JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Polyphenolic Profiles in Eight Apple Cultivars Using High-Performance Liquid Chromatography (HPLC)

RONG TSAO,* RAYMOND YANG, J. CHRISTOPHER YOUNG, AND HONGHUI ZHU

Food Research Program, Agriculture and Agri-Food Canada, 93 Stone Road West, Guelph, Ontario N1G 5C9, Canada

Polyphenolic compounds of apple may play an important role in physiologic functions related to human health. Different polyphenolics may have varied biological activities including antioxidant activity. The objective of this study was to investigate the profiles of polyphenolic compounds in different apple varieties and different parts of an apple. The total and individual polyphenolics differed significantly among the eight apple cultivars grown in Ontario, and the peels had higher concentrations than the fleshes. Among the tested cultivars, Red Delicious and Northern Spy had the highest concentrations and Empire the lowest. Five major polyphenolic groups with a total of 16 identified individual compounds were found, among which the dihydroxycinnamic acid esters, phloretin glycosides, and flavan-3-ols were found in both flesh and peel, whereas quercetin glycosides were almost exclusively found in the peel. Cyanidin 3-galactoside was unique to and found only in red apple peels. In both apple peel and flesh, the predominant group of polyphenolics was the procyanidins, followed by quercetin glycosides in the peel and hydroxycinnamic acid esters in the flesh. 3-Hydroxyphloretin 2′-xyloglucoside was newly identified in apple. The results obtained in this study will further the understanding of the polyphenolic composition of apples and their roles in health-promoting physiological functions.

KEYWORDS: HPLC; polyphenolics; flavonoids; procyanidins; anthocyanins; hydroxycinnamic acids; dihydrochalcones; apple; antioxidants

INTRODUCTION

The general perception that apples are good for health has encouraged many researchers to search for the "magic" ingredient in apple. Recent epidemiological studies have shown the inverse correlation between the consumption of apple and/or related products and many chronic diseases of humans. Most noticeably, it has been associated with lowered risk of cardiovascular disease, lung dysfunctions, and various cancers, particularly prostate, liver, colon, and lung cancers (*1*−*4*). This biological impact of apple, similar to that of many other fruits, may be due largely to the presence of antioxidants (*5*), which are considered to be from phytochemicals such as polyphenolics, rather than from vitamin C, vitamin E, or β-carotene (*2*, *6*−*8*). For example, in apple, vitamin C explains only 0.4% of the total antioxidant activity based on a total oxyradical-scavenging capacity (TOSC) assay. Most of the activity is attributed to the polyphenolics in apple (*2*).

Five major polyphenolic groups are found in various apple varieties: hydroxycinnamic acids, flavan-3-ols/procyanidins, anthocyanins, flavonols, and dihydrochalcones (*9*−*13*). Many of the phenolics are often associated with sugar moieties (*14*). The predominant sugar involved in glycosylation in apple is galactose, followed by glucose, rhamnose, xylose, and arabinose; the disaccharide rutinose is also associated with the phenolics in apple (*11*, *15*, *16*). Chlorogenic acid, quercetin 3-glycosides (galactoside, glucoside, xyloside, arabinoside, and rhamnoside), catechin and epicatechin and their dimers, phloridzin, and cyanidin 3-glycosides (mainly galactoside) are the major individual polyphenolics in apple (*11*, *17*−*19*).

Although the major polyphenolic groups and many individual polyphenolic compounds have been identified, due to the minute amount of some of these compounds, the lack of standards, and limitations in analytical methodology, there has not been a comprehensive study on the polyphenolic profile in different apple varieties. Furthermore, among the major polyphenolics of apple, only a few of the individual compounds including quercetin were studied for their health protective activity. Therefore, a comprehensive investigation is necessary because minor components may have stronger antioxidant and other bioactivities, and they may also play a synergistic role in the major bioactives. The profile of phenolic phytochemicals, which includes the chemical composition and concentration, is affected mainly by genetic variation, although it is also subjected to changes in growth period, growing season, and geographic location (*15*, *19*). In this paper, we report a comprehensive study of the phytochemical profiles of eight different apple varieties using an HPLC method with a diode array detector. Identities of polyphenolics were confirmed using LC-MS.

* Author to whom correspondence should be addressed [telephone (519) 829-2400; fax (519) 829-2600; e-mail caor@agr.gc.ca].

10.1021/jf0346298 CCC: $25.00 Published 2003 by the American Chemical Society
Published on Web 09/13/2003

Table 1. Standard Curves and Detection Limits of Standards Used for HPLC Quantification of Individual Phenolic Compounds in Apple

phenolic	retention time[a] (min)	detection wavelength (nm)	linear range (ppm)	calibration formula[b]	R^2	detection limit (ppm at 10 μL)
procyanidin B1	8.06	280	1.0–200	$Y = 2.82X - 6.62$	0.9982	0.75
catechin	12.25	280	1.0–200	$Y = 5.41X - 3.10$	0.9998	0.5
procyanidin B2	14.57	280	1.0–200	$Y = 4.60X - 4.51$	0.9997	0.75
chlorogenic acid	16.04	320	0.2–100	$Y = 26.74X - 10.71$	0.9999	0.1
cyanidin 3-galactoside	18.21	520	1.0–100	$Y = 20.04X + 8.34$	0.9997	0.5
epicatechin	22.41	280	1.0–200	$Y = 6.27X + 8.32$	0.9994	0.5
p-coumaric acid	29.24	320	0.2–100	$Y = 64.52X - 54.87$	0.9998	0.1
quercetin 3-galactoside	42.94	360	0.5–100	$Y = 14.84X - 7.74$	0.9988	0.1
quercetin 3-glucoside	44.74	360	0.5–100	$Y = 17.64X - 1.73$	0.9999	0.1
quercetin 3-rhamnoside	51.72	360	0.5–100	$Y = 21.93X - 2.29$	0.9996	0.1
phloridzin	55.52	280	0.1–200	$Y = 18.08X - 5.92$	0.9999	0.05

[a] Average of two runs. [b] In the calibration formula, *X* stands for the concentration of the analyte and *Y* is the peak area.

