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Comparison of Phenolic Compounds' Content and Antioxidant Activity between Some Native Iranian Apples and Standard Cultivar 'Gala'

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ABSTRACT

To determine the phenolic content and antioxidant activity in some native Iranian apple cultivars, some five cultivars namely: 'Bekran', 'Golab-e Kermanshah', 'Golab-e Kohanz' 'Golab-e Sheikhi' and 'Atlasi' were selected and compared with the standard cultivar 'Gala'. The profiles related to four phenolic compounds (chlorogenic acid, quercetin-3-galactoside, catechin, and phloridzin) were determined through HPLC for flesh and flesh+peel of the studied cultivars. Total Phenolic Content (TPC) through Folin-ciocalteu, and antioxidant activity applying DPPH radical (IC_{50}) were also evaluated for flesh *vs.* flesh+peel for each cultivar. The results of HPLC analysis revealed that catechin constituted the main phenolic compound of flesh and as well the flesh+peel in all the studied cultivars. The highest catechin concentrations in flesh+peel and in flesh were detected in 'Golab-e Kermanshah' (4,064.37 ug g^{-1} FW) and 'Bekran' (2,315.92 ug g^{-1} FW), recpectively. The second high concentration phenolic compound was quercetin-3 galactoside, with 'Atlasi' containing the highest content (833.96 ug g^{-1} FW). A high level of chlorogenic acid was detected in 'Golab-e Sheikhi' (276.106 ug. g^{-1} FW). The highest level of Total Phenolic Index (TPI) was observed in 'Golab-e Kermanshah' (4392.81ug g^{-1} FW). The highest TPCs were detected in 'Golab-e Kermanshah' and 'Atlasi'. Phenolic compounds were found as less abundant in the flesh than in the flesh+peel. The scavenging activity of DPPH radical (IC_{50}) revealed a higher antioxidant effect in flesh+peel than in (only) flesh. It can be concluded that in comparison with 'Gala', native Iranian cultivars contained higher concentrations of phenolic compounds, making them appropriate for use in breeding and being reintroduced into the production cycle. Also, it was revealed that whole fruit (flesh+peel) provides phytochemicals in levels far greater than the amounts provided by the fruit's (only) flesh.

Keywords: Fruit quality, Iranian apple germplasm, *Malus×domestica* Borkh., Phenolic compounds.

INTRODUCTION

Apple is known as one of the most commonly consumed fruits throughout the world. The fruit contains high levels of antioxidants (Francini and Sebastiani, 2013). Phenolic compounds, as some of the antioxidant components, naturally occurring in plant secondary metabolites, determine such outer and inner quality parameters of the fruit as appearance, flavor as well as health-promoting properties (Lee *et al.,* 2003; Sturm *et al.,* 2003). In apples, such several polyphenolic molecules as (+)-catechin and (−)-epicatechin, phloridzin,

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quercetin, cyanidin-3-*O*-galactoside, chlorogenic acid, and hydroxycinnamates were detected, using either Liquid Chromatography-Mass Spectrometry (LC-MS) or Gas Chromatography Mass Spectrography (GC-MS) analysis methods (Cuthbertson *et al.,* 2012; Vrhovsek *et al.,* 2004). Concentration of these phenolic compounds as well as their antioxidant activity differ with the cultivar, maturity stage, environmental conditions and as well the part of the fruit (Kjersti *et al.,* 2004; Kondo *et al.,* 2002; Van Der Sluice *et al.,* 2001).

Old time apple cultivars are of a wide range in flavour, aroma, sugar and acid-content and as well higher firmness, total polyphenol and flavonoid content, vitamin C concentration and antioxidative potential in comparison with those known as standard cultivars (Balík *et al.,* 2012; Donno *et al.*, 2012). Specific studies aimed at comparing phenolic compounds in commercial *vs*. anciently grown apple cultivars have been performed (Iacopini *et al.,* 2010; Minnocci *et al.,* 2010). One study revealed that two old grown varieties 'Ruggine' and 'Panaia' contained the highest levels of phenolic compounds as compared with 'Golden delicious' (Iacopini, *et al.,* 2010). Similar data have been obtained for two cultivars 'Diacciata' and 'Limoncella' in comparison with two modern commercially grown apples 'Gala' and 'Golden Delicious' (Minnocci *et al.,* 2010). These results suggest the relevance of ancient apple germplasm for providing the fruit with high polyphenolic content and as well for antioxidant scavenging properties.

