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Quantification of Phenolic Compounds in Peel and Pulp of 'Zonouz' Apple Cultivar from Iran

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Abstract

Composition of phenolic compounds in peel and pulp of 'Zonouz' apple cultivar was studied during three fruit growth stages (early, middle and harvesting stages) using HPLC. Based on the obtained results, pulp contained the highest amounts for total phenolic and flavonoid compounds as well as total phenolic acids content during early growth stage. In peel, total phenolic and flavonoids were decreased till mid-season and then were raised at commercial harvest time. Total phenolic acids concentration attained declining trend till end-season. Chlorogenic acid was the major peel phenolic acid constituent in all three stages. Peel flavanols had their highest amounts during mid-season with a variation pattern: early season < mid season < harvest time. Pulp flavonols achieved their maximum amount during early growth stage. Cyaniding-3- galactoside (63%) and pholridzin dehydrate (20%) were the main flavonoids of early growth stage in peel. Rutin hydrate (56%) dominated in pulp during early growth stage. During the second measurement i.e. mid-season, catechin was the major flavonoid component in both peel and pulp. Cyaniding-3- galactoside (67%) was the most abundant flavonoid compound in peel at harvest time.

Keywords: Analytical HPLC, Apple, Cyanidine-3-galactoside, Phenolc acids, Phenolic compounds

Introduction

Great climatologic and geographical diversification of Iran made it as one of the capable apple native origins and production areas in the world. Iran was the forth producer of apple in the world with 2660000 metric ton during 2009 [1]. Owing to increased population and the crucial needs for safe food and supplemental products, there is huge interest in introduction and cultivation of high-valued apple cultivars with especially elevated phenolic and flavonoid compounds.

'Zonouz' is a main native apple cultivar in Northwest Iran. This cultivar is an own rooted cultivar with up to 4 m height, late flowering habit , low flower and fruit abscission, and fruit bearing pattern mainly on spurs [2]. Considering the high production area (more than 300 hectares) of this cultivar in North-West Iran, identification and quantification of its phenolic compounds is nutritionally appreciated.

Phenolics are one of the main groups of plant secondary metabolites with great impact on organoleptics of fruits. Furthermore, they have long been used as natural preservatives in food industry. Strong antioxidant capacity of phenolic compounds made them excellent

natural products in coping with cardiac disorders and cancer [3]. Considerable differences between apple cultivars and different fruit parts have been reported regarding phenolic compounds. Generally, it is accepted that fruit peel contains more phenolic traces compared with pulp and core. Procyanidins, quercitin glycosides, anthocyanins and hydroxycinnamic esters have been found as the main phenolics of apple fruit [4]. Due to the suitable adaptation and expanding cultivation of 'Zonouz' in northwest Iran, significant role of phenolic compounds as food additives as well as un-substituted phenolic profile of apple fruit, we aimed to evaluate the phenolic compounds composition of 'Zonouz' apple.

Materials and Methods

HPLC analysis for quantification of phenolic compounds in the apple peel and pulp was done at the Pharmacognosy Laboratory of the Drug Applied Research Center of Tabriz University of Medical Sciences, Tabriz, Iran.

Plant material and extraction procedure: Fruits of 'Zonouz' apple cultivar were collected from Zonouz district in Northwest Iran. Appropriate horticultural procedures were employed for adequate light exposure of fruits at the period of development. In order to reduction of shading effects and homogeneity of harvested materials, fruits were spontaneously harvested from all peripheral sides of trees. Collected fruits were immediately transferred to the laboratory, rinsed with distilled water and finally dried with a clean towel. Apple fruits were peeled with a sharp knife and then dried in a dark place with appropriate ventilation. Afterward, the air-dried plant materials were grinded to obtain a fine grade powder. Lipids and waxy compounds of samples (1g of air-dried plant material) were eliminated using n-hexane (10 ml) for 20 min in an ultrasonic (Power Sonic 505, Korea) bath. Solvent was enforced to evaporation utilizing a rotary-evaporator (Heidolph, Germany) until dryness. The achieved extracts were treated by 100ml MeOH: H_2O (1:1) and then sonicated for 20 min. The acquired aqueous extracts were sequentially filtered and centrifuged (10 min) at 13000 rpm. Finally, extracts were assayed for phenolic compounds constituents by analytical HPLC.