MATERIALS AND METHODS

Chemicals and Solvents. Phenolic standards were purchased from different manufacturers. Gallic acid, chlorogenic acid, *p*-coumaric acid, catechin, epicatechin, quercetin, sodium carbonate (Na_2CO_3), and the Folin−Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, MO); quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-rhamnoside, and quercetin 3-rutinoside (rutin) were from Fluka Chemie GmbH (Buchs, Switzerland); quercetin 3-arabinoside and quercetin 3-xyloside were from Apin Chemicals Ltd. (Abingdon, U.K.); procyanidins B1 and B2, phloridzin, and cyanidin 3-galactoside were from Indofine Chemical Co. (Hillsborough, NJ). *p*-Coumaroylquinic acid was a gift from Dr. Juergenliemk (University of Muenster, Germany). Water used for HPLC analysis was purified in-house from distilled and deionized water using a NanoPure system (Dubuque, IA). All other solvents were of HPLC grade and were purchased from Caledon Laboratories Ltd. (Georgetown, ON).

Sample Preparation. Eight apple cultivars (10 apples each) including Golden Delicious, Red Delicious, McIntosh, Empire, Ida Red, Northern Spy, Mutsu, and Cortland were commercially harvested from the McCallum's Orchard (Woodstock, ON). All apples were peeled with a hand peeler (1−2 mm thickness). Approximately 10 g of peel and flesh was taken from each apple. The peels and the flesh of the 10 apples were pooled separately, immediately weighed, and ground in liquid nitrogen in a mortar. The ground sample was then transferred to a beaker with 70% aqueous methanol at a 1:1 (w/v) ratio. The mixture was homogenized using a Polytron blender (Brinkmann Instruments, Westbury, NY) and filtered first through a Whatman no. 1 filter paper under vacuum and then through a 0.45 μm Acrodisc syringe filter (Gelman Laboratory, Ann Arbor, MI). The final filtrate was stored at −20 °C before being analyzed.

Determination of Total Phenolic Content. A slightly modified Folin−Ciocalteu (FC) reagent method (*20*) was used to measure the total phenolic content. Briefly, 0.2 mL of sample or solvent blank, 1.0 mL of FC reagent, and 0.8 mL of Na_2CO_3 (7.5% aqueous solution) were mixed in a 20-mL vial and allowed to stand at room temperature for 30 min. Absorption was measured at 765 nm in a Varian Cary 3C spectrophotometer (Varian Analytical Instruments, Harbor City, CA). Gallic acid solutions were prepared for the generation of a standard curve. Results were expressed as micrograms of gallic acid equivalent (GAE) per milliliter of solution, from which the total phenolic contents in fresh apple samples were calculated. Concentrations beyond the highest point (500 μg/mL) of the linear range of the standard curve were diluted before final analysis.

HPLC Conditions. An HPLC system (Agilent Technology 1100 series, Palo Alto, CA) equipped with a quaternary pump, an inline degasser, a thermostatic autosampler, and a diode array detector (DAD) was used for the identification and quantification of various phenolic compounds in the samples. A Phenomenex Luna C18(2) analytical column (250 × 4.6 mm i.d.; particle size, 5 μm) with a C18 guard column (Phenomenex, Torrance, CA) was used for the separation. The binary mobile phase consisted of a 6% acetic acid in 2 mM sodium acetate buffer (solvent A, pH 2.55, v/v) and acetonitrile (solvent B), and 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. There was a 10-min postrun going back to the starting conditions for reconditioning. The flow rate was 1.0 mL/min for a total run time of 70 min. The injection volume was 10 μL for all samples. All standards except for anthocyanins were dissolved in methanol. The latter were dissolved in 1% HCl in methanol. The detector was set at 280, 320, 360, and 520 nm for simultaneous monitoring of the different groups of phenolic compounds.

LC-MS Conditions. LC-MS was performed using HPLC coupled to a photodiode array UV detector (Finnigan MAT Spectra System UV6000LP, San Jose, CA) equipped with a Finnigan LCQ Deca electrospray ionization mass spectrometer (HPLC-ESI-MS) operated in both negative and positive ion modes. The same separation conditions without 2 mM sodium acetate in solvent A as described in the above HPLC analysis were used in the LC-MS experiment. The instrument parameters were optimized against gallocatechin prior to sample analysis.

Acid Hydrolysis and Monosaccharide Analysis. HPLC fractions were hydrolyzed with 1 M H_2SO_4 at 90 °C for 1.5 h. The hydrolyzed product was diluted with water and filtered through a 0.45 μm syringe filter before analysis. Monosaccharides were analyzed in a Dionex (Sunnyvale, CA) DX-500 ion chromatograph equipped with Dionex CarboPac PA1 column (4 × 250 mm) with a Dionex PA guard column (4 × 25 mm) and pulsed amperometric detection (PAD) detector. A gold electrode was used as the working electrode and silver/silver chloride as the reference electrode. The mobile phase consisted of 100 mM NaOH (solvent A), 30 mM NaOH (solvent B), and water (solvent C). Prior to sample injection, 100% B was run for 15 min, followed by a mixture of 8% A and 92% C for 10 min. After injection, a mixture of 8% A and 92% C was run for 7 min followed by 100% C for 18 min. The column was held at 35 °C with a flow rate of 1 mL/min. The injection volume was 50 μL for all samples.

Quantification and Identification. Compounds were tentatively identified by congruent retention times and UV−vis spectra with those of standards. Confirmation of identity was achieved by comparing the retention times and UV spectra of both standards and samples determined by LC-MS. Some flavonoid glycosides for which no standards were available were hydrolyzed, and the aglycons and sugars were analyzed separately. The identity was also determined using spectral information from UV and LC-ESI-MS.