Iran enjoys a large variety of old age apple cultivars. These ancient germplasms constitute the potential source of genes for apple breeding programs through which polyphenols may be manipulated in apple fruit. The present study was carried out aimed at analyzing and recovering, the evaluation of the antioxidant properties and as well the phenolic profile in some of the mebtioned cultivars.

MATERIALS AND METHODS

Chemicals

In this study was methanol, acetic acid, Folin-Ciocalteu's phenol reagent, sodium carbonate, HPLC Grade Solvents were purchased from Merck. Gallic acid, DPPH ((2,2-dipheny l-1- picryl hydrazyl) and Trolox were purchased from Sigma-Aldrich. Other chemicals were: cholorogenic acid prepared from Cayman Chemical Co. Japan; quercetin-3-galactoside and (+)-cathechin from Extrasynthase, Lyon, France, and phloridzin from Sigma Chemical Co. St. Loius, MO, USA.

Plant Material

Six cultivars grown in Iran including one Iranian red fleshed apple ('Bekran'), four white fleshed cultivars ('Golab-e Kermanshah', 'Golab-e Kohanz', 'Golab-e Sheikhi' and 'Atlasi') and the standard cultivar 'Gala' were selected for the study. 'Bekran' is a very early ripening cultivar (April to May) and while 'Golab' cultivars are early ripening (July to August). Fruits were harvested at their commertially mature time. Three to five replicates including 3 fruits of each cultivar were sampled, washed (using distilled water), cut into several pieces in two parts of flesh+peel *vs.* sole flesh , frozen in liquid nitrogen and maintained at –80°C until being analyzed.

Extraction Procedure

Extraction of phenolic compounds was performed accordingly to the method described by Lister *et al.* (1994). Briefly, 2 grams of fruit sample were powdered in mortar and macerated overnight in a methanol: acetic acid (85:15 v/v) solution at 4°C. Then, samples were centrifuged for 10 minutes at 10,000 rpm. Supernatant was filtered through 0.45 µm filter.

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HPLC Analysis of Phenols

A 50 µl aliquot of the extractwas analyzed through HPLC. The HPLC assembly was coupled onto a PhotoDiode Array (PDA) detector (Waters 2478 Dual λ Absorbance). The solid phase was a Symmetry C18 column (4.6×150mm, 5µm; Waters, Dublin, Ireland). The detection was carried out at 280 nm for catechin and phloridzin, 320 nm for chlorogenic acid and 350 nm for quercetin-3-galactoside. The retention times (min) of the compounds were recorded as follows: 15.5 (catechin), 17.8 (chlorogenic acid), 25.7 (quercetin-3-galactoside), 31.3 (phloridzin). The identification was based upon matching the retention time with the retention time of the related standards under the same chromatographic conditions. The mobile phase was a mixture of A (95% water, 5% methanol) and B (5% water, 95% methanol) at a gradient of A: B from 90: 10 to 55: 45 in 15 minutes and 55: 45 in 30 minutes, then, isocratic mode continued for 30 minuts. All the samples were analyzed in triplicates.

Total Phenol Content

Total phenolic content for each extract was determined, Folin-Ciocalteu reagent (D' Angelo *et al.,* 2007) used. The reaction mixture was prepared using 25 µl of extract, 175 µl of water, and 1.0 ml of Folin-Ciocalteu's reagent diluted 10 times with distilled water. Then, 800 µl of sodium carbonate (7.5 %, w/v) was added. The samples were incubated in dark at room temperature for 2 hours and then assayed at 765 nm using UV-Vis spectrophotometer. Total phenolic content was expressed as mg Gallic acid equivalent/g using the equation obtained from the calibration curve for Gallic acid. Data are expressed as mean±SD of five replicates.

Evaluation of DPPH-Radical-Scavenging Activity

The same extract was used for a determination of DPPH radical scavenging. DPPH (2 mg) was dissolved in 50 ml of Methanol. Aliquots of the extract (20, 40, 60, 80 and, 100 µl) were added to 1 ml of Methanol plus 1 ml of DPPH solution and left at room temperature for 15 minutes. The blanks containing 1 ml Methanol and 1 mL of DPPH solution were kept in the dark for 15 minutes. A absorption was read at 517 nm and the scavenging effect percentage expressed as $I= (A_{DPPH}(t) - A_{sample}(t)/A_{DPPH}(t))\times 100$, where, $A_{DPPH}(t)$ stands for the absorbance of DPPH at time *t*, while $A_{sample}(t)$ representing the simultaneous absorbance of the sample.

