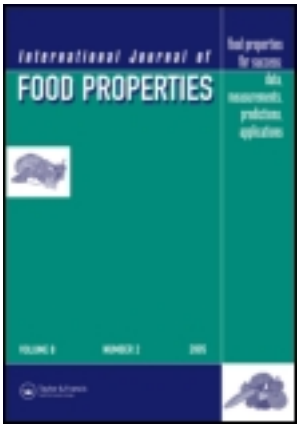

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NOVEL DATA ON THE POLYPHENOL COMPOSITION OF ITALIAN ANCIENT APPLE CULTIVARS

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The pulp polyphenol composition of some Italian ancient apple cultivars, Dominici, Giachetta, Grigia di Torriana, Pom d'Aram, Ronzè, and Ros Borsetta, was studied in comparison with a Golden Delicious commercial variety, over a three-year period. Polyphenols were analyzed by liquid chromatography coupled with diode array and mass spectrometer detectors. The results showed that ancient varieties, such as Grigia di Torriana, Ros Borsetta, and Giachetta, constitute good sources of polyphenols, even without the peel. It was demonstrated that some representative apple phenolics, such as chlorogenic acid and phloridzin, were clearly affected by the harvesting year, whereas others, such as catechins and procyanidins, did not.

Keywords: ***Apple, Pulp, Biodiversity, HPLC-DAD-MS, Polyphenols.***

INTRODUCTION

Polyphenols represent an extremely large and variegate group of secondary plant metabolites and constitute the majority of dietary antioxidants.[1] Due to this property, polyphenols have been shown to possess many potential beneficial effects on human health, such as lowered risk of cardiovascular diseases, inhibition of cancer cell proliferation *in vitro*, and protective effect against neurodegenerative diseases.[2] Apple fruit constitutes an important source of polyphenols,[3] and its consumption has been associated to a healthy diet and several health positive effects.[4,5] Five groups of phenolics are commonly found in apples: hydroxycinnamic acids, flavan-3-ols, anthocyanins, flavonols, and dihydrochalcones, which can be present in the form of esters, such as hydrocynnamic acids, or glycosides, such as flavonols, with galactose, glucose, rhamnose, arabinose, and xylose as predominant sugars.[6] Flavonols and anthocyanins were reported as main phenolics in the apple peel, while procyanidins, catechins, hydroxycinnamic acids, and dihydrochalcones accounted for the majority in the pulp.[7] Also, factors, such as genetic variation, growth period, growing season, and geographic location, could affect the apple phenolic concentration.[8,9]

The aim of this research was to define the polyphenolic composition of old (Dominici, Giachetta, Grigia di Torriana, Pom d'Aram, Ronzè, and Ros Borsetta) and commercial

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(Golden Delicious) apple cultivars, grown in Piedmont (Italy). Italy is the sixth largest apple producer in the world and Piedmont is one of its most productive regions. These varieties are mainly cultivated on the mountains and hills of Piedmont and are better adapted to tolerate the local climate than the international cultivars. Their cultivation also contributes to the preservation of the local biodiversity. The obtained fruits are available in the local market and are particularly appreciated for their typical tastes and genuineness. The valorization of this varietal patrimony has to include the determination of their nutraceutical properties, to which polyphenols greatly contribute. In order to get this goal, data of apple polyphenols were collected for 3 years.

MATERIALS AND METHODS

Chemicals and Solvents

Caffeic acid, chlorogenic acid, gallic acid, ferulic acid, (+)-catechin, (−)-epicatechin, (−)-epicatechin gallate, procyanidin B1, procyanidin B2, phloridzin, cyanidin-3-glucoside, quercetin-3-galactoside, quercetin-3-rhamnoside, HPLC grade methanol and acetonitrile, acetic acid, formic acid, and trifluoroacetic acid were purchased from Sigma-Aldrich (Milan, Italy). All chemicals were of analytical or higher grade and the aqueous solution were prepared by using ultra-pure water purified by Milli-Q System (Millipore, Milan, Italy).