The retention times, detection wavelengths, linearity, and detection limits of the 11 major polyphenolic standards are listed in **Table 1**. In the calibration formulas, *X* stands for the concentration of the analyte and *Y* is the peak area. The detection limit was defined as the concentration at which the signal-to-noise ratio (S/N) was ≥3. Quantification of those polyphenolics that had no standard to generate a curve was based on a representative standard of the same group. For instance, concentrations of unknown procyanidins (see Results and Discussion) were calculated by using the standard curve of procyanidin B2, and the concentration of *p*-coumaroylquinic acid was calculated using *p*-coumaric acid. Phloretin 2′-xyloglucoside, 3-hydroxyphloretin 2′-glucoside, and -xyloglucoside were quantified by using the phloridzin standard curve. All samples were prepared and analyzed in duplicate.

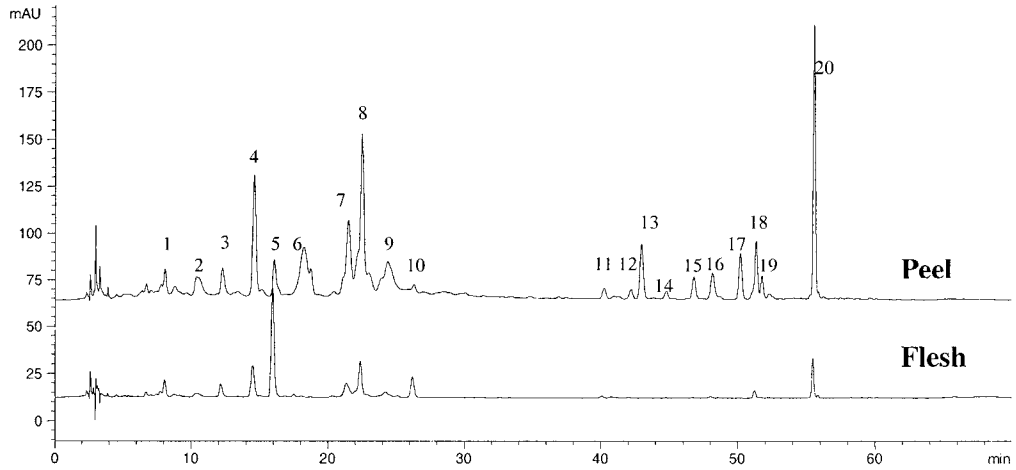

Figure 1. Chromatograms of Red Delicious peel and flesh extracts with 6% acetic acid in 2 mM sodium acetate buffer at a wavelength of 280 nm: 1, procyanidin B1; 2, unknown; 3, catechin; 4, procyanidin B2; 5, chlorogenic acid; 6, cyanidin 3-galactoside; 7, unknown procyanidin dimer; 8, epicatechin; 9, unknown procyanidin dimer; 10, *p*-coumaroylquinic acid; 11, unknown procyanidin dimer; 12, 3-hydroxyphloretin 2′-xyloglucoside; 13, quercetin 3-galactoside; 14, quercetin 3-glucoside; 15, quercetin 3-xyloside; 16, 3-hydroxyphloretin 2′-glucoside; 17, quercetin 3-arabinoside; 18, phloretin 2′-xyloglucoside; 19, quercetin 3-rhamnoside; 20, phloridzin.

RESULTS AND DISCUSSION

Separation and Identification. The HPLC chromatograms (**Figure 1**) of the peel and flesh extracts of the Red Delicious apple showed good resolution of most of the major phenolic compounds known to be in apple, including the closely related quercetin 3-glycosides that are often difficult to separate (*15*, *21*). An excellent HPLC method recently reported by Schieber et al. (*13*) achieved near baseline separation for 26 commonly found polyphenolics of fruit. More remarkably, the method separated 6 quercetin glycosides with the following elution order: quercetin 3-rutinoside (rutin), 3-galactoside, 3-glucoside, 3-xyloside, 3-arabinoside, and 3-rhamnoside. By using a mobile phase that contained sodium acetate buffer, a similar resolution was achieved in our study. It should be mentioned that not all polyphenolic compounds have been detected in any single study. For example, one of the quercetin 3-glycosides, rutin, was reported by Escarpa and Gonzalez (*24*) and Perez-Ilzarbe (*10*). It was not found in the present study, nor was it reported in other similar studies (*21*, *22*). The elution order of the quercetin 3-glycosides in this study followed the same elution pattern as was reported in most of the studies (*9*, *13*, *21*).