The antioxidant efficacy of the investigated apple extracts was also reported as Trolox Equivalents (TE, mM). In fact it was quantified by reference to a Trolox standard calibration curve prepared using different concentrations of the Vitamin E analogue (0.003, 0.006, 0.012, 0.025, 0.36, 0.05, 0.100 mM)

Titratable Acidity (TA) and Total Soluble Solids (TSS) Determination

The juice in three replicates was extracted by use of an electric juicer, to be used for determination of Titratable Acidity (TA) and Total Soluble Solids (TSS). TSS was assessed with the use of a refractometer (Japan) and expressed in °Brix according to Mitcham *et al.* (1996). TA was determined by titration against 0.1 N NaOH and expressed in percent malic acid per 100 ml of juice (Mitcham *et al*., 1996).

Statistical Analysis

All the analyses were performed in their either 3 or 5 replicates. The significance of the content of phenolic compounds was estimated through SAS version 9.1 software using Analysis O Variance (ANOVA). The differences between treatments were estimated using multiple range and Least Significant Difference (LSD) tests ($P\leq 0.05$).

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RESULTS

Phenolic Contents in Flesh+Peel

Figure 1 shows chromatograms related to the four investigated phenolic compounds as determined through HPLC of the studied apples. Chlorogenic acid was detected at 320 nm, and a Retention Time (RT) of 17.4 minutes, quercetin-3 galactoside at 350 nm with a *RT* of 25.5 minutes, phloridzin and catechin at 280 nm and *RT*s of 28.9 and 15.5 minutes, respectively (Figure 1).

Catechin was the most prominent phenolic metabolite in flesh+peels and as well in fleshes (Tables 1 and 2). The level of this compound in flesh+peel ranged from 215.74±23.84 to 4064.37±634.11 ug g^{-1} FW, with 'Golab-e Kermanshah' and 'Bekran' having the highest *vs.* the lowest concentrations, respectively.

Chlorogenic acid varied from 5.85±0.44 to 276.46±11.25 ug g^{-1} FW. The highest concentration of chlorogenic acid was detected in 'Golab-e Sheikhi; 'Gala' and 'Bekran' carried (significantly) the lowest concentration of chlorogenic acid. All the cultivars of Golab carried (significantly) different concentration, of chlorogenic acid.

Quercetin-3 galactoside ranged from 38.91± 3.46 to 833.96± 58.58 ug g^{-1} FW. 'Atlasi' carried the highest content of quercetin-3 galactoside, while 'Gala' and

