Research Article

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A comparison of the chemical composition and antioxidant activity of several new early- to mid-season apple cultivars for a warmer climate with traditional cultivars

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Abstract

BACKGROUND: New early- to mid-season apple cultivars are being developed to help address warmer growing seasons due to climate change. Free sugars, organic acids, total phenolic content, total flavonoid content, antioxidant activity and phenolic composition were determined in the pulp and peel of six new and six traditional apple cultivars. In addition, the phenolic profiles of apple peels were characterized using high-resolution mass spectrometry. Forty-eight polyphenol compounds were identified, by accurate mass, in apple peel.

RESULTS: Compared to Fuji apples, a new apple cultivar, Decobell, contained 2.6- and 1.4-fold higher levels of the sum of individual polyphenol levels in the peel and the pulp, respectively. Decobell apples showed similar sugar-to-acid ratio (0.27) to Fuji apples (0.25).

CONCLUSIONS: The results indicate that the Decobell cultivar could have the best quality characteristics in terms of sugar-to-acid ratios and health-promoting activities due to the phenolic profiles.

Supporting information may be found in the online version of this article.

Keywords: apple; phenolic; UHPLC-(ESI)-qTOF; peel; sugar; sugar/acid

INTRODUCTION

Phenolic compounds are of great interest to the food and health industries as they have potent antioxidant activity, and are able to modulate diseases such as cancer and cardiovascular diseases.[1–3] Apples are an important dietary source of phenolic compounds, including chlorogenic acid, caffeic acid, catechin and epicatechin.[4–6] Additionally, apple peels are rich sources of quercetin glycosides, which are absent or present at only trace levels in apple pulp.[7] The phenolic composition in apples depends on the genotype (i.e. cultivar/variety) and tissue evaluated (peel or pulp). For example, Lee *et al.* found a broad range of chlorogenic acid: 156–746 mg kg^{-1} DW in apple pulp and 53–450 mg kg^{-1} DW in apple peel.[5] Earlier studies also showed that apple peel had higher phenolic content than apple pulp.[8] Apple processing (e.g. juice processing, canning and drying) can generate a significant amount of co-product material (e.g. apple peel). Disposal of apple processing waste materials is expensive, and disposal costs are increasing. However, co-product material such as apple peel can be valuable sources of functional compounds such as phenolic compounds and there is increasing pressure on food manufactures to increase sustainability by utilizing co-product materials.

In addition to the genotype and tissue type, environmental factors (e.g. harvest season, temperature, different latitudes, soil, light exposure, etc.) and agricultural practices (e.g. organic *versus* conventional cultivation, open field *versus* protected cultivation, water management, etc.) also affect levels of phenolic compounds in fruits.[9–13] It is therefore important to identify environmental factors and agricultural practices that influence levels of phenolic compounds when developing cultivars for enhanced levels of these bioactive compounds.

The average surface temperature of the Earth has been increasing rapidly over the past century, a phenomenon called 'global warming'. The Earth's average surface temperature increased

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0.6–0.9 °C from 1906 to 2005, and the rate has doubled in the last 50 years (NASA Earth Observatory, 2018). Global warming causes earlier blooming of apples;[14] thus, new varieties of early- to mid-season apple cultivars, those harvested from late August to October, are now widely considered for cultivation. Consumers increasingly prefer small apples as a serving size, and early- to mid-season apple cultivars produce smaller apples than late-season cultivars.[15] It has been reported that apples that bloom earlier tend to taste sweeter.[16] Sweet taste in apples might be correlated with free sugar content.[17] Little information is available about the nutritional components, such as free sugars and organic acids, in these new apple cultivars. To the best of our knowledge, no information is available regarding the phenolic composition of new early- to mid-season apple cultivars.

Previous studies have identified and quantified the phenolic composition of apples using high-performance liquid chromatography (HPLC) and LC–ion-trap mass spectrometry (MS).[18,19] Although useful for general analysis, ion-trap MS is typically limited to measuring the unit mass of target compounds. However, quadrupole time-of-flight (qTOF) MS can measure the accurate mass of target compounds and provide high resolution and sensitivity that can separate isobaric ions.[7]

In the study reported here, we investigated the free sugar content, organic acid content, total phenolic content, total flavonoid content, antioxidant activity and phenolic composition of six new early- to mid-season apple cultivars (i.e. Arisoo, Decobell, Hwangok, Picnic, Ruby-S and Summer King), three traditional early- to mid-season apple cultivars (i.e. Hongro, Shinano Sweet and Yoko) and three traditional late-season apple cultivars (i.e. Aika No Kaori, Arkansas Black and Fuji). We also determined the phenolic compositions of apple peel using ultrahigh-pressure liquid chromatography (UHPLC) coupled to accurate mass qTOF MS with electrospray ionization (UHPLC-(ESI)-qTOF).

MATERIALS AND METHODS

Chemicals and reagents

Phenolic compound standards (catechin, chlorogenic acid, cyanidin-3-*O*-galactoside, cyanidin-3-*O*-arabinoside, epicatechin, rutin, quercetin-3-*O*-galacotoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-arabinoside, quercetin-3-*O*-rhamnoside, phloridzin and quercetin aglycone), sugars (glucose, fructose, sucrose and sorbitol) and organic acids (citric acid, malic acid and shikimic acid) were purchased from Extrasynthese (Lyon Nord, France) or Sigma-Aldrich (St Louis, USA). HPLC-grade formic acid and sodium hydroxide were purchased from Sigma-Aldrich (St Louis, USA). HPLC-grade methanol, LC/MS-grade water and acetonitrile were obtained from Burdick & Jackson (Muskegon, USA).

Apple samples

The 12 apple cultivars (i.e. Aika No Kaori, Arisoo, Arkansas Black, Decobell, Fuji, Hongro, Hwangok, Picnic, Ruby-S, Shinano Sweet, Summer King and Yoko) were harvested from the research orchard of Apple Research Institute, National Institute of Horticultural and Herbal Science (NIHHS), Rural Development Administration (RDA), Gunwi (36°16′39.9″ N, 128°27′47.8″ E), Republic of Korea in 2015. The age of the plants of Summer King, Ruby-S, Arisoo, Decobell, Hwangok and Picnic apples was 4–8 years old and their rootstock was M.9. The plant age of other apple cultivars was 15 years old and the rootstock was M.26. Similar compositions of phenolic compounds (e.g. chlorogenic acid (34–37%), quercetin glycosides (32–34%), catechin (14%), etc.) are reported in apple fruits grown in M.9 and M.26 roostocks.[20] At commercial ripeness (when fruits grew to average of their size with proper starch index), apple fruits were harvested from more than three apple plants. Right after harvesting 160 apple fruits per cultivar, 20 apple fruits were randomly selected per cultivar and were used to make a composite sample for each cultivar. Then, apple fruits were washed, peeled and separated into peel and pulp. The apple peel and pulp were frozen using liquid nitrogen, lyophilized and stored at −80 °C for 1–2 months until analyzed. The lyophilized peel or pulp were ground and passed through a mesh for a uniform powder size.

Determination of free sugar and organic acid in apple juice

The free sugar and organic acid contents in apple juice were investigated as previously described.[4] The supernatant of apple juice after centrifugation was used for free sugar and organic acid analyses. The supernatant was filtered through a 0.22 μm PTFE filter (GE Healthcare Life Sciences, Issaquah, WA, USA). The free sugars in the apple juice were analyzed using an Agilent 1260 Infinity HPLC system (Agilent Technologies, Santa Clara, CA, USA) with a refractive index detector. The mobile phase was a mixture of water and acetonitrile (25:75 v/v) with a flow rate of 1 mL min^{-1}. Free sugars were separated on a YMC-Pack Polyamine II (4.6 mm × 250 mm, 5 μm) column at 35 °C. The injection sample volume was 10 μL. Free sugars were identified and quantified using authentic standards of glucose, fructose, sucrose and sorbitol.

The organic acid content in apple juice was determined using a HPLC system (Jasco, Japan) equipped with a quaternary pump (PU-2089 plus), a thermostatically controlled column compartment (CO-2060plus), an autosampler (AS-2051-plus) and a UV detector (2075plus) at 210 nm. The mobile phase for this analysis was 25 mmol L^{-1} K_2HPO_4 (pH 2.8) at a flow rate of 0.8 mL min^{-1}. A Grace Prevail organic acid column (4.6 mm × 150 mm, 5 μm) was used with a column temperature of 40 °C and an injection volume of 20 μL. The organic acids in apple juice were identified and quantified using authentic standards of citric acid, malic acid and shikimic acid. Analytical measurements of free sugar and organic acid were done in triplicate ($n = 3$).

Extraction of phenolic compounds for analysis of total phenolic content, total flavonoid content and antioxidant activity

Freeze-dried apple powder (0.5 g) was homogenized for 1 min after adding 10 mL of 80% aqueous methanol. The homogenate was sonicated with ice for 15 min to keep the temperature cool and prevent degradation of the phenolic compounds.[21,22] After sonication, the samples were centrifuged at 10 621 × *g* at 5 °C for 15 min, and then the supernatants were filtered through a 0.22 μm PTFE filter (GE Healthcare Life Sciences, Issaquah, WA, USA). The samples were extracted three times for each analysis.

