Acta Sci. Pol., Technol. Aliment. 11(3) 2012, 259-264
pISSN 1644-0730 eISSN 1889-9594 www.food.actapol.net/

DEVELOPMENTAL VARIATION OF PHENOLIC COMPOUNDS IN FRUIT TISSUE OF TWO APPLE CULTIVARS

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ABSTRACT

Background. Qualitative and quantitative analyses of 'Zonouz' and 'Gala' apples phenolic compounds were accomplished by HPLC.
Material and methods. Samples for phenolics study were taken at three different growing stages [1 – fruit early growing stage, 2 – mid-stage of fruit development (80 days after full bloom), 3 – during commercial harvest time].
Results. The results showed qualitative differences between two apple cultivars regarding phenolic compounds. The highest amounts of total phenols, flavonoids, flavonols and phenolic acids content in both cultivars were recorded during the fruit early growing stage. The high phenolic acids content was due to increasing in chlorogenic acid content in fruits during early growing stage. The highest amount for flavanol content was recorded in 'Zonouz' peel at the harvest time. Descending pattern was recorded for phloridzin dihydrate content during the season. 'Gala' peel had the greatest amounts for cyanidin-3-galactoside at harvest time. Increasing in total flavonoid content was due to the great amounts of cyanidine-3-galactoside and epicatechin (in 'Gala' peel), rutin hydrate (in 'Zonouz' pulp) at fruit early growing stage, catechin (in 'Zonouz' peel) during mid-stage of fruit development and cyanidin-3-galactoside and quercetin-3-D-galactoside (in 'Gala' peel) at the harvest time in both apple cultivars.
Conclusions. There were meaningful quantitative differences between two cultivars, and 'Gala' was richer in phenolics than 'Zonouz'.

Key words: apple, HPLC, penolic compounds, flavonoids, phenolic acids

INTRODUCTION

Fruits and vegetables are rich sources of phenolic compounds. Apple is a valuable dietary fruit because of its phenolic compounds pool [Henriquez et al. 2010]. Plant phenolics are high-valued natural metabolites biosynthesized via the shikimate/phenylpropanoids pathways [Lattanzio et al. 2001, Cheynier 2005]. This group of compounds encompasses a wide array of structurally diverse constituents with some more biological activities [Lattanzio et al. 2001]. Plants employ phenolic compounds for pigmentation, growth, reproduction, pollination, protection against UV radiation, resistance to pathogens and defense mechanisms under different environmental stressful conditions such as wounding, infection and for many other biological functions [Lattanzio et al. 2001, Cheynier 2005]. Furthermor, these compounds are responsible for the

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organoleptic characteristic such as taste and colour of fruits during per and post harvest stages [Cheynier 2005]. Strong antioxidant capacity of phenolic compounds made them excellent natural products in coping with cardiac disorders and cancer [Lattanzio et al. 2001, Cheynier 2005, Lata et al. 2009]. Flavonoids have long been recognized to possess antiallergenic, anti-inflammatory, antiviral and antiproliferative activities [Lattanzio et al. 2001, Cheynier 2005, Lata et al. 2009]. Undoubtedly, different parts of apple fruit owe various concentrations of phenolic compounds. Noticeably, apple peel has the major affinity for the biosynthesis of aforementioned compounds compared with the other parts of the fruit and also has a high bioactivity potential [Henriquez et al. 2010].

Knowledge on the biosynthesis and accumulation pattern of phenolic compounds in this fruit will improve our maneuverability on the application of organic and orchard management practices for intensifying the intrinsic potential of plants for agglomeration of these high-valued dietary compounds in fruits. For this, the present experiment was conducted to evaluate the growth-stage related variation of fruits phenolic constituents in two apple cultivars in hope to the acquired data have the potential to be employed for the optimum orchard management in favour of phenolics rich fruit production.

MATERIAL AND METHODS

HPLC analysis for the identification and quantification of phenolic compounds in the fruits was conducted at the Pharmacognosy Laboratory of the Drug Applied Research Center of Tabriz University of Medical Sciences, Tabriz, Iran during 2009.

Plant material and extraction procedure

Fruits of 'Gala' and 'Zonouz' apple cultivars were collected during fruit development [1 – fruit early growing stage, 2 – mid-stage of fruit development (80 days after full bloom) and 3 – during commercial harvest time] from Bostanabad (37°51' N, 46°50' E, height above sea level: 1700 m, and the mean annual temperature: 8°C) and Zonouz (38°35' N, 46°51' E, height above sea level: 1700 m and, the mean annual temperature: 10.9°C) districts in Northwest Iran.