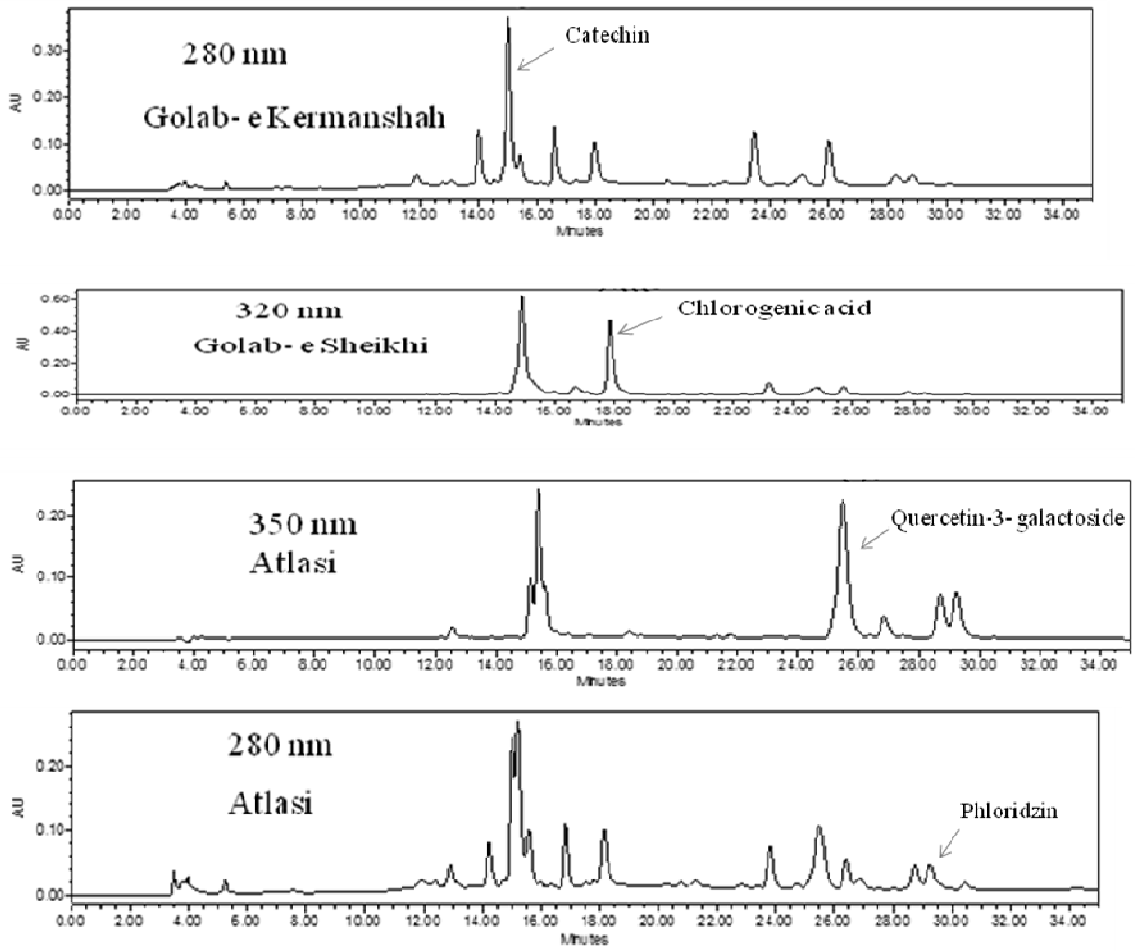

Figure 1. HPLC chromatograms of catechin (280 nm) chlorogenic acid (320 nm), quercetin-3-galactoside (350 nm) and phloridzin (280 nm). The indicated cultivars were those with the highest concentration of each compound. The Retentinon Times (RT) were recorded as 15.5, 17.4, 25.5 and 28.9 minutes, respectively

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Table 1. Concentration of chlorogenic acid, quercetin, phloridzin and catechin (ug g^{-1} FW), Total Phenolic Index and Total Phenolic Content (mg 100 g^{-1} gallic acid equivalent) in edible part (peel and flesh) of five native Iranian apple cultivars *vs.* the standard *cv*. 'Gala' (mean±standard deviation).

Cultivar	Chlorogenic acid	Quercetin	Phloridzin	Catechin	TPI [a]	TPC [b]
Atlasi	119.83± 13.43 bc	833.96± 58.58a	42.32± 1.64a	1667.23± 358.80 ab	2663.35± 109.17 ab	3596.05±242.27 b
Bekran	5.85± 0.44 d	91.23± 11.58 bc	23.26± 2.6 b	215.74± 23.84 b	336.10±0.08 b	4481.01± 378.69 a
Gala	14.40± 3.06 d	38.91± 3.46 c	10.47± 1.94cd	673.38± 95.05b	738.13± 75.97 b	2171.02±188.64 c
Golab-e Kermanshah	167.87±23.90 b	133.86±19.64bc	26.70±1.67b	4064.37±234.11a	4392.81±350.05 a	3358.82±362.67 b
Golab-e Kohanz	66.15±16.98 cd	354.96±89.36 b	22.58±2.85bc	1478.62±9.24 ab	1922.33±194.44 ab	2192.89±104.31 c
Golab-e Sheikhi	276.46±11.25 a	223.30±13.86bc	19.16± 1.13bc	1034.98 ± 133.811b	1553.56± 143.556 b	1863.13±108.62 d

Values followed by the same letter within a column are not significantly different as according to the Least Significant Difference (LSD) Test $P \leq 0.05$. [a] Total Phenolic Index, [b] Total Phenolic Content.

Table 2. Concentration of chlorogenic acid, quercetin, phloridzin and catechin (ug g^{-1} FW), Total Phenolic Index and Total Phenolic Content (mg 100 g^{-1} gallic acid equivalent) in flesh of five native Iranian apple cultivars *vs.* the standard *cv*. 'Gala' (mean±standard deviation).

