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

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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.
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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

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/cm², 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-20A₅ 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

wavelength was 280 or 320 nm. Phenolic standards were dissolved in methanol. The identified compounds were ordered by retention time (t_R) and plotted according to their maximum absorbance. Quantification was based on the peak areas and the external standard calibration curve, and concentrations were expressed as mg/100 g dry weight (mg/100 gDW).

The accuracy, which was described as the percentage recovery of all analytes, was evaluated at three concentration levels. The repeatability of the retention times and peak areas was determined and expressed by relative standard deviation (RSD) to assess the precision of the HPLC method.

2.5. Total phenolic content (TPC)

Total phenolic content was measured by the Folin–Ciocalteu method according to Singleton et al. (1999) with some modifications. One milliliter of an appropriately diluted sample was mixed with 5 mL of water and 1 mL of Folin–Ciocalteu reagent. After 3 min, 3 mL of 15% sodium carbonate in water was added to reach a final volume of 10 mL. The mixture was left to stand for 2 h at room temperature in the dark, and the absorbance was read at 765 nm in a UV mini 1240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The results were expressed as mg gallic acid equivalents (GAE)/gDW.

2.6. Determination of ferric reducing/antioxidant power

The FRAP assay was carried out according to Benzie and Strain (1996) with slight modifications. The FRAP reagent was freshly prepared by mixing acetate buffer (300 mM, pH 3.6), $FeCl_3 \cdot 6H_2O$ (20 mM), and TPTZ (10 mM in HCl, 40 mM) at a 10:1:1 (v/v/v) ratio. The mixture was heated to 37 °C and immediately used for measurements. 150 μL of standard solutions or samples were added to 3 mL of the working FRAP reagent. The change in absorbance from red to blue was read at 593 nm after 10 min of incubation at 37 °C. A Trolox calibration curve was performed between 200 and 1000 μM, and the obtained results were expressed in Trolox equivalents (μM TE/g FW [fresh weight]). Each experiment was performed in triplicate.

2.7. DPPH radical scavenging activity

The DPPH method was used according to Brand-Williams et al. (1995) with some modifications. Seed extracts were diluted 1:20 (v/v) with methanol; peel and flesh extracts were diluted 1:10 (v/v) with methanol. A 2-mL aliquot of diluted sample or standard compound was added to 2.0 mL of DPPH solution (0.1 mM in methanol). The mixture was incubated for 30 min in the dark. Absorbance was recorded at 517 nm after reaction against a blank (using methanol in place of the extract). The scavenging activity of DPPH was calculated as follows:

$$\text{Radical scavenging rate (\%)} = \frac{A_0 - A}{A_0} \times 100 \quad (1)$$

where A_0 was absorbance of the DPPH blank solution after 30 min of incubation, and A was the final absorbance of the tested sample after 30 min of incubation. The results were expressed as Trolox equivalents (μM TE/g FW) using standard curves. Trolox standard solutions were prepared at concentrations ranging from 100 to 1000 μM. The concentration which caused a half-maximal reduced DPPH radical level (IC_{50}) was determined. The IC_{50} values were calculated by linear regression where the abscissa represented the concentration of samples and the ordinate represented inhibition rate. Tea polyphenols (TP), Vitamin C (VC) and butylated hydroxytoluene (BHT) were used as positive controls. Each experiment was performed in triplicate.

2.8. ABTS radical scavenging activity

The ABTS method was used according to Re et al. (1999) with some modifications. ABTS was dissolved in distilled water at a final concentration of 7 mM and mixed with 88 μL of 140 mmol/L potassium persulfate solution. The mixture was left to stand for 12 h in the dark before use. For each experiment, $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Then, 25 μL of various concentrations of sample or Trolox was mixed with 2.0 mL of $ABTS^{\cdot+}$ solution. Absorbance was measured at 734 nm after incubation at room temperature for 6 min. A standard curve was obtained by using Trolox standard solution at concentrations from 200 to 2000 μM. ABTS radical scavenging activity of each extract was expressed as μmol TE/g FW. Each experiment was performed in triplicate. The scavenging rate was calculated using the formula (2).

$$\text{Radical scavenging rate (\%)} = \frac{A_0 - A}{A_0} \times 100 \quad (2)$$

where A_0 was the absorbance of ABTS blank solution, and A was the final absorbance of the tested sample after 6 min of incubation. The concentration which caused a half-maximal reduced ABTS radical level (IC_{50}) was determined. The IC_{50} values were calculated by linear regression where the abscissa represented the concentration of samples and the ordinate represented inhibition rate. Tea polyphenols (TP), Vitamin C (VC) and butylated hydroxytoluene (BHT) were used as positive controls. Each experiment was performed in triplicate.

