. Therefore, beside light, other factors are involved in anthocyanin synthesis. Anthocyanin accumulated in pulp might be synthesised in the leaves and then transferred to the fruit, which requires further study. So, red-fleshed apple fruit is a good material for studying factors involved in anthocyanin biosynthesis and accumulation.

REFERENCES

Alonso-Salces RM, Korta E, Barranco A, Berrueta LA, Gallo B, Vicente F (2001) Pressurized liquid extraction for the determination of polyphenols in apple. *Journal of chromatography A* **933**, 37-43

Awad MA, de Jager A (2002) Relationship between fruit nutrients and concentrations of flavonoids and chlorogenic acid in 'Elstar' apple skin. *Scientia Horticulturae* **92**, 265-276

Awad MA, de Jager A, van Westing LM (2000) Flavonoid and chlorogenic acid levels in apple fruit: Characterization of variation. *Scientia Horticulturae* **88**, 249-263

Awad MA, Wagenmakers PS, de Jager A (2001) Effects of light on flavonoid and chlorogenic acid levels in the skin of Jonagold apples. *Journal of Scientia Horticulturae* **88**, 289-298

Ayala-Zavala JF, Rosas-Dominguez C, Vega-Vega V, Gonzales-Aguilar GA (2010) Antioxidant enrichment and antimicrobial protection of fresh-cut fruits using their own byproducts: Looking for integral exploitation. *Journal of Food Science* **75 (8)**, R175-R181

Bakhshi D (2006) Study on the accumulation of phenenolic compounds in apple fruit. PhD thesis, Hirosaki University, Japan, 113 pp

Bakhshi D, Arakawa O (2006) Effects of UV-B irradiation on phenolic compound accumulation and antioxidant activity in 'Jonathan' apple influenced by bagging, temperature and maturation. *Journal of Food, Agriculture and Environment* **4 (1)**, 75-79

Bakhshi D, Arakawa O (2006b) Induction of phenolic compounds biosynthesis with light irradiation in the flesh of red and yellow apples. *Journal of Applied Horticulture* **8 (2)**, 101-104

Boyer J, Liu RH (2004) Apple phytochemicals and their health benefits. *Journal of Nutrition* 3.5

Carbone K, Giannini B, Picchi V, Lo Scalzo R, Cecchini F (2011) Phenolic composition and free radical scavenging activity of different apple varieties in relation to the cultivar, tissue type and storage. *Food Chemistry* **127**, 493-500

Ceymann M, Arrigoni E, Scharer H, Bozzi Nising A, Hurrell RF (2012) Identification of apples rich in health-promoting flavan-3-ols and phenolic acids by measuring the polyphenol profile. *Journal of Food Composition and Analysis* **26**, 128-135

Chinnici F, Bendini A, Gaiani A, Riponi C (2004) Radical scavenging activities of peels and pulps from cv. Golden Delicious apples as related to their phenolic composition. *Journal of Agricultural and Food Chemistry* **52**, 4684-4689

D'Abrosca B, Pacifico S, Cefarelli G, Mastellone C, Fiorentino A (2007) Limoncella apple, an Italian apple cultivar: Phenolic and flavonoid contents and antioxidant activity. *Journal of Food Chemistry* **104**, 1333-1337

Drogoudi PD, Michailidis Z, Pantelidis G (2008) Peel and flesh antioxidant content and harvest quality characteristics of seven apple cultivars. *Scientia Horticalturae* **115**, 149-153

Escarpa A, Gonzales MC (1998) High-performance liquid chromatography with diode-array detection for the determination of phenolic compounds in peel and flesh from different apple varieties. *Journal of Chromatography A* **823**, 331-337

Gosch C, Halbwirth H, Kuhn J, Miosic S, Stich K (2009) Biosynthesis of phloridzin in apple (*Malus domestica* Borkh.). *Journal of Plant Science* **176**, 223-231

Harris SA, Robinson JP, Juniper BE (2002) Genetic clues to the origin of the apple. *Trends in Genetics* **18**, 426-430

Jakopic J, Stampar F, Veberic R (2009) The influence of exposure to light on the phenolic content of 'Fuji' apple. *Scientia Horticulturae* **123**, 234-239

Khanizadeh Sh, Tsao R, Rekika D, Yang R, Charles MT, Vansantha HP (2008) Polyphenol composition and total antioxidant capacity of selected ap-

ple genotypes for processing. *Journal of Food Composition and Analysis* **21**, 396-401

Manach C, Scalbert A, Morand C, Remesy C, Jimenez L (2004) Polyphenols: Food sources and bioavailability. *The American Journal of Clinical Nutrition* **79**, 727-747

Mulabagal V, Van Nocker S, Dewitt DL, Nair MG (2007) Cultivars of apple fruits that are not marketed with potential for anthocyanin production. *Journal of Agricultural and Food Chemistry* **55**, 8165-8169

Robinson JP, Harris SA, Juniper BE (2001) Taxonomy of the genus *Malus* Mill. (Rosaceae) with emphasis on the cultivated apple, *Malus domestica* Borkh. *Plant Systematics and Evolution* **226**, 35-38

Shoji T, Akazome Y, Kanda T, Ikeda M (2004) The toxicology and safety of apple polyphenol extract. *Journal of Food and Chemical Toxicology* **42**, 959-967

Solovchenko A, Schmitz-Eiberger M (2003) Significance of skin flavonoids for UV-B-protection in apple fruits. *Journal of Experimental Botany* **54**, 1977-1984

Stevenson DE, Lowe T (2009) Plant-derived compounds as antioxidants for health - are they all really antioxidants? *Functional Plant Science and Biotechnology* **3 (Special Issue 1)**, 1-12

Van der Sluis AA, Dekkerm M, Skrede G, Jongen WMF (2002) Activity and concentration of polyphenolic antioxidants in apple juice. 1. Effect of existing production methods. *Journal of Agricultural and Food Chemistry* **50**, 7211-7219

Vieira FGK, Borges GDSC, Copetti C, Di Pietro PF, Nunes EC, Fett R (2011) Phenolic compounds and antioxidant activity of the apple flesh and peel of eleven cultivars grown in Brazil. *Scientia Horticulturae* **128**, 261-266

Wolfe K, Wu X, Liu RH (2003) Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry* **51**, 609-614