Apple Cultivars and Sample Preparation

Six old cultivars (Dominici, Giachetta, Grigia di Torriana, Pom d'Aram, Ronzè, and Ros Borsetta) and one commercial cultivar (Golden Delicious), were harvested at maturity during the 2007, 2008, and 2009 seasons at the experimental station of Bibiana (Turin, Italy). Bibiana collection arose in 1998; it is placed at 430 m above sea level, over a sandy soil. Plants were grown according to biological culture rules. Three bacthes of 1 kg of apples were prepared for each cultivar. Apples were rapidly peeled, sliced, frozen in liquid nitrogen, and lyophilized (LIO-5P, 97 Cinquepascal, Milan, Italy). After being freeze-dried, the fruits were powdered and used for polyphenol analysis.

Extraction of Phenolics

The extraction of phenolics was carried out on 100 mg of pulp powder by adding 5 mL of 80% acqueous methanol. The suspension was put into an ultrasonic bath (Bransonic 220, AS Strumenti Scientifici, Torino, Italy) for 30 min and then centrifuged at 10,000 rpm (16,800 $\times$ g) and 10°C. The residue was extracted again with the same solvent mixture. The extracts were combined, dried with a rotary evaporator (40°C, 50 mbar), and dissolved into 2 mL of acetonitrile/H_2O/trifluoroacetic acid (50:49:1, v/v/v). After filtering (0.45 μm), extracts were analyzed by high-performance liquid chromatography (HPLC). Three replicates were carried out for each sample.

HPLC-DAD Analysis

Phenolic acids, catechins, procyanidins, dihydrochalcones, and antocyanidins were identified and quantified by using a Thermo-Finnigan Spectra-System HPLC

(Thermo-Finnigan, Waltham, MA, USA), equipped with a P2000 binary gradient pump, a SCM 1000 degasser, an AS 100 automatic injector, an UV6000LP diode array detector (DAD), and the ChromQuest 4.2.34. software for data processing. The separation was achieved on a C_{18} RP Lichrosphere 250 × 4.6 mm, 5 μm (Merck, Milan, Italy) column, equipped with a C_{18} RP Lichrosphere guard column 5 μm (Merck). The mobile phase was composed of solvent A (2.5% acetic acid in water) and solvent B (acetonitrile). Flow rate was 0.8 mL/min and the injection volume was 20 μL. The elution program was as follows: A 95% as initial conditions, A 85% in 20 min, A 80% in 10 min, A 30% in 8 min, A 0% in 5 min, which was kept in isocratic for 1 min and A 95% in 10 min, which was kept in isocratic for 5 min. DAD spectra were recorded in full scan modality over the wavelength range of 210–360 nm and at a discrete wavelength of 520 nm. Retention times (Rt) and spectra were compared to those of authentic standards. Each compound was quantified as mg/kg dry weight (DW) by means of calibration with external standard.

