

Phytochemical Distribution Among Selected Advanced Apple Genotypes Developed for Fresh Market and Processing

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Abstract

The phenolic composition of the flesh and peel of five advanced apple genotypes developed for processing and 14 cultivars was investigated using high-performance liquid chromatography (HPLC). The total phenolic content (TPC) was investigated using the Folin-Ciocalteu method, and the total antioxidant capacity (TAC) was investigated using a ferric reducing antioxidant power (FRAP) assay. 'Floribunda Rosea' was found to possess the highest TPC, total phenolic index (TPI) and TAC values, whereas 'Eden™' (also known as 'SJCA38R6A74') had the lowest. The profiles of the phenolic compounds varied among the 19 genotypes, and the peel showed higher concentrations than the flesh. The apples studied were found to contain 14 individual phenolic compounds, with epicatechin and procyanidin B2 being the most abundant phenolic compounds in the peel, and chlorogenic acid being the most abundant phenolic compound in the flesh. Procyanidins were the most predominant group in both the flesh and the peel, and 'Eden™' was the only apple selection that did not contain any procyanidins in its flesh. No flavonols were detected in the flesh of some genotypes ('Cortland', 'McIntosh Summerland' and 'Spartan'). Cyanidins were found essentially in red apple peels ('Cortland', 'Primevert', 'SJC649' and 'SJC7123-1'). The significant variation in antioxidant capacity and total phenolic compounds clearly shows the potential value of certain new cultivars and advanced lines as parents in a breeding program.

Keywords: *HPLC; phenolics; flavonols; procyanidins; cyanidins; hydroxycinnamic acids; dihydrochalcones; apple; antioxidants*

Introduction

An increasing amount of work is being done nowadays to identify or develop specialty apple genotypes because of their potential high antioxidant capacity and their use for value-added food processing (Khanizadeh et al. 2006). Antioxidants help neutralize free radicals, which are

unstable molecules that are linked to the development of a number of degenerative diseases and conditions, including cancer, cardiovascular disease, cognitive impairment, immune dysfunction, cataracts and macular degeneration (Lea and Timberlake 1974; Lea 1990; Robards et al. 1999; Sanoner et al. 1999; Bushway et al. 2002; Van der Sluis et al. 2002; Folts 2002). Among the phytochemicals, phenols have received a great deal of attention because of their antioxidant properties (Tsuda et al. 1994). Significant amounts of phenolic compounds frequently occur in foods such as fruits and vegetables and are routinely consumed in our diet (Cao et al. 1996; Wang et al. 1996; Eberhardt et al. 2000). Phenolic compounds make an important contribution to the sensory qualities (colour, flavour and taste) of fresh fruits and vegetables and their products (Bushway et al. 2002; Robards et al. 1999; Van der Sluis et al. 2002). In addition, many phenolic phytochemicals have antioxidative, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory activities (Cao and Cao 1999; Eberhardt et al. 2000; Ito et al. 1998; Kawaii et al. 1999; Kim et al. 2000).

Apples are excellent sources of several polyphenols with high antioxidant capacities. Wang et al. (1996) ranked apples 9th out of 12 tested fruits, and Vinson et al. (2001) placed them 8th out of 20. The total extractable phenolic content was investigated and found to range from 110 to 357 mg/100 g⁻¹ fresh apple (Podsdek et al. 2000; Liu et al. 2001).

The distribution and composition of phenolic phytochemicals are affected by a number of factors: cultivar, fruit part, growing season, environmental conditions, horticultural practices, geographic origin, postharvest storage conditions and processing procedures (Burda et al. 1990; Amiot et al. 1992; Spanos and Wrolstad 1992; Robards et al. 1999; Awad and De Jager 2002; Awad et al. 2000; Van der Sluis et al. 2001; Wang et al. 2007). Alonso-Salces et al. (2004) identified and quantified the individual phenolic constituents of 31 Basque cider apple cultivars using a high-performance liquid chromatography (HPLC) method with a photo diode array detector (DAD) and reported considerable differences in total polyphenolic content among the tested cultivars. Several authors reported that apples had a higher “total oxyradical scavenging capacity” (TOSC) in the peel than in the flesh (Eberhardt et al. 2000; MacLean et al. 2003; Wolfe et al. 2003). The concentration of total phenolic compounds is not only much greater in the peel than in the flesh (Burda et al. 1990; Ju et al. 1996; Escarpa and Gonzalez 1998), but quercetin glycosides are also reported only in the peel (Escarpa and Gonzalez 1998; Van der Sluis et al. 2001; Tsao et al. 2003, 2005). Anthocyanins, which contribute to the red color of apple fruits, are found exclusively in the peel (Awad et al. 2000).