Sixteen polyphenolic compounds belonging to all five major polyphenolic groups were identified from the eight popular Ontario apple varieties. They are chlorogenic and *p*-coumaroylquinic acid (hydroxycinnamic acids); cyanidin 3-galactoside (anthocyanins); catechin, epicatechin, and procyanidins B1 and B2 (flavan-3-ols/procyanidins); quercetin 3-galactoside, -glucoside, -xyloside, -arabinoside, and -rhamnoside (flavonols); and two glycosides, glucoside and xyloglucoside, of phloretin and hydroxyphloretin (dihydrochalcones) (**Figure 2**). These individual polyphenolic compounds for which standards are available were initially identified by comparing their retention times and UV−vis spectra with those of the standards that were stored in the library created in-house using the ChemStation software and further confirmed by matching the mass spectra obtained from the LC-MS experiments. For peaks 12, 16, and 18 for which no standards were available (**Figure 1**), fractions containing the individual peaks were collected through multiple HPLC runs and hydrolyzed, and the sugars and aglycon were determined using HPLC-PAD and LC-MS. Two sugars, xylose and glucose, were detected after the hydrolysis of peaks 12 and 18; for peak 16, only glucose was found. The ESI-MS spectrum of peak 12 was characterized by ions at *m*/*z* 583 $[M - H]^-$, 643 $[M + CH_3COOH - H]^-$, 1167 $[2M - H]^-$, and 289 [M − xyloglucose − $H]^-$ in the negative ionization mode and by ions at *m*/*z* 585 $[M + H]^+$, 453 [M + H − xylose]$^+$, and 291 [M + H − xyloglucose]$^+$ in the positive ionization mode. The aglycon with an ion at 291 [M + H − xyloglucose]$^+$ was suggestive of hydroxyphloretin (*21*). Because there was no [M + H − glucose]$^+$ fragment, it was concluded that the two sugars were not independently attached to the aglycon. The loss of xylose indicated that glucose was the one that directly attached to the aglycon. Thus, peak 12 was tentatively identified as 3-hydroxyphloretin 2′-xyloglucoside. Peak 16 showed *m*/*z* 451 $[M - H]^-$, 511 $[M + CH_3COOH - H]^-$, 903 $[2M - H]^-$, and 289 [M − glucose − $H]^-$ in the negative ionization mode and *m*/*z* 453 $[M + H]^+$ and 291 [M + H − glucose]$^+$ in the positive ionization mode; therefore, it was similarly given a tentative structure of 3-hydroxyphloretin 2′-glucoside. Peak 18 had *m*/*z* 567 $[M - H]^-$, 627 $[M + CH_3COOH - H]^-$, 1135 $[2M - H]^-$, and 273 [M − xyloglucose − $H]^-$ in the negative ionization mode and *m*/*z* 569 $[M + H]^+$, 437 [M + H − xylose]$^+$, and 275 [M + H − xyloglucose]$^+$ in the positive ionization mode. Similarly, this compound was temporarily identified as phloretin 2′-xyloglucoside. 3-Hydroxyphloretin 2′-glucoside has been identified by Lu and Foo (*21*). However, some other studies indicated that it was phloretin 2′-glucuronide, which has an identical molecular mass and similar UV spectra (*10*, *23*−*25*). When the UV spectra of these closely related compounds were carefully examined, it was found that the above three dihydrochalcones all showed a UV_{max} of 286 nm, which was the same as that of phloridzin, but the two hydroxyphloretin derivatives, peaks 12 and 16, had a shoulder at 246 and 242 nm in their spectra. Further study on structure elucidation of these compounds will be carried out to reconfirm their identities. When confirmed, this would be the first report of 3-hydroxyphloretin 2′-xylglucoside in apple.

Quantification of Polyphenolics. The high sensitivity of the method was achieved by using wavelengths at the maximum UV absorption (λ_{max}) for the different groups of polyphenolics. All standards gave high linearity within the serial dilution range (**Table 1**).

For the eight most popular apple cultivars grown in Ontario, the total polyphenolics determined by HPLC ranged from 1016.5

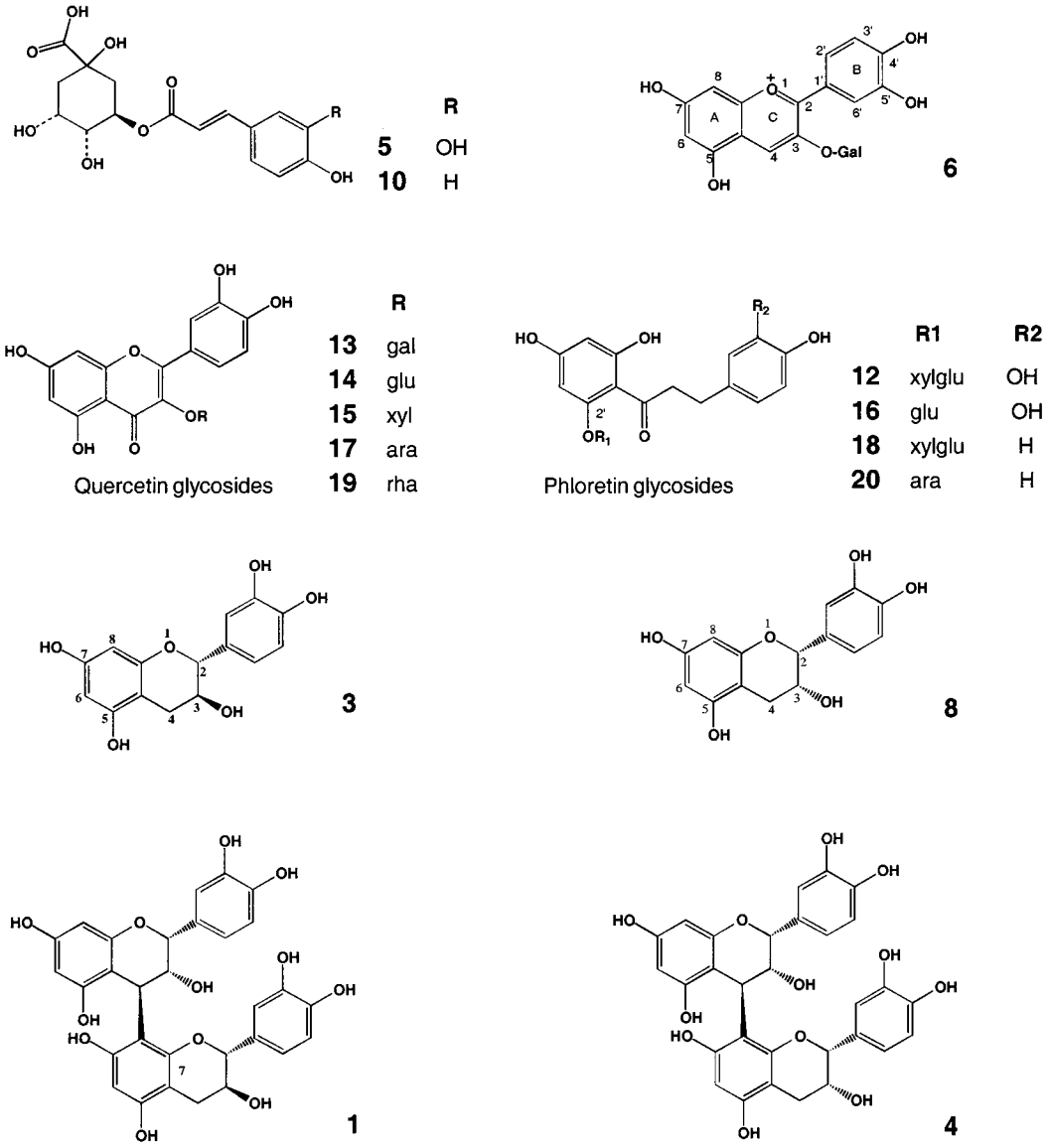

Figure 2. Polyphenolic compounds identified in eight Ontario apples (numbers correspond to peaks in **Figure 1**).