Collected fruits were immediately transferred to the laboratory, rinsed with distilled water to removing of dust or external debris and finally dried with a clean towel. Apple fruits were peeled with a sharp knife and then dried at 25°C 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 (1 g 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 100 ml MeOH:H_2O (1:1) and then sonicated for 20 min. The acquired aqueous extracts were sequentially filtered and centrifuged (10 min) at 13 000 rpm. Finally, extracts were assayed for phenolic compounds constituents by analytical HPLC.

Total phenolics, flavonoids, flavanols, flavonols and phenolic acids were the sum of all the related individual components beyoned their measerment with analytical HPLC.

High performance liquid chromatography (HPLC) analysis

Phenolic compounds were quantified according to the method described by Lata et al. [2009] with some modifications. Separation of phenolics 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 same time 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 phenolcarbonic acids (except for chlorogenic acid). For separation of flavonols (rutin hydrate, quercetin-3--β-D-glucoside and quercetin-3-D-galactoside) 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 1 ml/min and 20 µl, respectively. Phenolic compounds constituents were detected at 280 nm in short running time of chromatography (30 min). The separated compounds were identified by comparing their retention time (R_t) and UV spectra with those of authentic standards. Quantification was based on an external standard calibration curve. All reference reagents and solvents were afforded from Sigma, Sigma Aldrich and Merck companies.

Statistical analysis

The data of two replications were analysed 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.

RESULTS AND DISCUSSION

The result showed that there were quantitative differences regarding individual phenolics and flavonoids compounds content between 'Gala' and 'Zonouz' apple peel and pulp extracts during the growing season (Figs 1, 2; $P \leq 0.01$). In both cultivars, peel phenolics content was the higher during the early season but, it had the constant amounts at the commercial harvest time (Fig. 1). Variations in phenolic compounds were recorded in the pulp of both cultivars during the season (Fig. 1). Similarly, the high content of phenolic compounds content in 'Gala' peel rather than 'Zonouz' was as a result of increasing in cyaniding-3-galactoside and quercetin-3-D-galactoside contents at the harvest time (Table 1; $P \leq 0.01$). In addition, it is well characterized that beyond the first stage of fruit development, phenolics content falls may be due to the reduced biosynthesis and/or dilution process [Amer et al. 2002, Satisha et al. 2008]. Accurately, the high water intake potential of developing fruits as well as the hydrolysis of phenolics have been defined as the keys reasons for such a decrease in total phenolic content during fruit fast growing stage [Satisha et al. 2008]. Individual phenolic compounds profile variations experienced between two apple cultivars are possibly due to diverse genetic makeup of plant, and is more likely attributed to the different climatic conditions and/or orchard management practices.

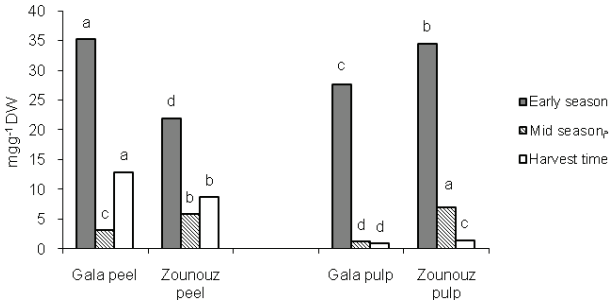

Fig. 1. Changes in total phenolics content in peel and pulp of 'Gala' and 'Zounoz' apple cultivars during the fruit development. Different letters on bars show significant difference based on Duncan's multiple range test at $P \leq 0.01$

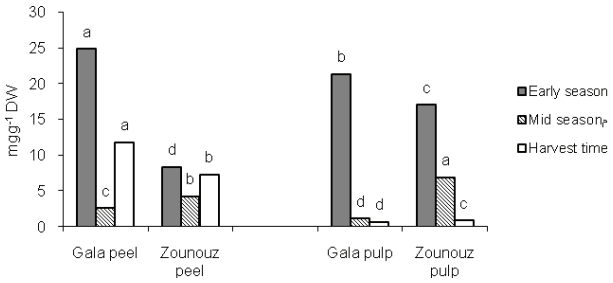

Fig. 2. Changes in total flavonoids content in peel and pulp of 'Gala' and 'Zounoz' apple cultivars during the fruit development. Different letters on bars show significant difference based on Duncan's multiple range test at $P \leq 0.01$

The data revealed a considerable decreasing amount in flavonols content of 'Zonouz' pulp during commercial harvest time (Fig. 3; $P \leq 0.01$). Contrarily, at the harvest time, flavonol content of 'Gala' peel was increased due to the high amounts of quercetin-3-D-galactoside (Table 1; $P \leq 0.01$). Rutin hydrate was the predominant phenolic compound in 'Zonouz' pulp at the first step of growth (Table 1). Seemingly, rare accumulation of quercetin-3-D-galactoside may be a criterion adversely affects storage life of 'Zonouz' apple. In such a way Oleszek et al. [1998] concluded that the high level of quercetin-3-D-galactoside was the determinant factor enhances the storability of apple fruit mainly due to the prevention of β-galactosidases activity.