Various apple varieties contain five major groups of polyphenolic compounds: hydroxycinnamic acids, procyanidins, cyanidins, flavonols and dihydrochalcones (Lee et al. 2003; Sun et al. 2002). Chlorogenic acid, neochlorogenic acid, *p*-coumaroylquinic acid, catechin, epicatechin, procyanidin B1, procyanidin B2, cyanidin-3-galactoside, quercetin-3-galactoside, quercetin-3-xyloside, quercetin-3-arabinoside, quercetin-3-rhamnoside, phloridzin and phloretin-3-xyloglucoside are the major individual polyphenolics in apple fruits (Oleszek et al. 1988; Guyot et al. 1998; Sanoner et al. 1999; Tsao et al. 2003; Alonso-Salces et al. 2004).

The objectives of the present study were to quantify the individual phenolic compounds in selected advanced apple lines and to determine their total phenolic content (TPC) and total antioxidant capacity (TAC) in comparison with known cultivars. The future goal is to determine the effect of these chemical compositions on processing, including the selection of lines for fresh-cut apples (e.g. non-browning apples for apple slices), cider, juice (e.g. non-browning juice) and ice cider.

Materials and methods

Chemicals

Gallic acid, chlorogenic acid, *p*-coumaric acid, catechin, epicatechin, quercetin, sodium carbonate (Na₂CO₃) and the Folin-Ciocalteu (FC) reagent were obtained from Sigma Chemical Co. (St.

Louis, Missouri). Quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-rhamnoside and quercetin-3-rutinoside (rutin) were obtained from Fluka Chemie GmbH (Buchs, Switzerland). Quercetin-3-arabinoside and quercetin-3-xyloside were obtained from Apin Chemicals Ltd. (Abingdon, England). Procyanidins B1 and B2, phloridzin and cyanidin-3-galactoside were obtained from Indofine Chemical Company Inc. (Hillsborough, New Jersey). *p*-coumaroylquinic acid was provided by Dr. Jürgenliemk (University of Münster, Germany). The water used for the HPLC analysis was purified in-house from distilled and deionized water using a NanoPure® system (Dubuque, Iowa). All other HPLC-grade solvents were obtained from Caledon Laboratories Ltd. (Georgetown, Ontario).

Sample preparation and extraction procedure

The 19 apple genotypes and cultivars were picked at commercial maturity during the 2003 harvest season at the Agriculture and Agri-Food Canada experimental station in Frelighsburg, Quebec (45°N, 72°W). For the fruit samples, two batches of 10 apples per genotype were randomly selected, peeled (2 to 3 mm thickness), cored, cut into small pieces, frozen immediately in liquid nitrogen, and stored at –80°C until analysis.

From each apple genotype, 10 g frozen peel and flesh were pooled separately and ground in liquid nitrogen in a mortar. The ground sample and 70% aqueous methanol (1:1, w/v) were homogenized in a beaker using a Polytron® blender (Brinkmann Instruments, New York). The mixture was filtered through filter paper (Whatman No. 1) and a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, Michigan). The final filtrate was stored at –20°C before analysis.

Polyphenol analysis using HPLC

The phenolic composition of apples was measured using an Agilent Technology 1100 Series HPLC system equipped with a quaternary pump, a degasser, a thermostatic autosampler and a diode array detector (DAD) (Tsao and Yang 2003). Briefly, the polyphenolics were separated using a Phenomenex Luna C18 (2) analytical column (250 × 4.6 mm i.d.; particle size: 5 µm) with a C18 guard column. The binary mobile phase was a mixture of 6% acetic acid and acetonitrile (solvent B), pumped at flow rate of 1 ml min⁻¹ for a total run time of 70 min. The gradient program was as follows: 0% B to 15% B in 45 min, 15% B to 30% B in 15 min, 30% B to 50% B in 5 min, and 50% B to 100% B in 5 min. The injection volume was 10 µl for all samples. The detector was set at 280, 320, 360 and 520 nm for simultaneous monitoring of the different groups of phenolic compounds. Compounds were tentatively identified by comparing retention times and UV-vis spectra with those of the standards in the library that were built using the in-line DAD with a three-dimensional feature.

The total phenolic index (TPI) was calculated according to Tsao and Yang (2003) and Tsao et al. (2005). All samples were prepared and analyzed in duplicate. The results were expressed as µg g⁻¹ fresh-frozen weight.