Determination of total phenolic content and total flavonoid content

To determine the total phenolic content, 50 μL of 1 N Folin–Ciocalteu reagent and 160 μL of 2% sodium carbonate aqueous solution were added to 40 μL of diluted apple peel or pulp extract, and the mixture was allowed to stand for 30 min

in the dark. The absorbance of the mixture was measured by a spectrophotometer at 700 nm (Multiskan go, Thermo Scientific, Waltham, USA). The total phenolic contents of the apple peel and pulp are reported as micrograms of gallic acid equivalent (ge) per gram DW.

The total flavonoid content in the apple samples was determined by modifying the method of Moreno *et al.*[23] An amount of 500 μL of diluted apple peel or pulp extract was mixed with 100 μL of a 10% aluminium nitrate solution, 100 μL of 1 mol L^{-1} potassium acetate and 4.3 mL of methanol. The mixture was allowed to stand for 40 min, and then its absorbance was measured at 415 nm. The total flavonoid content is reported as micrograms of quercetin equivalent (qe) per gram DW.

Antioxidant activity (ABTS and FRAP)

The 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging method was used to determine antioxidant activity.[24,25] Briefly, 40 μL of apple extract was mixed with 300 μL of diluted ABTS solution and allowed to react for 10 min in the dark, and then the absorbance was measured at 734 nm. ABTS radical scavenging activity is reported as micrograms Trolox equivalent (te) per gram DW.

Also, a modified method for the ferric reducing ability (FRAP) assay was used in this study:[26] 80 μL of diluted apple extract was reacted with 240 μL of FRAP reagent for 30 min in the dark, and then the absorbance at 593 nm was measured. The results are reported as micrograms te per gram DW.

Quantification of phenolics in peel and pulp of apple samples using UHPLC

Individual phenolic compounds were determined by analyzing the peel and pulp of apple samples using a modification of the method of Jakobek *et al.*[27,28] After adding 10 mL of 80% aqueous methanol to 300 mg of freeze-dried apple powder, we sonicated the mixture with ice for 15 min to prevent phenolic compound degradation. The extract was centrifuged at 10 621 × *g* at 5 °C for 10 min, and the supernatant was dried *in vacuo*. The dried extract was re-dissolved in water with 1 mL of 1% formic acid and filtered through a 0.22 μm PVDF filter (Millipore, MA, USA). The samples were extracted three times. The phenolic composition in the peel and pulp samples was determined using an Acquity UPLC H-class UHPLC system (Waters, Eschborn, Germany) at 280 nm for catechin, epicatechin and phloridzin, 320 nm for chlorogenic acid, 360 nm for quercetin glycosides and aglycone (i.e. rutin, quercetin-3-*O*-arabinoside, quercetin-3-*O*-rhamnoside and quercetin-3-*O*-galactoside) and 499 nm for cyanidin glycosides (i.e. cyanidin-3-*O*-arabinoside and cyanidin-3-*O*-galactoside) and aglycone. The injection volume was 10 μL, and the phenolic compounds in the extracts were separated on an Acquity HSS T3 column (2.1 mm × 150 mm, 1.8 μm, Waters, Eschborn, Germany). The mobile phases were 1% formic acid in water (A) and 1% formic acid in acetonitrile (B), and the gradient conditions were as follows: 0–3 min, 5–18% (B); 3–6 min, 18–20% (B); 6–9 min, 20% (B), 9–15 min, 20–45% (B); 15–17 min, 45–95% (B). The flow rate was 0.4 mL min^{-1}.

To determine the accuracy of the measurement, known amounts of catechin, phloridzin, chlorogenic acid, cyanidin-3-*O*-galactoside, quercetin-3-*O*-galactoside and quercetin-3-*O*-arabinoside were spiked into apple powder before extraction, and the recovery rate (%) was calculated. The added amount was based on values previously reported in apples.

Identification of apple phenolics by UHPLC-(ESI)-qTOF MS analysis

The identification of phenolic compounds in apple peel was performed on an Agilent 1290 Infinity UHPLC system coupled to a 6530 accurate mass qTOF mass spectrometer (UHPLC-(ESI)-qTOF MS/MS) with ESI via Jet Stream Technology (Agilent Technologies, Santa Clara, CA, USA). The methanolic extract of apple peel was used for UHPLC-qTOF MS analysis. The samples were extracted three times. The phenolic compounds in the apple peel samples were separated with a Poroshell C18 column (2.1 mm × 100, 2.7 μm, Agilent Technologies) at 30 °C. The mobile phase consisted of a gradient of 0.1% formic acid in water (A) and acetonitrile (B) for ESI negative mode and a gradient of 1% formic acid in water (A) and acetonitrile (B) for ESI positive mode. Injection volume was 5 μL. The other LC-(ESI)-MS methods followed the protocols given in Lee *et al.*[7]

A database of all phenolic compounds possibly contained in apples, based on the literature, was developed. The database includes the theoretical *m/z* of the possible phenolic compounds based on their molecular formulas. Then the acquired qTOF MS1 data were used to search for the phenolic compounds found in the apple peel extracts and identified them by comparing the theoretical mass and isotope patterns (i.e. isotope abundance and spacing) with the observed mass and isotope patterns, using Mass Hunter Qualitative Analysis (Agilent Technologies, Santa Clara, CA, USA). The identification of 19 compounds was confirmed with authentic standards by comparing their mass, isotope patterns and retention times. The database was updated with the retention times of the 19 phenolic compounds identified with authentic standards. For MS/MS fragment interpretation of the phenolic compounds identified in MS1 mode, MS2 data were acquired using the Auto MS/MS mode with a collision energy of 20 eV. MS2 data were acquired at an MS/MS scan rate of three spectra per second and two maximum precursors per cycle over a range of *m/z* 100–1000. Integrations and peak alignments were performed using Mass Profiler Professional (Agilent Technologies, Santa Clara, CA, USA). The relative quantification of each phenolic compound in the apple peel extract was determined by qTOF in the MS1 mode using the ion peak area extracted at its retention time.

Statistical analysis

All statistical analyses were performed using IBM SPSS statistics software 23 (v. 23.0, SPSS, Inc., Chicago, IL). Significant differences between the peel and pulp of a single cultivar were tested using a paired *t*-test at $P < 0.05$. Significant differences among apple cultivars in the same tissue (pulp or peel) were determined using one-way ANOVA followed by a *posthoc* test, a Tukey's HSD test, at $P < 0.05$.

RESULTS AND DISCUSSION

A total of 12 apple cultivars were compared in these studies and data for fruit peel color, registration year, genetic crossings, harvest season and fruit weight are presented in Table 1. Apples evaluated include six new cultivars and six cultivars that have been in production since 2001 and even as far back as 1870 (Arkansas Black). Images of these apples are shown in Fig. 1.

Free sugars and organic acids in apple juice

The free sugars and organic acids in the juice obtained from the apples are presented in Table 2. The total sugar content

Table 1. Apple sample information

Cultivar	Peel color	Registration year	Genetic information	Average fruit weight (g)	Harvest season	New/ traditional
Summer King	Yellow	2010	Fuji × Golden Delicious	250	Early to mid (early Aug)	New
Ruby-S	Red	2014	Alps Otome (IT 249906) × Sansa (IT 225509)	69	early to mid (late Aug)	New
Arisoo	Red	2011	Yoko × Shensu	258	Early to mid (early Sept)	New
Decobell	Red	2013	Sansa (IT225509) × Jay Darling (IT225830)	23	Early to mid (early Sept)	New
Hwangok	Yellow	2009	Hongwol × Yataka Fuji	216	Early to mid (mid Sept)	New
Picnic	Red	2008	Fuji × Sansa (IT 225509)	203	Early to mid (late Sept)	New
Hongro	Red	1988	Supr Earliblaze × Supr Golden Delicious	300	Early to mid (mid Sept)	Traditional
Shinano Sweet	Red	1993	Fuji × Sugaru	300	Early to mid (early Oct)	Traditional
Yoko	Red	1981	Golden Delicious × unknown pollen parent (possibly Jonathan)	300	Early to mid (early Oct)	Traditional
Aika No Kaori	Red	2001	Fuji × unknown pollen parent (possibly Tsugaru)	450	Late (mid/late Oct)	Traditional
Fuji	Red	1962	Rall's Janet × Red Delicious	300	Late (late Oct)	Traditional
Arkansas Black	Dark Red	1870	–	250	Late (early Nov)	Traditional

Bold type means new apple cultivars (early- to mid-season apples).

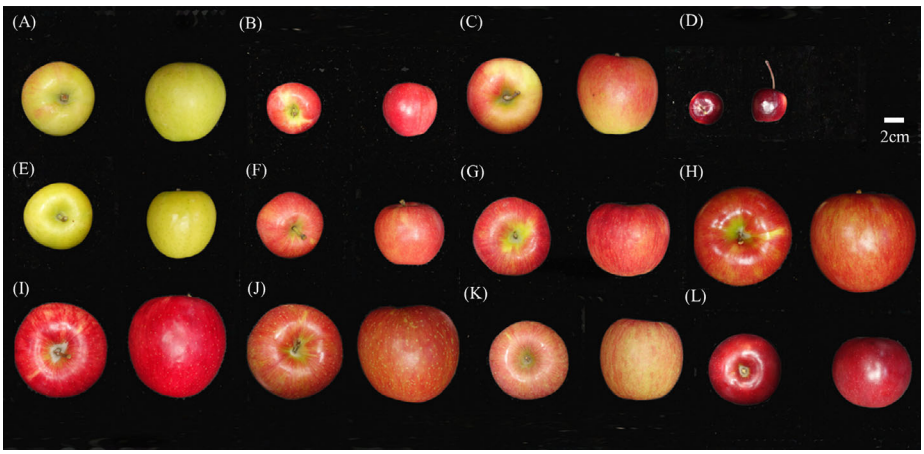

Figure 1. Images of the 12 apple cultivars: (A) Summer King, (B) Ruby-S, (C) Arisoo, (D) Decobell, (E) Hwangok, (F) Picnic, (G) Hongro, (H) Shinano Sweet, (I) Yoko, (J) Aika No Kaori, (K) Fuji, (L) Arkansas Black.

