

THE COMPARISON OF PHENOLIC COMPOUNDS CONTENT AND ANTIOXIDANT CAPACITY OF SOME SELECTED IRANIAN APPLE GENOTYPES FOR PROCESSING TECHNOLOGY

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ABSTRACT. *The interest in the consumption of apple fruits is, to a large extent, due to its content of bioactive compounds and their importance as dietary antioxidants. In this study, the phenolic compounds especially flavonoids such as catechin and chlorogenic acid, total phenols and antioxidant activity of nine apple fruit genotypes were investigated. The results showed a significant difference for antioxidant components among the apple genotypes. The fruits of 'Soltanie Shabestar', 'Heidarzade' and 'Hajie Karaj' showed the highest total phenols and flavonoids content. The richest genotype in catechin and chlorogenic acid content was 'Soltanie Shabestar'. Although apple genotypes with the higher total phenols and flavonoids showed the higher antioxidant activity, but there was no significant correlation between antioxidant capacity with catechin or chlorogenic acid. At last, the genotypes IRI1, IRI4 and IRI6 with the lowest total flavonoid, catechin and chlorogenic acid contents exhibited the lowest flesh browning index which would be suitable for fresh cut and dried apple slices.*

KEYWORDS: *antioxidant capacity, chlorogenic acid,
phenolic composition*

INTRODUCTION

An important field of research today is the control of 'redox' status by consuming foods with high antioxidant properties. Natural antioxidants present in the diet increase the resistance to oxidative stress and they may have a substantial impact on human health (Dimitrios 2006). Phenolic compounds, especially phenolic acids and flavonoids are considerably present in vegetables and fruits; thus they are an integral part of the human diet. Recently, they have received much attention, since many epidemiological studies suggest that consumption of polyphenol-rich foods and beverages is associated with a reduced risk of cardiovascular diseases, stroke and certain forms of cancer (Prior & Cao 2000, Kaur & Kapoor 2001). These protective effects have partly been ascribed to the antioxidant properties. Apple fruits are an excellent source of several phenolic compounds and also possess high total antioxidant capacity. Sun et al. (2002) found that apples had the highest soluble free phenolics content, when compared to 10 other commonly consumed fruits. The distribution of phenolic compounds varies considerably among different cultivars, and also within different tissues of fruits. Alonso-Salces (2004) identified and quantified individual phenolic constituents of 31 Basque cider apple cultivars and reported considerable differences in the content of total polyphenolics among tested cultivars. When selecting cultivars for apple production, special attention is paid to the polyphenols content and profile, since they contribute to color, bitterness, and astringency (Lea 1990). Several polyphenols play an important role in browning characteristics of apple products. Chlorogenic acid was found as the most important substrate of polyphenol peroxidase (PPO). In the presence of oxygen and PPO, chlorogenic acid is converted into O-quinone, which further reacts with other phenolic compounds, resulting in the formation of yellow and brown pigments (Oszmianski & Lee 1990). Furthermore, catechin is also involved in enzymatic browning and particularly its degree of polymerization is responsible for bitterness and astringency of apple juices and ciders (Khanizadeh et al. 2006). Flavonoids are also involved in browning processes by the action of the enzyme PPO (Nicolas et al. 1994,

Khanizadeh et al. 2006). The aim of this study was to assess flavonoids compounds and antioxidant activity of selected Iranian apple genotypes for used in processing technology.

MATERIALS AND METHODS

Plant materials and treatments

The fruits of apple genotypes; 'Hajje karaj', 'Soltanie Shabestar', 'Golshahi', 'IRI1', 'IRI4', 'Heidarzade', 'IRI6', 'Khorsijaan' and 'Shafii' which randomly were picked from the orchard of Seed and Plant Breeding Research, Institute, Karaj at commercial maturity and the following characteristics were analyzed.