HPLC-MS Analysis

HPLC-MS analysis was carried out to confirm the identity of the phenolics detected by DAD and to quantify the quercetin glycosides. A Thermo-Finnigan Spectra-System HPLC (Thermo-Finnigan, Waltham, MA, USA), equipped with a P2000 binary gradient pump, a SCM 1000 degasser, an AS 3000 automatic injector, a Finnigan MAT LCQ ion trap mass spectrometer with an electrospray ionization (ESI) source, and the Xcalibur™ software was used. The separation was achieved on a Luna C_{18}, 150 × 2.0 mm, 5 μm (Phenomenex, Castel Maggiore, Italy). For the phenolic acids, the mobile phase was composed of solvent A (0.1% formic acid in water) and solvent B (methanol). Flow rate was 0.2 mL/min and with an injection volume of 20 μL. The elution program was as follows: A 80% as initial conditions, A 70% in 6 min, which was kept in isocratic for 14 min, A 50% in 2 min, A 30% in 28 min, A 0% in 10 min, which was kept in isocratic for 5 min, and A 80% in 5 min, which was kept in isocratic for 25 min. For the other phenolic compounds, the mobile phase consisted of solvent A (2% formic acid in water) and solvent B (methanol). Flow rate was 0.2 mL/min and the injection volume was 20 μL. The following gradient conditions were used: A 80% as initial conditions, A 70% in 6 min, which was kept in isocratic for 14 min, A 50% in 2 min, A 30% in 28 min, A 0% in 10 min, which was kept in isocratic for 5 min, and A 80% in 5 min, which was kept in isocratic for 25 min. Negative electrospray mode was used for the ionization of molecules with spray voltage at 3.50 kV and capillary temperature at 200°C. For phenolic acids, the negative masses were monitored in the selected ion monitoring mode in three segments: m/z 169 from Rt 0 to 7 min; m/z 179 from Rt 7 to 24 min; m/z 193 from Rt 24 to 31 min. For the other phenolic compounds, the negative masses were monitored in the selected ion monitoring mode in 4 segments: m/z 289, 353, 447, and 577 from Rt 0 to 11 min; m/z 441 from Rt 11 to 15 min; m/z 463 from Rt 15 to 29 min; m/z 435 and 447 from Rt 29 to 31 min. Phenolic identification was obtained by comparing the Rt and mass spectra with those of authentic standards. In addition, MS^2 experiments were carried out using helium as a collision gas. Collision-induced dissociation spectra were obtained with an isolation width of 1 m/z for parent mass and a normalized collision energy of 21% for chlorogenic acid, 24% for procyanidin B1, procyanidin B2, (+)-catechin, (−)-epicatechin, 25% for cyanidin-3-O-glucoside and quercetin-3-O-rhamnoside, 27% for gallic acid, (−)-epicatechin gallate, and quercetin-3-O-galactoside, 28% for caffeic acid

and ferulic acid. Quantification of quercetin glycosides was achieved by using external calibration.

Statistical Analysis

Data statistical analysis (one-way and two-way analysis of variance) was performed by using SPSS software (version 12.0 for Windows; SPSS Inc., Chicago, IL, USA) and Tukey's HSD test was used to see significant differences within mean values.

RESULTS AND DISCUSSION

The pulp polyphenolic compounds found in the seven examined apple varieties are listed in Table 1. Polyphenols were identified by the comparison with the Rt and UV-Vis spectra of authentic standards. The identity was confirmed by matching structures to standard mass spectra using fragmentation patterns (Table 1). Phenolic compounds, such as hydroxybenzoic acids (gallic acid), hydroxycinnamic acids (caffeic acid and ferulic acid) and their esters (chlorogenic acid), flavan-3-ol monomers ((+)-catechin, (−) -epicatechin and (−)-epicatechin gallate), and polymers (procyanidin B1 and B2), cyanidin glycosides (cyanidin-3-glucoside), dihydrochalcones (phloridzin), and quercetin glycosides (quercetin-3-galactoside and quercetin-3-rhamnoside), were found (Table 2). The total polyphenol content (TP), calculated as the sum of all identified phenolic compounds, ranged from 445.2 ± 23.1 mg/kg for Ronzè to 4996.1 ± 371.6 mg/kg for Grigia di Torriana. This variety showed the highest TP values in all three sampling years. TP values, as those exhibited by Grigia di Torriana variety in 2008 and 2009 (4177.8 ± 891.7 and 4996.1 ± 371.6 mg/kg, respectively), and by Dominici in 2008 (4043.1 ± 52.7 mg/kg), were relatively high, if compared with data published on TP of whole apples.[10] In fact, the highest value of total polyphenols found by these authors from the analysis of the

Table 1 Phenolic compounds detected and identified in the apple pulps.