2.9. Statistical analysis

Each treatment was replicated three times, and results were expressed as means ± SD. One-way analysis of variance (ANOVA) with Duncan's test was performed to assess the significance of differences between means using SPSS ver. 17.0 at the 0.01 and 0.05 significance levels. Pearson correlations (0.01 and 0.05 significance levels) were performed in SPSS, and principal component analysis (PCA) was applied to separate the cultivars according to phenolic composition and antioxidant activity using SPSS ver. 17.0 and Minitab ver. 15.0.

3. Results

3.1. Phenolic profiles in apple seed extracts

Polyphenol standards were scanned from 190 to 400 nm to obtain the maximum absorption wavelength. Chlorogenic acid, caffeic acid, and ferulic acid had maximum absorption at 320 nm, ellagic acid had maximum absorption at 254 nm, and other polyphenols had maximum absorption at 280 nm. Considering the uniformity, the detection wavelengths were selected at 280 nm and 320 nm for polyphenols.

HPLC analysis of apple seed extracts revealed the presence of phloridzin, hyperin, chlorogenic acid, (−)-epicatechin, quercetin, protocatechuic acid, (+)-catechin and caffeic acid (Fig. 2), consistent with the result described by Lu and Yeap (1998). Gallic acid (peak 1 in Fig. 1), ferulic acid (peak 8 in Fig. 1), and ellagic acid (peak 11 in Fig. 1) were not detected, and peaks U1, U2, U3 and U4 were unidentified compounds. As suggested by Fromm et al. (2012), compounds U3 and U4 appeared to be phloretin derivatives, such as 3-hydroxyphloridzin and phloretin-O-xyloglucoside.

HPLC method was performed to measure phenolic contents in apple seeds. RSDs of the results for the retention times and peak areas were 0.69–1.51%, 0.87–1.25%, respectively, proving the precision of the HPLC method (less than 2%), while the recoveries were 95.2–101.4% for all analytes, proving the method's accuracy. As shown in Table 1, phloridzin was

the predominant phenolic compound in apple seeds. Phloridzin contents varied from 240.45 mg/100 gDW for Golden Delicious to 864.42 mg/100 gDW for Honeycrisp. Assuming the seed oil content of 20%, it was estimated that phloridzin contents varied from 300 to 1080 mg/100 g defatted dry matter, which was consistent with phloridzin contents of 326–2235 mg/100 g defatted dry matter in the seed reported by Fromm et al. (2012). Moreover, phloridzin contents accounted for 36.6–56.1% of the total phenolic content determined by the Folin–Ciocalteu assay, which was lower than the values reported by Fromm et al. (2013), who found that phloridzin comprised approximately half of TPC. These differences might be associated with variations caused not only by genetic diversity, different growth periods, and geographic areas, but also by analytical methods. In addition, phloridzin content in the seed (240.45–864.42 mg/100 gDW) was higher than
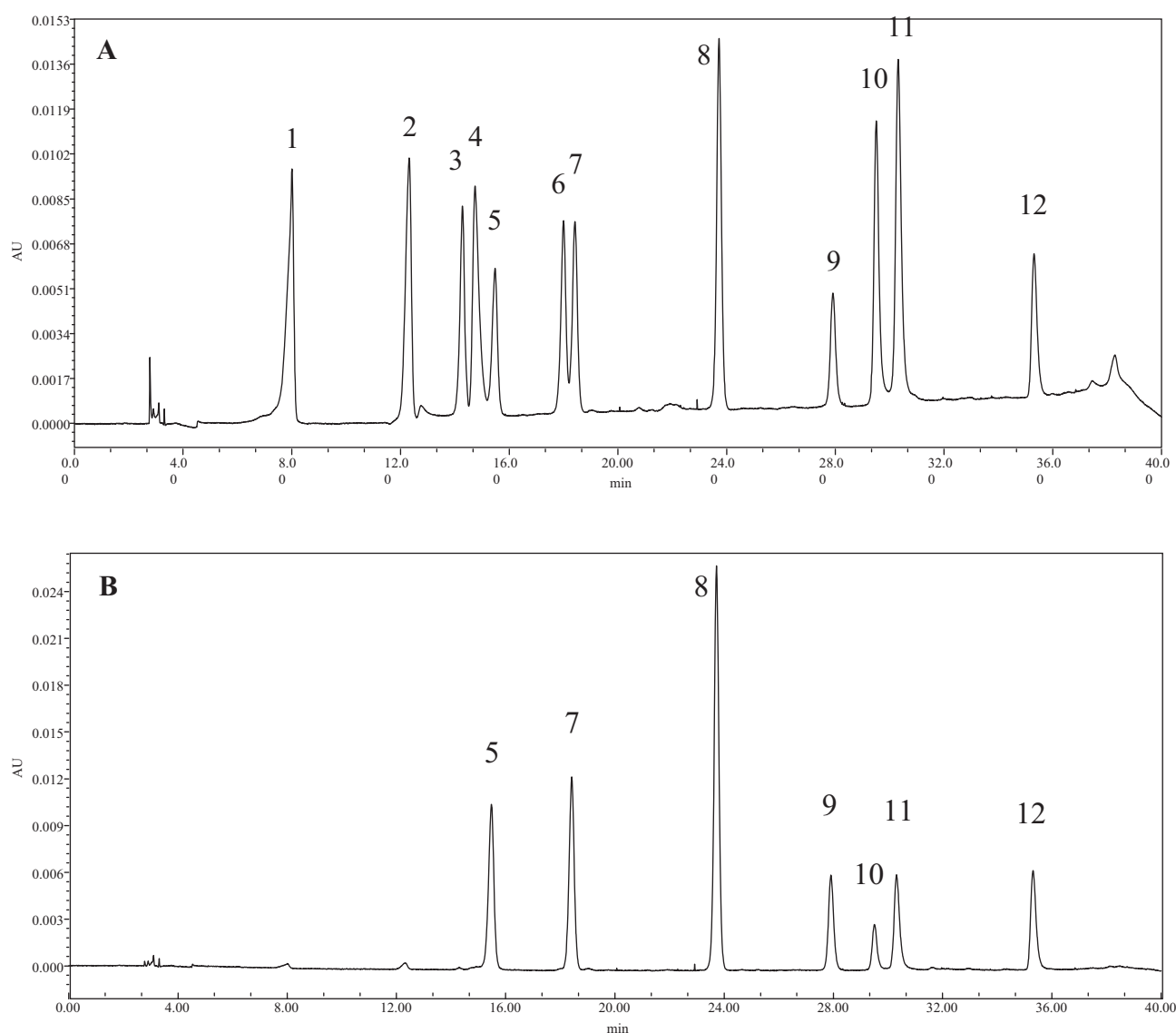