Total phenolic content

Total phenolics were analyzed spectrophotometrically using the modified Folin–Ciocalteu colorimetric method as described by Ghasemnezhad et al. (2011), with some modifications. Each sample (1 g) was extracted with 10 mL methanol, and then 150 μ L of the methanolic extract were mixed with 350 μ L of distilled (DI) water in a test tube followed by addition of 2.5 mL of 10 % Folin–Ciocalteu reagent and allowed to stand for 6 min. Then, 2 mL of 7.5% sodium carbonate solution were added. Each sample was allowed to stand for 90 min at room temperature in darkness and the absorbance was measured at 760 nm using an UV/Vis spectrophotometer model PG Instrument +80₇ (Leicester, UK). Results are expressed as mg gallic acid (GAE)/100 g FW.

Total flavonoids content

Total flavonoid content was measured according to Blasa et al. (2005). 1 mL aliquot of standard solution of catechin at different concentrations (0–100 mg/L) or sample was added to 10 mL volumetric flasks containing 4 mL water. At the onset of the experiment, 0.3 mL of 5% NaNO₂ was added to the flask. After 5 min, 0.3 mL of 10% AlCl₃ was added. At 6 min, 2 mL of 1 M NaOH was added to the mixture. Immediately, the solution was diluted to a final volume of 10 mL with water and mixed thoroughly. Absorbance of the mixture was determined at 510 nm using an UV/Vis spectrophotometer model PG Instrument +80, (Leicester, UK). Total flavonoid content was expressed as mg catechin equivalents per 100 g fresh weight.

HPLC analysis of flavonoids

Flavonoids were determined using high-performance liquid chromatography (HPLC). For polyphenols extraction, 1ml of solvent (methanol/acetic acid, 85:15, v/v) was added to 1 g of apple tissue. The samples were kept in a refrigerator for 24 h and centrifuged for 10 min at 10000 rpm. The supernatants of centrifuged samples were filtered through disposable 0.45µm syringe filter. Fifty microlitres of the filtered sample were injected in HPLC (Waters, 1525, Milford, USA) equipped with a UV-Visible detector (Waters Dual λ Absorbance 2487), using C18 column: Waters Symentery C18 5µm 4.6×150mm (Waters, Dublin, Ireland), and detection at 280 and 350 nm. The flavonoids were identified by comparing their UV spectra and retention times with those of the corresponding standards and by the spiking of samples with the appropriate standard. Catechin standard was purchased from Sigma-Aldrich Canada Ltd and chlorogenic acid from Cayman Chemical Japan (Bakhshi & Arakawa 2006b)

Antioxidant activity

The antioxidant activity was evaluated by 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method according to the procedure of Ghasemnezhad et al. (2011), with some modifications. Briefly, 150 µL of apple extracts were added to 850 µL of DPPH radical. The solution was vortexed and allowed to stand at room temperature in darkness. The absorbance of the samples was measured at 515 nm after 15 min using an UV/Vis spectrophotometer model PG Instrument +80, Leicester, United Kingdom. For each sample, three separate determinations were carried out. The antioxidant activity was expressed as percentage of absorbance decrease, relative to the control, corresponding to the percentage of DPPH that was scavenged. The percentage of DPPH, which was scavenged (% DPPHsc), was calculated using %DPPHsc:

$$\%DPPHsc = (A_{cont} - A_{sam}) \times 100 / A_{cont}$$

Statistical analyses

The experiment was conducted using a completely randomized design with three replications. A preliminary test was run prior to the main experiment reported here. Data were analyzed as a two-factor linear model via PROC GLM procedure by SAS software (ver. 9.1 2002-2003), where treatments and storage time were the factors.

RESULTS AND DISCUSSION

Total phenolics content

Significant difference was for total phenolics content among the tested genotypes (Figure 1). The flesh tissue of 'Hajie karaj' and 'Heidarzade' have the highest phenolic content, while 'IRI1', 'IRI4' and 'IRI6' showed the lowest ones. Previous studies have also confirmed the difference between apple varieties for total phenolics (Khanizadeh et al. 2008, Wolfe et al. 2005). The difference for total phenolics content was also found in other fruits such as red grapes and plum varieties (Rupasinghe et al. 2006, Russell et al. 2002).