		R_t (min)	$[M\text{-}H]^-$ (m/z)	Fragment ions (m/z)
	Phenolic acids			
1	Gallic acid	5, 2	169	125
2	Caffeic acid	22, 5	179	109
3	Ferulic acid	29, 3	193	149, 178
	Other phenolic compounds			
1	Procyanidin B1	3, 5	577	451, 425
2	(+)-Catechin	5, 0	289	245, 205
3	Procyanidin B2	5, 7	577	451, 425
4	(-)-Epicatechin	8, 9	289	245, 205
5	Cyanidin-3-O-glucoside	10, 01	447	285
6	Chlorogenic acid	10, 45	353	191
7	(-)-Epicatechin gallate	13, 5	441	331, 289
8	Quercetin-3-O-galactoside	28, 2	463	301, 343, 179
9	Phloridzin	30, 0	435	
10	Quercetin-3-O-rhamnoside	31, 4	447	179, 151

The polyphenols are listed according to the elution order obtained with HPLC-MS analysis, on the Luna C_{18}, 150 × 2.0 mm, 5 μm column.

Table 2 Pulp polyphenol composition of Italian ancient and commercial apple cultivars studied over three sampling years.

		Concentration (mg/kg DW)						
	Phenolic compound	Dominici	Giachetta	Golden Delicious	Grigia di Torriana	Pom d'Aram	Ronzè	Ros Borsetta
2007								
Hydroxybenzoic acids	Gallic acid	20.2 ± 1.2^{b}	21.6 ± 3.8^{bc}	nd	16.2 ± 0.1^{b}	9.5 ± 2.5	8.7 ± 1.4	27.7 ± 4.0^{c}
Hydroxycinnamic acids and their esters	Caffeic acid	nd	33.9 ± 5.9^{c}	nd	nd	6.7 ± 1.7^{ab}	5.9 ± 1.6^{ab}	8.3 ± 2.3^{b}
	Ferulic acid	nd	nd	5.6 ± 0.8^{b}	11.0 ± 1.4^{c}	nd	nd	1.1 ± 0.0^{a}
	Chlorogenic acid	130.3 ± 66.4^{a}	672.9 ± 392.5^{ab}	489.6 ± 219.5^{ab}	868.4 ± 148.3^{b}	366.3 ± 114.5^{ab}	366.6 ± 7.5^{ab}	461.9 ± 258.9^{ab}
Flavan-3-ols	Procyanidin Bl	nd	nd	nd	76.6 ± 25.9^{b}	nd	nd	22.8 ± 12.7^{a}
	Procyanidin B2	175.9 ± 56.4^{c}	55.0 ± 23.0^{b}	17.5 ± 6.4^{a}	429.4 ± 199.2^{d}	nd	36.5 ± 14.2^{b}	67.7 ± 16.2^{b}
	(+)-Catechin	57.5 ± 3.0^{c}	28.9 ± 5.5^{bc}	15.5 ± 5.0^{ab}	57.4 ± 25.8^{c}	13.6 ± 0.8^{ab}	nd	nd
	(−)-Epicatechin	134.6 ± 4.6^{a}	54.7 ± 25.3^{a}	50.0 ± 9.8^{a}	279.8 ± 119.0^{b}	43.2 ± 14.4^{a}	21.0 ± 5.6^{a}	22.7 ± 12.9^{a}