Figure 1 Chromatogram of 12 polyphenols with PDA detected at 280 nm (A) and 320 nm (B). Peaks: 1 – gallic acid; 2 – protocatechuic acid; 3 – catechin; 4 – proanthocyaninB$_2$; 5 – chlorogenic acid; 6 – epicatechin; 7 – caffeic acid; 8 – ferulic acid; 9 – hyperin; 10 – phloridzin; 11 – ellagic acid; 12 – quercitin.
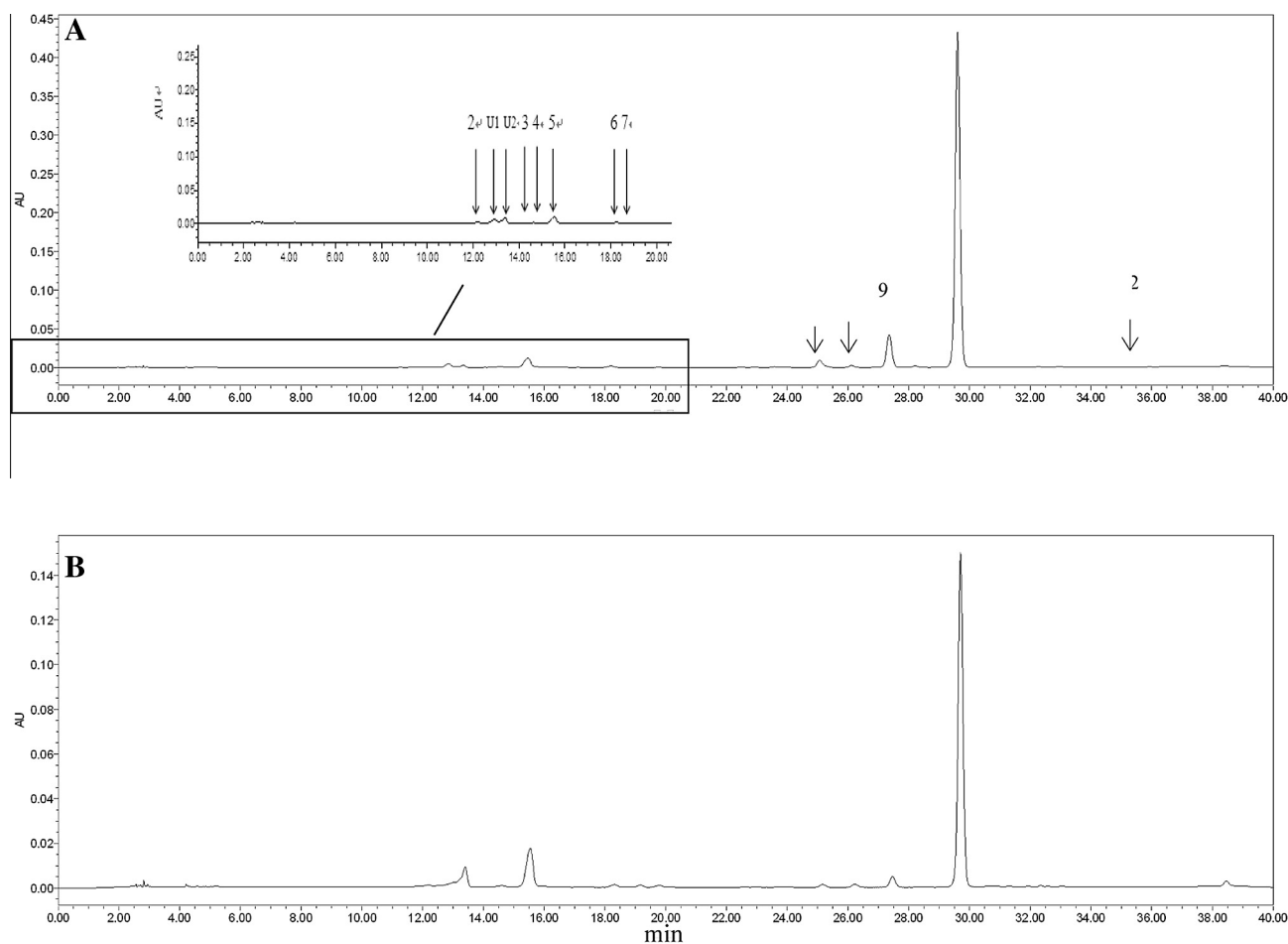
Figure 2 Chromatograms of Honeycrisp apple seed polyphenols detected at 280 nm (A) and 320 nm (B).