to 2350.4 μg/g of fresh weight in the peel. The Red Delicious apple had the highest polyphenolic concentration in the peel, followed by Northern Spy, Ida Red, Cortland, McIntosh, Golden Delicious, Mutsu, and Empire (**Table 2**). Although the total polyphenolic concentrations in the peel were not exactly the same as the total phenolic contents measured by using the FC method, they correlated well, and the order stayed the same among the eight varieties except for an order switch between Golden Delicious and McIntosh (**Table 2**). In the flesh, the total polyphenolic concentrations analyzed by HPLC were significantly lower compared to that of the peel, ranging from 177.4 to 933.6 μg/g (**Table 3**). Northern Spy had the highest total polyphenolic content in its flesh, followed by Red Delicious, Cortland, Ida Red, McIntosh, Golden Delicious, Mutsu, and Empire (**Table 3**). The total phenolic concentrations by HPLC and FC did not follow the same pattern, due possibly to the close concentrations among all varieties. Nonetheless, the varieties with the highest (Northern Spy) and lowest (Empire) phenolic contents were the same for both analytical methods (**Table 3**).

Among the five major groups, the procyanidins predominated in both the peel (59.7%) and the flesh (55.7%) polyphenolic profiles (**Tables 2** and **3**). Two monomers, catechin and epicatechin, and two dimers, procyanidins B1 and B2, were identified from the peel and flesh of apple. Other procyanidins ($[M - 1]^- = 577$), peaks 2, 7, 9, and 11 (**Figure 1** and **Table 2**), were recognized and tentatively identified on the basis of their UV and MS spectra, and further work is required for final identification of the structures. The total procyanidins ranged from 151.3 to 1654.8 μg/g in the peel and from 0 to 583.0 μg/g in the flesh. Red Delicious and Northern Spy had the highest in the peel and flesh, respectively, whereas Empire had the lowest concentrations of procyanidins in both apple portions. The Empire flesh was the only apple variety that contained no detectable procyanidins (**Tables 2** and **3**). The high concentration of procyanidins in Red Delicious peel was also reported elsewhere (*15*) and may explain its slight astringency and bitterness. Procyanidin B2 and epicatechin were the two most predominant individual compounds in both the peel and flesh of most apple varieties, with the highest concentrations found in the peel of Red Delicious (**Table 2**). These two compounds were reported as the major phenolic compounds in apple skin (*10*, *24*). We found that epicatechin and procyanidin B2 were responsible for the high total procyanidin content in all apple varieties studied, and there was a general trend that higher

Table 2. Concentrations (Micrograms per Gram of Fresh Weight) of Phenolic Compounds in the Peel of Different Apple Cultivars[a]

	Empire	McIntosh	Cortland	Mutsu	Red Delicious	Northern Spy	Golden Delicious	Ida Red	mean	%
chlorogenic acid	176.9	135.6	19.3	134.3	44.6	233.6	149.0	195.3	136.1	8.5
p-coumaroylquinic acid	5.4	33.6	14.2	7.5	5.5	13.8	9.6	9.3	12.4	0.8
total hydroxycinnamics	182.3	169.2	33.5	141.8	50.1	247.4	158.6	204.6	148.5	9.3
catechin	ND[b]	112.8	123.9	43.0	81.9	112.8	38.1	79.3	74.0	4.6
epicatechin	78.1	232.7	293.5	165.6	591.6	439.1	207.2	290.4	287.3	17.9
procyanidin B1	ND	254.4	153.0	50.7	183.8	201.4	46.6	201.2	136.4	8.5
procyanidin B2	73.2	196.5	251.1	205.5	468.1	460.3	276.8	269.9	275.2	17.2
other procyanidins[c]	ND	218.9	243.8	110.1	329.4	242.8	140.0	197.1	185.3	11.5
total procyanidins	151.3	1015.3	1065.3	574.9	1654.8	1456.4	708.7	1037.9	958.2	59.7
cyanidin 3-galactoside	208.2	42.9	159.8	ND	148.9	17.4	ND	111.0	86.0	5.4
total anthocyanins	208.2	42.9	159.8	0	148.9	17.4	0	111	86	5.4
quercetin 3-galactoside	81.3	57.8	101.1	98.2	90.1	62.9	72.5	100.9	83.1	5.2
quercetin 3-glucoside	78.0	65.8	89.2	32.6	15.2	11.8	24.9	18.0	42.0	2.6
quercetin 3-xyloside	44.9	37.8	34.8	25.6	37.1	31.9	20.3	42.7	34.4	2.1
quercetin 3-arabinoside	81.8	82.1	73.1	49.6	69.4	75.4	43.5	103.3	72.3	4.5
quercetin 3-rhamnoside	63.9	57.3	35.6	67.4	32.3	91.4	59.1	44.0	56.4	3.5
total flavonols	349.9	300.8	333.8	273.4	244.1	273.4	220.3	308.9	288.2	17.9
3-hydroxyphloretin 2′-xylglucoside	6.7	4.1	ND	5.8	ND	3.8	7.7	ND	3.5	0.2
3-hydroxyphloretin 2′-glucoside	16.0	ND	ND	5.6	29.3	ND	6.4	4.5	7.7	0.5
phloretin 2′-xylglucoside	31.2	46.2	28.4	39.3	51.2	29.6	79.4	16.4	40.2	2.5
phloridzin	70.9	58.0	37.6	48.5	172.0	44.7	67.5	79.2	72.3	4.5
total dihydrochalcones	124.8	108.3	66	99.2	252.5	78.1	161	100.1	123.7	7.7
total polyphenolics (HPLC)[a]	1016.5	1636.4	1658.5	1089.4	2350.4	2072.7	1248.5	1762.6	1604.4	100
total phenolic content (FC)[e]	781.6	1163.4	1322.8	1016.9	2011.5	1548.3	1265.2	1478.8	1323.6	

