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Original Scientific Paper

Variations in some quality attributes of air-dried fruit of two apple cultivars from Iran

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ABSTRACT: Apple is known as an available natural source of phenolic compounds that have a great effect on human health. Quantitative analyses of 'Zonouz' and 'Gala' apple phenolic compounds were carried out by HPLC and colorimetric methods during commercial harvest time. For this, fruits were harvested, separated into peel and pulp, and air-dried for measurements of pH, total soluble solids (TSS) and total acidity (TA) during their development. The results showed no differences between two apple cultivars regarding pH. 'Gala' had higher TSS content compared to 'Zonouz'. Also, colorimetric methods revealed that the highest amount of total phenolics, anthocyanins, proanthocyanidins and flavonoid compounds were recorded in 'Gala' and 'Zonouz' apple peel, respectively. By contrast, 'Zonouz' pulp contained higher amounts of these phenolic compounds than 'Gala' pulp. HPLC analysis showed that the higher quantities of flavanols were in 'Zonouz' fruit (peel and pulp), due to much higher amounts of epicatachin in 'Zonouz' compared with 'Gala'.

Key words: apple, HPLC, anthocyanin, flavonoid, phenolic compounds

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INTRODUCTION

Epidemiological evidence has shown that people who regularly consume a diet rich in vegetables and fruits have substantially lower risks of cardiovascular diseases, type two diabetes and some forms of cancer (Lata *et al.* 2009). Protective roles of fruits and vegetables are in the main part due to the occurrence of phenolic compounds (Lister *et al.* 1994). Apple fruit contains a wide array of phenolic compounds with huge antioxidant and therapeutic activities (Lister *et al.* 1994; Mayer *et al.* 1995). Anthocyanins, flavanols and condensed tannins are the main secondary metabolites of higher plants and also apple fruits, that all belong to flavonoides (Schieber *et al.* 2001; Vargas *et al.* 2008; Hanlin & Downey 2009). Anthocyanin is responsible for blue, red and purple colors in plants. Also, it is a criterion of fruit ripening in horticulture (Bate-Smith 1975; Markakis 1982). The brown color of seed-coats is due to the intrinsic oxidation of condensed tannins (proanthocyanidins). These compound are also responsible for the herbivore deterrent characteristic and astringent taste of fruits (Hanlin & Downey 2009). Flavonols are colorless compounds which protect plants against UV radiation (Vieira *et al.* 2009). Furthermore, those compounds as antioxidant materials in the diet have promising effects on human health (Vieira *et al.* 2009).

Some registered and un-registered apple cultivars are

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frequently cultivated in Iran, from which 'Zonouz' is one of the common and valuable native ones from the Azarbaijan district in Northwest Iran. This cultivar has a red-yellow colored waxy skin, delicious pulp and fragile texture with medium shelf-life potential (Rokhafrouz 1997). In recent decades, orchards of 'Zonouz' have gradually been replaced with 'Gala', which has received the special attention of apple fruit growers in the main producing areas of the world and Iran (Rokhafrouz 1997). However, to our knowledge no comparative study has been accomplished on this shift of cultivars based on phytochemical assays. Therefore, in the present study we investigated the quality characteristics of 'Gala' and 'Zonouz' apple fruit with emphasis on some phenolic compounds.

MATERIALS AND METHODS

Analyses for the identification and quantification of phenolic compounds were 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 'Gala' and 'Zonouz' apple cultivars were collected from Bostanabad and Zonouz districts in Northwest Iran during commercial harvest time. To achieve typically representative plant material, fruits were harvested from all peripheral sides of trees. Collected fruits were immediately transferred to the laboratory, rinsed with distilled water to remove dust or external debris and finally dried with a clean towel. The fruits were peeled with a sharp knife and then peel and pulp of fruits were separately mixed and dried in a dark place with ambient ventilation at a temperature of 30-35^{0}C for about 48 h till constant weight. Afterwards, the air-dried mixed plant materials were ground to obtain a fine grade powder. Lipids and waxy compounds of samples (1g of air-dried plant material) were extracted using n-hexane (10 ml) for 20 min in an ultrasonic (Power Sonic 505, Korea) bath. Solvent was evaporated utilizing a rotary-evaporator (Heidolph, Germany) until dryness. Two replicate dried plant samples were used for extraction of phenolics. Extracts were treated with 100ml MeOH: H_2O (1:1) and then sonicated for 20 min. The subsequent aqueous extracts were sequentially filtered and centrifuged (10 min) at 13000 rpm. Finally, the two extracts were assayed for phenolic compounds by 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 phenolic compounds was carried out with an 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). The first binary solvent system of the mobile phase consisted of 2% acetic acid in water/methanol, with a gradient of 10-100% for the separation of flavanols. For separation of quercetin-3-D-galactoside the second binary solvent system was 0.25 mMol phosphate buffer, pH 2.5/acetonitrile, with a gradient of 10-30%. Cyanidin-3-galactoside was separated by the third binary solvent system of 0.1% formic acid in water/methanol, with a gradient of 10-100%. The flow rate and injection volume were 1ml/min and 20µl, respectively. Phenolic compounds were detected at 280 nm. The separated compounds were identified by comparing their retention time (R_t) with those of authentic standards. All samples were analyzed in duplicate and the mean is presented on a dry weight basis.

