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Original Research Article

Phenolic and mineral profiles of four Balkan indigenous apple cultivars monitored at two different maturity stages

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ABSTRACT

Some primary and secondary metabolites, as well as mineral nutrients in pulp, peel and juice obtained from four Serbian indigenous apple cultivars (Kožara, Kolačara, Budimka and Šumatovka) collected at two different developmental stages were studied. With advanced maturation soluble solids content, total and reducing sugars increased, while L-ascorbic acid content and titratable acidity decreased. Thirteen phenolic compounds were quantified using LC–MS/MS. The total phenolic content (TPC) ranged from 9.37 to 1440 mg/100 g fw, and 0.83 to 7.84 mg/100 g fw in peel and pulp samples, respectively. Quercetin derivatives were the major detected polyphenolic group. Majority of determined phenolic compounds were influenced by cultivar and the best sources were cultivars Kolačara and Budimka. The content of flavonols (with the exception of quercitrin) varied significantly depending on maturity at harvest. With regard to mineral analysis, K was the most abundant ranging from 104 to 158 mg/100 g fw in peel, 74.4 to 93.3 mg/100 g fw in pulp, and 77.1 to 91.5 mg/100 g fw in juice samples. Obtained results provide detailed information on nutritional potential and chemical composition of tested apple cultivars and thereby could encourage their wider cultivation and consumption.

1. Introduction

Apples are one of the most extensively produced and consumed fruits worldwide. Available on the market for the whole year, they represent significant part of the diets and an excellent source of nutrients (Lata et al., 2009). Epidemiological studies showed an inverse correlation between the consumption of apples and/or related products and the risk of cardiovascular diseases, lung dysfunctions, and various cancers, particularly prostate, liver and colon (Tsao et al., 2003). The health-protecting properties of apples have been mainly attributed to the presence of polyphenols (Lamperi et al., 2008). Generally, five major polyphenolic groups are found in apple cultivars: hydroxycinnamic acids, flavan-3-ols/procyanidins, anthocyanins, flavonols, and dihydrochalcones (Wojdylo et al., 2008). Recent studies have shown that the content of phenolic compounds in apples varies considerably among different cultivars (Lata et al., 2009; Veberič et al., 2005; Panzella et al., 2013) and also within different apple parts (Lata et al., 2009; Veberič et al., 2010). Maturity stage is another important factor that influences the compositional quality of apples. In fact, harvesting at the proper maturity stage is essential for optimum quality and often for the maintenance of this quality after harvest and storage (Tavarini et al., 2008).

Besides phytochemicals, an important part of the nutritional information is concentration of essential elements. Lacking metal ions may have a significant impact on human health, since function of more than one-third of all human proteins depend on them (Konczak and Roulle, 2011). As with polyphenols, factors that influence mineral content in fruits include cultivar and maturity at harvest (Ekholm et al., 2007).

Many apple cultivars, showing different organoleptic and nutritional characteristics, are available for the whole year. Due to the globalization of the apple trade and its effect on farm cultivar composition, a few global cultivars are dominant on markets. This not only threatens the maintenance of biodiversity, but could also lead to worldwide epidemics of certain pest and pathogens (Tóth et al., 2013). By contrast, indigenous cultivars represent the local

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germplasm cultivated mainly in the marginal areas. Generally, they show a good adaptability to the local environment and represent a valuable source for the crop genetic variability, resistance to biotic and abiotic stresses as well as for phenological and quality characteristics. Traditional apple cultivars are not cultivated for large scale production (Panzella et al., 2013) because they usually do not meet some of the appearance standards and struggle to withstand the transport and storage demands. Therefore, indigenous apple cultivars are economically less important (Goland and Bauer, 2004) although some of them could be more nutritious than newer cultivars.

A number of studies have been made on the characteristic polyphenolic compounds of apples (Panzella et al., 2013; Lata et al., 2009; Mikulič Petkovšek et al., 2009); however, they are often confined to a few cultivars, which are very popular with customers. Little attention has been given to indigenous apple cultivars (Begić-Akagić et al., 2011; Wojdylo et al., 2008), while there are no data on polyphenolic compounds in indigenous cultivars grown in Serbia. Owing to its geographical location, the Republic of Serbia has favorable natural conditions and areas for planting a high number of fruit species and varieties of high-quality fruit (Bulatović et al., 2013). Apple production has great importance for the agriculture of the Republic of Serbia for direct consumption as well as the processing industry. Standardization and globalization in Serbian apple marketing have significantly reduced the numbers of local cultivars and most of them have nearly disappeared (Mratinić and Fotirić-Akšić, 2011).

For this study, four Serbian indigenous cultivars: Kožara, Kolačara, Budimka and Šumatovka were chosen. The aim of this study was chemical analysis of some primary and secondary metabolites, as well as mineral nutrients in pulp, peel and juice obtained from these cultivars collected at two different developmental stages. This work is important for understanding the nutritional potential of Serbian indigenous species and for expansion of their cultivation and use.