High performance liquid chromatography (HPLC) analysis: Phenolic compounds were quantified according to the method described by Lata *et al* [5] with some modifications. Separation of phenolic compounds was carried out with a HPLC system (Cecil Company, English) equipped with a binary pump (CE 4100), Cecil in-line degasser and UV/Vis detector (CE 4201). Phenolic compounds were separated on a symmetry C_{18} column (250×4.6 mm with 5 µm packing, Dr. Masch GmbH, Germany) protected with a corresponding guard column (symmetry $C_{18,}$ 5 µm, 5×4 mm). To avoid the analogous elution of some compounds from column encountered in the primary experiments, three different binary solvent systems were employed. The first binary solvent system of the mobile phase consisted of 2% acetic acid in water/methanol, with gradient of 10-100% for the separation of flavanols and phenolic acids (except for chlorogenic acid). For separation of flavonols the second binary solvent system was 0.25 mMol phosphate buffer, pH = 2.5/acetonitril, with gradient of 10-30%. Chlorogenic acid, cyanidin-3-galactoside and phloridzin dehydrate were separated by the third binary solvent system of 0.1% formic acid in water/methanol, with gradient of 10-100%. The flow rate and injection volume were 1ml/min and 20µl, respectively. The column temperature was adjusted at 20^{0C}. Phenolic compounds constituents were detected at 280 nm. The separated compounds were indentified by comparing their retention time (R_t) and UV spectra with those of authentic standards. Quantification was based on an external standard calibration curve. Total phenolic and flavonoids content were the quantitative sum of the related identified phenolic compounds.

Reference reagents and solvents: Gallic acid, rutin hydrate, phloridzin dehydrate, chlorogenic acid, quercetine-3-β-D-glucoside, quercetine-3-D-galactoside, P-coumaric acid, (-)-epicatechin and cyanidin-3-galactoside were purchased from Sigma. Ferulic acid, trans-2-hydroxycinnamic acid, caffeic acid and (+) catechin hydrate were afforded from Sigma Aldrich. Methanol (Caledon, Canada), acetonitril, n-hexane and formic acid (Merck, Germany) were of HPLC grade.

Statistical analysis: The data of two replications were analyzed by SPSS (Ver. 15) according to one-way ANOVA based on completely randomized design. Mean comparisons were carried out by Duncan's multiple range test at $P \leq 0.01$ probability level.

Result and Discussion

Total phenolics and flavonoids: The highest amounts for total phenolic and flavonoid compounds during early season belonged to fruit pulp (Figure 2) ($P \leq 0.01$). A gradual decrease in total phenolics and flavonoids was recorded till mid-season in both fruit tissues (Figures 1 and 2). During the harvest time, those compounds acquired their highest sum in fruit peel (Figures 1) ($P \leq 0.01$). Our results are in agreement with the finding of Lister *et al* [6] reporting about the concurrent progress in fruit development and increase in total flavonoids content. The overall results showed that during harvest time phonolics content of peel was six-fold higher than pulp. Elevated amounts of flavonoid compounds during early growth stage were due to high contents of pulp rutin hydrate. Pulp containing phloridzin dehydrate proved a descending depict during growing season. At the same time, there was no trace of this compound in peel during the mid-season. In contrast, peel contained very low amounts of this compound at the harvest time (Table 1) ($P \leq 0.01$). The increase in peel flavonoids content during early growth stage and harvest time was probably due to the agglomeration of cyaniding-3-galactoside. Meanwhile, early growth stage held the highest quantity for peel cyaniding-3- galactoside. Yuri *et al* [7] noted that total phenolic contents and antioxidant capacity of apple peel were three to ten times higher than pulp mainly due to greater amounts of cyaniding-3-galactoside and quercetin glycosides. Furthermore, in 'Epagri F5P283' apple, positive correlation was recoded between total phenolic content and antioxidant capacity of fruit extracts [8]. Comparative results revealed that 'Zonouz' peel at harvest time was rich in total phenolics compared with pulp seemingly with higher nutritive value.

Flavanol compounds: Flavanols possessed the greatest sum ($P \leq 0.01$) in peel during mid growth stage and were decreased until harvest time in both peel and pulp (figure 1 and 2). There was no difference between peel and pulp catechin content in early fruit growth stage (Table 1). In peel, catachin content was increased till mid-season but decreased toward late season and/or harvest time. Epi-catechin was not traced in peel and pulp of fruit in early growth stage. Epi-catechin had the highest amount at harvest time in both tissues (Table 1) ($P \leq 0.01$). Mayr *et al* [9] stated that in apple there was correlation between scab desease tolerance and flavanol and hydroxycinnamic acid content.

Flavonols: The highlighted signs for pulp flavonols were recorded in early stage of fruit growth (Figure 2) ($P \leq 0.01$). Rutin hydrate was recorded as the only flavonol in this growth stage (Table 1) and then pulp flavonols were diminished till harvest time (Table 1) ($P \leq 0.01$). Quercetine-3-β-D-glucoside was the major flavonol constituent in peel during early growth stage and in pulp at the middle growth stage (Table 1) ($P \leq 0.01$). Quercetine-3-β-D-glucoside ($P \leq 0.01$) was only cued in peel at harvest time (Table 1). Takos *et al* [10] reported that flavonol content of 'Crips red' apple was high till 32 days after full bloom, but with further increase in fruit development its amount showed reducing figure.