Total phenolic content

The TPC was determined using the FC method (Slinkard and Singleton 1977) with slight modifications. Briefly, 0.2 ml standard or sample extract, 1.0 ml FC reagent and 0.8 ml Na₂CO₃ (7.5%) were mixed in a 20 ml vial and allowed to stand for 30 min at room temperature. Absorption was determined at 765 nm in a Varian Cary® 3C spectrophotometer (Varian Analytical Instruments, Harbor City, California). Gallic acid solutions were prepared for the generation of a standard curve. Results were expressed as gallic acid equivalent (GAE) in µg g⁻¹ fresh-frozen weight. Concentrations beyond the highest point (500 µg ml⁻¹) of the linear range of the standard curve were diluted before final analysis.

Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) was estimated according to the method described by Benzie and Strain (1996) with modifications for the 96-well microplate reader as described previously (Tsao and Yang 2003). Briefly, standard ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) or sample extract (10 μl) was added to 300 μl ferric-TPTZ reagent in the wells. The reagent contained 300 mM acetate buffer, pH 3.6, plus 10 mM TPTZ in 40 mM HCl and 20 mM FeCl_3 at a ratio of 10:1:1 (v/v/v). The plate was warmed at 37°C for the duration of the reaction. The absorbance was measured at 593 nm at 0 and 4 min using a visible-UV kinetic microplate reader (EL 340, BioTek Instruments, Inc., Winooski, Vermont). The FRAP value of the samples was expressed on the basis of 500 μM L-ascorbic acid.

Statistical analysis

The data were analyzed using the ANOVA and GLM procedures of SAS (1989), and the means were separated using the least significant difference (LSD) test at the 0.05 level.

Results

Phenolic composition (HPLC)

A total of 14 polyphenolic compounds belonging to five major polyphenolic groups were identified in apples (Tables 1 and 2). They are chlorogenic acid, neochlorogenic acid and *p*-coumaroylquinic acid (hydroxycinnamic acids); catechin, epicatechin, procyanidin B1 and procyanidin B2 (procyanidins); quercetin-3-galactoside, quercetin-3-xyloside, quercetin-3-arabinoside and quercetin-3-rhamnoside (flavonols); phloridzin and phloretin-3-xyloglucoside (dihydrochalcones); and cyanidin-3-galactoside (cyanidins).

The mean total phenolic concentration (TPI, measured by HPLC) in the peel was 2.6 times greater (1465.2 $\mu\text{g g}^{-1}$) than the concentration in the flesh (564.1 $\mu\text{g g}^{-1}$), with 'Floribunda Rosea' and 'Eden™', a newly released cultivar (Khanizadeh et al. 2006), containing the highest and the lowest TPI values, respectively, in both the flesh and the peel (Tables 1 and 2).

Among the five groups, procyanidins were the most predominant phenolic group and contributed to 49.7% and 48.7% of the TPI of the flesh and peel, respectively (Tables 1 and 2). The total procyanidins ranged from 93.9 to 3989.7 $\mu\text{g g}^{-1}$ in the peel and from 0 to 1928.5 $\mu\text{g g}^{-1}$ in the flesh. In both the peel and the flesh, 'Floribunda Rosea' had the highest total procyanidin concentrations and 'Eden™' had the lowest. 'Eden™' was also the only apple selection that contained no procyanidins in its flesh (Table 2). In addition, procyanidin B1 was detectable in the flesh of certain apple genotypes only ('Floribunda Rosea', 'Macspur' and 'McIntosh Summerland') (Table 2). In all genotypes, epicatechin (18.9%) and procyanidin B2 (14.4%) were the most abundant phenolic compounds in the peel (Table 1).

Hydroxycinnamic acids were the second most abundant group in the flesh with 44.0% of total phenolics, ranging from 37.0 to 1607.5 $\mu\text{g g}^{-1}$ (Table 2). In the peel, hydroxycinnamic acids accounted for only 11.8% and ranged from 33.4 to 831.3 $\mu\text{g g}^{-1}$ (Table 1). 'Floribunda Rosea' presented the richest composition in the flesh and the peel (Tables 1 and 2). Three hydroxycinnamic acid compounds were found in these apple genotypes, and their concentrations generally appeared in the following order: chlorogenic acid (35.4% of TPI in the flesh and 8.6% of TPI in the peel), *p*-coumaroylquinic acid (5.9% and 1.5%) and neochlorogenic acid (2.7% and 0.9%). In all genotypes, chlorogenic acid was the most abundant phenolic compound in the flesh (35.4%), ranging from 15.6 $\mu\text{g g}^{-1}$ ('Eden™') to 1374.7 $\mu\text{g g}^{-1}$ ('Floribunda Rosea') (Table 2).