Reference reagents and solvents. Quercetine-3-D-galactoside, (-)-epicatechin, catechin and cyanidin-3-galactoside were purchased from Sigma (Spain). Methanol (Caledon, Canada), acetonitrile, n-hexane and formic acid (Merck, Germany) were of HPLC grade.

TA (titrable acidity), pH and TSS (total soluble solids) assay. The pH of fruit pulp extract was assayed by the method of Kafkas *et al.* (2007) using a pH meter (HANNA, 209). TA and TSS were determined according to the methods described by Kafkas *et al.* (2007). For this, TA was calibrated against malic acid and TSS was measured using a hand refractometer (ERMA, Japan).

pH, TA and TSS were measured at three fruit growth stages i.e. early growth stage (32 days after full-bloom), unripe developed fruit stage during the initial anthocyanin accumulation period (90 days after full-bloom) and commercial harvest time.

Total anthocyanin contents. Total anthocyanin contents were measured by the method of Markakis (1982). Extraction was accomplished with ethanol and HCl (85:15) mixture. Absorbance was recorded at 535 nm using a Beckman DU50 spectrophotometer.

Total proanthocyanidin contents. Total proanthocyanidins were quantified using the spectrophotometric method detailed by Bate–Smith (1975).

Total phenolics and total flavonoid contents. Total phenolic contents were determined as gallic acid equivalents by the Folin-Ciocalteu method previously described by Kim *et al.* (2006). The method of Wang *et* *al.* (2008) was employed for the quantification of total flavonoid compounds using rutin hydrate for standard curve calibration. Total anthocyanins, procyanidins, phenolics and flavonoids as well as pH, TSS and TA were measured in triplicate and one-way ANOVA was employed for the analysis of data. Mean comparisons were carried out using Duncan's multiple range test at P≤1% and P≤5%.

RESULTS AND DISCUSSION

The results showed that titratable acidity and pHs declined during fruit development in both cultivars (table 1) (P≤1%). This was in agreement with the findings of Kovac *et al.* (2010) in 'Golden Delicious' and 'Crispin' apple cultivars. Moreover, Rutkowski *et al.* (2008) reported an inverse relationship between TA and anthocyanin content in 'Golden Delicious' apple. TSS in both cultivars increased during fruit development (table 1) (P≤1%). Kubota *et al.* (2000) found no relationship between TSS, total phenolics content and antioxidant activity in peach fruits. 'Gala' contained more TSS than 'Zonouz'. . In contras, Fournand *et al.* (2006) showed that in grapevine, anthocyanin accumulation was correlated with TSS, and our results from HPLC and colorimetric methods are in good agreement with the above reports (fig.3).

Total phenolics content. There were quantitative differences between the two cultivars regarding total phenolics contents (48 mg.g^{-1}DW in 'Gala' versus 38 mg.g^{-1}DW for 'Zonouz' apple peel) (fig. 1) (P≤0.05). It seems that the majority of this difference was due to the occurrence of higher amounts of quercetin-3-D-galactoside in 'Gala'. Moreover, total phenolics contents in both apple peels were much higher than in pulps (fig. 1) (P≤0.05). Previous studies have shown that total phenolics contents are positively associated with antioxidant potential in apple fruit (Vieira *et al.* 2009). Our results indicated that 'Gala' is a valuable source of phenolic compounds ready-to-use as a nutritious pool of those high-value natural products.