2. Materials and methods

2.1. Chemicals

(+)-Catechin (≥99%, HPLC), (−)-epicatechin (≥90%, HPLC), chlorogenic acid (≥95%, titration), rutin hydrate (≥94%, HPLC), hyperoside (≥97%, HPLC), isoquercitrin (≥90%, HPLC), phloridzin (≥99%, HPLC), phloretin (≥99%, HPLC), gallic acid (≥97.5%, titration), *p*-coumaric acid (≥98%, HPLC), caffeic acid (≥98%, HPLC) and arbutin (≥96%, HPLC) were purchased from Sigma Aldrich (St. Louis, MO, USA) and quercitrin (≥98.5%, HPLC) and protocatechuic acid (≥90%, HPLC) from Extrasynthèse (Genay, France). Acetonitrile (99.8%) and formic acid (98–100%) were supplied from Sigma-Aldrich (Steinheim, Germany). Water was deionized by using a MilliQ system (Millipore, Bedford, MA, USA).

2.2. Plant material

The collection orchard of apple cultivars (*Malus domestica*) was planted in 2004 at the Experimental Station "Radmilovac" of the Faculty of Agriculture in Belgrade. Planting distance was 4 m × 1.5 m. The trees were trained as a slender spindle (Ferree, 1980), under non-irrigated standard cultural practices. The study included four indigenous autumn type cultivars: Kolačara, Budimka, Šumatovka and Kožara. Their yield per tree decreased in following order: Kožara, Budimka, Šumatovka, Kolačara (approximately 40 kg, 37 kg, 31 kg and 28 kg, respectively). Five apples from each of five trees of corresponding cultivars were harvested at commercial (the stage of development needed for the market when a plant will continue development even if detached) and full maturity stage (the stage of development when a plant fully develops) and stored at 4 °C prior to analysis, no longer then one month.

For the determination of ripening stage, the Streif index (SI) considering starch, sugar and firmness was implemented to reduce subjectivity (Streif, 1996).

Depending on cultivar, intervals between the two stages were 14 and 20 days. The time of apple collection is presented in Table 1.

2.3. Sample preparation

Fresh apple fruits were washed with tap water to remove adhered dirt and dust particles, dried with paper towels, cut into pieces with a stainless steel knife. Seeds and stems were removed and peel was mechanically separated from the pulp. Apple juice was obtained from a whole fruit by using an electric juicer. Peel or pulp was blended to obtain a paste. Then 100 μL of arbutin in methanol (internal standard) were added to 1 g of the paste or juice and further homogenized with 4 mL of methanol. The mixture was centrifuged (4000 rpm at 25 °C) for 20 min. The supernatant was filtered through a 0.45-μm cellulose filter (Millipore, Billerica, MA, USA) into a vial for LC–MS/MS analysis.

2.4. Fruit quality

During the research period, the technological properties such as fruit weight (FW), firmness, maturity stage, soluble solids content, total acid, total and invert sugars and ascorbic acid of fruits were monitored. Fruit firmness was measured using a Wagner FT penetrometer (Greenwich, CT, USA) and maturity stage was determined with iodine-starch test (Streif, 1996). Total soluble solids content (TSS) was determined by a PAL-1 refractometer (Atago, Tokyo, Japan) in °Brix. Titratable acidity (TA) was measured by neutralization with 0.1 N NaOH; data are given as g/L of malic acid. Total sugar content (TS) was determined by Luff-Schoorl method in %. An iodometric titration method was performed for the determination of ascorbic acid (Harris, 2000) and the results were expressed as mg/100 g FW.

2.5. LC–MS/MS analysis

Various phenolic compounds in the investigated samples were tentatively identified by comparing the retention times, absorption spectra (200–400 nm) and mass spectra (in scan mode) of unknown peaks with the reference standards. LC–MS analysis was performed on an Agilent MSD TOF coupled to an Agilent 1200 Series HPLC (Agilent Technologies, Palo Alto, CA, USA). Mobile phase A was water, mobile phase B acetonitrile, and mobile phase C 1% formic acid in water. The injection volume was 1.5 μL, and elution at 0.5 mL/min with gradient program (0–13.3 min 5–16% B and 10% C, 13.3–16 min 16–28% B and 10% C, 16–22 min 28–40% B and 10% C, 22–23 min 40–100% B and 10% C, 23–25 min 100% B, 25–25.1 min 100–5% B and 0–10% C, 25.1–33 min 5% B and 10% C). The mass spectrometer was operated in positive and negative ESI mode. Drying gas (N_2) flow was 800 L/h; drying gas temperature

Table 1
Date of harvesting at commercial and full maturity for apple cultivars in year 2012.

Cultivar	Date of harvest	
	1st maturity stage (CM)	2nd maturity stage (FM)
Kolačara	21-09	08-10
Budimka	19-09	06-10
Šumatovka	02-10	21-10
Kožara	30-09	18-10