Flavonols were the group with the second highest concentration in apple peel with 21.7% of TPI (Table 1). The total flavonol content in the peel varied between 90.6 and 487.6 $\mu\text{g g}^{-1}$, with 'Primevert' and 'Reinette Russet' showing the highest and lowest concentrations, respectively.

Table 1. Phenolic composition ($\mu\text{g g}^{-1}$ fresh-frozen weight) in the peel of selected advanced apple lines compared to selected standard cultivars.

	Cortland	Eden™	Eistar	Floribunda Rosea	Gala	Golden Delicious	GoldRush	Macspur	McIntosh Summerland	Paula Red	Primevert	Reinette Russet	SJC649	SJC7123-1	SJC7713-1	SJC8234-1	SJCA16R5A15	Spartan	SuperMac	Mean	%	LSD
Chlorogenic acid	11.4	11.0	14.1	757.8	112.1	99.3	100.6	85.8	122.7	113.8	36.2	277.2	125.3	95.3	31.9	250.9	24.5	122.5	4.3	126.1	8.6	23.7
Neochlorogenic acid	33.6	6.3	7.9	ND	0.0	5.7	12.2	16.6	18.8	15.6	ND	27.6	23.4	22.2	ND	20.7	5.5	17.9	14.6	13.0	0.9	6.6
<i>p</i> -Coumaroylquinic acid	19.0	11.7	13.8	73.5	14.2	11.2	3.7	38.1	44.5	32.3	14.9	69.6	5.0	4.7	6.3	24.8	3.5	13.6	3.3	21.4	1.5	7.3
Other hydroxycinnamic acids	32.3	9.8	ND	ND	ND	10.8	16.6	27.8	33.5	25.2	ND	22.2	ND	50.2	ND	18.7	4.1	ND	11.2	13.8	0.9	8.3
Total hydroxycinnamic acids	96.3	38.8	35.8	831.3	126.3	127.0	133.1	168.3	219.5	186.9	51.1	396.6	153.7	172.4	38.2	315.1	37.6	154.0	33.4	174.4	11.8	-
Catechin	40.9	21.0	13.2	318.9	13.9	6.7	92.7	26.0	23.7	104.0	23.5	69.9	24.2	42.4	25.9	43.4	5.4	39.6	69.0	52.8	3.6	20.5
Epicatechin	165.1	44.6	303.4	1380.8	269.4	203.3	351.4	130.4	195.0	277.1	445.2	214.8	211.2	116.1	264.8	242.6	149.4	207.4	99.1	277.4	18.9	66.5
Procyanidin B1	21.4	0.0	33.6	210.1	32.3	19.4	34.2	32.0	185.7	44.7	49.8	59.2	28.1	24.0	39.4	31.0	23.2	43.3	32.6	49.7	3.4	98.9
Procyanidin B2	111.1	23.8	239.7	1236.6	208.4	168.6	302.7	92.6	115.7	148.1	282.5	228.1	115.2	70.4	118.2	172.8	153.3	142.1	77.0	210.9	14.4	41.6
Other procyanidins	43.7	4.5	92.1	843.3	83.9	57.5	116.6	57.4	97.1	114.8	173.1	158.3	80.9	37.8	102.7	62.2	37.4	123.1	52.0	123.1	8.4	28.6