(sum of glucose, fructose, sucrose and sorbitol) ranged from 98.4 to 199.2 g kg^{-1} FW. These results are in good agreement with previously reported values in apples (115.0–160.0 g kg^{-1} FW).[29] Summer King, Ruby-S, Hwangok, Shinano Sweet, Yoko, Aika No Kaori and Arkansas Black cultivars contained similar total sugars (101.2–129.8 g kg^{-1} FW) to the Fuji cultivar (114.7 g kg^{-1} FW), one of the most popular traditional apple cultivars consumed worldwide. Total sugars in Fuji apples in this study were lower than those in Fuji apples grown in Europe (150 g kg^{-1} FW)[29] and higher than those in Fuji apples grown in New Zealand (72.2–75.28 g kg^{-1} FW).[30] Among yellow-skinned apple cultivars, Summer King apples contained lower total sugars (101.2 g kg^{-1} FW) and Hwangok apples contained similar total sugars (129.8 g kg^{-1} FW), compared with Golden Delicious apples (with yellow skin) grown in Italy (135.6–147.9 g kg^{-1} FW).[31] Hongro apples had significantly higher total sugars (199.2 g kg^{-1} FW) than the other cultivars ($P < 0.05$). Fructose and sucrose were the major sugars in all apple cultivars (47.5–98.4 g fructose kg^{-1} FW and 14.6–46.0 g sucrose kg^{-1} FW).

The total organic acid content (sum of malic, shikimic and citric acids) was 1629.0–4431.9 mg kg^{-1} FW. Arkansas Black apples contained significantly more of the organic acids (4431.9 mg kg^{-1} FW) than the other apple cultivars ($P < 0.05$). Among the early- to mid-season cultivars, Summer King apples had the highest organic acid content (3985.5 mg kg^{-1} FW) ($P < 0.05$). Hongro and Shinano Sweet apples had significantly lower organic acid content (1629.0–1718.9 mg kg^{-1} FW) than the other cultivars ($P < 0.05$). Of those organic acids, malic acid was predominant in all the apple cultivars. Fuji apples had 2479.7 mg malic acid kg^{-1} which is similar to Fuji apples grown in Europe (2264–3302 mg kg^{-1} FW).[32] Interestingly, Decobell apples contained more citric acid (551.2 mg kg^{-1} FW) than the other apple cultivars (32.9–98.6 mg kg^{-1} FW).

The sugar-to-acid ratio is used to determine the sweetness and sourness of apples. In the cultivars tested herein, the sugar-to-acid ratios had up to a fivefold difference, with Hongro apples showing the highest sugar-to-acid ratio and Summer King apples showing the lowest. Thus, Hongro apples may taste sweeter

Table 2. Sugar and organic acid contents in apple juice

Cultivar	Free sugar (g kg^{-1} apple fresh weight)					Organic acid (mg kg^{-1} apple fresh weight)				Sugar/acid ratio
	Fructose	Glucose	Sucrose	Sorbitol	Total sugars	Malic acid	Shikimic acid	Citric acid	Total organic acids	
Summer King	62.1 ± 3.9de	15.8 ± 0.8b	14.6 ± 0.0a	8.7 ± 0.4 cd	101.2 ± 5.8ab	3882.1 ± 172.1 g	4.8 ± 1.5ef	98.6 ± 10.5 g	3985.5 ± 183.7 g	62.1 ± 3.9e
Ruby-S	67.9 ± 0.4ef	25.0 ± 0.7ef	17.3 ± 0.0a	14.8 ± 0.3 g	125.0 ± 2.1cde	3619.4 ± 82.1f	3.0 ± 0.1bcd	61.1 ± 4.2de	3683.5 ± 85.7f	67.9 ± 0.4f
Arisoo	70.4 ± 0.4 fg	20.7 ± 0.3 cd	38.3 ± 0.0e	10.1 ± 0.2ef	139.4 ± 1.0ef	3077.8 ± 113.9e	2.6 ± 0.1bc	54.9 ± 2.2d	3135.3 ± 115.6e	70.4 ± 0.4f
Decobell	49.0 ± 0.9ab	17.5 ± 0.7bc	22.8 ± 0.6b	9.2 ± 0.0de	98.4 ± 0.8a	2182.4 ± 12.1b	0.9 ± 0.0a	551.2 ± 1.9 h	2724.5 ± 14.0 cd	49.0 ± 0.9ab
Hwangok	62.5 ± 0.6de	26.8 ± 0.8f	29.6 ± 0.0c	11.0 ± 0.3f	129.8 ± 2.1de	2768.3 ± 208.8d	2.1 ± 0.1b	48.3 ± 4.8bcd	2818.7 ± 211.1d	62.5 ± 0.6e
Picnic	75.5 ± 1.3 g	25.1 ± 0.9ef	37.9 ± 0.0e	14.0 ± 0.4 g	152.6 ± 2.8f	2643.9 ± 95.9 cd	4.4 ± 0.3ef	63.4 ± 7.2de	2711.7 ± 94.2 cd	75.5 ± 1.3 g
Hongro	98.4 ± 7.5 h	38.7 ± 3.1 g	46.0 ± 0.0f	16.2 ± 1.0 h	199.2 ± 15.2 g	1542.0 ± 65.5a	2.0 ± 0.4ab	85.0 ± 8.1 fg	1629.0 ± 73.9a	98.4 ± 7.5 h
Shinano Sweet	56.2 ± 5.2bcd	22.4 ± 2.8de	35.4 ± 0.5de	8.4 ± 0.7 cd	122.4 ± 12.7 cd	1677.1 ± 12.5a	2.9 ± 0.1bcd	39.0 ± 1.9abc	1718.9 ± 11.6a	56.2 ± 5.2 cd
Yoko	47.5 ± 0.5a	6.5 ± 0.2a	45.7 ± 2.0f	6.4 ± 0.3a	106.1 ± 1.3ab	2579.5 ± 117.9 cd	3.8 ± 0.4 cde	51.1 ± 11.5 cd	2634.4 ± 126.9 cd	47.5 ± 0.5a
Aika No Kaori	52.8 ± 0.4abc	17.7 ± 1.5bc	32.5 ± 1.5 cd	7.7 ± 0.4bc	110.7 ± 1.7abc	2029.8 ± 55.2b	4.0 ± 0.2def	32.9 ± 3.6a	2066.8 ± 57.3b	52.8 ± 0.4bc
Fuji	60.0 ± 0.6 cd	22.0 ± 0.4de	24.9 ± 0.0b	7.9 ± 0.3bc	114.7 ± 0.6bcd	2479.7 ± 41.0c	5.1 ± 0.4f	33.4 ± 3.0ab	2518.2 ± 42.2c	60.0 ± 0.6de
Arkansas Black	70.0 ± 2.9 fg	25.0 ± 2.0ef	21.4 ± 4.2b	6.9 ± 0.4ab	123.3 ± 6.3 cd	4353.1 ± 68.9 h	4.6 ± 0.2ef	74.2 ± 6.9ef	4431.9 ± 72.6 h	70.0 ± 2.9f

Bold type means new apple cultivars (early- to mid-season apples). Mean values followed by different letters indicate significant cultivar differences at $P < 0.05$.

than the others, and Summer King apples may taste more sour. Three new apple cultivars, Decobell, Hwangok and Picnic apples, showed similar sugar-to-acid ratio (0.27–0.28) to that of Fuji apples (0.25).

Total phenolic content, total flavonoid content and antioxidant activity in apple peel and pulp

The total phenolic content, total flavonoid content and antioxidant activity (FRAP and ABTS) in the peel and pulp of the 12 apple cultivars are presented in Table 3. Generally, the apple peels contained more total phenolic compounds and total flavonoids and had higher antioxidant activity than the apple pulp. The total phenolic and total flavonoid contents in the peel and pulp differed significantly by cultivar ($P < 0.05$). Decobell apples had significantly higher total phenolic content (150.8 μg ge g^{-1} DW in the peel and 89.6 μg ge g^{-1} DW in the pulp) than the other apple cultivars (47.0–124.7 μg ge g^{-1} DW in the peel and 12.0–50.6 μg ge g^{-1} DW in the pulp). Summer King apples contained the lowest total phenolic content in the peel. Interestingly, the apple cultivar with the lowest total phenolic content in the pulp was Arisoo, not Summer King. The total flavonoid content also varied by genotype and differed in the peel (104.3–196.7 μg qe g^{-1} DW) and pulp (15.6–31.8 μg qe g^{-1} DW). Arkansas Black, Hongro and Yoko (191.6–196.7 μg qe g^{-1} DW) had significantly higher total flavonoid content in the peel than the other apple cultivars ($P < 0.05$).