Cultivar	Chlorogenic acid	Quercetin	Phloridzin	Catechin	TPI [a]	TPC [b]
Atlasi	99.98±14.45 ab	23.33±7.12 ab	7.31±0.53a	1552.74±116.89 b	1683.37±123.00 b	3106.46 ±146.37a
Bekran	21.90±0.75b	37.61±9.99 ab	7.16±1.21 a	2315.92±324.38 a	2382.60±511.15a	2965.13±169.92 a
Gala	38.03±1.72b	56.87±12.36 c	9.30±2.69 a	758.89±90.71b	863.09±146.48 c	1775.65±262.46 c
Golab-e Kermanshah	238.18±85.50 a	2.83±0.48 b	5.31±0.97 a	1624.16±127.16b	1870.49±43.68 b	3020.65±394.5a
Golab-e Kohanz	60.86±9.06 ab	6.58±1.07b	4.48±0.78 a	496.02±96.91 b	567.95±64.02 c	2122.23±87.48 b
Golab-e Sheikhi	179.68±11.70ab	31.92±5.06 ab	12.69±1.27 a	1470.01±108.45b	1694.31±154.96 b	1701.62 ±80.75 c

[a] Values followed by the same letter within a column are not significantly different as according to the Least Significant Difference (LSD) Test $P \leq 0.05$. [a] Total Phenolic Index, [b] Total Phenolic Content.

'Bekran' (significantly) the lowest concentrations of the compound. Among the Golab cultivars, only 'Golab-e Kermanshah' carried a significantly lower concentration of the compound.

The concentration of phloridzin varied from 10.47± 1.94 to 42.32± 1.64 ug g^{-1} FW. 'Atlasi' and 'Gala' contained the highest *vs.* the lowest contents of this dihydrochalcone, respectively. There were no significant differences observed among Golab cultivars as regards this phenolic compound.

Total Phenolic Index (TPI) ranged from 33.10 to 4392.81±350.05 ug g^{-1} FW, with the highest value going to the 'Golab-e Kermanshah'. 'Bekran' had (significantly) the lowest concentration. There were no significant differences observed among other cultivars as regarded TPI (Table 1).

Total Phenolic Content (TPC) ranged from 1863.13±108.62 to 4481.01± 378.69 mg GAE 100 g^{-1} FW in flesh+peel. The highest level was found in 'Bekran' while 'Golab-e Sheikhi' bore (significantly) the lowest TPC (Table 1).

Phenolic Contents and Their Comparison with Those in the Fruit's flesh+peel

The present study's results indicate that phenolic compounds were less abundant in the fruit's flesh than in the flesh+peel (Tables 1 and 2). There were not much differences observed among cultivars as regards the studied phenolic compounds, TPI, TPC and DPPH (Tables 3 and 4).

The ratio between the level of individual phenolic content in the flesh+peel and that in the flesh (concentration in flesh+peel/concentration in flesh) was obtained (Table 3). In the apple flesh, catechin was present at its highest concentration, ranging from 496.02±436.91 in 'Golab-e Kohanz' to 2315.92±1524.38 ug g^{-1} FW in 'Bekran'. The concentration of catechin in flesh+ peel was from 0.09 to 2.98 times higher than that in the flesh (Table 3). Quercetin levels in flesh+peel of 'Golab-e Kohanz', 'Golab-e Kermanshah' and 'Atlasi' were respectively 53.89, 47.27 and 35.73 times the levels in the flesh. 'Golab-e Kohanz' had the highest TPI ratio (3.38) too, (Table 3).

Antioxidant Activity of DPPH, TSS and TA of Flesh+Peel

Flesh+peel of fruits carried a higher DPPH scavenging potential than their flesh parts (Tables 1 and 2). On the basis of calculated DPPH radical IC_{50} values, flesh+peel of 'Bekran', 'Gala' and 'Golab-e Kermanshah' bore a lower scavenging potential than the flesh+peel from 'Atlasi', 'Golab-e Sheikhi', and 'Golab-e Kohanz' cultivars did. 'Atlasi' exhibited the highest DPPH antiradical scavenging potential (46.42 ug ml^{-1} FW) (Table 1).