Table 1 Contents of individual phenolic compounds in the seeds of seven apple cultivars (mg/100 gDW).

Polyphenols	Cultivars						
	C1	C2	C3	C4	C5	C6	C7
Protocatechuic acid	3.29 ± 0.06d	9.41 ± 0.34a	4.93 ± 0.22c	6.50 ± 0.47b	4.28 ± 0.18c	3.09 ± 0.16d	2.43 ± 0.20e
(+)-Catechin	2.48 ± 0.09c	2.92 ± 0.10ab	2.14 ± 0.07d	2.72 ± 0.11bc	1.73 ± 0.05e	3.04 ± 0.13a	2.12 ± 0.08d
Proanthocyanin B2	2.89 ± 0.05e	7.52 ± 0.25b	6.23 ± 0.31c	6.79 ± 0.31c	2.25 ± 0.17f	3.62 ± 0.24d	9.72 ± 0.38a
Chlorogenic acid	21.43 ± 1.57e	32.90 ± 2.76a	26.23 ± 2.03c	30.65 ± 2.78b	17.40 ± 1.56f	22.17 ± 2.43d	15.74 ± 1.64 g
Caffeic acid	1.09 ± 0.01	1.01 ± 0.01	1.03 ± 0.01	nd	nd	1.04 ± 0.01	nd
(−)-Epicatechin	6.31 ± 0.23d	7.29 ± 0.31ab	6.54 ± 0.35 cd	6.94 ± 0.41bc	4.11 ± 0.12f	5.83 ± 0.32e	7.43 ± 0.28a
Quercetin	3.46 ± 0.18d	4.82 ± 0.22b	3.05 ± 0.16e	5.51 ± 0.28a	4.14 ± 0.22c	4.90 ± 0.20b	2.73 ± 0.12e
Hyperin	54.72 ± 2.58b	38.95 ± 1.32c	75.25 ± 4.45a	33.75 ± 2.32d	31.06 ± 2.49e	29.65 ± 1.89ef	28.20 ± 1.45f
Phloridzin	413.45 ± 4.54c	282.74 ± 3.63e	864.42 ± 7.96a	423.34 ± 4.32b	411.74 ± 4.32c	240.45 ± 3.08e	314.12 ± 5.33d
Total	509.12	387.56	989.82	516.11	476.71	313.79	382.48

C1 – Gale Gala; C2 – Starking; C3 – Honeycrisp; C4 – Fuji; C5 – Qinguan; C6 – Golden Delicious; C7 – Qinyang. Mean ± SD, n = 3; Nd – not detected.

72–217 mg/100 gDW in the peel reported by Łata et al. (2009). Phloridzin content in the seed was also higher than 15.94–59.47 mg/100 gDW in the pomace measured by Diñeiro et al. (2009). It can be inferred that apple seeds had higher phloridzin content compared to peel and pomace.

Hyperin (quercetin 3-O-galactoside) which is attributed to flavonol glycosides was the second rich phenolic compounds in the seed, making up 4.1–5.5% of TPC measured by the Folin–Ciocalteu assay. Hyperin contents in the seed ranged from 28.20 to 75.25 mg/100 gDW, with Honeycrisp showing

the highest value and Qinyang showing the lowest value. Lavelli and Kerr (2012) found that the pomace (a mixture of Red Delicious and Golden Delicious varieties) contained 203.2 mg/100 gDW flavonols (quercetin glycosides), among which quercetin 3-O-galactoside (32%) was the main component. It indicated that Hyperin content in the seed was comparative with that in apple pomace (65.02 mg/100 gDW, calculated according to Lavelli and Kerr (2012)).