Table 1. Proportional amounts of individual phenolic compounds in peel and pulp of 'Gala' and 'Zounoz' apple cultivars during the fruit development

		Flavonoids					Phenolic acids	
		flavanol		anthocyanin	flavonol			
		catechin	epicatechin	cyanidin-3-galactoside	rutin hydrate	quercetine-3-d-galactoside	chlorogenic acid	caffeic acid
Gala peel	Stage 1	–	10.4a	4b	4.7a	2.7b	8.5a	0.21b
	Stage 2	0.43a	–	1.5c	0.006c	0.024c	0.33b	0.012c
	Stage 3	0.3b	0.96b	4.97a	0.07b	5.3a	0.2c	0.77a
Gala pulp	Stage 1	1.7a	8.9a	–	8.1a	–	4.8a	0.13a
	Stage 2	1b	–	–	–	–	0.11b	0.0004c
	Stage 3	0.19c	0.35b	0.12a	–	–	0.06c	0.077b
Zonoz peel	Stage 1	1.7a	–	3b	–	–	11.25a	1.25a
	Stage 2	1.5b	0.37b	1c	0.011b	–	1.3b	0.057c
	Stage 3	0.6c	1.3a	4.78a	0.12a	0.13a	1c	0.17b
Zonouz pulp	Stage 1	1.7b	–	1.5a	10a	–	15.5a	1.16a
	Stage 2	6.2a	0.13b	–	–	–	0.054c	0.029c
	Stage 3	0.3c	0.46a	0.012b	0.049c	–	0.09b	0.25b

Stage 1 – fruit early growing stage, stage 2 – mid-stage of fruit development (80 days after full bloom), and stage 3 – commercial harvest time.
Different letters shows significant difference between two apple cultivars based on Duncan's multiple range tests at $P \leq 0.01$.

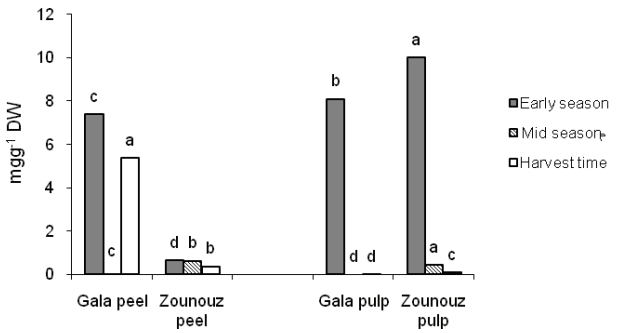

Fig. 3. Change in total flavonols content in peel and pulp of 'Gala' and 'Zounoz' apple cultivars during the fruit development. Different letters on bars show significant difference based on Duncan's multiple range test at $P \leq 0.01$

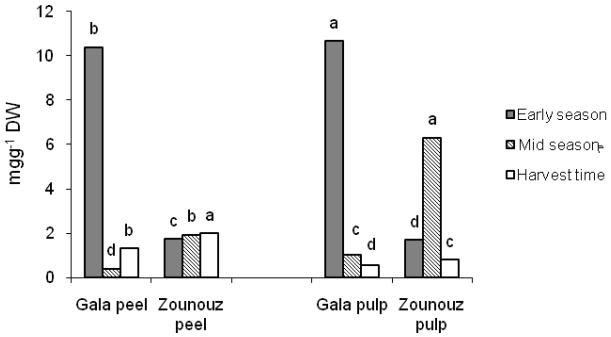

Fig. 4. Changes in total flavanols content in peel and pulp of 'Gala' and 'Zounoz' apple cultivars during the fruit development. Different letters on bars show significant difference based on Duncan's multiple range test at $P \leq 0.01$

The high level of flavanol (Fig. 4; $P \leq 0.01$) content in 'Zonouz' peel was due to the great amounts of epicatechin at the harvest time (Table 1). In contrast, 'Zonouz' pulp showed the highest content for flavanol compounds during mid-season (Fig. 4). Takos et al. [2006] reported that in 'Crips red' apple, proanthocyanidin dilution during the growing season was a time-cause response of plant to the environmental and developmental stimuli. Seemingly, astringent taste of 'Zonouz' fruit might be in part due to the high concentration of phenolic acids, flavanols and phloridzin dehydrate.