	(−)-Epicatechin gallate	nd	nd	nd	12.7 ± 1.7^{c}	1.9 ± 0.1^{a}	nd	10.3 ± 0.8^{b}
Dihydrochalcones	Phloridzin	32.2 ± 11.4^{bc}	45.4 ± 7.2^{c}	nd	121.0 ± 6.8^{d}	16.8 ± 7.4^{ab}	6.0 ± 0.6^{a}	22.8 ± 17.9^{abc}
Quercetin glycosides[MS]	Quercetin-3-galactoside	2.0 ± 0.9^{b}	0.6 ± 0.1^{a}	0.4 ± 0.1^{a}	0.5 ± 0.0^{a}	0.5 ± 0.2^{a}	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}
	Quercetin-3-rhamnoside	26.7 ± 20.3^{a}	9.2 ± 0.1^{a}	22.5 ± 8.2^{a}	nd	nd	nd	nd
Cyanidin glycosides	Cyanidin-3-glucoside	2.9 ± 0.4^{b}	1.7 ± 0.0^{a}	nd	nd	nd	nd	nd
	TP	582.1 ± 25.9^{a}	923.8 ± 451.6^{a}	601.3 ± 248.3^{a}	1873.1 ± 525.7^{b}	458.4 ± 113.0^{a}	445.2 ± 23.1^{a}	645.5 ± 311.8^{a}
	Phenolic compound	Dominici	Giachetta	Golden Delicious	Grigia di Torriana	Pom d'Aram	Ronzè	Ros Borsetta
2008								
Hydroxybenzoic acids	Gallic acid	22.1 ± 2.7^{d}	17.6 ± 2.6^{cd}	nd	11.6 ± 0.3^{bc}	12.2 ± 3.9^{bcd}	7.0 ± 4.3^{ab}	33.8 ± 6.5^{e}
Hydroxycinnamic acids	Caffeic acid	nd	nd	nd	nd	3.9 ± 1.3^{a}	nd	22.3 ± 9.9^{b}
and their esters	Ferulic acid	nd	69.3 ± 10.9^{b}	10.4 ± 2.1^{a}	1.9 ± 0.7^{a}	nd	nd	2.4 ± 1.0^{a}
	Chlorogenic acid	2082.1 ± 273.4^{b}	1521.7 ± 447.2^{b}	405.0 ± 72.2^{a}	1475.7 ± 327.8^{b}	400.4 ± 34.7^{a}	366.6 ± 7.5^{a}	1486.4 ± 475.6^{b}
Flavan-3-ol	Procyanidin Bl	272.5 ± 1.4^{c}	62.0 ± 10.0^{a}	nd	248.7 ± 58.7^{c}	29.7 ± 0.8^{a}	nd	180.2 ± 16.0^{b}
	Procyanidin B2	934.1 ± 118.4^{b}	196.5 ± 43.1^{a}	82.1 ± 9.3^{a}	1143.8 ± 237.3^{b}	nd	nd	274.4 ± 64.1^{a}
	(+)-Catechin	111.1 ± 0.5^{c}	36.0 ± 5.5^{ab}	31.7 ± 1.1^{ab}	194.7 ± 44.8^{d}	nd	78.6 ± 48.1^{bc}	112.3 ± 17.7^{c}
	(−)-Epicatechin	503.5 ± 116.8^{b}	122.0 ± 6.3^{a}	159.2 ± 20.0^{a}	898.1 ± 199.7^{c}	20.1 ± 10.2^{a}	144.5 ± 99.8^{a}	234.9 ± 32.6^{ab}
	(−)-Epicatechin gallate	19.4 ± 1.1^{b}	30.4 ± 1.5^{c}	nd	35.0 ± 6.9^{c}	5.5 ± 0.5^{a}	nd	7.9 ± 4.6^{a}

(*Continued*)

Table 2 (Continued).