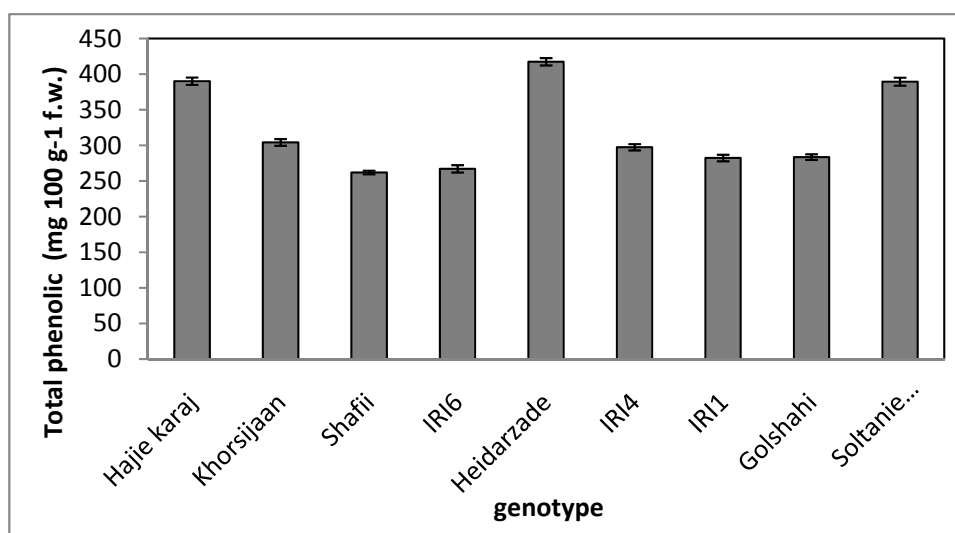


Figure 1. Total phenolic content of fruits of different apple genotypes.

Total flavonoid content

There also was significant difference for total flavonoid content between the tested apple genotypes fruit (Figure 2). The flesh tissue of 'Heidarzade' and 'Soltanie shabestar' fruits had the highest content of total flavonoid, while 'IRI1' and 'IRI4' had the lowest ones. Differences in the amount of total

flavonoid in different apple genotypes have been reported in previous studies (Khanizade et al. 2008, Wolf et al. 2003). McGhie et al. (2005) showed the location that apple trees grow in it, can influence on the type of phenolic compounds of tissue apple, but amount of total flavonoids dependent on the variety. Since apple genotypes have been grown in the same condition, therefore, the difference of total flavonoids could be due to genetic variation.