Total procyanidins	382.2	93.9	682.0	3989.7	607.9	455.5	897.6	338.4	617.1	688.7	974.1	730.3	459.6	290.7	551.0	552.0	368.7	555.5	329.7	713.8	48.7	-
Cyanidin-3-galactoside	144.0	63.7	70.1	95.2	77.9	0.0	0.0	51.3	46.5	77.4	168.4	0.0	237.0	63.9	171.3	85.3	0.0	105.4	35.6	78.6	5.4	75.9
Cyanidin-3 galactoside equiv.	7.0	0.0	0.0	0.0	5.0	0.0	0.0	4.2	0.0	0.0	10.4	0.0	43.0	0.0	8.3	0.0	0.0	4.5	0.0	4.3	0.3	10.6
Total cyanidins	151.0	63.7	70.1	95.2	82.9	0.0	0.0	55.5	46.5	77.4	178.8	0.0	280.0	63.9	179.6	85.3	0.0	109.9	35.6	82.9	5.7	-
Quercetin-3-galactoside	89.6	46.7	77.0	141.9	173.5	117.4	66.5	84.9	115.6	54.5	86.7	16.1	84.1	98.1	58.2	104.3	66.8	66.1	64.6	84.8	5.8	50.2
Quercetin-3-xyloside	50.8	24.0	21.8	27.8	31.3	23.0	29.7	75.5	100.9	26.7	33.6	13.0	28.7	32.3	22.3	32.4	17.8	20.3	36.2	34.1	2.3	16.3
Quercetin-3-arabinoside	27.3	18.0	20.8	54.2	34.7	30.6	44.3	40.3	51.5	38.2	72.1	16.4	33.6	28.9	28.6	53.2	46.5	20.6	26.0	36.1	2.5	17.6
Quercetin-3-rhamnoside	68.1	38.4	4.5	10.6	12.1	4.4	9.3	91.3	108.6	45.6	11.6	29.7	76.0	56.9	53.3	12.6	6.2	44.5	59.8	39.1	2.7	30.6
Other flavonols	79.1	43.2	57.2	161.8	170.8	144.0	119.1	113.3	137.7	55.0	283.6	15.4	179.1	134.4	151.8	321.5	46.9	42.6	75.3	122.7	8.4	59.6
Total flavonols	314.9	170.3	181.3	396.3	422.4	319.4	268.9	405.3	514.3	220.0	487.6	90.6	401.5	350.6	314.2	524.0	184.2	194.1	261.9	317.9	21.7	-
Phloridzin	38.0	26.7	48.3	288.7	31.6	73.5	99.8	43.3	43.5	49.8	86.3	668.6	31.2	40.8	78.4	146.8	19.5	40.0	29.5	99.1	6.8	42.8
Phloretin derivative	20.6	25.7	53.4	158.1	49.6	57.1	81.9	31.0	29.5	26.7	24.6	485.2	21.3	39.7	37.0	103.6	26.0	22.1	39.5	69.9	4.8	15.6
Extra peaks	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	132.9	ND	ND	ND	ND	ND	ND	ND	7.0	0.5	-
Total dihydrochalcones	58.6	52.4	101.7	446.8	81.2	130.6	181.7	74.3	73.0	76.5	110.9	1286.7	52.5	80.5	115.4	250.4	45.5	62.1	69.0	176.3	12.0	-
Total phenolics index (TPI) ^a	1002.5	418.9	1070.6	6349.7	1320.5	1031.9	1481.0	1041.7	1470.1	1249.2	1802.3	2504.0	1346.7	957.8	1198.0	1726.5	636.5	1075.3	729.3	1465.2	100	361.4