Chlorogenic acid was the third major phenolic compound, accounting for 1.5–4.3% of TPC measured by the Folin–Ciocalteu assay. The highest chlorogenic acid content in the seed was found in Starking, whereas the lowest value was detected in Qinyang. Chlorogenic acid content in the seed (15.74–32.90 mg/100 gDW) was lower than 26–136 mg/100 gDW in the peel reported by Łata et al. (2009).

Furthermore, (−)-epicatechin, quercetin, protocatechuic acid, proanthocyanin B2 and (+)-catechin were minor phenolic constituents, and caffeic acid was a trace polyphenol. These results indicated that apple seeds provide a good source of phloridzin, hyperin and chlorogenic acid.

3.2. Total phenolic content

As is apparent from Fig. 3, TPCs of apple seed extracts differed significantly among the seven cultivars ($p < 0.05$); TPC was the highest in Honeycrisp, followed (in decreasing order) by Gale Gala, Fuji, Starking, Qinguan, Qinyang, and Golden Delicious, indicating a cultivar-dependent difference in phenolic compounds. Honeycrisp was the cultivar with the highest TPC (17.44 mgGAE/gDW) in the seed among all cultivars under study, whereas the lowest value (5.74 mgGAE/gDW) was observed in Golden Delicious. It means that TPC in the seed is related to cultivar, but not related to the difference of maturation period, since both Honeycrisp and Golden Delicious are middle-maturity cultivars. In addition,
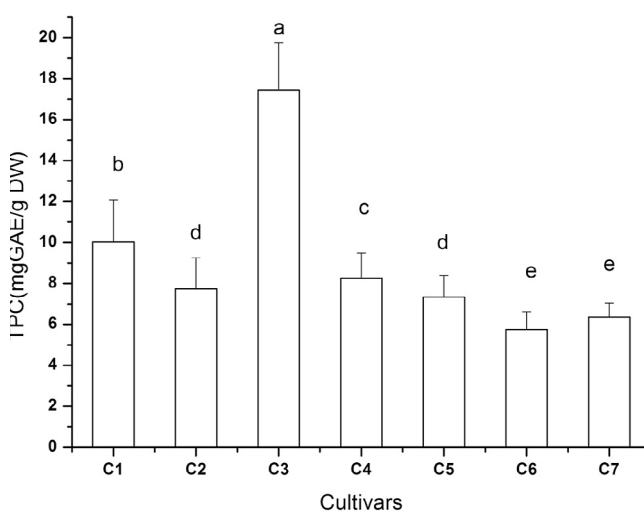

Figure 3 TPCs of apple seed extracts of seven cultivars. Bars represent mean ± standard error ($n = 3$). Different letters denote significant difference according to one way ANOVA and Duncan's test ($p < 0.05$), identical letters indicate that the samples are not significantly different from one another. C1, C2, C3... represent the cultivar as mentioned in Table 1.

it was highlighted that TPC of Honeycrisp seed extracts was 1.7-fold higher than that of Gale Gala, which had the second-highest content. Honeycrisp was produced from a cross between Macoun and Honeygold and was named in 1992 by the Department of Horticultural Science at the University of Minnesota (USA). Notable features of Honeycrisp apples include exceptionally crisp and juicy texture, low acidity, mildly aromatic flavor, and excellent storage life. Attention could be paid to Honeycrisp for apple researchers and growers owing to its remarkable features and antioxidant activity (mentioned in Table 2).

3.3. Evaluation of antioxidant activity (AOA) in apple seeds

Three in vitro antioxidant assays were carried out to evaluate antioxidant activity (AOA) in apple seeds, peels, and flesh of the seven cultivars (shown in Table 2). Significant differences were observed among cultivars for FRAP and ABTS ($p < 0.05$) but not for DPPH ($p > 0.05$). FRAP values in the seed varied from 57.59 ± 4.87 to 397.70 ± 14.27 µM TE/g FW, with a decreasing order of Honeycrisp > Gale gala > Fuji > Starking > Qinguan > Golden Delicious > Qinyang. DPPH values in the seed were between 37.56 ± 4.65 and 64.31 ± 6.43 µM TE/g FW, in a decreasing order of Honeycrisp > Gale gala > Fuji > Golden Delicious > Qinyang > Qinguan > Starking. ABTS values in the seed were in the range of 220.52 ± 10.44 and 708.02 ± 18.67 µM TE/g FW, with a decreasing order of Honeycrisp > Fuji > Starking > Qinguan > Gale gala > Qinyang > Golden Delicious. It should be emphasized that Honeycrisp showed the highest antioxidant activity in the seed for all three assays, which was mainly due to the highest TPC (mentioned in Fig. 3). Furthermore, the ratio of AOA in the seed to that in the peel ranged from 0.67 to 3.70 (FRAP assay), 2.09 to 3.59 (DPPH assay), and 0.65 to 2.22 (ABTS assay). The ratio of AOA in the seed to that in the flesh ranged from 1.33 to 4.91 (FRAP), 2.08 to 3.56 (DPPH), and 1.27 to 2.58 (ABTS). Our data were consistent with the results reported by Duda-Chodak et al. (2011), who found that antioxidant potential of Idared seeds were 2.84 times higher than the value in the peel for ABTS assay and 2.80 times higher than that in the peel for DPPH assay. Hence, these results indicated that apple seeds exhibited stronger antioxidant activity than the peel and flesh.

