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3 AUTHORS, INCLUDING:

Aneta Wojdyło
Wrocław University of Environmental and …

Jan Oszmiański
Wrocław University of Environmental and …

Article

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Polyphenolic Compounds and Antioxidant Activity of New and Old Apple Varieties

ANETA WOJDYŁO,*,† JAN OSZMIAŃSKI,† AND PIOTR LASKOWSKI‡

Department of Fruit and Vegetable Technology, Wrocław Environmental and Life Science University, 25 Norwida Street, 50-375 Wrocław, Poland, and Research Station for Cultivar Testing in Zybiszów near Wrocław, 55–080 katy Wrocławskie, Poland

There is considerable evidence to show that a greater intake of apple contributes to improved health by reducing the risk of diseases, such as cardiovascular disease and some forms of cancer. Apple fruit is a major source of phenol compounds, because its consumption is widespread in many countries and it is available on the market for the whole year. The phenolic composition of 67 varieties of apple cultivars (new and old varieties) was examined for the concentration of some important phytochemicals and antioxidant activity. For the first time, we have looked at the correlation and compared polyphenolic coumpounds in Golden Delicious variety and new varieties grown from it. Up to 18 compounds, including catechin, procyanidin, hydroxycinnamates, flavonols, anthocyanins, and dihydrochalcones, were analyzed by high-performance liquid chromatography with diode array detection analysis of crude extracts and after thiolysis and LC−MS. The mean content of total polyphenols lay between 523.02 and 2723.96 mg/100 g dw and depending upon the apples variety. Flavanols (catechin and oligomeric procyanidins) are the major class of apple polyphenols, representing more than 80%, followed by hydroxycinnamic acids (1−31%), flavonols (2−10%), dihydrochalcones (0.5−5%), and in red apples, anthocyanins (1%). In this study, the best correlation was found for the total polyphenols and ABTS method, with a lower correlation for FRAP and DPPH methods (r = 0.871, 0.839, and 0.804, respectively). The presented data clearly demonstrated that new varieties, i.e., Ozark Gold, Julyred, and Jester, of apple had the same or higher value of bioactive compounds in comparison to the old varieties, i.e., Golden Delicious, Idared, and Jonagold.

KEYWORDS: Apple (*Malus domestica*); bioactive compounds; procyanidins; cultivars; HPLC; LC−MS

INTRODUCTION

During the last few decades, consumption of fruits and vegetables has received growing interest because many epidemiological and biochemical studies have demonstrated that they possess beneficial effects on human health. There is increasing evidence that the consumption of fruit and vegetables, which are rich sources of vitamins and fiber (*1*), is associated with a reduced risk of degenerative diseases, such as cancer, cardiovascular diseases, and cataracts (*2*). This association is often attributed to the antioxidants present in fruits and vegetables, which prevent free-radical damage, such as vitamins C and E, carotenoids, and polyphenols (*3*).

Apple fruit (*Malus domestica* Borkh.) is a major source of phenol compounds (*4*), because its consumption is widespread in many countries and it is available on the market for the whole year; therefore, it represents a major source of dietary antioxidants. A reduction in the risk of lung cancer, asthma, type-2 diabetes, thrombotic stroke, ischemic heart disease, and proliferation activities have been attributed to apple consumption (*5–8*).

Generally, five major polyphenolic groups are found in various apple varieties: hydroxycinnamic acids, flavan-3-ols/procyanidins, anthocyanins, flavonols, and dihydrochalcones. The flavan-3-ols can be found in their monomers (catechin and epicatechin), oligomers, and polymers (procyanidins); flavonols are often associated with sugar moieties (predominant sugar is galactose, glucose, rhamnose, arabinose, and xylose), whereas dihydrochalcones are mainly associated with glucose and xyloglucose. The complexity of the chemical profile and the variations are caused by growth period, growing season, geographical location, and most importantly, genetic variation (*9, 10*).

Apples are the most important fruits in Poland and several other countries in Europe and America. The major part of the production is consumed fresh, while a lesser part is processed into juices, concentrates, and purees. A number of studies have been made on the characteristic polyphenolic compounds; however, they are often restricted to a few cultivars, which are very popular with customers. Phenolic compounds have been tested in table apples and apples used for cider production and in their peel. Several characterization studies of different apple

* To whom correspondence should be addressed. Telephone: (48 71) 320-5474. Fax: (48 71) 320-5477. E-mail: aneta@wnoz.up.wroc.pl.
† Wrocław Environmental and Life Science University.
‡ Research Station for Cultivar Testing in Zybiszów.