[a] Data are the average of duplicates determined by HPLC method. [b] Not detectable. [c] Sum of peaks, 2, 7, 9, and 11, calculated as procyanidin B2 equivalent. [d] Calculated on the basis of the total phenolics measured from HPLC. [e] Total phenolic content measured by FC method.

Table 3. Concentrations (Micrograms per Gram of Fresh Weight) of Phenolic Compounds in the Flesh of Different Apple Cultivars[a]

	Empire	McIntosh	Cortland	Mutsu	Red Delicious	Northern Spy	Golden Delicious	Ida Red	mean	%[c]
chlorogenic acid	158.6	205.5	103.1	132.6	125.0	308.0	153.6	231.9	177.3	36.8
p-coumarylquinic acid	3.8	29.9	29.1	9.0	11.7	20.0	11.0	11.1	15.7	3.3
total hydroxycinnamics	162.4	235.4	132.2	141.6	136.7	328	164.6	243	193	40.1
catechin	ND[b]	20.2	41.7	1.1	25.4	55.2	1.1	21.2	20.7	4.3
epicatechin	ND	70.1	133.8	27.6	122.5	142.3	65.8	51.6	76.7	16.0
procyanidin B1	ND	64.2	37.9	32.4	96.0	172.8	31.9	67.3	62.8	13.1
procyanidin B2	ND	81.9	140.1	91.0	122.3	212.6	121.4	90.3	107.5	22.3
total procyanidins	0	236.4	353.5	152.1	366.2	582.9	220.2	230.4	267.7	55.7
quercetin 3-rhamnoside	ND	ND	ND	ND	3.7	ND	6.4	ND	1.3	0.3
total flavonols	0	0	0	0	3.7	0	6.4	0	1.3	0.3
phloretin-2′-xyloglucoside	3.3	7.2	4.1	5.0	3.3	6.5	7.5	2.3	4.9	1.0
phloridzin	11.7	9.2	8	14.3	24.6	16.1	17.9	13.7	14.4	3.0
total dihydrochalcones	15.0	16.4	12.1	19.3	27.9	22.6	25.4	16	19.3	4.0
total polyphenolics (HPLC)[c]	177.4	488.1	497.7	313.0	534.4	933.6	416.6	489.3	481.3	100
total phenolic content (FC)[d]	158.6	428.1	536.5	329.0	445.8	755.2	370.3	413.1	429.6	

[a] Data are the average of duplicates determined by HPLC method. [b] Not detectable. [c] Calculated on the basis of the total phenolics measured by HPLC. [d] Total phenolic content measured by FC method.

epicatechin concentration led to higher procyanidin B2 (R^2 = 0.94) (**Tables 2** and **3**).

Quercetin glycosides were the only flavonols found in apples analyzed. These compounds consisted of 17.9% of the total polyphenolics (second largest group), ranging from 220.3 to 349.9 μg/g in the peels of the eight varieties (**Table 2**). Five quercetin glycosides were found in the peel, and their concentrations generally followed the order of quercetin 3-galactoside, 3-arabinoside, 3-rhamnoside, 3-glucoside, and 3-xyloside (**Table 2**). Quercetin 3-rhamnoside was also detected in the flesh of Red Delicious and Golden Delicious at 3.7 and 6.4 μg/g, respectively (**Table 3**), which is different from other similar studies (*9*, *10*, *15*, *19*, *24*).

Two hydroxycinnamic acid esters with quinic acid, chlorogenic acid, and *p*-coumaroylquinic acid were found in both peel and flesh of all apple varieties (**Tables 2** and **3**). Although the total hydroxycinnamic acids was only a small portion of the total polyphenolic profile of the peel (9.3%), they comprised the second major group in the flesh (40.1%). Chlorogenic acid was not only the major hydroxycinnamic acid but also the single principal compound in the flesh of all apple varieties, particularly Northern Spy (**Table 3**). Chlorogenic acid was nearly 90% of all polyphenolics detected in the flesh of Empire. Similar results were found in other studies (*10*, *26*, *27*).

Phloretin 2′-glucoside (phloridzin) was the predominant dihydrochalcone together with phloretin 2′-xyloglucoside found

and identified in all apple peel and flesh samples. Red Delicious apple contained the most of these two compounds (**Tables 2** and **3**). The average concentrations of phloridzin in the peel and flesh were 72.3 and 14.4 μg/g of fresh weight, respectively, and those of the 2′-xyloglucoside were 40.2 and 4.9 μg/g of fresh weight, respectively (**Tables 2** and **3**). Two hydroxyphloretin glycosides, 3-hydroxyphloretin glucoside and xyloglucoside, were found in the peel of all apples studied except Cortland (**Table 2**). These two hydroxyphloretin derivatives had much lower concentrations than those of phloretin derivatives. Phloridzin and phloretin 2′-xyloglucoside were the two major dihydrochalcones reported in apple (*9*, *10*, *12*, *15*, *22*, *28*). Other phloretin derivatives such as 3-hydroxyphloridzin and phloretin have occasionally been found in apple at trace amounts (*13*, *21*). Although the dihydrochalcones exist at relatively low concentrations, due to their uniqueness to the apple and their varied profiles among different cultivars, they have been used to distinguish apple from a number of other fruits (*12*, *26*, *27*, *29*) and to identify apple cultivars (*30*).