		Concentration (mg/kg DW)						
	Phenolic compound	Dominici	Giachetta	Golden Delicious	Grigia di Torriana	Pom d'Aram	Ronzè	Ros Borsetta
Dihydrochalcones	Phloridzin	86.3 ± 13.1^{b}	nd	11.2 ± 0.1^{a}	160.3 ± 20.9^{c}	10.5 ± 3.1^{a}	1.4 ± 0.1^{a}	57.4 ± 27.0^{b}
Quercetin glycosides^{MS}	Quercetin-3-galactoside	1.0 ± 0.0^{b}	0.4 ± 0.0^{a}	0.6 ± 0.1^{a}	1.0 ± 0.1^{b}	0.4 ± 0.1^{a}	0.5 ± 0.2^{a}	0.5 ± 0.2^{a}
	Quercetin-3-rhamnoside	7.5 ± 3.1^{a}	33.3 ± 8.2^{b}	42.6 ± 4.6^{b}	5.1 ± 4.0^{a}	nd	nd	nd
Cyanidin glycosides	Cyanidin-3-glucoside	3.5 ± 0.2^{a}	nd	nd	1.9 ± 0.1^{b}	nd	nd	nd
	TP	4043.1 ± 52.7^{c}	2089.3 ± 382.8^{b}	742.9 ± 105.3^{a}	4177.8 ± 891.7^{c}	482.7 ± 50.3^{a}	601.0 ± 158.5^{a}	2412.5 ± 619.4^{b}
	Phenolic compound	Dominici	Giachetta	Golden Delicious	Grigia di Torriana	Pom d'Aram	Ronzè	Ros Borsetta
2009								
Hydroxybenzoic acids	Gallic acid	nd	nd	nd	nd	nd	nd	nd
Hydroxycinnamic acids	Caffeic acid	nd	nd	nd	nd	nd	nd	nd
and their esters	Ferulic acid	nd	nd	nd	nd	nd	nd	nd
	Chlorogenic acid	338.8 ± 23.3^{a}	1279.5 ± 53.1^{b}	347.4 ± 61.6^{a}	1258.1 ± 115.9^{b}	1150.5 ± 166.3^{b}	1213.6 ± 183.9^{b}	1235.1 ± 291.3^{b}
Flavan-3-ols	Procyanidin Bl	88.9 ± 13.6^{a}	5.3 ± 0.4^{a}	75.3 ± 2.8^{a}	338.0 ± 25.3^{b}	132.4 ± 17.4^{a}	318.8 ± 114.9^{b}	297.7 ± 47.2^{b}
	Procyanidin B2	211.7 ± 8.4^{a}	458.0 ± 42.0^{a}	472.9 ± 108.1^{a}	2087.5 ± 187.1^{b}	857.6 ± 40.0^{a}	826.4 ± 87.7^{a}	1618.9 ± 619.2^{b}
	(+)-Catechin	nd	32.5 ± 5.8^{ab}	nd	134.8 ± 15.7^{c}	34.7 ± 4.5^{ab}	56.4 ± 1.6^{b}	140.7 ± 40.1^{c}
	(−)-Epicatechin	86.2 ± 6.4^{a}	132.4 ± 5.7^{a}	146.1 ± 28.0^{a}	912.5 ± 111.6^{c}	284.9 ± 26.1^{ab}	249.4 ± 39.8^{a}	531.1 ± 213.5^{b}
	(−)-Epicatechin gallate	nd	9.3 ± 4.0^{a}	6.1 ± 0.6^{a}	nd	nd	nd	14.4 ± 0.2^{b}
Dihydrochalcones	Phloridzin	254.4 ± 46.9^{b}	49.0 ± 7.9^{a}	54.6 ± 6.9^{a}	263.2 ± 84.2^{b}	108.8 ± 12.5^{a}	25.4 ± 0.4^{a}	75.3 ± 30.7^{a}
Quercetin glycosides^{MS}	Quercetin-3-galactoside	0.6 ± 0.1^{a}	0.6 ± 0.0^{a}	0.3 ± 0.0^{a}	0.4 ± 0.0^{a}	0.4 ± 0.1^{a}	1.0 ± 0.3^{b}	0.5 ± 0.0^{a}
	Quercetin-3-rhamnoside	8.0 ± 0.9^{a}	16.7 ± 2.6^{b}	6.8 ± 2.9^{a}	nd	nd	6.6 ± 3.6^{a}	nd
Cyanidin glycosides	Cyanidin-3-glucoside	1.7 ± 0.2^{b}	2.9 ± 0.7^{bc}	nd	1.6 ± 0.1^{ab}	2.6 ± 0.2^{bc}	3.8 ± 1.0^{c}	9.0 ± 1.0^{d}
	TP	990.2 ± 68.3^{a}	1986.1 ± 101.9^{abc}	1109.7 ± 191.3^{ab}	4996.1 ± 371.6^{e}	2571.9 ± 267.1^{bcd}	2701.3 ± 430.5^{cd}	3922.8 ± 1241.2^{de}

MS: quantified by HPLC-MS analysis; nd: not detected; TP: total phenolics.