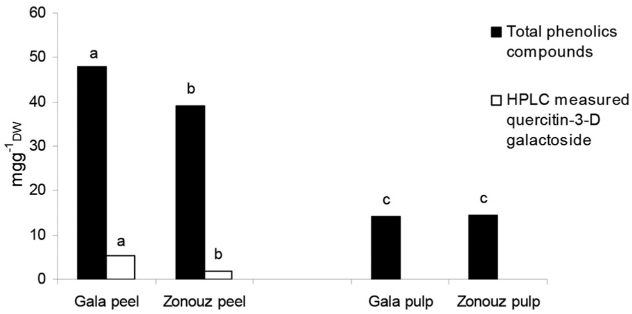

Fig 1. Total phenolics and quercetin-3-D-galactoside contents in 'Gala' and 'Zonouz' apple at harvest time.
Different letters on columns indicate a significant difference between apple cultivars based on Duncan's multiple range test at P≤0.05.

Total flavonoid contents. 'Zonouz' peel and pulp had higher total flavonoid contents than 'Gala' (fig. 2) (P≤0.05). Increase in proanthocyanidin content was the chief reason for elevated amounts of total flavonoids in 'Zonouz' apple (fig. 4) (P≤0.05). Our results are similar to the findings of Lister *et al.* (1994) in terms of increasing total flavonoied contents with fruit development. Moreover, Schieber *et al.* (2001) found that there was an inverse relationship between the consumption of fruit with high flavonoid contents and the risk of heart disease and cancer.

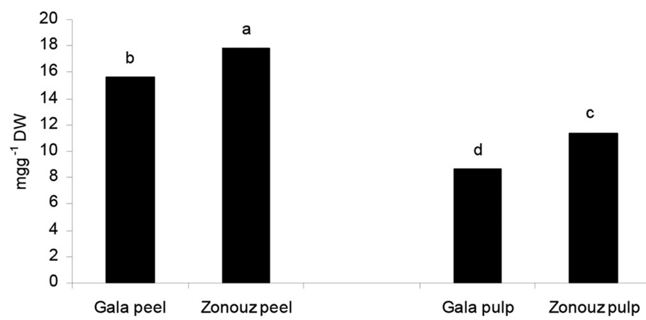

Fig 2. Total flavonoid contents in 'Gala' and 'Zonouz' apple at harvest time.
Different letters on columns indicate a significant difference between apple cultivars based on Duncan's multiple range test at P≤0.05.

Table 1. Titratable acidity (TA), pH and percentage total soluble solids (TSS) in 'Gala' and 'Zonouz' apple cultivars during fruit development. Different letters in columns show significant differences based on Duncan's multiple range test at P≤0.01.

	TSS (%)		TA (%)		pH	
	'Zonouz'	'Gala'	'Zonouz'	'Gala'	'Zonouz'	'Gala'
Early stage of fruit development	3.3b	3.8b	0.44a	0.37a	4.9a	4.7a
Developing fruit at the beginning of anthocyanin accumulation	9.2a	15.4a	0.28b	0.23b	4b	3.6b
Harvest time	13a	18.8a	0.26b	0.21b	3.7b	3.7b

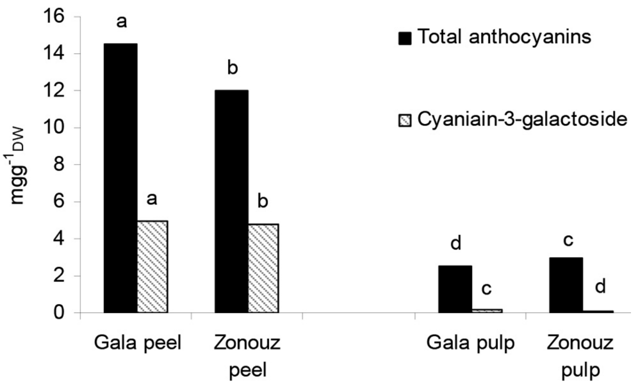

Fig 3. Total anthocyanin and cyanidine-3-galactoside contents in 'Gala' and 'Zonouz' apple.
Different letters on columns indicate a significant difference between apple cultivars based on Duncan's multiple range test at P≤0.05.