Table 1: Flavonoid constituents in peel and pulp of 'Zonouz' apple during growing season.

		Flavanol		Flavonol				Dihydrochalcone
		Catechin	Epicatechin	Quercetine -3-β-D-glucoside	Quercetine-3-D-galactoside	Rutin hydrate	Cyaniding-3-galactoside	Phloridzin dehydrate
Early stage	peel	1.7b	-	0.64a	-	-	9a	2.9b
	pulp	1.7b	-	-	-	10a	2.5c	3.8a
Mid-season	peel	2.56a	-	-	-	-	-	-
	pulp	1.58c	0.37c	0.53b	-	0.01d	0.41d	0.72c
Harvest time	peel	0.61d	1.3a	0.12c	0.13a	0.12b	4.78b	0.12d
	pulp	0.31e	0.46b	0.03d	-	0.04c	0.012e	-

Different letters in columns show significant difference between diverse fruit parts for each compound based on Duncan's multiple range test at P≤0.01.

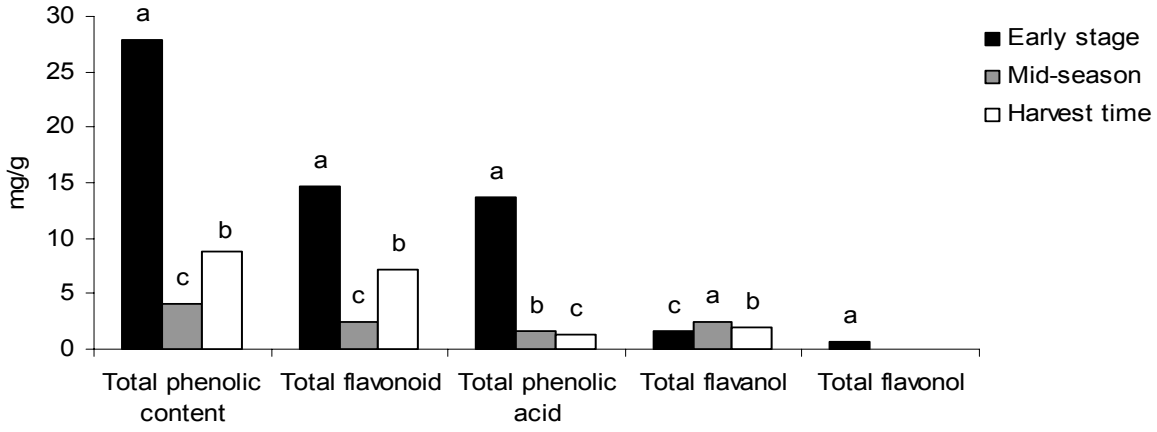

Figure 1. Total phenolics, flavonoids, phenolic acids, flavanols and flavonols of 'Zonouz' apple peel from Iran.
Different letters on bars show significant difference between diverse fruit parts for each compound based on Duncan's multiple range test at P≤0.01.

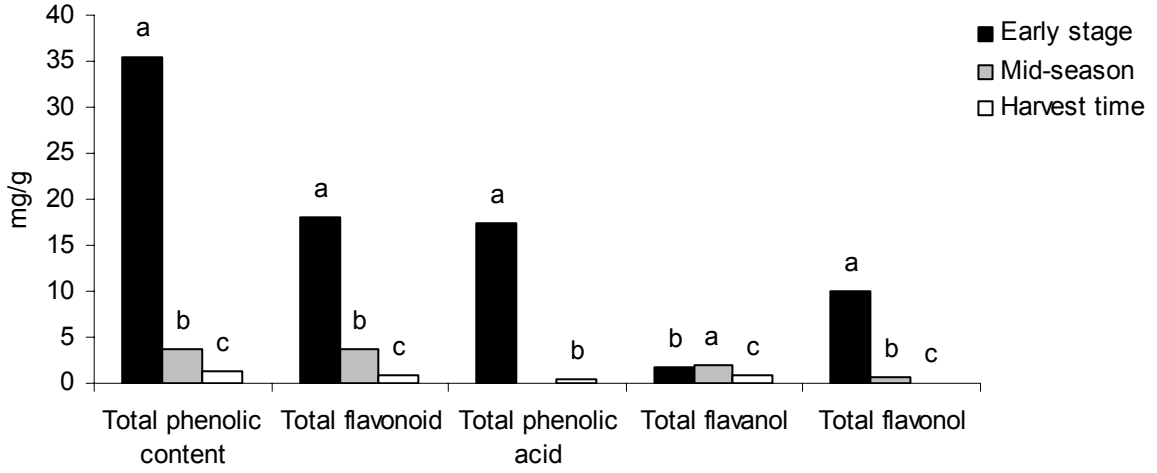

Figure 2. Total phenolics, flavonoids, phenolic acids, flavanols and flavonols of 'Zonouz' apple pulp from Iran. Different letters on bars show significant difference between diverse fruit parts for each compound based on Duncan's multiple range test at P≤0.01.