[a–e]Values within a row by the same letters are not significantly different at $p \leq 0.05$. Values are the means ± SD ($n = 3$).

whole fruit was 272.4 mg/100 g DW. All over the sampling period, the commercial cultivar Golden Delicious was among the varieties with the lowest TP content. Most of the ancient varieties contained gallic acid, while Golden Delicious did not. Its content ranged between 7.0 ± 4.3 and 33.8 ± 6.5 mg/kg, the maximum value being registered for Ros Borsetta and the lowest for Ronzè in 2008. However, this compound was not detected in 2009. Some authors reported that its presence in edible fruits might be due to the hydrolysis of gallotannins.[11] Among the hydroxycinnamic acids, caffeic acid and ferulic acid were detected in a relatively low level, the range being 3.9 ± 1.3 to 22.3 ± 9.9 mg/kg for caffeic acid and 1.1 to 69.3 ± 10.9 mg/kg for ferulic acid. From a comparison with literature data, it can be seen that caffeic acid was detected in the Golden Delicious with contents ranging from 1.8 to 2.9 mg/kg fresh weight in the flesh[12] and at a content of 2.43 mg/l in the whole fruit.[13] In our work, both ferulic and caffeic acids were not detected in Golden Delicious and Ronzè cultivars and in 2009 ferulic acid was absent in all cultivars. These results are in agreement with those obtained by other authors[6,7] on Golden Delicious phenolic acid composition. Instead, chlorogenic acid was the most abundant phenolic compound in all varieties, in every sampling year. It was reported as the main apple compound also by other authors.[6,7] In the seven examined varieties, chlorogenic acid ranged from 130.3 ± 66.4 mg/kg of Dominici to 2082.1 ± 273.4 mg/kg of the same variety. It is worth noting that values obtained in this work were quite aligned with those reported in the literature for some old varieties although these referred to whole apples.[10] Differently from other authors,[6,7,10,14] we did not detect the neochlorogenic and the *p*-coumaroylquinic acids. This could be related to the difference in varieties and climate, which can strongly influence the fruit chemical composition.[7] Procyanidins represent the second most abundant class in all varieties, followed by catechins. The content of procyanidin B1 varied from 5.3 ± 0.4 mg/kg of Giachetta to 338.0 ± 25.3 mg/kg of Grigia di Torriana cultivar. Procyanidin B2 ranged from 17.5 ± 6.4 mg/kg of Golden Delicious to 2087.5 ± 187.1 mg/kg of Grigia di Torriana. (+)-Catechin and (−)-epicatechin were present in the lowest amounts (13.6 ± 0.8 mg/kg and 20.1 ± 10.2 mg/kg, respectively) in Pom d'Aram cultivar, while the highest were found in Grigia di Torriana (194.7 ± 44.8 mg/kg and 912.5 ± 111.6 mg/kg, respectively). Golden Delicious was among the varieties that contained the lowest levels of both procyanidins and catechins (Table 2). Also (−)-epicatechin gallate was found in some of the studied cultivars at a range of 1.9 ± 0.1 to 35.0 ± 6.9 mg/kg. The highest content was found for Grigia di Torriana in 2008, while the minimum was for Pom d'Aram in 2007. This compound was not detected in Ronzè variety. Although (−)-epicatechin gallate is a main polyphenol component of green tea, and also in fruit, such as apples, cherries, and pears, have been reported to contain limited amounts of this component.[14] However, to our knowledge, (−)-epicatechin gallate did not result in any apple fruit phenolic composition. Among dihydrochalcones, only phloridzin was identified. As the peel has been discarded, probably most of these compounds were removed.[6,7,15] In this investigation, phloridzin was found in apple pulps at contents ranging between 1.4 ± 0.1 mg/kg for Ronzè in 2008 and 263.2 ± 84.2 mg/kg for Grigia di Torriana in 2009. This last variety was characterized by the highest phloridzin level every year. Instead, Golden Delicious and Ronzè were among the most poor in this compound.