Total anthocyanin contents. Anthocyanins and cyanidine-3-galactoside were the principal phenolic compounds in 'Gala' peel and pulp (fig. 3) (P≤0.05). Lister *et al.* (1994) showed that anthocyanins were also the predominant phenolic compounds in 'Splendour' apple during harvest time. Anthocyanin biosynthesis and accumulation is the main visual quality characteristic in apple fruit. Moreover, high occurrence of anthocyanins, especially cyanidine-3-galactoside, gives apple fruit a valuable rank regarding both the therapeutic point of view and the availability of high-value antioxidant nutritious compounds commonly imported into cells.

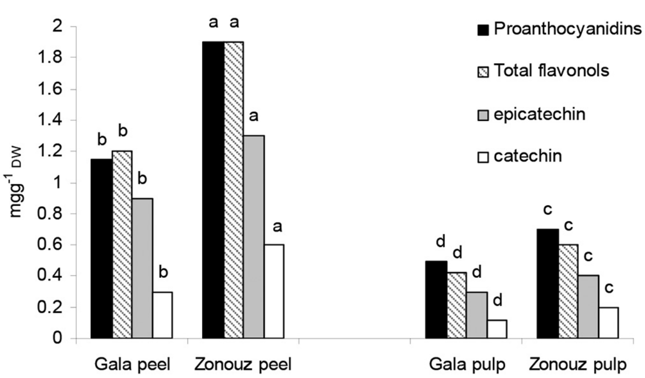

Fig 4. Total proanthocyanidins, total flavonols, epicatechin and catechin contents in 'Gala' and 'Zonouz' apple.
Different letters on columns indicate a significant difference between apple cultivars based on Duncan's multiple range test at P≤0.05.

Total proanthocyanidin contents. 'Zonouz' fruit (peel and pulp) was superior to 'Gala' regarding proanthocyanidin contents (fig. 4) (P≤0.05). The higher proanthocyanidin contents were related to higher amounts of flavonols (quantified by HPLC). Moreover, epicatechin and catechin contents in 'Gala' and 'Zonouz' fruits showed variations in their contents similar to proanthocyanin contents (fig. 4) (P≤0.05). Lister *et al.* (1994) showed that in 'Splendour' apple during harvest time monomeric flavonols tend to polymerize as proanthocyanins consistent with our findings,

CONCLUSION

Identification and quantification of apple phenolic and flavonoid compounds have gained great importance in horticulture and fruit production due to the unique role of those compounds in visual and organoleptic characteristics of fruits, storage life of fruit and also in human health. Various methodologies have been employed for the identification and quantification of these compounds. Methods used in the present experiment (HPLC and colorimetric method) showed their efficiency for the quantification of phenolics and flavonoid compounds in different parts of the apple fruit. The main difference between the two apple cultivars ('Zonouz' and 'Gala') was due to the high occurrence of quercetin-3-D-galactoside in 'Gala' peel. Overall, it seemed that 'Zonouz' may be a good native alternative for well-known apple cultivars considering phenolic and flavonoid compounds.

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REZIME

Variranje kvaliteta suve jabuke dva kultivara iz Irana

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Jabuke su poznate kao izvor fenolnih jedinjenja sa uticajem na ljudsko zdravlje. U ovom radu analiza kvaliteta dve sorte Zonouz i Gala vršeno je uz pomoć HPLC tehnike i kolorimetrijskih metoda u vreme branja. Vreme branja odredjeno je prema merenju pH, TSS i TA parametara za vreme njihovog razvoja. Izmedju plodova dva kultivara nije primećena razlika u pH vrednostima, dok je Gala sorta imala veću TSS vrednost u odnosu na Zonouz. Kolorimetrijska metoda pokazala je da je sadržaj ukupnih fenola, antocijanina, proantocijanidina i flavonoidnih jedinjenja viši kod Gala sorte i najviši u kori kod obe sorte. Zanouz pulpa ima veći sadržaj navedenih jedinjenja od Gala sorte.

HPLC analiza je pokazala najviši sadržaj flavanola kod plodova sorte Zanouz i u kori i u pulpi. Visok nivo sadržaja epikatahina je razlog visokog sadržaja flavanola kod sorte Zanouz u odnosu na sortu Gala.

Ključne reči: jabuka, HPLC, antocijanin, flavonoid, fenolna jedinjenja