Phenolic acids: During the early growth stage, phenolic acids had their highest amounts in peel (Figure 1) ($P \leq 0.01$). A similar decreasing pattern as for total phenolics was recorded for phenolic acid of pulp (Figure 2) ($P \leq 0.01$). In contrast, peel phenolic acid content variation was the same as pulp flavonoids (Figure 1). Awdad *et al* [11] reported a descending of phenolic acids contents during growing season in 'Elstar' and 'Jonagold' apple cultivars. A raised amount of chlorogenic acid in peel and pulp was the principal reason for increased phenolic acids of small fruits (early growth stage). However, progress in fruit development led to decrease in chlorogenic acid content (Table 2). Early studies by Mayr *et al* [9] in 'Golden delicious' and Burda *et al* [12] with 'Golden delicious' and 'Empire' apples revealed that peel chlorogenic acid content had continuous lessening model till late season. Nutritionally, chlorogenic acid is a high–valued phenolic constituent because of its great role in enzymatic browning of processed fruits [13]. Furthermore, Petkovsek *et al* [14] documented that chlorogenic acid content of fruits is a reasonable indicator in fungal disease resistance. The maximal amounts for gallic, caffeic and p-comaric acids in apple peel were measured during early growth stage (Table 2). Both peel and pulp were deprived of trans-2-hydroxyl cinnamic acid during mid-season (Table 2). Pulp contained no traces of p-coumaric acid at mid-season growth stage as well (Table 2). Kobayashi *et al* [15] gave an account that antioxidant potential of *Asimina triloba* was related to fruit phenolic compounds especially its related to gallic and chlorogenic acid content. Moreover, there was positive correlation between powdery mildew resistance in grape fruits gallic acid and catechin content [16]. Ferrulic and p-cumaric acids content of 'Zonouz' peel decreased during growing season (Table 2). Meanwhile, in pulp, ferrulic acid was declined till mid-season and then raised up toward late season. Caffeic acid variation pattern in pulp and peel were the same as total phenolic compounds of peel (Table 2). Our results are in well conformity with the finding of Satisha *et al* [16] and Zhang *et al* [17] in pear. In addition, studies of Petkovsek *et al* [14] in 'Jonagold' apple displayed scarce variation in p-comaric, ferrulic and caffeic acids during growing season. P-comaric, ferrulic and caffeic acids have been characterized as the initial precursors of lignin biosynthesis in plant taxa. Regarding the high contents of above mentioned phenolic acid constituents in 'Zonouz' peel it seems that lignin reservoir of 'Zonouz' peel might be high enough to potentiate the handling and transportation of this high-valued apple cultivar.

Table 2: Phenolic acid constituents in peel and pulp of 'Zonouz' apple during growing season

		Chlorogenic acid	P-coumaric acid	Caffeic acid	Ferulic acid	trans-2-Hydroxy cinnamic acid	Gallic acid
Early stage	peel	11.25b	0.41a	1.25a	0.67a	0.009b	0.05a
	pulp	15.25a	0.21b	1.16b	0.54b	-	0.03b
Mid-season	peel	1.3c	0.067c	0.057e	0.21c	-	0.0005e
	pulp	0.054f	-	0.029f	0.0087f	-	0.000025f
Harvest time	peel	1.01d	0.016e	0.172d	0.15d	0.007c	0.005c
	pulp	0.09e	0.064d	0.25c	0.07e	0.04a	0.0006d

Different letters in columns show significant difference between diverse fruit parts for each compound based on Duncan's multiple range test at $P \leq 0.01$.

Conclusions

Our results have conclusively showed that there were considerable quantitative differences concerning phenolic compounds in our cultivar and in previously reported diverse apple cultivars from different region of the world. Except for chlorogenic acid, 'Zonouz' peel had higher phenolic compounds content at harvest time compared to pulp. Variation in

phenolic compounds content in peel and pulp of apple fruits depends mainly upon cultivar. Despite of low proportional percentage of apple peel in fruit total weight (maximum 7-8%) this tissue contains considerable amounts of flavanols and flavonols. Phytochemical assays of phenolic and flavonoid compounds of apple have been in focus of interest due to great impacts of these compounds on fruit quality characteristics and healthcare systems. The results of the present work revealed that 'Zonouz' owns huge amounts of phenolics during harvest time. Therefore, it seems that with improvements in orchard management (suitable rootstock and appropriate training and pruning systems as well as proper cultivation practices) we would be able to increase the biosynthesis and accumulation of phenolic and flavonoid (especially light–dependent) compounds in favor of quality characteristics of fruits.

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