There was a high correlation observed

Table 3. Relationship between the edible parts (peel+flesh) and flesh (concentration in edible parts/concentration in the flesh) as regard the content of phenolic compounds, Total Phenolic Index and Total Phenolic Content in five native Iranian apple cultivars *vs.* the standard *cv*. 'Gala'.

Cultivar	Chlorogenic acid	Quercetin	Phloridzin	Catechin	TPI[a]	TPC[b]	DPPH IC_{50}
Atlasi	1.20	35.73*	5.79	1.07	1.58	1.15	1.13
Bekran	0.27	2.42	3.24	0.09	0.14	1.51	1.55
Gala	0.38	0.7	1.13	0.89	0.86	1.22	2.31
Golab-e Kermanshah	0.70	47.27*	5.03	2.50	2.35	1.11	1.44
Golab-e Kohanz	1.09	53.89*	5.03	2.98	3.38	1.03	1.16
Golab-e Sheikhi	1.54	7.00	1.51	0.70	0.92	1.09	1.24

[a] TPI: Total Phenolic Index, [b] TPC**:** Total Phenolic Content.

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between Total Phenol Index (TPI) and catechin and also between Total Phenol Content (TPC) and DPPH activity (r= 0.97).

Titratable Acidity (TA) and Total Solid Soluble (TSS) ranged from 0.36 to 2% of malic acid and 13.03 to 15.50 °Brix, respectively. 'Bekran' carried the highest content of TSS (15.50) and TA (2.00, Table 5). Compared with 'Gala', all the studied cultivars had (significantly) lower TAa, except 'Bekran' which had the highest (Table 5). TSS levels were not significantly different from each other, except for 'Golab-e Kohanz' which had a (significantly) lower *vs.* 'Bekran', which bore a significantly higher value. TSS: TA ratio was not significantly different among 'Golab-e Kohanz', 'Golab-e Kermanshah' and 'Atlasi' the three of which had the highest values, followed by 'Golab-e Sheikhi', 'Gala', and 'Bekran' carrying (significantly) lower values. TSS: TA ratio in 'Bekran' was (5.6 times) lower than that in 'Golab-e Kohanz' (Table 5).

DISCUSSIONS

Antioxidant activity and phenolic contents vary with the part of fruit, the kind of polyphenolic compound, cultivar, maturity stage, as well as environmental conditions (Kjersti *et al.,* 2002; Kondo *et al.,* 2004; Van Der Sluice *et al.,* 2001). Throughout the ongoing study, there were significant differences observed in the kind of polyphenolic compounds and their content in fruit's flesh *vs.* its flesh+peel, and as well among cultivars. Catechin was the most prominent phenolic observed in both flesh and flesh+peel (Francini and Sebastiani, 2013). The highest content of catechin in flesh+peel was found in 'Golab-e Kermanshah' (4,064.37±234.11ug g^{-1} FW). Our findings are in line with data reported from D'Abrosca *et al.* (2007), who observed that phloretin-2'-xylo glucoside, and catechin are the main phytochemical constituents of 'Limoncella' apple. In particular, they found that these metabolites were mostly present in the peel of the

Table 4. DPPH ($IC_{50\,\%}$) and Trolox equivalent in flesh+peel *vs*. flesh in the six studied apple cultivars.

Cultivars	Flesh+Peel		Flesh	
	DPPH (IC_{50} %) (ug ml^{-1})	Trolox eq [a] (ug ml^{-1})	DPPH (IC_{50} %) (ug ml^{-1})	Trolox eq (ug ml^{-1})
Atlasi	28.21	2.07	24.795	1.83
Bekran	59.055	6.33	37.965	2.945
Gala	62.13	7.08	26.835	1.97
Golab-e Kermanshah	54.34	5.34	37.725	2.92
Golab-e Kohanz	37.53	2.90	32.33	2.40
Golab-e Sheikhi	42.12	3.4	33.77	2.53

[a] Trolox equivalent

Table 5. Correlation between content of phenolic compounds, Total Phenolic Index, Total Phenolic Content and DPPH in the six studied apple cultivars.[a]

	Chlorogenic acid	Quercetin	Phloridzin	Catechin	TPI	TPC
TPI	0.22*	ns	0.49*	0.97**	1	
TPC	0.38*	ns	0.23*	0.49**	0.49**	1
DPPH	0.11*	ns	ns	0.21*	0.37*	0.74*

[a] ns and the superscrips * and ** stand for non significant or significant at $P \leq 0.05$ and 0.01, respectively. TPI: Total Phenolic Index, and TPC**:** Total Phenolic Content.