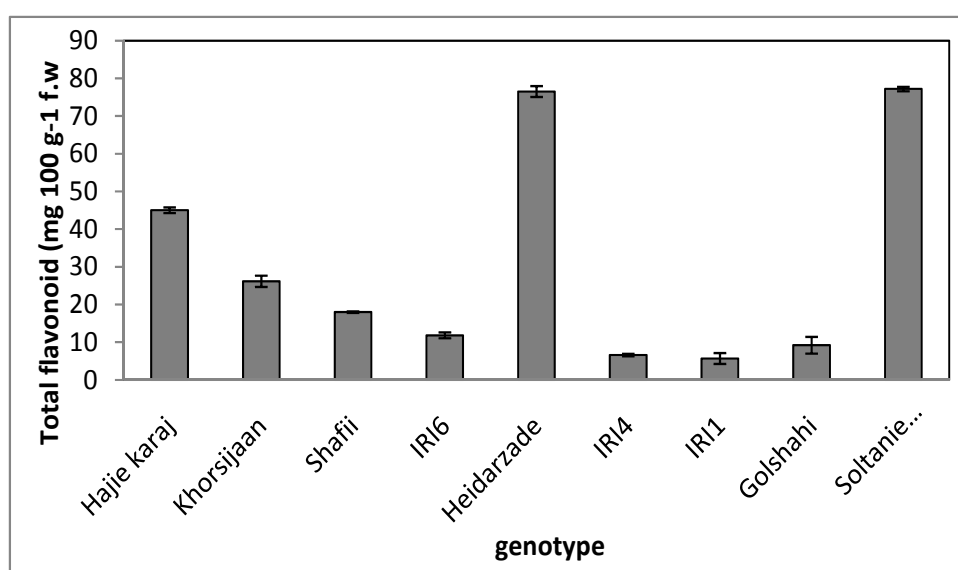


Figure 2. Total flavonoid content of different apple genotypes fruits.

Flavonoids composition

The results showed a significant difference for flavonoids composition such as catechin and chlorogenic acid among apple genotypes. 'Soltanie Shabestar' had the highest and 'Golshahi' had lowest catechin content among cultivars (Figure 3, 4). Catechin is one of the building blocks of phenolic polymers that are causing astringency in fruit. Fruits such as apples that have less catechin are more favorable to consumers (Joshi et al. 2007). Previous studies showed that the catechins in different varieties