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varieties grown in New Zealand (*11*), Italy (*12*), United States (*13*), and Canada (*14*) and apple ciders from Spain (*15*), France (*16, 17*), and United Kingdom (*18*) have been carried out on the basis of their polyphenolic profiles.

The varieties chosen for this study included the old cultivars, i.e., Beforest, Bramley's Seeding, Fuji, George Cave, Herrnhut Beauty, Jonathan, Meris, Reneta Sudecka, Papirovka, Paulared, and Titovka. However, Cortland, Idared, Jonagold, Golden Delicious, and Kosztela are the most widely cultivated varieties in Poland. The rest of varieties, especially Shampion, Ligol, Topaz, Gala, Fuji, and Jonafree, are new varieties, but their importance has been growing most rapidly in Eastern and Western Europe because they are appreciated by consumers (*19*). The new varieties chosen for this survey represented ~60% of the total production of the dessert apple in Poland and other countries in Europe over last few years. It is known that old varieties, whose production is decreasing, had a higher content of total polyphenols on average but still there was limited information about polyphenol compounds and antioxidant activity of other varieties, especially new apple varieties.

Therefore, the aim of this work was to evaluate the polyphenolic profiles (hydroxycinnamic acids, flavan-3-ols/procyanidins, anthocyanins, flavonols, and dihydrochalcones) of 67 varieties of apples (new and old varieties grown in Western Europe) in relation to their antioxidant activity measured by different methods. Correlations between the antioxidant activity and total and individual phenolic compounds were examined. For the first time, we have looked at the correlation and compared polyphenolic coumpounds in Golden Delicious variety and new varieties grown from it.

MATERIALS AND METHODS

Chemicals. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), chlorogenic acid, phloridzin, acetic acid, benzyl mercaptan (toluene α-thiol), and methanol were purchased from Sigma-Aldrich (Steinheim, Germany). Acetonitrile were purchased from Merck (Darmstadt, Germany). (−)-Epicatechin, (+)-catechin, procyanidins B2, and quercetin 3-glucoside were purchased from Extrasynthese (Lyon, France).

Apple Cultivars. A total of 28 apple cultivars examined in 2005 and 41 cultivars examined in 2006 were obtained from the Research Station for Cultivar Testing in Zybiszǒw near Wrocław. Apples were harvested successively as they were ripening from October 6 to 27 and from July 20 to October 20 of 2005 and 2006, respectively (**Table 1**). After harvest, the whole apple (each variety represented by five apples from the same tree, and then samples were taken and analyzed in three separate replications) was cut directly in liquid nitrogen and freeze-dried (24 h). The homogeneous powder was obtained by crushing the dried tissues with the use of closed laboratory mill to avoid hydration. Powders were kept in desiccators in darkness during analysis not longer than 24 h.

Extraction Procedure. Grounded dry plant materials (1 g) were weighed into a test tube. A total of 20 mL of 80% of aqueous methanol with 1% of HCl was added, and the suspension was slightly stirred. Tubes were sonicated for 15 min twice and left for 24 h at room temperature in darkness (~20 °C). The extract was centrifuged for 10 min (10 min, 20.878*g*), and supernatants were collected at 4 °C to be used within 24 h.

Identification of Polyphenols by the Liquid Chromatography−Mass Spectrometry (LC−MS) Method. Identification of polyphenol apple extracts was conducted using a LC−MS system consisting of a Waters 2690 gradient high-performance liquid chromatography (HPLC) separation module, an autoinjector, a 996 diode array ultraviolet−visible (UV−vis) absorbance detector (Waters Corp., Milford, MA), and a quadrupole iontunnel mass spectrometer (Quattro Ultima, Micromass Ltd., Manchester, U.K.) equipped with a ZQ-spray electrospray ionization (ESI) source. Separations were carried out using a Symmetry C18 (Waters Corp., Milford, MA) 5 μm column (150 × 4.6 mm i.d.) at 20 °C. The mobile phase was composed of solvent A (10% formic acid) and solvent B (100% of acetonitrile). The program began with isocratic elution with 95% A (0−1 min), and then a linear gradient was used until 41 min, lowering A to 0%; from 42 to 51 min, a decrease to 0% A. The flow rate was set at 0.5 mL/min. Analysis was carried out using full scan, data-dependent MS scanning from *m/z* 100 to 1000. The capillary temperature was 300 °C; the sheath gas and auxiliary gas were 50 and 5 units, respectively; and the source voltage was 3 kV for negative ionization and 0.1 kV for positive ionization.