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cultivar. A maximum value of catechin in the flesh was detected in 'Bekran' (2,315.92±324.38 ug g^{-1} FW, Table 2). Catechin was identified as the major phenolic compound in the pulp for all the cultivars studied by Veberic *et al.* (2005), while they identified chlorogenic acid, rutin, quercetin-3-rhamnoside and phloridzin in the fruit's peel.

Organically produced apples showed higher contents of phenolic substances in the apple pulp than the apple cultivars of integrated production (Veberic *et al.,* 2005). In the present study, 'Bekran' as a red flesh apple was an organically produced apple cultivar which had the highest level of catechin in its flesh, Total Phenol Index (TPI) and Total Phenol Content (TPC) also in the flesh *vs.* the highest value of TSS (Total Solid Soluble) and TA (Titratable Acidity) in the flesh+peel. Another of the integratedly produced apple cultivars was 'Golab-e Kermanshah' with a maximum of catechin, TPI and TPC in its flesh+peel. It is stated that peel constitutes, at a maximum, 10% of the whole fruit, therefore, the phenols in the flesh are of greater importance to the consumer than those found in the peel. Antioxidant activity (scavenging of DPPH radical) in the flesh + peel of 'Bekran', 'Golab-e Kermanshah' and 'Gala' was higher than those in the flesh of the other cultivars. The present study's results indicate higher figures than those reported by Jelodarian *et al.* (2012).

Results also showed that the highest contents of chlorogenic acid in flesh and in flesh+peel were respectively found in the 'Golab-e Kermanshah' and 'Golab-e Sheikhi' cultivars (Tables 1 and 2). Tsao *et al.* (2003) reported that the level of chlorogenic acid within the peel (in eight different apple cultivars) was on the average 13.6 mg·g^{-1}. In flesh+peel, the highest levels of quercetin-3-galactoside and phloridzin were observed in 'Atlasi'. Quercetin and phloridzin are of ample antioxidative properties (Lee *et al.,* 2003) making a host resistant against apple scab caused by *Venturia inaequalis* (Hock and Elstner, 1988). In the present study, the average total phenolic content in flesh+peel ranged from 1863.13±108.62 to 4481.01±378.69 mg 100 g^{-1} FW and in flesh it ranged from 1,701.62 ±80.75 to 3,106.46 ±146.37 mg 100 g^{-1} FW galic acid, whereas for the plum, total phenol content amounted to 368.7 mg g^{-1} FW galic acid (Chun and Kim, 2004). In comparison with 'Gala', (a known asstandard cultivar), Iranian apple cultivars are of high contents of phenolic compounds in their flesh+peel.

'Golab-e Kermanshah' was characterized with high phenolic compounds with its flesh getting a kind of brown coloration quickly once cut into slices. Golab cultivars are early ripening Iranian cultivars of high fruit quality, mild taste as well as desirable flavor. These characteristics are suggested in the name chosen for the cultivar. 'Golab' is an Iranian name, composited of the words 'Gol' (flower) and 'Ab' denoting fragrance (Faramarzi *et al.,* 2014).

TA level recordings were in agreement with those in other studies (Mitre *et al.,* 2009; Jemrić *et al.,* 2013). The only exception was 'Bekran' having a very high TA level, which might come out to be practically useful in future breeding programs. TA can be affected by such other factors, as low crop load (Saei *et al.,* 2011) or harvest date, but such a high level of TA [2% of malic acid (Table 6)] must have been the result of genetic factors too.

TSS levels were found as similar to those in old and ancient apple cultivars found in Romania (Mitre *et al.,* 2009) and in Croatia (Jemrić *et al.,* 2012), but higher than local cultivars found in Turkey (Pirlak *et al.,* 2003) and in Czech Republic (Balík *et al.,* 2012).

High differences (in TSS: TA ratio) between 'Bekran' and 'Golab-e Kohanz' offers possibilities for breeding apple cultivars with diverse tastes and sugar: acid ratio balance, and suitable for adoption to different apple markets.