Fig. 4(A) shows DPPH radical scavenging activity of seed polyphenols (SP) compared with other typical antioxidants. Seed polyphenols showed a very strong concentration-inhibition effect, with a rapid increase in inhibition as concentration increased. At concentration of 5 µg/mL, SP exhibited the highest inhibition, even concentration continued to increase, the inhibition being kept constant. The inhibitory concentration of 50% (IC_{50}) of SP, Tea polyphenols (TP), Vitamin C (VC) and butylated hydroxytoluene (BHT) was 0.34 ± 0.01, 3.71 ± 0.82, 6.03 ± 0.73 and 29.35 ± 1.26 µg/mL, respectively. The result implied that seed polyphenols exhibited the strongest DPPH radical scavenging activity as compared to TP, VC and BHT. As shown in Fig. 4(B), IC_{50} of SP, TP, VC, and BHT were 20.10 ± 0.41, 87.50 ± 0.82, 174.21 ± 1.73 and 549.26 ± 2.47 µg/mL, respectively. The result also implied that seed polyphenols showed the strongest ABTS radical scavenging activity as compared to TP, VC and BHT.

Table 2 Comparison of AOA of apple peel, flesh and seed extracts measured by FRAP, DPPH and ABTS methods (μM TE/g FW).

Antioxidant index	Cultivars						
	C1	C2	C3	C4	C5	C6	C7
FRAP							
Peel	75.94 ± 5.56d	107.85 ± 7.33b	107.38 ± 7.51b	102.09 ± 7.78c	64.89 ± 4.29e	110.96 ± 8.68a	52.90 ± 3.34f
Flesh	36.55 ± 3.76e	74.07 ± 8.02b	81.08 ± 7.48a	76.72 ± 6.55b	54.15 ± 5.98c	53.22 ± 4.89c	43.26 ± 4.78d
Seed	161.70 ± 8.76b	105.92 ± 6.32d	397.70 ± 14.27a	146.70 ± 7.62c	95.94 ± 6.32e	74.69 ± 5.11f	57.59 ± 4.87 g
Seed/peel	2.13b	0.98e	3.70a	1.44c	1.48c	0.67f	1.09d
Seed/flesh	4.42b	1.43d	4.91a	1.91c	1.77c	1.40d	1.33d
DPPH							
Peel	17.87 ± 1.78	17.98 ± 1.89	17.90 ± 1.67	18.01 ± 1.72	17.94 ± 1.86	17.98 ± 0.95	17.94 ± 1.43
Flesh	16.16 ± 1.02b	18.05 ± 1.67a	18.09 ± 1.57a	18.12 ± 1.79a	18.09 ± 143a	18.09 ± 1.54a	18.05 ± 1.65a
Seed	52.23 ± 5.44b	37.56 ± 4.65f	64.31 ± 6.43a	47.58 ± 4.77c	39.01 ± 3.44	44.08 ± 6.22d	40.34 ± 3.78e
Seed/peel	2.92b	2.09d	3.59a	2.64c	2.17d	2.45c	2.25d
Seed/flesh	3.23b	2.08e	3.56a	2.63c	2.16e	2.44 cd	2.23de
ABTS							
Peel	245.17 ± 10.32e	324.69 ± 12.11c	318.70 ± 13.43d	331.86 ± 13.58b	187.78 ± 9.41f	340.83 ± 10.22a	180.00 ± 9.56 g
Flesh	128.64 ± 7.45 g	261.31 ± 8.54b	274.47 ± 11.23a	251.15 ± 10.62c	176.42 ± 9.43d	160.28 ± 7.90e	147.72 ± 8.34f
Seed	298.24 ± 10.32e	332.31 ± 11.32c	708.02 ± 18.67a	357.45 ± 15.74b	316.84 ± 14.32d	220.52 ± 10.44 g	253.62 ± 11.52f
Seed/peel	1.22c	1.02d	2.22a	1.08d	1.69b	0.65f	1.41e
Seed/flesh	2.32b	1.27e	2.58a	1.42d	1.80c	1.38d	1.72c