Anthocyanin biosynthesis in the peel of' 'Gala' had two peak times; first, at the early season and later during the harvest time (Table 1). Physiologicaly, the accumulation of anthocyanin in plant tissue apart from its major biological function is also related to the protection of protochlorophyl and chloroplasts from UV radiation at the early stages of fruit development [Gould et al. 2009]. Moreover, the biosynthesis of anthocyanins is commercially valuable at the harvest time, since these compounds are unique colour donors in fruit crops and also possess diverse therapeutic qualities especially from nutraceutical viewpoint in human diet [Takos et al. 2006]. Phloridzin dihydrate content of both apples was the least during the season (Fig. 5; $P \leq 0.01$). Song et al. [2007] noted that the bitter taste traces in apple fruit was in main part due to the high amounts of phloridzin dehydrate. 'Zounoz' peel had higher amounts for Phloridzin dehydrate than 'Gala' at the harvest time (Fig. 5). As mentioned before, one may consider that, phloridzin dyhedrate may be the principal constituent responsible for the astringent taste of 'Zounoz' fruit. Moreover, it is likely that the high contents of phloridzin dihydrate may be the case of intense alternate bearing potential in 'Zounoz' apple. This compound inhibits the flower bud formation (flower induction and initiation) in apple trees [Grochowska 1966].

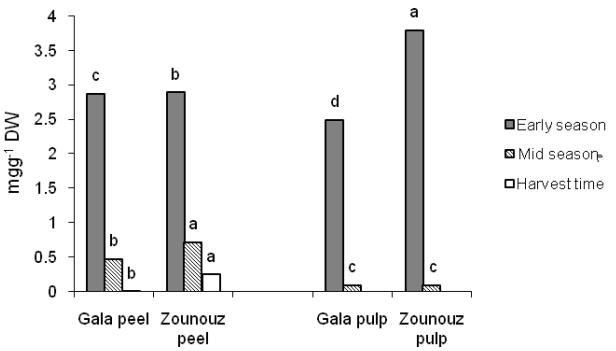

Fig. 5. Changes in phloridzin dihydrate content in peel and pulp of 'Gala' and 'Zounoz' apple cultivars during the fruit development. Different letters shows significant difference based on Duncan's multiple range test at $P \leq 0.01$

Total phenolic acids content was the highest in both 'Gala' and 'Zounoz' fruits during early season (Fig. 6; $P \leq 0.01$). Chlorogenic acid was the principal phenolic acid component in both apples during the early stage of fruit growth and later caffeic acid had the highest amounts at the harvest time in 'Gala' fruit (peel+ pulp) and 'Zounoz' pulp, respectively (Table 1; $P \leq 0.01$). There is evidence that, flavanol and phenolic acids content are the highlighted criteria affecting the resistance of apple fruits against scab disease. These compounds are also inversely related with the powdery mildew incidence in apple orchards [Mayer et al. 2008].

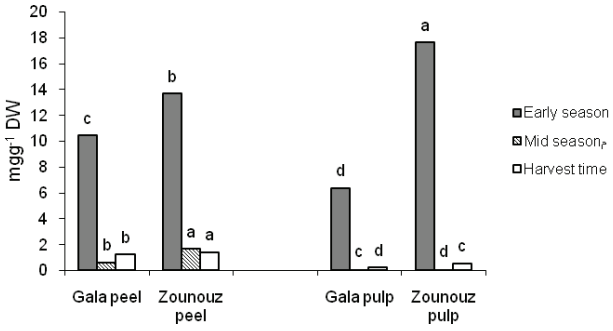

Fig. 6. Change in total phenolic acids content in peel and pulp of 'Gala' and 'Zounoz' apple cultivars during the fruit development. Different letters shows significant difference based on Duncan's multiple range test at $P \leq 0.01$

CONCLUSIONS

Our results have conclusively shown that there were considerable quantitative differences concerning phenolic compounds in 'Zonouz' and 'Gala' apple cultivars, and that 'Gala' had higher phenolic compounds than 'Zonouz'. Nearly all the compounds and phenolic constituents were the highest during early season

seemingly, due to the protection of sensitive fruits from harsh conditions. Then, during the harvest time, principally the organoleptic compounds and some others with conservative action and also storage related constituents were predominant but, with constant levels. Since, phenolic compounds have an important role in organoleptic attributes of fruits, and the biosynthesis of these compounds especially anthocyanins have a great impact on the commercial harvest time, so, knowledge on these compounds accumulation during the season will help us to update gardens management for the production of value-added fruits.

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Received – Przyjęto: 2.01.2012

Accepted for print – Zaakceptowano do druku: 14.03.2012

For citation – Do cytowania

Mehrabani L.V., Hassanpouraghdam M.B., 2012. Developmental variation of phenolic compounds in fruit tissue of two apple cultivars. Acta Sci. Pol., Technol. Aliment. 11(3), 259-264.