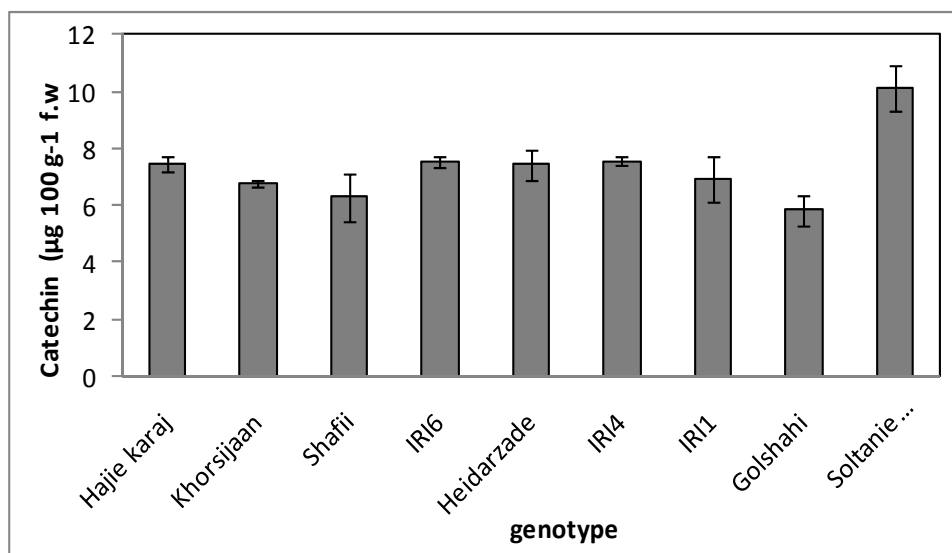


Figure 3. Content of catechin in different apple genotypes

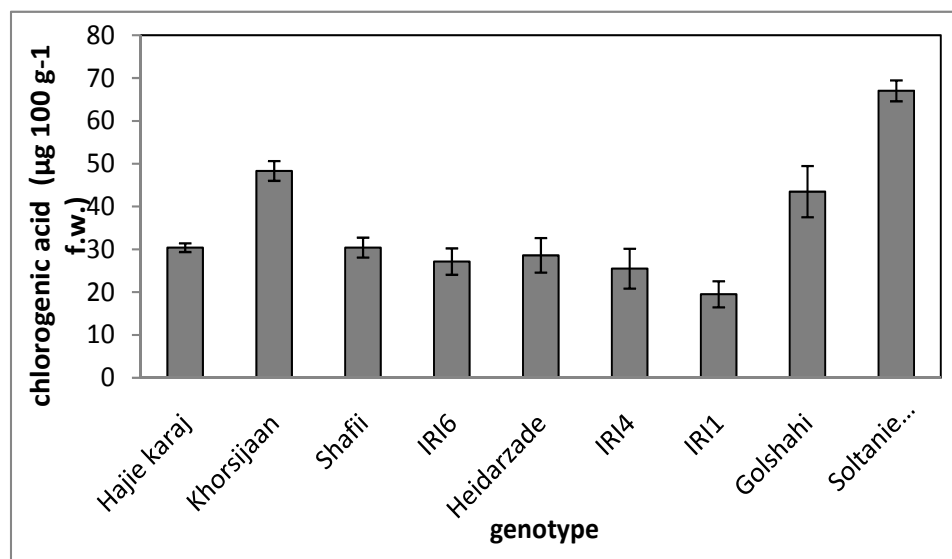


Figure 4. Content of chlorogenic acid in different apple genotypes.

of apples which grown in different places, showed significant differences over time. Awad et al. (2000) showed that the difference between the amount of catechins in fruits grown in inner and outer part of the crown is missing. Since the fruits used in this study were grown in the same location, differences are mostly due to genetics. Amount of chlorogenic acid in the flesh varied between 67.04 and 19.51 $\mu\text{g/g}$ fresh weight. The highest chlorogenic acid content was found in cultivar 'Soltanie Shabestar' and the lowest in cultivar 'IRI1' (Figure 4).

The significant differences between apple genotypes can be the role of genetics in the synthesis and amount the chlorogenic acid that correspond with findings of Chinnici et al. (2004) and Sanoner et al. (1999). Lata et al. (2009) also reported the amount of chlorogenic acid in different genotype depends on various factors such as growth period, geographic location and genetic diversity. Burda et al. (1990) stated chlorogenic acid is one of the important phenolic compounds in the browning that rapidly reduced during growth to maturity. Composition and concentrations of the major phenolics such as catechin and chlorogenic acid of apples studied are shown in (Figure 3, 4). All the nine apple genotypes flavonoid composition was different, however, the concentrations of chlorogenic acid were higher than catechin. 'Soltanie Shabestar' had the highest concentration of chlorogenic acid and catechin. The genotypes 'IRI1' and 'IRI4' have been showed the lowest concentration of chlorogenic acid and catechin followed by the lowest phenolic content. Catechin and chlorogenic acid are the substrates with greater affinity to PPO enzyme activity (Janovitz-Klapp et al. 1990, Oszmianski & Lee 1990). Joshi et al. (2007) reported that the absence of catechin or lower concentration of epicatechin can thus be expected to significantly contribute towards antibrowning properties of Eden™ apple genotypes.

Antioxidant activity

There was a significant difference for antioxidant activity between the studied apple genotypes. The Khorsijaan' had the highest, while 'Shafii' had lowest antioxidant capacity as compared to other genotypes (Figure 5). Khanizadeh et al. (2008), Chinnici et al. (2004) and Tsao et al. (2005)

showed that the antioxidant capacity of apple fruits were significant differences among varieties that they have examined. Leccese et al. (2009) showed that antioxidant capacity measurements in apple fruit with ABTS, FRAP and DPPH methods have significant differences among cultivars and suggested that differences in the cultivars are due to developmental period, location, season, method of extraction and more importantly genetic differences between cultivars.