In summary, the results finally indicate that the consumption of whole fruit provides phytochemicals in amounts far more abundant than those provided by the (single)

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Table 6. Titratable Acidity (TA), Total Soluble Solids (TSS) and TSS: TA ratio in in five native Iranian apple cultivars *vs.* in standard *cv.* 'Gala'.[a]

Cultivar	TA (%as malic acid)	TSS (%Brix)	TSS: TA ratio
Atlasi	0.36±0.04 c	13.67±0.90 bc	38.35±6.62 a
Bekran	2.00±0.20 a	15.50±1.80 a	7.80±1.29 d
Gala	0.60±0.05 b	14.60±0.60 ab	24.48±0.98 c
Golab-e Kermanshah	0.37±0.01 c	14.53±1.28 abc	39.34±5.14 a
Golab-e Kohanz	0.37±0.3 c	13.03±0.72 c	41.34±7.47 a
Golab-e Sheikhi	0.38±0.03 c	13.73±0.25 bc	35.74±2.63 b

Values followed by the same letter within a column are not significantly different according to the Least Significant Difference (LSD) Test $P \leq 0.05$.

flesh component. Compared with the known as standard cultivar 'Gala', native Iranian cultivars benefit from higher concentrations of phenolic compounds, making them suitable for use in breeding. The main disadvantage for such a cultivar of 'Bekran', (as a red flesh apple), is its small fruit size, but this is a common problem in old and indigenous apple cultivars. Fruit size is an important trait for fruits aimed for fresh consumption but is of not much importance in fruits aimed for being processed into juices, jams, etc. Further research must determine their response to vegetative rootstocks along with an optimization of other management practices before their being introduced into the production cycle.

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مقایسه ترکیبات فنلی و فعالیت آنتی اکسیدانتی بین تعدادی از ارقام سیب ایرانی و رقم تجاری 'گالا'

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چکیده

به منظور تعیین محتوای فنلی و فعالیت آنتی اکسیدانتی تعدادی از ارقام سیب ایرانی، ۵ رقم سیب ایرانی شامل 'بکران'،'گلاب کرمانشاه'، 'گلاب کهنز' ، 'گلاب شیخی' و ` اطلسی انتخاب شد و با رقم 'گالا' به عنوان رقم استاندارد تجاری مقایسه شد. چهار ترکیپ فنلی (کلروژنیک اسید، کورستین-۳-گالاکتوزید، کاتچین و فلوریدزین) به وسیلهٔ HPLC در گوشت و گوشت + پوست ارقام مورد مطالعه اندازه گیری شد. میزان فنل کل به وسیله فولین- سیوکالتو و فعالیت آنتی اکسیدنتی به وسیله رادیکال DPPH برای گوشت و گوشت + پوست هر یک از ارقام اندازه‌گیری شد. نتیجه حاصل از آنالیز HPLC نشان داد که کاتچین مهمترین ترکیب فنلی در گوشت و گوشت + پوست همه ارقام مورد مطالعه بود. بیشترین مقدار کاتچین در گوشت + پوست و گوشت مربوط به ارقام 'گلاب کرمانشاه' و 'بکران' (بترتیب ۴۰۶۴/۳۷ و۹۲/ ۲۳۱۵ میکروگرم برگرم وزن تر میوه) بود. دومین ترکیب فنلی که مقدار بالایی داشت، کورستین-۳-گالاکتوزید بود که بیشترین میزان آن در اطلسی (۸۳۳/۹۶ میکروگرم برگرم وزن تر میوه) وجود داشت. بیشترین میزان کلورژنیک اسید در رقم 'گلاب شیخی' (۲۷۶/۱۰۶) وجود داشت. بیشترین میزان شاخص فنل کل مربوط به رقم 'گلاب کرمانشاه' (۴۳۹۲/۸۱) بود. ارقام 'گلاب کرمانشاه' و 'اطلسی' دارای بیشترین میزان فنل کل بودند. در کل، ترکیبات فنلی در گوشت این ارقام نسبت به گوشت + پوست کمتر بود. میزان مهار رادیکال آزاد نیز نشان داد که فعالیت آنتی اکسیدانتی در گوشت + پوست بیشتر از گوشت است. در این مطالعه، می‌توان نتیجه گیری کرد در مقایسه با رقم 'گالا'، ارقام ایرانی دارای مقادیر بیشتری از ترکیبات فنلی هستند که آنها را برای برنامه‌های اصلاحی و ورود به بخش تولید درخور توجه می‌سازد. همچنین، مصرف سیب با پوست ترکیبات آنتی اکسیدانتی بیشتری تامین می‌کند.

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