Both the peel and pulp of Decobell apples showed significantly higher antioxidant activity in both the FRAP assay (2100 μg te g^{-1} in the peel and 1463 μg te g^{-1} in the pulp) and the ABTS assay (7301 μg te g^{-1} in the peel and 5872 μg te g^{-1} in the pulp) than those of the other cultivars ($P < 0.05$). Summer King and Arisoo apples had significantly lower antioxidant activity in the peel and pulp, respectively, than the other cultivars.

Method validation for phenolic compound quantification using UHPLC

Phenolic compounds (catechin, chlorogenic acid, cyanidin-3-*O*-galactoside, cyanidin-3-*O*-arabinoside, epicatechin, rutin, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-arabinoside, quercetin-3-*O*-rhamnoside, phloridzin and quercetin aglycone) were quantified in apple peel and pulp using UHPLC. Quantification was performed using authentic standards of all of the phenolic compounds. Linear dynamic ranges of the phenolic compounds were between 5 and 50 μg mL^{-1}, with R^2 of over 0.996.

To determine the accuracy of the phenolic compound quantification in the apple samples, we added known amounts of catechin, phloridzin, chlorogenic acid, cyanidin-3-*O*-galactoside, quercetin-3-*O*-galactoside and quercetin-3-*O*-arabinoside to apple samples (based on the literature) and measured their recoveries. Recovery rates ranged from 94 to 101%.

Phenolic compound composition in the peel and pulp of apples using UHPLC

Table 4 presents the quantified phenolic compounds in the peel and pulp of the 12 apple cultivars as determined by UHPLC. The total phenolic compound levels (i.e. sum of individual phenolic compounds) from the peels of all apple cultivars differed significantly from those in the pulp from the same cultivars ($P < 0.05$), which agrees with the results of earlier studies.[33] Total phenolic compound levels ranged from 1157 (Summer King) to 5119 μg g^{-1}

Table 3. Total phenolic content (TPC; μg ge g^{-1} DW), total flavonoid content (TFC; μg qe g^{-1} DW) and antioxidant activity of peel and pulp of 12 apple cultivars

	Peel				Pulp			
Cultivar	TPC	TFC	FRAP	ABTS	TPC	TFC	FRAP	ABTS
Summer King	47 ± 1a*	104 ± 3a*	552 ± 33a*	2331 ± 60a*	24 ± 1c	23 ± 1bc	379 ± 28c	1624 ± 69de
Ruby-S	66 ± 3c*	153 ± 7bc*	736 ± 45b*	2578 ± 60a*	18 ± 1b	31 ± 0de	303 ± 19b	775 ± 41b
Arisoo	57 ± 1b*	118 ± 4a*	659 ± 36ab*	2601 ± 65a*	12 ± 0a	15 ± 1a	233 ± 17a	462 ± 13a
Decobell	151 ± 9 k*	165 ± 3 cd*	2100 ± 25 g*	7301 ± 12f*	90 ± 2j	32 ± 1e	1463 ± 59 h	5872 ± 191i
Hwangok	92 ± 4ef*	158 ± 6 cd*	1013 ± 49 cd*	3752 ± 20b*	36 ± 2gh	16 ± 1a	553 ± 32e	2133 ± 103 fg
Picnic	91 ± 2e*	120 ± 3a*	1071 ± 63d*	3798 ± 96b*	36 ± 1 h	32 ± 0e	584 ± 30e	1621 ± 64 cd
Hongro	102 ± 4gh*	192 ± 9e*	1214 ± 79e*	4759 ± 33 cd*	51 ± 1i	31 ± 1e	665 ± 38f	3232 ± 36 h
Shinano Sweet	107 ± 3hi*	139 ± 5b*	1084 ± 68d*	4550 ± 18c*	31 ± 1e	29 ± 1d	477 ± 33d	1509 ± 48 cd
Yoko	111 ± 5i*	192 ± 10e*	1199 ± 81e*	5182 ± 23de*	34 ± 1 fg	16 ± 0a	552 ± 39e	1747 ± 81ef
Aika No Kaori	97 ± 2 fg*	174 ± 5d*	1115 ± 69de*	5272 ± 89e*	28 ± 1d	17 ± 0a	453 ± 23d	1343 ± 32c
Fuji	80 ± 4d*	119 ± 5a*	959 ± 65c*	3964 ± 57b*	33 ± 1ef	21 ± 1b	477 ± 29d	1824 ± 55ef
Arkansas Black	125 ± 4j*	197 ± 4e*	1482 ± 89f*	5590 ± 27e*	49 ± 1i	24 ± 1c	733 ± 43 g	1968 ± 90 fg

Bold type means new apple cultivars (early- to mid-season apples). Mean values followed by different letters indicate significant cultivar differences for the same tissue (peel or pulp) at $P < 0.05$.
*Significant difference between apple peel and apple pulp from the same cultivar at $P < 0.05$.

DW (Decobell) in the peel and from 423 (Aika No Kaori) to 1534 μg g^{-1} DW (Hongro) in the pulp. Fuji is one of the most commercially important apple cultivars in the world. Compared to Fuji, Decobell had 2.6-fold and 1.4-fold more total phenolic content in the peel and pulp, respectively. Decobell apples also contained significantly higher levels of the 12 phenolic compounds in both the peel and pulp than the other apple cultivars ($P < 0.05$). Decobell apples also showed higher total phenolic content and antioxidant activity in the peel and pulp than the other apple cultivars ($P < 0.05$).

In all apple cultivars, epicatechin and the quercetin glycosides (e.g. quercetin-3-*O*-galactoside and quercetin-3-*O*-arabinoside) were predominant (*ca* 68% of the sum) in the peel, whereas catechin, epicatechin and chlorogenic acid were the major phenolic compounds (97% of the sum) in the pulp. This agrees with the literature.[8,18,34–36] Chlorogenic acid, catechin and epicatechin were reported as major phenolic compounds in apple pulp,[8,18] whereas, epicatechin and quercetin glycosides were reported as major phenolic compounds in apple peel.[34–36]

Some apple cultivars contained higher contents of specific phenolic compounds in the peel. For example, Hongro apples contained higher epicatechin content than other cultivars, and 5.5–6.1 times higher than the lowest-content cultivars (i.e. Arisoo, Summer King and Ruby-S) in the peel. The peels of Fuji, Hongro and Picnic apples had higher epicatechin levels than quercetin-3-*O*-galactoside levels. The peel of Hongro apples also had the highest quercetin-3-*O*-rhamnoside levels among the apple cultivars we tested ($P < 0.05$), which might have contributed to the high total flavonoid content in the peel of Hongro apples. Fuji apples contained higher epicatechin levels (451.9 μg g^{-1} DW) than Fuji apples grown in Europe (250 μg g^{-1} DW) in the peel, after correcting FW to DW on the basis of a 90% estimated moisture content [37]. Yellow-skinned apple cultivars Summer King and Hwangok apples contained higher epicatehin levels (184.8 and 405.7 μg g^{-1} DW, respectively) than Golden Delicious apples grown in the USA (71.2 μg g^{-1} DW) and China (172.8 μg g^{-1} DW) in the peel.[38,39] The peel of Fuji apples contained lower catechin levels (242.4 μg g^{-1} DW) than the peel of Fuji apples grown in Europe (290–750 μg g^{-1} DW) [40] and higher catechin levels than the peel of Fuji apples grown in China (17.3 μg g^{-1} DW),[41] after correcting FW to DW on the basis of a 90% estimated moisture content.

Quercetin-3-*O*-galactoside levels ranged from 329.8 to 1048.8 μg g^{-1} DW in the peel of the 12 apple cultivars in this study. The values agree with previously reported quercetin-3-*O*-galactoside values (192–1011 μg g^{-1} DW) in the peel of apple cultivars grown in the USA and Canada, although the tested cultivars were different from those of this study.[8,39] Currently, there is little information on quercetin-3-*O*-galactoside concentrations in the peel of apples. Most studies evaluated whole fruits.[42–44] Among yellow-skinned apple cultivars, Hwangok apples contained higher quercetin-3-*O*-galatoside levels (755.3 μg g^{-1} DW) than Summer King apples (431.8 μg g^{-1} DW) ($P < 0.05$). Golden Delicious apples grown in Canada and in the USA contained 725 and 420 μg g^{-1} DW, respectively, in the peel.[8,39] Cyanidin-3-*O*-galactoside levels ranged from n.d. (not detected; Hwangok) to 915.0 μg g^{-1} DW (Decobell) in the peel and this is in good agreement with previously reported values (n.d.–922 μg g^{-1} DW) of Golden Delicious, Red Delicious and Gala apples.[45,46] Previously reported values in the literature were calculated as micrograms per gram DW after correcting FW to DW on the basis of a 90% estimated moisture content. Anthocyanin is a major component of red pigment in apple peel, and red apples appear to contain primarily cyanidin-3-*O*-galactoside and additional anthocyanins such as cyanidin-3-*O*-arabinoside were identified and quantified in the apple peel with authentic standards. Cyanidin-3-*O*-arabinoside ranged in the apple peel from n.d. (Summer King and Hwangok) to 55.7 μg g^{-1} DW (Decobell).

