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ORIGINAL ARTICLE

Variation in phenolic compounds and antioxidant activity in apple seeds of seven cultivars

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KEYWORDS

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Abstract Polyphenols are the predominant ingredients in apple seeds. However, few data are available on the phenolic profile or antioxidant activity in apple seeds in previous researches. In this study, low-molecular-weight phenolic compounds and antioxidant activity in seeds, peels, and flesh of seven apple cultivars grown in northwest China were measured and analyzed using HPLC and FRAP, DPPH, ABTS assays, respectively. HPLC analysis revealed phloridzin as the dominant phenolic compound in the seeds with its contents being 240.45–864.42 mg/100 gDW. Total phenolic content (TPC) measured by the Folin–Ciocalteu assay in apple seed extracts of seven cultivars ranged from 5.74 (Golden Delicious) to 17.44 (Honeycrisp) mgGAE/gDW. Apple seeds showed higher antioxidant activity than peels or flesh; antioxidant activity in seeds varied from 57.59 to 397.70 μM Trolox equivalents (TE)/g FW for FRAP, from 37.56 to 64.31 μM TE/g FW for DPPH, and from 220.52 to 708.02 μM TE/g FW for ABTS. TPC in apple seeds was significantly correlated with all three assays. Principal component analysis (PCA) indicated that Honeycrisp was characterized with high contents of total polyphenols and phloridzin. Our findings suggest that phenolic extracts from apple seeds have good commercial potential as a promising antioxidant for use in food or cosmetics.

1. Introduction

The Guanzhong region, in Central Shaanxi Province, China, has environmental conditions that are well suitable to apple growth. As a result, Guanzhong is a dominant apple-producing area and accounts for approximately one-third of the total apple yield in China. In 2012, approximately 9 million tons of apples were produced in Guanzhong, of which about 30% were processed into juice, cider, jam, purees, and dried products. Large quantities of residual pomace are generated during

Abbreviations: FRAP, ferric reducing/antioxidant power; TPC, total phenolic content; PCA, principal component analysis; AOA, antioxidant activity.

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the processing of apple juice concentrate. Apple pomace represents 25–30% of the original fruit weight and consists primarily of peel and residual flesh (95%), with minor amounts of stem (1%) and seed (2–4%) material (Bhushan et al., 2008; Vendruscolo et al., 2008).

Apple pomace was traditionally used as animal feed or as fertilizer (Lavelli and Kerr, 2012). These uses were not economically efficient and led to environmental problems. In recent years, research efforts have been devoted to the studies on composition and physiological function of apple pomace because of its putative health benefits (Lu and Yeap, 2000; Schieber et al., 2001; Cetkovic et al., 2008; Diñeiro et al., 2009; Suárez et al., 2010; Grigoras et al., 2013). The health-protective effects of apple pomace are attributed to phenolic compounds that may reduce the risk of obesity, diabetes, cardiovascular disease, and cancer through protection against oxidative damage (Drogoudi et al., 2008; Pontais et al., 2008; Wolfe et al., 2008).

Apple seeds are a rich source of polyphenols, especially phloridzin (Ehrenkranz et al., 2005). These polyphenols mainly consist of dihydrochalcones; hydroxycinnamic acids; flavan-3-ols which are present both in monomeric ((+)-catechin and (−)-epicatechin) and oligomeric (proanthocyanin B2) or even polymeric forms; and flavonols (Fromm et al., 2012, 2013). Phloridzin, a derivative of chalcone, is the characteristic apple polyphenol and is a phytoalexin that provides resistance to plant pathogens such as *Venturia inaequalis* (Cke.) Wint. and *Erwinia amylovora* (Mikulic-Petkovšek et al., 2007, 2008; Muthuswamy and Rupasinghe, 2007). It has been suggested that the antioxidant activity of phloridzin can inhibit lipid peroxidation (Lu and Yeap, 2000; Rupasinghe and Yasmin, 2010; Dugé de Bernonville et al., 2010). In addition to its antioxidant activity, phloridzin has been recognized as a potential anti-diabetes agent for its ability to limit intestinal and renal absorption of glucose by inhibiting sodium-linked glucose transporters 1 and 2 (Dudash et al., 2004; Manzano and Williamson, 2010).