Data were analyzed by ANOVA and different letters within each line indicate significant differences by Duncan's test at $p < 0.05$; C1, C2, C3… represent the cultivar as mentioned in Table 1. 'seed/peel' means the ratio of AOA in the seed to that in the peel, 'seed/flesh' means the ratio of AOA in the seed to that in the flesh.
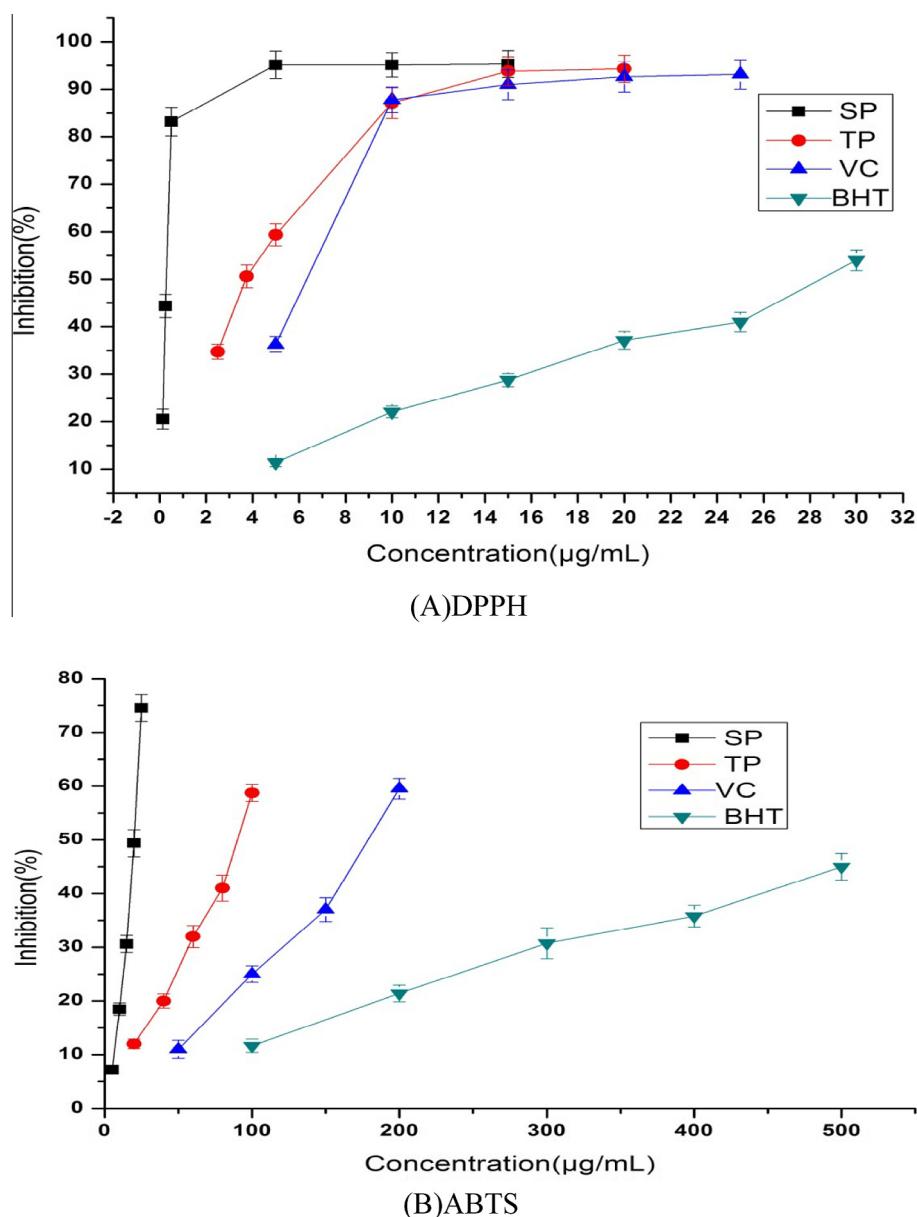
(A)DPPH

(B)ABTS

Figure 4 DPPH (A) and ABTS (B) radical scavenging activities of seed polyphenols (SP) compared with tea polyphenols (TP), Vitamin C (VC) and butylated hydroxytoluene (BHT). Each value is expressed as mean ± SD ($n = 3$).

4. Discussion

In the present work, TPC values in apple seed extracts of seven cultivars ranged from 5.74 to 17.44 mgGAE/gDW, whereas the value in apple pomace extract was only 2.4 mgGAE/gDW in a study by Schieber et al. (2003) and 1.48 mgGAE/gDW in a study by Bai et al. (2013), suggesting that the TPC of apple seeds was higher than that of apple pomace.

Based on Table 1 and Fig. 3, the TPC values calculated from individual polyphenols quantified by HPLC were lower than those measured by the Folin–Ciocalteu assay. This difference might have occurred because only low-molecular-weight phenolic compounds were extracted with methanol (high-molecular-weight compounds such as procyanidins were not extractable under these conditions). The content of

low-molecular-weight phenolic compounds represented 38.3–54.4% of that measured by the Folin–Ciocalteu assay, so only the low-molecular-weight phenolic profile is shown in Fig. 2. Fromm et al. (2012) reported that thiolysis of crude apple seed extracts prior to HLPC analysis could make procyanidins degrade to the monomeric constitutive units (−)-epicatechin or (+)-catechin, which could be determined by HPLC.