The major anthocyanins in apple are cyanidin glycosides, among which the 3-galactoside is the leading individual anthocyanin (*11*, *19*, *22*). Anthocyanins were found only in red apple peels (Red Delicious, McIntosh, Empire, Ida Red, Northern Spy, and Cortland), and only cyanidin 3-galactoside was identified in this study. The concentrations ranged from 42.9 to 208.2 μg/g (**Table 2**). Although the total anthocyanins were the smallest group among the major polyphenolics in apple peels, for Empire, cyanidin 3-galactoside was the only leading polyphenolic compound in its peel (**Table 2**), an observation consistent with that reported by Spanos et al. (*12*).

The FC method has been widely used to estimate the total phenolic content in various samples, and it is considered to have limited interferences from other plant constituents (*31*). However, it suffers from the disadvantage of not being able to measure the concentrations of individual phenolic compounds. The HPLC method, which is more sophisticated, separates and quantitatively measures the concentrations of each individual compound. Although the techniques used by these two methods are different, the correlation coefficient was $R^2 = 0.87$ for the two results in the peel (**Table 2**), and it was even higher for that in the flesh ($R^2 = 0.94$) (**Table 3**). It should be noted, however, that whereas in this study the FC estimation was less than our HPLC calculations, in almost all past studies the estimation by the FC method was greater (*12*, *32*−*34*). This may have been caused by the different standards used in calculating procyanidin concentrations. Often, only catechin or epicatechin is used as standard for the HPLC analysis of procyanidins, which leads to the underestimation of the total procyanidins (*12*, *32*−*34*). The reversal may also be due to the larger number of individual polyphenolic compounds separated and identified in this HPLC study.

Conclusion. The total polyphenolic contents, as well as the major groups and individual compounds, varied significantly among the eight studied apple cultivars grown in Ontario. In general, the peels have a much higher concentration of polyphenols than does the flesh, and different cultivars had significantly different levels of total polyphenolic concentrations. Red Delicious and Northern Spy had the highest concentrations among the eight cultivars, and Empire had the lowest. Five major polyphenolic groups with a total of 16 identified individual compounds were found in the apples studied, among which the dihydroxycinnamic acid esters, phloretin glycosides, and flavan-3-ols were found in both flesh and peel, whereas quercetin glycosides were almost exclusively found in the peel. Cyanidin 3-galacoside was unique to and found only in red apple peels. In both apple peel and flesh, the predominant group of polyphenolics was the procyanidins, followed by quercetin glycosides in the peel and hydroxycinnamic acid esters in the flesh. Flavonoids (polyphenolics excluding the hydroxycinnamic acids) composed 90% of the total polyphenolics in the peel and 60% in the flesh. The dihydrochalcones were a unique group of compounds to apple, and this is the first time that 3-hydroxyphloretin 2′-xyloglucoside is found in apple. Empire was low in procyanidins; however, it had relatively high chlorogenic acid and cyanidin 3-galactoside concentrations. The results obtained in this study will help further the understanding of the polyphenolic composition of apple and the roles of these compounds in health-promoting physiological functions.

ACKNOWLEDGMENT

We thank Yolanda Brummer, Jason McCallum, and Emily Sockovie for their technical assistance and the McCallum farm (Woodstock, ON) for supplying fresh apples. We also thank Dr. Juergenliemk (University of Muenster, Germany) for his gift of *p*-coumaroylquinic acid. We acknowledge Ann-Marie Honkanen for proof-reading the manuscript.

LITERATURE CITED

(1) Knekt, P.; Jarvinen, R.; Reunanen, A.; Maatela, J. Flavonoid intake and coronary mortality in Finland: a cohort study. *Br. Med. J.* **1996**, *312*, 478−81.

(2) Eberhardt, M. V.; Lee, C. Y.; Liu, R. H. Antioxidant activity of fresh apples. *Nature* **2000**, *405*, 903−904.

(3) Le-Marchand, L.; Murphy, S. P.; Hankin, J. H.; Wilkens, L. R.; Kolonel, L. N. Intake of flavonoids and lung cancer. *J. Natl. Cancer Inst.* **2000**, *92*, 154−160.

(4) Xing, N.; Chen, Y.; Mitchell, S. H.; Young, C. Y. F. Quercetin inhibits the expression and function of the androgen receptor in LNCaP prostate cancer cells. *Carcinogenesis* **2001**, *22*, 409−414.

(5) Hyson, D.; Studebaker-Hallman, D.; Davis, P. A.; Gershwin, M. E. Apple juice consumption reduces plasma low-density lipoprotein oxidation in healthy men and women. *J. Med. Food* **2000**, *3*, 159−166.

(6) Bors, W.; Werner, H.; Michel, C.; Saran, M. Flavonoids as antioxidants: determination of radical scavenging efficiencies. *Methods Enzymol.* **1990**, *186*, 343−355.

(7) Hanasaki, Y.; Ogawa, S.; Fukui, S. The correlation between active oxygen scavenging and antioxidative effects of flavonoids. *Free Radical Biol. Med.* **1994**, *16*, 845−850.

(8) Wang, H.; Cao, G.; Prior, R. L. Total antioxidant capacity of fruits. *J. Agric. Food Chem.* **1996**, *44*, 701−705.

(9) Oleszek, W.; Lee, C. Y.; Jaworski, A. W.; Price, K. R. Identification of some phenolic compounds in apples. *J. Agric. Food Chem.* **1988**, *36*, 430−432.

(10) Pefes-Ilzarbe, J.; Hernandez, T.; Estrella, I. Phenolic compounds in apples: varietal difference. *Z. Lebensm. Unters. Forsch.* **1991**, *192*, 551−554.