Fuji apples contained lower chlorogenic acid levels (296.0 μg g^{-1} DW) than Fuji apples grown in the USA (449.0 μg g^{-1} DW) and higher chlorogenic acid levels than Fuji apples grown in China (243.5 μg g^{-1} DW) in the peel.[5,41] Summer King and Hwangok apples had lower chlorogenic acid levels (57.3 and 27.8 μg g^{-1} DW, respectively) than Golden Delicious apples grown in Europe and the USA in the peel (84.8–370 μg g^{-1} DW), after correcting FW to DW on the basis of a 90% estimated moisture content.[5,39,47]

In the pulp, catechin is the predominant phenolic compound in most apple cultivars (i.e. Arkansas Black, Decobell, Hongro,

Table 4. Phenolic compounds in the peel and pulp of 12 apple cultivars (μg g^{-1} DW) determined by UHPLC

	Flavan-3-ols		Flavonols						Dihydrochalcone	Phenolic acid	Anthocyanins		
	Cat	Epi	Rut	Q3gal	Q3glc	Q3ara	Q3rha	Q	Phl	CA	C3gal	C3ara	Total
Peel													
Summer King	82.8 ± 4.2a*	184.8 ± 4.3a*	Below LOQ	431.8 ± 6.4c	70.1 ± 1.2bc	166.9 ± 4.4a*	80.8 ± 0.8a*	Below LOQ	82.0 ± 5.0a*	57.3 ± 0.5b*	Below LOQ	ND	1156.6 ± 17.5a*
Ruby-S	107.2 ± 4.5a*	174.0 ± 1.3a*	Below LOQ	459.8 ± 9.7d	71.4 ± 4.8bc	366.9 ± 7.1f*	124.4 ± 8.4bc*	Below LOQ	137.7 ± 3.2b*	325.4 ± 2.4e*	435.9 ± 14.5e	42.7 ± 1.8bc	2245.5 ± 34.3d*
Arisoo	139.3 ± 3.9b*	166.7 ± 4.9a*	Below LOQ	333.9 ± 11.7a	45.0 ± 2.1a	187.3 ± 8.9ab*	151.4 ± 8.0 cd*	Below LOQ	95.2 ± 1.1a*	63.8 ± 4.9bc*	76.6 ± 4.4a	Below LOQ	1259.1 ± 9.3b*
Decobell	498.9 ± 10.4 g*	793.3 ± 23.7 g*	161.2 ± 8.2f	954.2 ± 8.1 h	342.1 ± 19.0f	491.0 ± 5.4 g*	245.6 ± 13.2f*	Below LOQ	268.8 ± 15.8e*	392.9 ± 5.8f*	915.0 ± 38.8f	55.7 ± 3.7c	5118.6 ± 86.6j*
Hwangok	98.7 ± 5.5a*	405.7 ± 19.2b*	52.1 ± 2.2c	755.3 ± 12.0f	108.3 ± 1.6d	364.0 ± 9.9f*	342.9 ± 2.2 g*	Below LOQ	105.1 ± 10.3a*	27.8 ± 4.0a*	ND	ND	2259.8 ± 20.3d*
Picnic	374.4 ± 16.1f*	621.6 ± 9.9f*	17.0 ± 1.3a	370.2 ± 7.2b	53.5 ± 1.7ab	225.7 ± 8.2c*	169.3 ± 11.7de*	Below LOQ	89.1 ± 2.7a*	464.0 ± 15.2 g	168.0 ± 9.7c	Below LOQ	2564.0 ± 15.3e*
Hongro	482.8 ± 25.1 g*	1015.2 ± 18.9 h*	28.3 ± 0.7b	859.2 ± 8.2 g	124.5 ± 3.9de	573.4 ± 9.9 h*	469.7 ± 12.9 h*	Below LOQ	225.8 ± 7.2d*	465.9 ± 5.5 g*	295.3 ± 19.4d	26.0 ± 1.5a	4565.9 ± 70.8i*
Shinano Sweet	188.3 ± 9.1c*	497.6 ± 8.7d*	66.1 ± 0.9d	670.6 ± 8.4e	83.1 ± 3.7c	260.8 ± 6.3d*	270.2 ± 4.8f*	Below LOQ	84.2 ± 4.4a*	496.5 ± 9.5 h*	181.1 ± 11.0c	Below LOQ	2813.8 ± 26.5f*
Yoko	187.5 ± 4.8c*	579.1 ± 3.5e*	67.6 ± 5.0d	1048.8 ± 6.0i	73.2 ± 3.6c	314.0 ± 11.1e*	261.1 ± 17.0f*	Below LOQ	75.1 ± 1.4a*	83.5 ± 0.4c*	327.5 ± 10.1d	27.9 ± 1.0a	3045.2 ± 11.9g*
Aika No Kaori	224.8 ± 5.9d*	592.2 ± 12.8ef*	79.2 ± 2.9e	866.2 ± 16.0 g	131.7 ± 6.1e	513.7 ± 13.2 g*	333.5 ± 35.1 g*	Below LOQ	183.0 ± 5.1c*	36.2 ± 0.5a*	112.8 ± 5.7ab	Below LOQ	3073.4 ± 24.2 g*
Fuji	242.4 ± 6.5de*	451.9 ± 13.2c*	50.6 ± 2.7c	329.8 ± 9.2a	37.4 ± 2.1a	206.5 ± 3.0bc*	100.4 ± 4.6ab*	Below LOQ	100.1 ± 1.9a*	296.0 ± 7.7d*	121.7 ± 5.7b	Below LOQ	1936.8 ± 8.2c*
Arkansas Black	265.0 ± 9.5e*	622.5 ± 4.6f*	31.6 ± 2.4b	1040.6 ± 3.3i	118.1 ± 5.9de	345.6 ± 4.3f*	202.6 ± 15.9e*	Below LOQ	565.9 ± 29.0f*	344.7 ± 7.0e*	422.8 ± 6.5e	25.4 ± 0.7a	3984.9 ± 19.5 h*
Pulp													
Summer King	198.0 ± 3.0C	36.1 ± 0.0A	ND	ND	ND	ND	Below LOQ	ND	21.5 ± 0.7A	198.8 ± 4.2C	ND	ND	454.5 ± 7.3AB
Ruby-S	650.5 ± 3.6H	33.4 ± 0.9A	ND	ND	ND	ND	Below LOQ	ND	Below LOQ	474.9 ± 2.7GH	ND	ND	1158.7 ± 6.9E
Arisoo	231.5 ± 18.6D	Below LOQ	ND	ND	ND	ND	Below LOQ	ND	Below LOQ	232.4 ± 21.8D	ND	ND	463.9 ± 40.4B
Decobell	624.4 ± 13.2G	453.9 ± 17.5F	ND	ND	ND	Below LOQ	ND	ND	Below LOQ	433.3 ± 4.6F	ND	ND	1511.6 ± 35.1G
Hwangok	203.1 ± 3.0C	167.5 ± 3.7C	ND	ND	ND	ND	ND	ND	36.3 ± 0.9CD	202.0 ± 2.7C	ND	ND	608.8 ± 8.8C
Picnic	647.2 ± 3.3GH	190.6 ± 1.8D	ND	ND	ND	ND	Below LOQ	ND	22.0 ± 1.0A	466.4 ± 2.0G	ND	ND	1326.2 ± 6.4F
Hongro	698.0 ± 5.2I	291.3 ± 3.6E	ND	ND	ND	ND	18.0 ± 0.4	ND	38.8 ± 2.5D	488.1 ± 1.3H	ND	ND	1534.2 ± 8.9G
Shinano Sweet	562.2 ± 3.6F	144.8 ± 3.4B	ND	ND	ND	ND	Below LOQ	ND	46.9 ± 0.9E	414.9 ± 2.1F	ND	ND	1168.8 ± 9.3E
Yoko	143.7 ± 11.0B	168.7 ± 10.7C	ND	ND	ND	ND	Below LOQ	ND	Below LOQ	131.9 ± 7.4B	ND	ND	454.3 ± 28.8AB
Aika No Kaori	99.5 ± 0.2A	204.1 ± 2.0D	ND	ND	ND	ND	Below LOQ	ND	33.5 ± 1.6C	85.3 ± 0.4A	ND	ND	422.4 ± 3.2A
Fuji	492.8 ± 6.2E	187.8 ± 0.7D	ND	ND	ND	ND	ND	ND	27.8 ± 1.6B	379.9 ± 0.8E	ND	ND	1088.3 ± 5.3D
Arkansas Black	544.8 ± 5.3F	190.8 ± 3.3D	ND	ND	ND	ND	Below LOQ	ND	50.1 ± 1.0E	391.2 ± 1.2E	ND	ND	1176.9 ± 9.0E

Bold type means new apple cultivars (early- to mid-season apples).
* Significant difference between apple peel and apple pulp for the sample phenolic compound at $P < 0.05$.
Mean values followed by different lower case and capital letters indicate significant cultivar differences for peel or pulp, respectively, at $P < 0.05$. ND, not detected; below LOQ, below the limit of quantification. Cat, Epi, Rut, Q3gal, Q3glc, Q3ara, Q3rha, Q, Phl, CA, C3gal and C3ara are catechin, epicatehin, rutin, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-arabinoside, quercetin-3-*O*-rhamnoside, quercetin aglycone, phloridizin, chlorogenic acid, cyanidin-3-*O*-galactoside and cyanidin-3-*O*-arabinoside.