The correlation analysis revealed that TPC in apple seeds was significantly correlated with FRAP, DPPH, and ABTS assays ($p < 0.05$, $r = 0.984$, 0.886 and 0.808, respectively). Positive correlations between TPC and AOA have been observed by other researchers (Drogoudi et al., 2008; Vieira et al., 2011; Panzella et al., 2013; Carbone et al., 2011). Vieira et al. (2011) found a significant positive relationship between TPC and AOA measured by FRAP, DPPH, and
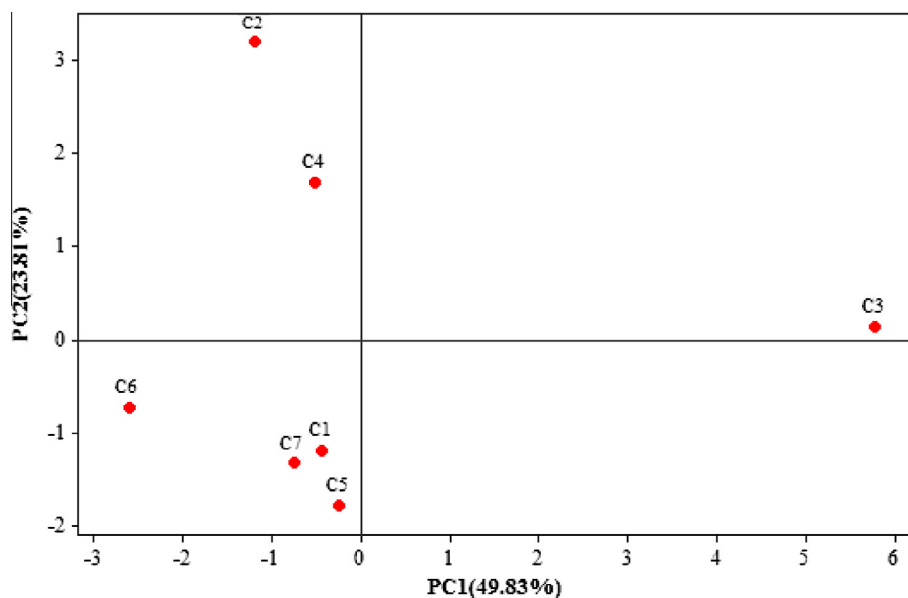
Figure 5 Projection of samples and variables on the factorial plane formed by the first two principle components. C1, C2, C3... represent the cultivar as mentioned in Table 1.

ABTS for flesh samples from 11 apple cultivars. These results indicate that the three assays are comparable and interchangeable for characterizing seed antioxidant activity.

To better discriminate among the cultivars under investigation, principal component analysis (PCA) was performed on the combined TPC data, individual polyphenols, and antioxidant parameters (13 total variables) (Fig. 5). The first two principal components (PCs) accounted for 73.6% of the total variance, with PC1 (49.8%) explaining 2 times as much as PC2 (23.8%), while PC3 explained another 13.0% of the variation. Gale Gala (C1), Qinguan (C5), and Qinyang (C7) were very close on the fourth axe; the other four cultivars were clearly separated. Honeycrisp (C3) was distant from all other cultivars on the right side as a result of its high contents of total polyphenols and phloridzin. It can be inferred that the classification of cultivars according to PCA was not correlated with time of fruit maturation. The eigenvectors indicating association between variables and PCs are presented in

Table 3. The bigger the eigenvectors, the higher the correlations between variables and PCs. Total polyphenols, FRAP, phloridzin, ABTS scavenging rate and DPPH scavenging rate were highly positively associated with PC1, whereas quercetin was negatively associated with PC1. Chlorogenic acid was positively associated with PC2.

5. Conclusions

It could be achieved that the significant diversity in the polyphenol contents and in the antioxidant activity for apple seeds was closely associated with cultivar under the condition of the same geography, climate, cultivation, and the particular part of fruit. Moreover, the results of this study indicate that apple seeds, an agro-industrial byproduct, have potential to be a promising source of antioxidants and functional food ingredients. Studies on the AOA of this valuable byproduct may lead to significant economic gains and prevent or decrease environmental problems caused by accumulation of apple pomace.

Table 3 Eigenvectors of the included variables in PCA of Fig. 5 on PC1 and PC2.

Component	PC1	PC2
Protocatechuic acid	.025	.765
Catechin	−.300	.805
Proanthocyanin B_2	.021	.471
Chlorogenic acid	.199	.894
Caffeic acid	.251	.420
Epicatechin	.094	.728
Quercitin	−.831	.313
Hyperin	.356	.082
Phloridzin	.969	−.134
Total phenol	.997	.032
FRAP	.990	.069
DPPH	.920	−.004
ABTS	.955	.078

The eigenvectors indicate association between variables and PCs.

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