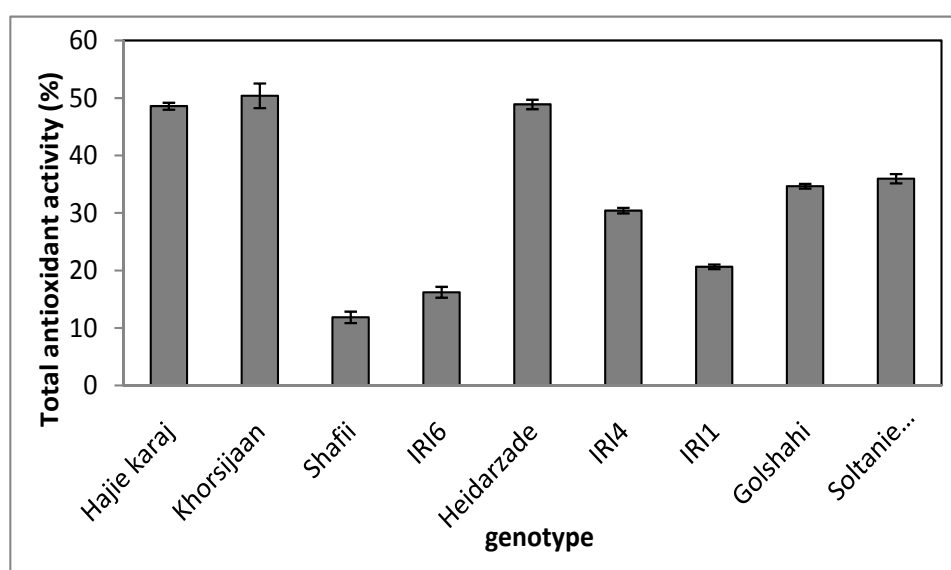


Figure 5. The antioxidant capacity of fruits of different apple genotypes

No significant correlation was found between antioxidant capacity and the flavonoids content ($R^2 = 0.16$ for catechin and $R^2 = 0.35$ for chlorogenic acid) (Table 1). Also, no significant correlation was found between total phenols content and flavonoids (Table 1). According to the results obtained in this study, we found a strong correlation between the total phenolic content and antioxidant capacity ($R^2 = 0.73$) (Table 1). Total phenolic content was high in 'Hajiekaraj', 'Soltanie shabestar', 'Heidarzade' while 'IRI1', 'IRI4' and 'Shafii' showed lower total phenolic content (Figure 1).

Table 1. The correlation between different biochemical parameters and antioxidant capacity in 9 apple genotypes

	Antioxidant capacity	Total phenolic content	Total flavonoids	Catechin	Chlorogenic acid
Antioxidant capacity	1	0.73	0.57	0.16	0.35
Total phenolic content	0.73	1	0.91	0.57	0.31
Total flavonoids	0.57	0.91	1	0.66	0.50
Catechin	0.16	0.57	0.66	1	0.52
Chlorogenic acid	0.35	0.31	0.50	0.52	1

** The significant different in level of 1%

* The significant different in level of 5%

ns: No significant different

In the study of Chinnici et al. 2004 on the antioxidant activity of apple cultivar 'Golden Delicious', they found a significant correlation between antioxidant activity and total phenolics, but no correlation between antioxidants and components flavonoids. This study showed that the antioxidant activity was significantly correlated with total phenolic content but no significant correlation between phenolic components and antioxidant activity. A particular combination does not determine the antioxidant activity alone, but the series of compounds will determine the antioxidant activity. of compounds of a series of compounds in tissue antioxidant activity determines because the different compounds in fruits can have the Intensify effect (synergistic) on total antioxidant activity (Martinez-Valverde et al. 2002). The total phenolic content among various cultivars is highly variable (Lee et al. 2003, Lata et al. 2005) and differences in phenolic content are suggested as a cause of the differences in the browning intensity among cultivars (Russell et al. 2002). Besides playing a major role in enzymatic

browning, the phenolic compounds present in apples act as a source of dietary antioxidants that may reduce the risk of many chronic disorders, including cancer (Boyer & Liu 2004). Therefore, there has been a growing interest in apples for their use in value added food products, such as functional beverages and healthy snack products. Song Ye et al. (2005) reported that phenolic compounds depend on the type of the cultivar, while total polyphenol apples effect on apple fruit quality. Generally there are many factors that affect the amount and type of phenolic compounds present in apples, which the role of genetics in controlling the phenolics composition in apples is very important.

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