Hwangok, Fuji, Picnic, Ruby-S and Shinano Sweet). Hongro apples contained higher catechin concentrations (698.0 μg g^{-1} DW) than the other cultivars ($P < 0.05$), followed by Ruby-S (650.5 μg g^{-1} DW) and Picnic (647.2 μg g^{-1} DW). Epicatechin was the predominant phenolic compound in the pulp of Aika No Kaori and Yoko apples, whereas chlorogenic acid predominated in the pulp of Summer King and Arisoo. Catechin levels ranged from 99.5 μg g^{-1} DW (Aika No Kaori) to 698.0 μg g^{-1} DW (Hongro) in apple pulp. This agreed with previously reported values (6.7 to 156 μg g^{-1} DW) in the pulp of various apple cultivars.[36,48] Fuji apples contained higher catechin concentrations (492.8 μg g^{-1} DW) than Fuji apples grown in Europe (124.9–133.0 μg g^{-1} DW) in the pulp, after correcting FW to DW on the basis of a 90% estimated moisture content.[44,49]

Chlorogenic acid in apple pulp ranged from 85.3 (Aika No Kaori) to 488.1 μg g^{-1} DW (Hongro). This agreed with reported chlorogenic acid values (157.0–745.8 μg g^{-1} DW) of Fuji, Pink Lady, Golden Delicious and Granny Smith apples grown in the USA.[5] Fuji apples contained lower chlorogenic acid levels (379.9 μg g^{-1} DW) than Fuji apples grown in the USA (745.8 μg g^{-1} DW) in the pulp, after correcting FW to DW on the basis of a 90% estimated moisture content.[5] The two yellow-skinned apple cultivars Summer King and Hwangok apples contained similar chlorogenic acid levels (198.8 and 202.0 μg g^{-1} DW, respectively) ($P > 0.05$) and the values were lower than previously reported values (290–570 μg g^{-1} DW) of Golden Delicious apples in the pulp, after correcting FW to DW on the basis of a 90% estimated moisture content.[47]

Epicatechin levels ranged from 33.4 (Ruby-S) to 453.9 μg g^{-1} DW (Decobell). This agrees with reported epicatechin values (6–124 μg g^{-1} DW) of apple cultivars grown in Europe after correcting FW to DW on the basis of a 90% estimated moisture content.[37] Especially, Fuji apple pulp contained higher epicatechin concentrations (187.8 μg g^{-1} DW) than previously reported values of European Fuji apple pulp (37 μg g^{-1} DW) after correcting FW to DW on the basis of a 90% estimated moisture content.[37] Phloridzin in Fuji apple pulp was 27.8 μg g^{-1} DW and is in good agreement with reported phloridzin values of Fuji apple pulp (20.4 μg phloridzin g^{-1} DW).[44]

It is difficult to compare previously reported phenolic compound levels in apples with our data, because each study used a different analysis method (e.g. different extraction solvents) and different varieties of apples. Le Bourvellec *et al.* concluded that apple genotype determines phenolic composition rather than agricultural practices (e.g. conventional *versus* organic cropping).[50] To date, only phenolic compositions of traditional apple cultivars such as Golden Delicious apples or local apples or old apples are available.[27,36,45,51] The characterization of phenolic compounds in new early- to mid-season apple cultivars is new. So, it is difficult to compare phenolic composition of the newly early- to mid-season apple cultivars with previously reported values of traditional apple cultivars. To date, there is also little information available on the phenolic composition of apple co-products such apple peel as most studies evaluate the phenolic content of pulp (i.e. flesh) or whole apple fruit.

Moreover, each study used apples grown in a different geographic location. Some studies used apples purchased from a local market (growing region is unknown) or apples harvested in different geographic locations across a country.[52,53] Growing region also affects phenolic concentrations in apple fruits for the sample genotype.[54] In addition to genotype and growing region, other factors such as cropping year, agricultural practices, postharvest treatment, storage and ripening stage may affect the phenolic content in apple fruits with the same genotype.[4,43,50] For example, 1-methylcyclopropene-treated apples contained higher total phenolic content than untreated apples from long-term storage.[4] Unripe apples contain higher phenolic content than ripe apples and the phenolic content decreases as apple fruits grow.[43] In this study, apple samples were grown with the same agricultural practices and harvested at commercial maturity in the same year with the same postharvest treatment. Apple samples were not stored for analysis. Apple samples were washed, frozen using liquid nitrogen and freeze-dried right after harvest and chemical composition was determined within 1–2 months. Rootstocks of several apple plants in this study were different (i.e. M.9. and M.26); however, a previous study reported that the phenolic composition was similar for the rootstocks used in this study.[20] This study included apple trees with different ages; however, it is unknown if the tree age will affect fruit metabolites. Future studies may be extended to consider this factor.

Previous studies provided phenolic compositions for only late-blooming apple cultivars.[55–57] However, as production of early- to mid-season apple cultivars increases in response to warmer climates so does the need to evaluate the composition of phenolic compounds in these apples, and determine how levels compare with more traditional cultivars.

Our study demonstrates that several of the new cultivars of early- to mid-season apples contained phenolic compounds at levels similar to or higher than those found in traditional late-season apple cultivars. These results demonstrate that new early- to mid-season apples are competitive with traditional varieties in terms of providing a large range of key bioactive compounds.

Phenolic compound identification in apple peels using UHPLC-(ESI)-qTOF

Apple peels demonstrated a higher phenolic content than apple pulp in all cultivars. Therefore, the phenolic compounds in apple peel were further characterized using UHPLC-(ESI)-qTOF. A representative total ion chromatogram of apple peel is shown in Fig. S1. Initially, both negative and positive ionization modes were performed for all samples. In general, negative mode resulted in better sensitivity, with the exception of anthocyanins which showed a greater response in the positive mode. Therefore, the positive mode was used for anthocyanin analysis, and the negative mode was used for analysis of the rest of the phenolic compounds in single MS and MS/MS modes.

Table 5 presents the 48 phenolic compounds identified in apple peels using high-resolution MS with a mass error of less than 5.8 ppm. The identification of protocatechuic acid, chlorogenic acid, catechin, 4-hydroxybenzoic acid, cyanidin-3-*O*-galactoside, epicatechin, cyanidin-3-*O*-arabinoside, eriodictyol-7-glucoside, quercetin-3-*O*-galactoside, rutin (quercetin3-*O*-rutinoside), quercetin-3-*O*-glucoside, quercetin-3-*O*-xyloside, quercetin-3-*O*-arabinoside, quercetin-3-*O*-rhamnoside, isorhamnetin-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, phloridzin, quercetin, kaempferol and isorhamnetin was confirmed with authentic standards. A tentatively identification of all other compounds was achieved based on their accurate mass and isotope pattern in single MS1 mode. UHPLC-(ESI)-qTOF MS/MS spectra of 35 peaks (Fig. S2) were also used to identify compounds. However, no fragment ion was found for 3-hydroxybenzoic acid isomers, (epi)gallocatehin, 2 procyanidin trimer B types, dihydroxybenzoic acid, butein, cyanidin-*O*-hexoside, isorhamnetin-*O*-glucuronide, quercetin-3-*O*-rhamnoside, isorhamnetin-3-*O*-rutinoside or kaempferol because of a low abundance of their precursor ions.

Table 5. Polyphenols identified in apple peels. Assignments are based on ESI and high-resolution Q/TOF MS data. All *m/z* are based on protonated or deprotonated pseudomolecular ions