The aim of this study was to determine the phenolic composition and assess the in vitro antioxidant activity of apple seed extracts, in comparison with peel and flesh extracts, based on FRAP, DPPH, and ABTS assays. This work will provide a basis for the use of apple seeds as a functional food ingredient that can replace synthetic compounds.

2. Materials and methods

2.1. Chemicals

Commercial standards for gallic acid, protocatechuic acid, (+)-catechin, proanthocyanin B_2, chlorogenic acid, (−)-epicatechin, caffeic acid, ferulic acid, hyperin (quercetin-3-galactoside), phloridzin, ellagic acid, and quercetin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Folin–Ciocalteu reagent, DPPH (1,1-diphenyl-2-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetrame-thylchroman-2-carboxylic acid, ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid), and TPTZ (2,4,6-tris-(2-pyridyl)-s-triazine were supplied from Amresco (Boise, ID, USA). Tea polyphenols was purchased from Shanghai Yuanye Biotechnology Company. HPLC-grade methanol was provided from Tedia (Fairfield, USA), and HPLC-grade water was purified with a Milli-Q system (Millipore, Bedford, MA, USA). All other chemicals and reagents used were of analytical grade.

2.2. Apple samples

Apples are various in variety and classified by appearance and characteristics of fruits. For example, there are two varieties, dessert and cider apples according to commercial purpose, and there are three varieties, early-maturity, middle-maturity and late-maturity in terms of maturation period. Given the comprehensive and reliability of the data in this study, seven apple cultivars were selected: Gale Gala (C1), Starking (C2), Honeycrisp (C3), Fuji (C4), Qinguan (C5), Golden Delicious (C6), and Qinyang (C7). Qinyang and Gale Gala are early-maturity cultivars; Honeycrisp, Golden Delicious, and Starking are middle-maturity cultivars; Fuji and Qinguan are late-maturity cultivars.

Ripe apple fruits were picked at random from 12-year-old apple trees grafted on the M9 rootstock in the experimental orchard of Northwest Agriculture & Forestry University (Yangling, Shaanxi, China) in August and September, 2013. In this orchard, apple trees were cultivated according to the guidelines for integrated fruit production. Apples were harvested at commercial maturity: flesh firmness 7–8 kg/cm^2, sugar 12–14 °Brix, starch index 6–7. The 6 defect-free apples were collected for each cultivar. Since all of samples originated from the same geographical location and were harvested at full maturity, the impact of climate or maturity degree should be excluded.

The peel was removed from the flesh as thin as possible (approximately 1 mm thick). The flesh was separated into small slices. The seeds were manually removed from the cores. The peel samples were combined for each cultivar and divided into three replicate groups, as were flesh and seed samples. All samples were wrapped in a tinfoil paper, immediately frozen in liquid nitrogen, and stored at −80 °C until use.

2.3. Extraction of phenolic compounds from apple tissue

Extraction of phenolic compounds was performed as described previously (Ran et al., 2013) with some modifications. Samples (1.0 g) of frozen apple peel, flesh, or seeds were ground in a mortar and extracted with 30 mL of methanol in a KQ-2500E ultrasonic bath (Kunshan Corporation, Jiangsu, China) for 30 min. The extract was concentrated in a RE-52 rotary evaporator (Yarong Corporation, Shanghai, China) under reduced pressure at 30 °C. The solution was diluted to 10 mL with methanol, passed through a 0.45-μm membrane filter (Millipore, Bedford, MA, USA), and analyzed by HPLC.

2.4. Quantification of individual polyphenols by HPLC

HPLC analysis was performed on a LC-20AT HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with an SPD-M20A photodiode array detector, a WondaSil™ C18 reversed-phase column (5 μm, 250 × 4.6 mm, GL Sciences Corporation), a CTO-20A column oven, a SIL-20A autosampler, and a DGU-$20A_5$ degasser. The elution solvents consisted of 1% (v/v) acetic acid in water (eluent A) and 100% methanol (eluent B). The gradients were as follows: 0–10 min, 5–30% B; 10–25 min, 30–50% B; 25–35 min, 50–70% B; 35–40 min, 70–5% B. The column was maintained at 30 °C. The sample injection volume was 20 μL. The flow rate and time of one separation were 1 mL/min and 40 min, respectively. The detection