^a Data are the average of duplicates; ND: not detected.

Table 2. Phenolic composition ($\mu\text{g g}^{-1}$ fresh-frozen weight) in the flesh of selected advanced apple lines compared to selected standard cultivars

	Cortland	Eden™	Elstar	Floribunda Rosea	Gala	Golden Delicious	GoldRush	Macspur	McIntosh Summerland	Paula Red	Primevert	Reinette Russet	SJC649	SJC7123-1	SJC7713-1	SJC8234-1	SJCA16R5A15	Spartan	SuperMac	Mean	%	LSD
Chlorogenic acid	62.4	15.6	47.4	1374.7	137.7	135.8	124.2	165.9	226.0	201.3	96.0	275.2	48.9	170.3	112.4	328.6	85.0	156.3	28.3	199.5	35.4	34.1
Neochlorogenic acid	25.6	4.0	2.4	84.2	2.8	1.2	4.1	10.3	10.3	6.6	1.4	7.4	90.0	21.8	0.0	8.3	0.0	9.0	3.6	15.4	2.7	15.0
<i>p</i> -Coumaroylquinic acid	46.1	26.8	21.0	148.6	22.8	22.2	6.2	57.0	55.1	47.2	26.3	44.0	3.2	7.9	9.5	46.4	9.9	22.5	5.1	33.0	5.9	14.4
Total hydroxycinnamic acids	134.1	46.4	70.8	1607.5	163.3	159.2	134.5	233.2	291.4	255.1	123.7	326.6	142.1	200.0	121.9	383.3	94.9	187.8	37.0	248.0	44.0	-
Catechin	26.3	0.0	18.4	252.1	23.2	15.5	25.2	32.2	26.1	55.3	26.9	62.2	24.9	28.1	23.6	34.3	11.7	43.0	27.0	39.8	7.0	28.4
Epicatechin	56.4	0.0	84.9	957.5	63.3	63.1	91.4	50.5	52.9	86.1	90.1	119.5	42.6	45.5	76.0	100.0	54.6	77.7	56.4	114.1	20.2	36.3
Procyanidin B1	0.0	0.0	0.0	119.7	0.0	0.0	0.0	17.6	15.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.1	1.4	18.0
Procyanidin B2	62.5	0.0	88.4	150.4	66.4	79.3	110.3	64.7	68.5	103.8	98.4	133.8	59.5	65.1	71.3	122.3	69.0	88.9	69.7	82.7	14.7	25.6
Other procyanidins	10.7	0.0	9.6	448.8	18.3	17.9	19.2	20.7	12.6	10.1	12.7	24.8	11.7	7.7	15.4	9.3	9.3	7.2	13.1	35.7	6.3	14.3
Total procyanidins	155.9	0.0	201.3	1928.5	171.2	175.8	246.1	185.7	175.9	255.3	228.1	340.3	138.7	146.4	186.3	265.9	144.6	216.8	166.2	280.4	49.7	-
Cyanidin-3-galactoside	ND	ND	ND	15.4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.8	0.1	-
Total cyanidins	ND	ND	ND	15.4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.8	0.1	-
Quercetin-3-rhamnoside	0.0	2.6	2.6	16.1	2.3	6.4	8.7	4.6	0.0	4.3	6.8	3.2	3.5	4.4	6.8	3.4	2.6	0.0	2.9	4.3	0.8	1.6
Total flavonols	0.0	2.6	2.6	16.1	2.3	6.3	8.7	4.5	0.0	4.3	6.8	3.2	3.5	4.4	6.8	3.4	2.6	0.0	2.9	4.3	0.8	-
Phloridzin	7.3	5.6	6.7	20.3	5.7	11.9	11.5	7.3	11.0	3.8	12.3	16.8	5.0	6.2	9.0	14.0	4.6	9.3	5.6	9.1	1.6	4.1
Phloretin-3-xyloglucoside	12.8	13.6	10.4	92.8	10.1	16.0	18.8	16.7	17.3	18.1	10.8	68.2	8.9	11.2	14.8	40.1	5.9	9.5	13.3	21.5	3.8	4.1
Total dihydrochalcones	20.1	19.2	17.1	113.1	15.8	27.9	30.3	24.0	28.3	21.9	23.1	85.0	13.9	17.4	23.8	54.1	10.5	18.8	18.9	30.6	5.4	-
Total phenolics index (TPI) ^a	309.9	68.1	291.6	3680.6	352.4	369.0	419.4	447.3	495.4	536.2	381.6	755.0	298.0	368.1	338.5	706.3	252.4	423.2	224.8	564.1	100	113.3

^a Data are the average of duplicates; ND: not detected.

The prevailing flavonols in the peel were quercetin-3-galactoside (5.8%), with ample amounts of quercetin-3-rhamnoside (2.7%), quercetin-3-arabinoside (2.5%), and quercetin-3-xyloside (2.3%) also present. Quercetin-3-rhamnoside, the only compound of the flavonol class detected in the flesh, was below the detection limit in some genotypes ('Cortland', 'McIntosh Summerland' and 'Spartan') (Table 2).

Dihydrochalcones were found in only minor amounts, 12.0% and 5.4% of TPI in the peel and the flesh, respectively (Table 1 and 2). In all genotypes, the amount of total dihydrochalcones varied between 10.5 and 113.1 $\mu\text{g g}^{-1}$ in the flesh and between 45.5 and 1286.7 $\mu\text{g g}^{-1}$ in the peel. In both the flesh and the peel, 'Floribunda Rosea' and 'Reinette Russet' had the most dihydrochalcones and 'SJCA16R5A15' had the fewest. Both phloretin-3-xyloglucoside and phloridzin were found in the flesh tissues, whereas phloridzin and phloretin derivatives were detected in the peel.

Cyanidins were found almost exclusively in the peel and represented less than 6% of TPI (Table 1). 'Floribunda Rosea' was the only genotype that contained cyanidins in the flesh (Table 2). The cyanidin content in the peel ranged from 0 to 280.0 $\mu\text{g g}^{-1}$, with the highest concentration observed in the 'SJC649' genotype (Table 1).

Total phenolic content

The TPC values (determined using the FC method and expressed as μg gallic acid equivalents [GAE] g^{-1} fresh weight) of the 19 selected apple genotypes are shown in Table 3. The peel of 'Floribunda Rosea' had the highest TPC (12358.7 $\mu\text{g GAE g}^{-1}$), whereas 'EdenTM' had the lowest (less than 300 $\mu\text{g g}^{-1}$). The remaining genotypes were intermediate, with the TPC varying between 458.1 and 1147.6 $\mu\text{g GAE g}^{-1}$. In the flesh, the TPC ranged from 45.5 to 4426.4 $\mu\text{g GAE g}^{-1}$, with the highest amount measured in 'Floribunda Rosea' and the lowest amount measured in 'EdenTM'.