Peak no	tR	Compound assigned	Molecular formula	Predicted MS1 *m/z*	Fragment *m/z*	Error (ppm)	Subclass
1	2.3	Protocatechuic acid*	$C_7H_6O_4$	153.0193	109	−3.9	Hydroxybenzoic acid
2	2.4	Hydroxybenzoic acid	$C_7H_6O_3$	137.0244		2.9	Hydroxybenzoic acid
3	3.3	Catechin-*O*-hexoside	$C_{21}H_{24}O_{11}$	451.1246	289	0.4	Flavan-3-ol
4	3.5	Hydroxybenzoic acid	$C_7H_6O_3$	137.0244		−5.8	Hydroxybenzoic acid
5	3.7	Catechin-*O*-hexoside	$C_{21}H_{24}O_{11}$	451.1246	289	0.4	Flavan-3-ol
6	3.7	Procyanidin dimer B type	$C_{30}H_{26}O_{12}$	577.1351	289	0.3	Proanthocyanidins
7	4.2	Chlorogenic acid*	$C_{16}H_{18}O_9$	353.0878	191	−0.3	Hydroxycinnamic acid
8	4.7	Catechin*	$C_{15}H_{14}O_6$	289.0718	245	−0.7	Flavan-3-ol
9	4.7	4-Hydroxybenzoic acid*	$C_7H_6O_3$	137.0244		2.2	Hydroxybenzoic acid
10	5.0	(Epi)gallocatechin	$C_{15}H_{14}O_7$	305.0667		4.3	Flavan-3-ol
11	5.3	Procyanidin trimer B type	$C_{45}H_{38}O_{18}$	865.1985		0.9	Proanthocyanidins
12	5.7	Coumaroylquinic acid	$C_{16}H_{18}O_8$	337.0929	173	−1.8	Hydroxycinnamic acid
13	6.1	Cyanidin-3-*O*-galactoside*	$C_{21}H_{20}O_{11}$	449.1078†	287	0.9	Anthocyanin
14	6.1	Coumaroylquinic acid	$C_{16}H_{18}O_8$	337.0929	191	−0.3	Hydroxycinnamic acid
15	6.2	Procyanidin dimer B type	$C_{30}H_{26}O_{12}$	577.1351	289	0.7	Proanthocyanidins
16	6.6	Coumaroylquinic acid	$C_{16}H_{18}O_8$	337.0929	173	−0.6	Hydroxycinnamic acid
17	6.9	Dihydroxybenzoic acid	$C_7H_6O_4$	153.0193		1.3	Hydroxybenzoic acid
18	7.1	Epicatechin*	$C_{15}H_{14}O_6$	289.0718	245	2.4	Flavan-3-ol
19	7.4	Cyanidin-3-*O*-arabinoside*	$C_{20}H_{18}O_{10}$	419.0973†	287	1.4	Anthocyanin
20	8.3	Coumaroylquinic acid	$C_{16}H_{18}O_8$	337.0929	191	1.8	Hydroxycinnamic acid
21	8.5	Myricetin-*O*-hexoside	$C_{21}H_{20}O_{13}$	479.0831	316, 300	0.2	Flavonol
22	8.9	Procyanidin trimer B type	$C_{45}H_{38}O_{18}$	865.1985		0.5	Proanthocyanidins
23	9.1	Quercetin-*O*-dihexoside	$C_{27}H_{30}O_{17}$	625.1410	463, 301	0.3	Flavonol
24	9.5	Cyanidin-*O*-pentoside	$C_{20}H_{18}O_{10}$	419.0973†	287	1.4	Anthocyanin
25	10.6	Quercetin-*O*-hexose pentoside	$C_{26}H_{28}O_{16}$	595.1305	300	1.0	Flavonol
26	10.7	Butein	$C_{15}H_{12}O_5$	271.0612		2.6	Chalcone
27	10.8	Naringenin-*O*-hexoside	$C_{21}H_{22}O_{10}$	433.1140	271	5.1	Flavanone
28	11.1	Quercetin-*O*-hexose pentoside	$C_{26}H_{28}O_{16}$	595.1305	300	1.7	Flavonol
29	11.8	Eriodictyol-7-glucoside*	$C_{21}H_{22}O_{11}$	449.1089	287	1.6	Flavanone
30	11.9	Quercetin-3-*O*-galactoside*	$C_{21}H_{20}O_{12}$	463.0882	300	0.6	Flavonol
31	12.1	Rutin*	$C_{27}H_{30}O_{16}$	609.1461	463, 301	0.5	Flavonol
32	12.6	Quercetin-3-*O*-glucoside*	$C_{21}H_{20}O_{12}$	463.0882	300	0.9	Flavonol
33	13.3	Quercetin-3-*O*-xyloside*	$C_{20}H_{18}O_{11}$	433.0776	300	1.4	Flavonol
34	14.0	Cyanidin-*O*-hexoside	$C_{21}H_{20}O_{11}$	449.1078†		0.7	Anthocyanin
35	15.1	Quercetin-3-*O*-arabinoside*	$C_{20}H_{18}O_{11}$	433.0776	301	1.6	Flavonol
36	15.5	Isorhamnetin-*O*-glucuronide	$C_{22}H_{20}O_{13}$	491.0831		1.4	Flavonol
37	15.8	Isorhamnetin-*O*-hexoside	$C_{22}H_{22}O_{12}$	477.1038	314, 300	0.6	Flavonol
38	16.1	Quercetin-3-*O*-rhamnoside*	$C_{21}H_{20}O_{11}$	447.0933		1.1	Flavonol
39	16.2	Isorhamnetin-3-*O*-rutinoside*	$C_{28}H_{32}O_{16}$	623.1618		0.0	Flavonol
40	16.2	Isorhamnetin-*O*-hexoside	$C_{22}H_{22}O_{12}$	477.1038	314, 300	0.8	Flavonol
41	16.4	Phloretin-*O*-hexose pentoside	$C_{26}H_{32}O_{14}$	567.1719	273	0.9	Dihydrochalcone
42	16.5	Isorhamnetin-3-*O*-glucoside*	$C_{22}H_{22}O_{12}$	477.1038	314, 300	0.8	Flavonol
43	17.0	Cyanidin-*O*-rhamnoside*	$C_{21}H_{20}O_{10}$	433.1129†	287	0.2	Anthocyanin
44	17.0	Phloridzin*	$C_{21}H_{24}O_{10}$	435.1297	273	0.5	Dihydrochalcone
45	17.3	Quercetin deoxyhexose hexoside	$C_{30}H_{26}O_{14}$	609.1250	463, 301	1.3	Flavonol
46	17.6	Quercetin*	$C_{15}H_{10}O_7$	301.0354	273, 151	2.0	Flavonol
47	18.1	Kaempferol*	$C_{15}H_{10}O_6$	285.0405		1.4	Flavonol
48	18.2	Isorhamnetin*	$C_{16}H_{12}O_7$	315.0510	300	1.3	Flavonol

*Identified with authentic standard.
†*m/z* are based on protonated pseudomolecular ions ($[M+H]^+$) and other *m/z* are based on deprotonated pseudomolecular ions ($[M-H]^-$).

Anthocyanins

All anthocyanins (Table 5, peaks 13, 19, 24, 34, 43) were identified as cyanidin glycosides in the ESI(+) ion mode. Cyanidin-3-*O*-galactoside (peak 13), cyanidin-3-*O*-arabinoside (peak 19) and cyanidin-3-*O*-rhamnoside (Table 5, peak 43) were identified by comparing the HPLC retention time and MS data with those of authentic anthocyanin standards. Cyanidin-*O*-pentoside and cyanidin-*O*-hexoside (Table 5, peaks 24 and 34) had pseudomolecular ions at *m/z* 419.0973 and 449.1078 $[M+H]^+$, respectively. Cyanidin glycosides (Table 5, peaks 13, 19, 24, 43) produced a characteristic fragment ion of *m/z* 287.0550 $[M+H]^+$, corresponding to the loss of conjugated sugar moieties.

Dihydrochalcones

Two phloretin-related conjugates (phloretin-*O*-hexose pentoside (Table 5, peak 41) and phloridzin (Table 5, peak 44)) were identified in the apple peel samples. The identification of phloridzin was confirmed with an authentic standard. The qTOF MS/MS spectra of phloretin-*O*-hexose pentoside and phloridzin (Table 5, peaks 41 and 44) are characterized by a strong fragment ion at *m/z* 273.0768 $[M-H]^-$, corresponding to the loss of sugars (hexose pentoside and a glucose moiety, respectively). Phloretin-*O*-hexose pentoside may be phloretin-2′-xyloglucoside. Previous study reported phloretin-2′-xyloglucoside in Limoncella apples.[33]

Flavan-3-ols (monomers and proanthocyanidins)

Monomers of flavan-3-ols identified in apple peel samples include catechin (Table 5, peak 8), epicatechin (Table 5, peak 18) and their conjugate forms (Table 5, peaks 3, 5 and 10). Oligomeric forms of (epi)catechin (i.e. proanthocyanidins) were also identified (Table 5, peaks 6, 11, 15 and 22). Catechin and epicatechin showed a pseudomolecular ion at *m/z* 289.0718 $[M-H]^-$. Catechin and epicatechin (Table 5, peaks 8 and 18) produced fragment ion at *m/z* 245.0819, as reported in a previous study [53]. Two catechin-*O*-hexoside peaks (Table 5, peaks 3 and 5) produced a strong fragment ion at *m/z* 289.0718 $[M-H]^-$, resulting from the loss of a hexose moiety (Table 5, peaks 3 and 5). The qTOF MS2 spectra of two dimers (Table 5, peaks 6 and 15) are characterized by a predominant fragment ion at *m/z* 289.0718, corresponding to the (epi)catechin unit. The MS/MS spectra of catechin and epicatechin show characteristic fragmentation patterns, which was also confirmed by comparing them with the fragmentation pattern of their authentic standards.

Flavanones and flavonols

Two flavanones, naringenin-*O*-hexoside and eriodictyol-7-glucoside (Table 5, peaks 27 and 29), produced strong fragment ions at *m/z* 271.0612 $[M-H]^-$ and 287.0561 $[M-H]^-$, respectively, resulting from the loss of the conjugated sugar moiety.

The quercetin-*O*-dihexoside peak (Table 5, peak 23) produced fragment ions at *m/z* 463.0882 $[M-H-162.0528]^-$ and *m/z* 301.0354 $[M-H-162.0528-162.0528]^-$, corresponding to the sequential loss of two hexose moieties.