Commonly, cyanidin glycosides are located in apple peels.[10] Yet, in this study, small amounts of cyanidin-3-glucoside were found in apple pulps of some of the old examined varieties. Its content ranged from 1.6 ± 0.1 mg/kg of Grigia di Torriana to 9.0 ± 1.0 mg/kg of Ros Borsetta. Cyanidin-3-glucoside was never detected in the commercial cultivar, Golden Delicious. Apple flavonols are essentially constituted by quercetin glycosides,

Table 3 Effect of the sampling year, cultivar, and their interaction on total phenolics and the content of each phenolic compound.

	Cultivar (A)	Year (B)	AB
TP	***	*	***
Gallic acid	***	***	***
Cyanidin-3-glucoside	***	***	***
Procyanidin B2	***	ns	***
(+)-Catechin	***	ns	***
Chlorogenic acid	***	***	***
Caffeicacid	***	***	***
Procyanidin Bl	***	ns	***
(−)-Epicatechin	***	ns	***
Ferulic acid	***	***	***
Quercetin-3-galactoside	***	ns	***
(−)-Epicatechin gallate	***	***	***
Phloridzin	***	***	***
Quercetin-3-rhamnoside	***	***	***

ns: not significant.
*,***Significantly different at $p = 0.05$ and $p = 0.001$, respectively.

which are mainly located in the peel.[6,7,15] In this work, the identification and the quantification of quercetin glycosides by DAD was not possible due to their low concentrations and co-elution with other molecules. Then, both qualitative and quantitative analyses of these compounds were carried out on HPLC-MS. Two quercetin glycosides were found in apple pulps: quercetin-3-galactoside (or hyperoside) and quercetin-3-rhamnoside (or quercitrin). Hyperoside content ranged from a minimum of 0.3 ± 0.0 mg/kg to a maximum of 2.0 ± 0.9 mg/kg. The lowest value was found in Golden Delicious, the highest in Dominici. Quercitrin was not as ubiquitous as hyperoside. In fact, only Dominici, Giachetta, and Golden Delicious varieties contained it over all of the sampling period. Its range was between 5.1 ± 4.0 mg/kg and 42.6 ± 4.6 mg/kg, the minimum concentration being recorded in Grigia di Torriana and the highest in Golden Delicious. Generally, variability of data is consistent with that reported from other authors.[16] In agreement with other authors,[7,17] we pointed out that the cultivar clearly affected the content of each detected phenolic compound and the TP content (Table 3). Further, the year was also found to be influent, but depending on the phenolic component. In fact, the content of a large number of phenolic compounds, except procyanidins, (+)-catechin, (−)-epicatechin, and hyperoside, are significantly affected by the sampling year (Table 3).

CONCLUSIONS

Our study provides data on the polyphenolic profiles of important ancient apple cultivars cultivated in Piedmont (Italy). Some of these varieties, such as Grigia di Torriana, Ros Borsetta, and Giachetta, proved to be an interesting source of bioactive compounds, especially in comparison with the commercial Golden Delicious. In particular, these fruits contain a large amount of polyphenols even though the peel was discarded before eating. It was shown that some representative apple phenolics, such as chlorogenic acid and phloridzin, are clearly affected by the harvest year, whereas others, including catechins and

procyanidins, are not. Thus, this factor should be taken into account in the correct definition of the polyphenolic profiles of apple fruits.

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