Total antioxidant capacity

Great differences in TAC expressed as FRAP (μg ascorbic acid equivalents [AAE] g^{-1}) were found among the apple genotypes (Table 3). The ferric reducing ability of the peels was greater than that of the flesh for all genotypes. 'Floribunda Rosea' peel showed the greatest antioxidant capacity, with 6680.9 $\mu\text{g g}^{-1}$, compared to 'EdenTM', with 517.1 $\mu\text{g g}^{-1}$. In the flesh, the greatest antioxidant capacity was also found in 'Floribunda Rosea', with a FRAP value of 729.1 $\mu\text{g g}^{-1}$, while 'EdenTM' had the lowest activity with 102.9 $\mu\text{g g}^{-1}$.

Discussion

Within the selected apple genotypes, great variation was observed in the levels of total phenolics measured by both TPC and TPI as well as in the distribution and composition in the flesh and peel of measured phenolic compounds. Polyphenolics are powerful antioxidants, and apple fruits contain large amounts of phenolic compounds (Hamazu et al. 1999). Hydroxycinnamic acids, procyanidins, flavonols, dihydrochalcones and cyanidins were the five groups of phenolic compounds that were detected in the peel and flesh of the 19 selected advanced apple lines and commercial cultivars studied.

Wolfe et al. (2003) reported that apple peel is a rich source of antioxidants. Our results are similar to previous studies that showed that apple peel possesses a higher phenolic compound content and a higher antioxidant capacity than apple flesh (Escarpa and Gonzalez 1998; Lee et al. 2003; Awad et al. 2000; Alonso-Salces et al. 2004; Oleszek et al. 1988; Clifford 1999). Phenolic compounds tend to accumulate in the dermal tissues of plant bodies because of the compounds' potential roles in protecting against ultraviolet radiation, acting as attractants in fruit dispersal, and acting as defence chemicals against pathogens and predators (Liu et al. 2005).

Table 3. Total phenolic contents and antioxidant capacities (FRAP) of 19 advanced apple lines and cultivars.

Genotype	Total phenolic content ^a ($\mu\text{g g}^{-1}$)		FRAP - AAE ^b ($\mu\text{g g}^{-1}$)	
	Flesh	Peel	Flesh	Peel
Cortland	76.2	695.7	322.3	1158.7
Eden™	45.5	228.2	102.9	517.1
Elstar	162.0	719.8	295.1	999.6
Floribunda Rosea	4426.4	12358.7	729.1	6680.9
Gala	155.2	881.0	355.3	1299.9
Golden Delicious	147.5	737.3	385.6	1250.0
GoldRush	178.2	1029.4	415.3	1700.8
Macspur	181.7	671.7	333.6	778.6
McIntosh	197.1	688.6	411.2	1235.3
Summerland				
Paula Red	213.2	497.1	444.9	1209.5
Primevert	175.4	1147.6	388.8	1869.5
Reinette Russet	381.6	1092.7	592.3	1535.8
SJC649	107.5	881.0	358.4	1589.6
SJC7123-1	136.7	472.1	354.3	1033.5
SJC7713-1	164.3	869.9	374.2	1294.2
SJC8234-1	188.6	806.9	542.6	1536.6
SJCA16R5A15	96.7	487.2	326.5	979.7
Spartan	153.4	585.4	398.0	898.7
SuperMac	102.9	458.1	267.6	723.4
LSD _{0.05}	69.0	280.4	47.8	406.5

Values are means of 4 replicates.

^a Total phenols expressed as micrograms gallic acid equivalent (GAE) per gram fresh-frozen weight.

^b FRAP: Ferric Reducing Antioxidant Power expressed as micrograms ascorbic acid equivalent (AAE) per gram fresh-frozen weight.

Among the five groups, procyanidins was the most predominant phenolic group in both the flesh and the peel. According to previous reports, epicatechin and procyanidin B2, which are part of this group, were found in higher amounts in apple peel (Escarpa and Gonzalez 1998; Tsao et al. 2003; Alonso-Salces et al. 2004). In our results, epicatechin and procyanidin B2 were the most abundant phenolic compounds in the peel. Hydroxycinnamic acids were the second highest group in the flesh, whereas flavonols were the second highest group in the peel in terms of concentration. Chlorogenic acid is the main hydroxycinnamic acid compound as well as the most abundant phenolic compound in apple flesh (Bushway et al. 2002; Spanos and Wrolstad 1992; Oleszek et al. 1988; De Simon et al. 1992; Tsao et al. 2003).