Quercetin aglycone (Table 5, peak 46) and isorhamnetin aglycone (Table 5, peak 48) showed pseudomolecular ions at *m/z* 301.0354 $[M-H]^-$ and 315.0510 $[M-H]^-$, respectively. The MS/MS spectra of quercetin aglycone show the characteristic fragmentation pattern of quercetin aglycone (Table 5, peak 46), as reported previously.[58] Rutin (quercetin-3-*O*-rutinoside) and quercetin-3-*O*-arabinoside (Table 5, peaks 31 and 35) are characterized by a strong fragment ion at *m/z* 301.0354 $[M-H]^-$, resulting from the loss of rutinoside and arabinoside, respectively. The fragmentation pattern agreed with a previous study.[34] Quercetin deoxyhexose hexoside (Table 5, peak 45) had predominant peak ions at *m/z* 463.0882 and 301.0354 $[M-H]^-$, corresponding to the sequential loss of hexose and deoxyhexose moieties. It is predicted that quercetin glycosides produce quercetin aglycone (*m/z* 301.1354 $[M-H]^-$) after the loss of glycosyl moieties in the ESI negative mode. However, two quercetin-*O*-hexose pentoside isomers, quercetin-3-*O*-galacoside, quercertin-3-*O*-glucoside and quercetin-3-*O*-xyloside (Table 5 peaks 25, 28, 30, 32 and 33) produced a strong fragment ion at *m/z* 300.0276 $[M-H]^-$ in addition to a fragment ion at *m/z* 301.0354 $[M-H]^-$. The fragment ion at *m/z* 300.0276 $[M-H]^-$ could have been produced by the formation of deprotonated radical aglycone during the heterolytic cleavage of glycoside or by proton loss and quinone formation.[58]

Myricetin-*O*-hexoside (Table 5, peak 21) produced a fragment ion at *m/z* 317.0303 $[M-H]^-$ after the loss of the conjugated hexose moiety and an additional fragment ion at *m/z* 316.0225 $[M-H]^-$. To the best of our knowledge, we are the first to identify myricetin-*O*-hexoside in apples, although a previous study did report myricetin-*O*-glycoside in cashew apples (*Anacardium occidentale*).[59] The MS/MS spectra of two isorhamnetin-*O*-hexoside isomers and isorhamnetin-3-*O*-glucoside (Table 5, peaks 37, 40 and 42) show a fragment ion at *m/z* 315.0510 $[M-H]^-$, which corresponds to a conjugated sugar loss, and a fragment ion at *m/z* 314.0432 $[M-H]^-$. The fragment ions at *m/z* 316.0225 $[M-H]^-$ for myricetin-*O*-hexoside (Table 5, peak 21), *m/z* 314.0432 $[M-H]^-$ for isorhamnetin hexoside (Table 5 peaks 37, 40 and 42) and *m/z* 300.0276 $[M-H]^-$ for isorhamnetin aglycone (Table 5, peak 48) could have been produced by the same reaction as the fragment ion at *m/z* 300.0276 $[M-H]^-$ produced by the quercetin glycoside peaks (Table 5 peaks 25, 28, 30, 32 and 33). The identification of eriodictyol-7-glucoside, quercetin-3-*O*-galactoside, rutin, quercetin-3-*O*-glucoside, quercetin-3-*O*-xyloside, quercetin-3-*O*-arabinoside, quercetin-3-*O*-rhamnoside, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, quercetin aglycone and isorhamnetin aglycone was performed using authentic standards.

Phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids)

Protocatechuic acid produced a weak pseudomolecular ion at *m/z* 153.1093 $[M-H]^-$ and a base peak at *m/z* 109.0295 $[M-H]^-$, corresponding to the loss of the CO_2 moiety from carboxylic acid (Table 5, peak 1).[5] The MS/MS spectrum of chlorogenic acid is characterized by a strong fragment ion at *m/z* 191.0562 $[M-H]^-$, resulting from the loss of the caffeoyl moiety (Table 5, peak 7). Two coumaroylquinic acid isomers (Table 5, peaks 12 and 16) had a weak pseudomolecular ion at *m/z* 337.0929 and produced a base peak ion at *m/z* 173.0459, corresponding to the loss of coumaroyl and an H_2O moiety. The fragmentation pattern of chlorogenic acid (Table 5, peak 7) and coumaroylquinic acid isomers (Table 5, peaks 12 and 16) is in good agreement with a previous study.[5] Other coumaroylquinic acid isomers (Table 5, peaks 14 and 20) had a weak pseudomolecular ion at *m/z* 337.0929 and produced a base peak ion at *m/z* 191.0565, resulting from the loss of the coumaroyl moiety alone. Identification of protocatechuic acid, chlorogenic acid and 4-hydroxybenzoic acid was performed using their authentic standards.

Relative quantification of phenolic compounds in apple peels using UHPLC-(ESI)-qTOF

We used extracted ion chromatograms (EICs) from the UHPLC-(ESI)-qTOF MS1 data to find the peak areas of each

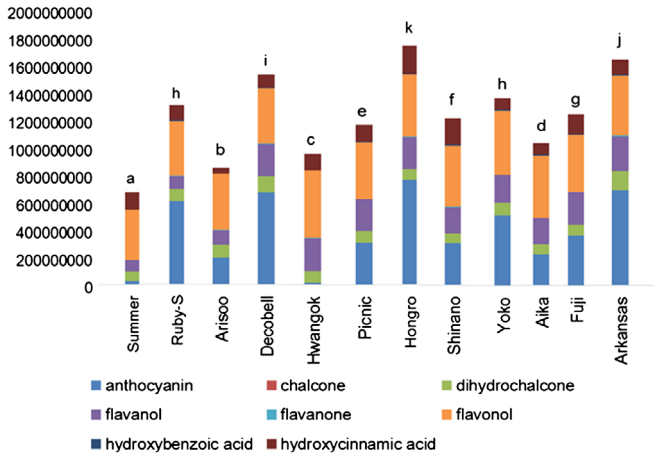

Figure 2. Average extracted ion chromatogram (EIC) peak areas of phenolic compounds in the peels of 12 apple cultivars from the MS1 data of UHPLC-(ESI)-qTOF. Significant differences between cultivars at $P < 0.05$ are denoted by different letters.

compound with the chosen *m/z*, based on protonated pseudomolecular ions ($[M+H]^+$) in the ESI positive mode and deprotonated pseudomolecular ions ($[M-H]^-$) in the ESI negative mode. The EIC peak areas of each phenolic compound in the apple peel extracts were used to compare each phenolic compound among the different apple cultivars (Fig. 2 and Table S1). For a simple interpretation of the UHPLC-(ESI)-qTOF data, the 48 identified phenolic compounds were grouped into the following subclasses: anthocyanins, chalcones, dihydrochalcones, flavanols, flavanones, flavonols, hydroxybenzoic acids and hydroxycinnamic acids (Table 5). Anthocyanins and flavonols comprised the two predominant subclasses of phenolics for all apple cultivars compared. Significant variability in the phenolic subclasses was observed across cultivars. For example, Arkansas Black, Decobell and Hongro had the highest levels of anthocyanins as compared to other cultivars examined. This result agrees with the HPLC data (Table 4). Ruby-S and Decobell apples showed significantly higher sum of peak areas for the identified compounds than Fuji apples.

Hongro and Ruby-S apple peels had relatively large peak areas for cyanidin-*O*-hexoside (Table S1, peak 34) and cyanidin-*O*-pentoside (Table S1, peak 24). The sum of the peak area for two (epi)catechin-*O*-hexoside isomers (Table S1, peaks 3 and 5) resulted in a 13-fold difference among the different apple cultivars (from 754 977 in Arisoo to 9 935 140 in Shinano Sweet). Using this same approach, the sum of the four proanthocyanin isomers (Table S1, peaks 6, 11, 15 and 22) ranged between 53 947 400 (Summer King) and 154 529 475 (Hwangok). Eriodictyol-7-*O*-glucoside had a higher area in Arkansas Black (8 492 343) and Hongro (2 944 385) than in the other apple cultivars (n.d.–891 684). Arkansas Black (22 393 820) had a significantly higher area for isorhamnetin conjugates than the other cultivars (1 397 309–12 716 690) ($P < 0.05$).

In addition to chlorogenic acid, identified using HPLC, nine additional phenolic acids were identified and quantified using UHPLC-(ESI)-qTOF. When the areas of the coumaroylquinic acid isomers were summed, we found a 9.0-fold difference among the apple cultivars (13 019 301 in Arisoo peel to 117 121 232 in Shinano Sweet peel). Among the hydroxybenzoic acids, 4-hydroxybenzoic acid was detected only in Aika No Kaori, Arkansas Black, Decobell, Fuji, Shinano Sweet and Yoko apple peel extracts. When the areas of the hydroxybenzoic acid isomers were summed, Arisoo, Fuji, Hwangok, Ruby-S, Yoko and Summer King showed a significantly lower area (823 660–1 252 032) than the other apple cultivars (1 648 444–2 850 869) ($P < 0.05$). There were 21.9-fold differences among the cultivars (218 068–4 776 082) in the area of protocatechuic acid in the apple peel extract.

CONCLUSIONS

Decobell, Hwangok and Picnic apples showed similar sugar-to-acid ratios to Fuji apples (one of the most popular apple cultivars worldwide). The peel and pulp of Decobell apples showed the highest total phenolic content and antioxidant activity among the 12 apple cultivars. Phenolic compounds known to facilitate antioxidant activity were identified and quantified in apple peel/pulp using HPLC. The sum of phenolic content was also higher in Decobell apples than in the other cultivars in this study. Using UHPLC-(ESI)-qTOF, 48 phenolic compounds were identified in apple peels. Decobell apples showed higher sum of peak areas of 48 identified phenolic compounds than Fuji apples ($P < 0.05$). Thus, Decobell apples may have the best quality characteristics based on their sugar-to-acid ratios and antioxidative phenolic contents among the new early- to mid-season apple cultivars.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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