(11) Mazza, G.; Velioglu, Y. S. Anthocyanins and other phenolic compounds in fruits of red-flesh apples. *Food Chem.* **1992**, *43*, 113−117.

(12) Spanos, G. A.; Wrolstad, R. E. Phenolics of apple, pear, and white grape juices and their changes with processing and storage—a review. *J. Agric. Food Chem.* **1988**, *36*, 430−432.

(13) Schieber, A.; Keller, P.; Carle, R. Determination of phenolic acids and flavonoids of apple and pear by high-performance liquid chromatography. *J. Chromatogr. A* **2001**, *910*, 265−273.

(14) Merken, H. M.; Beecher, G. R. Measurement of food flavonoids by high-performance liquid chromatography: A review. *J. Agric. Food Chem.* **2000**, *48*, 577−599.

(15) McRae, K. B.; Lidster, P. D.; De Marco, A. C.; Dick, A. J. Comparison of the polyphenol profiles of the apple fruit cultivars by correspondence analysis. *J. Sci. Food Agric*. **1990**, *50*, 329−342.
(16) Guyot, S.; Marnet, N.; Laraba, D.; Sanonerm, P.; Drilleau, J. F. Reversed-phase HPLC following thiolysis for quantitative estimation and characterization of the four main classes of phenolic compounds in different tissue zones of a French cider apple variety (*Malus domestica* var. Kermerrien). *J. Agric. Food Chem.* **1998**, *46*, 1698−1705.
(17) Lancaster, J. E.; Dougall, D. K. Regulation of skin color in apples. *Crit. Rev. Plant Sci.* **1992**, *10*, 487−502.
(18) Nicolas, J. J.; Richard-Forget, F. C.; Goupy, P. M.; Amiot, M. J.; Aubert, S. Y. Enzymatic browning reactions in apple and apple products. *Crit. Rev. Food Sci. Nutr.* **1994**, *34*, 109−57.
(19) Awad, M. A.; de Jager, A.; van Westing, L. M. Flavonoid and chlorogenic acid levels in apple fruit: characterization of variation. *Sci. Hortic.* **2000**, *83*, 249−263.
(20) Slinkard, K.; Singleton, V. L. Total phenol analysis: automation and comparison with manual methods. *Am. J. Enol. Vitic*. **1977**, *28*, 49−55.
(21) Lu, Y.; Foo, L. Y. Identification and quantification of major polyphenols in apple pomace. *Food Chem.* **1997**, *59*, 187−194.
(22) Lister, C. E.; Lancaster, J. E.; Sutton, K. H. Developmental changes in the concentration and composition of flavonoids in skin of a red and a green apple cultivar. *J. Sci. Food Agric.* **1994**, *64*, 155−161.
(23) Perez-Ilzarbe, J.; Hernandez, T.; Estrella, I.; Vendrell, M. Cold storage of Granny Smith. *Z. Lebensm. Unters. Forsch. A* **1997**, *204*, 52−55
(24) Escarpa, A.; Gonzalez, M. C. High-performance liquid chromatography with diode-array detection for the determination of phenolic compounds in peel and pulp from different apple varieties. *J. Chromatogr. A* **1998**, *823*, 331−337.
(25) Sakakibara, H.; Honda, Y.; Nakagwa, S.; Ashida, H.; Kanazawa, K. Simultaneous determination of all polyphenols in vegetables, fruits and teas. *J. Agric. Food Chem.* **2003**, *51*, 571−581.
(26) De Simon B. F.; Perez-Ilzarbe, J., Hernandex, T.; Gomez-Cordoves, C.; Estrella, I. Importance of phenolic compounds for the characterization of fruit juices. *J. Agric. Food Chem.* **1992**, *40*, 1531−1535.
(27) Versari, A.; Barbanti, B. D.; Farnell, P. J. Adulteration of Fruit Juices: Dihydrochalcones as Quality Markers for Apple Juice Identification. *Lesbensm. Wiss. -Technol.* **1997**, *30*, 585−589.
(28) Dick, A. J.; Redden, P. R.; DeMarco, A. C.; Lidster, P. D.; Grindley, T. B. Flavonoid glycosides of Spartan apple peel. *J. Agric. Food Chem.* **1987**, *35*, 529−531.
(29) Silva B. M.; Andrade, P. B.; Valentao, P.; Mendes, G. C.; Seabra, R. M.; Ferreira, M. A. Phenolic profile in the evaluation of commercial quince jellies authenticity. *Food Chem.* **2000**, *71*, 281−285
(30) Tomas-Barberan, F. A.; Clifford, M. N. Flavanones, chalcones and dihydrochalcones—nature, occurrence, and dietary burden. *J. Sci. Food Agric.* **2000**, *80*, 1073−1080.
(31) Escarpa, A.; Gonzalez, M. C. Approach to the content of total extractable phenolic compounds from different food samples by comparison of chromatographic and spectrophotometric methods. *Anal. Chim. Acta* **2001**, *427*, 119−127.
(32) Pearson, D. A.; Tan, C. H.; German, J. B.; Davis, P. A.; Gershwin, M. E. Apple juice inhibits human low-density lipoprotein oxidation. *Life Sci.* **1999**, *64*, 1913−1920.
(33) Kondo, S.; Tsuda, K.; Muto, N.; Ueda, J. Antioxidative activity of apple skin or flesh extracts associated with fruit development on selected apple cultivars. *Sci. Hortic.* **2002**, *96*, 177−185.
(34) Tsao, R.; Yang, R. Optimization of a New Mobile Phase To Know the Complex and Real Polyphenolic Composition: Toward a Total Phenolic Index Using HPLC. *J. Chromatogr. A* **2003**, in press.

Received for review June 13, 2003. Revised manuscript received July 14, 2003. Accepted July 31, 2003. This is scientific publication S142 of the Food Research Program, Agriculture and Agri-Food Canada, Guelph.

JF0346298