A significant difference was observed between phenolic distribution in the peel and phenolic distribution in the flesh. Chlorogenic acid, neochlorogenic acid, *p*-coumaroylquinic acid, catechin, epicatechin, procyanidin B1, procyanidin B2, cyanidin-3-galactoside, quercetin-3-rhamnoside, phloridzin and phloretin-3-xyloglucoside were detected in the flesh, while the peel possessed all of the above except for phloretin-3-xyloglucoside. The peel also had additional flavonols that were not found in the flesh, such as quercetin-3-galactoside, quercetin-3-xyloside and quercetin-3-arabinoside; this finding was in keeping with the results presented in the literature (Burda et al. 1990; Escarpa and Gonzalez 1998; Golding et al. 2001; Van der Sluis et al. 2001). Our results are similar to those reported previously to the effect that glycosylated quercetins are essentially located in apple peel (Awad and Jager 2002; Alonso-Salces et al. 2004); however, low concentrations were detected in the flesh, which is consistent with the findings of other researchers (Price et al. 1999; Van der Sluis et al. 2001).

Phloridzin and phloretin-3-xyloglucoside are the two major dihydrochalcones reported in apple (Dick et al. 1987; Oleszek et al. 1988; Pérez-Illarbe et al. 1991; McRae et al. 1990; Lister et al. 1994). In the present study, phloridzin was detected in both the flesh and the peel, whereas phloretin-3-xyloglucoside was found only in flesh tissues. Although the dihydrochalcones exist at relatively low concentrations, dihydrochalcones, because of their uniqueness to apple and their varied profiles among different cultivars, have been used to distinguish apple from a number of other fruits (Spanos and Wrolstad 1988; De Simon et al. 1992; Versari et al. 1997; Silva et al. 2000) and to identify apple cultivars (Tomas-Barberan and Clifford 2000).

Cyanidins were essentially located in apple peel. It was interesting to note that 'Floribunda Rosea' was the only genotype that contained cyanidins in the flesh, a finding that set it apart completely from the other genotypes and confirmed previous literature reports. The measured cyanidin content of the apple peels was related to their appearance. The red color of apple peels is due to the presence of cyanidin-3-galactoside (Mazza and Velioglu 1992; Tsao et al. 2003; Awad et al. 2000; Lister et al. 1994). The 'Cortland', 'Primevert', 'SJC649' and 'SJC7123-1' genotypes were dark red in color and had the most cyanidin-3-galactoside. No detectable cyanidins were found in the peels of 'Reinette Russet', 'GoldRush (Coop 38)', 'SJCA16R5A15' or 'Golden Delicious', as expected given their lack of red pigmentation. An extra peak was detected in the peel of 'Reinette Russet'; this peak was absent in all other cultivars and might have been due to the cultivar's bronze russet-coloured skin (Khanizadeh et al. 2003).

In contrast to TPC (FC method), TPI as determined using the HPLC method provides a full picture of the quantity and quality of phenolic constituents in the extract. In almost all past studies, TPC was greater than TPI (Spanos and Wrolstad 1988; Pearson et al. 1999; Kondo et al. 2002; Tsao et al. 2003), whereas in the present study, the FC estimation was less than our HPLC calculations, with the exception of 'Floribunda Rosea', where TPC was greater than TPI in both the flesh and the peel. This may have been caused by the different standards used in calculating procyanidin concentrations (Spanos and Wrolstad 1988; Pearson et al. 1999; Kondo et al. 2002; Tsao et al. 2003). The reversal may also have been due to the larger number of individual polyphenolic compounds separated and identified in this HPLC study.

The higher TPC in apple fruits resulted in higher TAC (Liu et al. 2001). Different methods were used in the literature to assess the TAC of apples: TOSC, oxygen radical absorbance capacity

(ORAC) and FRAP (Vinson et al. 2001; Wang et al. 1996; Eberhardt et al. 2000). Our results clearly indicate that apples are an excellent source of phenolics and therefore possess an extremely high TAC similar to those reported previously (Wang et al. 1996; Lee et al. 2003; Tsao et al. 2003; Sun et al. 2002; Imeh and Khokhar 2002; Khanizadeh et al. 2007).

Procyanidins were the most predominant group in both the flesh and the peel, with epicatechin and procyanidin B2 being the most abundant in the peel and chlorogenic acid the most abundant in the flesh. Among the dihydrochalcone compounds, phloridzin was detected in both the flesh and the peel, whereas phloretin-3-xyloglucoside was found only in flesh tissues. Cyanidin-3-galactoside was unique to and found only in red apple.

The variability of the antioxidant capacities and phenolic compounds in different advanced lines can be used as a marker to breed specific apples for processing and/or fresh market, as reported previously (Tsao et al. 2006; Khanizadeh et al. 2006)

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