Quantification of Polyphenols by the HPLC Method. The analysis of flavan-3-ols, hydroxycinnamates, dihydrochalcones, anthocyanin, and flavonol glycosides were carried out on a Merck-Hitachi L-7455 liquid chromatography with a diode array detector (DAD) and quaternary pump L-7100 equipped with a D-7000 HSM multisolvent delivery system (Merck-Hitachi, Tokyo, Japan) and autosampler L-7200. Separation was performed on a Synergi Fusion RP-80A 150 × 4.6 mm (4 μm) Phenomenex (Torrance, CA) column. Oven temperature was set to 30 °C. The mobile phase was composed of solvent A (2.5% acetic acid) and solvent B (acetonitrile). The program began with a linear gradient from 0% B to 36 min 25% B, followed by washing and reconditioning the column. The flow rate was 1.0 mL/min, and the runs were monitored at the following wavelengths: flavan-3-ols and dihydrochalcones at 280 nm, hydroxycinnamates at 320 nm, flavonol glycosides at 360 nm, and anthocyanins at 520 nm. Photo diode array (PAD) spectra were measured over the wavelength range of 200−600 nm in steps of 2 nm. Retention times and spectra were compared to those of pure standards within 200−600 nm. The calibration curves were made from (−)-epicatechin, (+)-catechin, quercetin-3-*O*-rhamnoside, chlorogenic acid, phloretin 2′-*O*-glucoside, and procyanidin B2, C1, and B1 as standards. Thus, ploretin-2-xyloglucoside was quantified as phloridzin; *p*-coumaroylquinic acid was quantified as coumaric acid; and quercetin derivatives was quantified as quercetin-3-*O*-rhamnoside.

Procyanidin Analysis by Thiolysis. The direct thiolysis of freeze-dried apples was performed as described by Guyot et al. (*20*). Portions (50 mg) of freeze-dried apple powders were precisely weighed in 2.0 mL Eppendorf vials, and then 400 μL of acidic methanol [3.3% HCl (v/v)] and 800 μL of toluene α-thiol (5% in methanol) were added. Vials were closed and incubated at 40 °C for 30 min with agitation on a vortex every 10 min. Next, the vials were cooled in ice water and centrifuged immediately at 4 °C at 20000*g* during 10 min. Samples were stored at 4 °C until RP-HPLC analysis. All incubations were performed in triplicate. Thiolysis products were separated on a Merck Purospher RP 18 end-capped column 250 × 4 mm, 5 μm (Merck, Darmstadt, Germany). The liquid chromatograph was a Waters (Milford, MA) system equipped with DAD and scanning fluorescence detectors. The solvent A [aqueous acetic acid, 2.5% (v/v)] and solvent B (acetonitrile) were used as the following gradient: initial, 3% B; 0−5 min, 9% B linear; 5−15 min, 16% B linear; and 15−45 min, 50% B linear, followed by washing and reconditioning of the column. Flow rate of 1 mL/min and oven temperature of 30 °C were used. The compounds for which reference standards were available (synthesized or isolated previously) were identified on chromatograms according to their retention times and UV−vis spectra. Fluorescence was recorded at an excitation wavelength of 278 nm and emission wavelength of 360 nm. Calibration curves were established using flavan-3-ol and benzylthioether standards prepared in our laboratory. The average degree of polymerization (DP) was measured by calculating the molar ratio of all of the flavan-3-ol units (thioether adducts + terminal units) to (−)-epicatechin and (+)-catechin corresponding to terminal units.

Ferric Reducing/Antioxidant Power (FRAP) Assay. The total antioxidant potential of a sample was determined using a ferric-reducing ability of plasma FRAP assay by Benzie et al. (*21*) as a measure of the antioxidant power. The assay was based on the reducing power of a compound (antioxidant). A potential antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 μM, pH 3.6), a solution of 10 μM TPTZ in 40 μM HCl, and 20 μM $FeCl_3$ at 10:1:1 (v/v/v). The reagent (300 μL) and sample solutions (10 μL)

Table 1. Origin of Apple Varieties and Harvest Time in Years 2005 and 2006

cultivars	origin of the apple[a]	harvest time (year)	new/old[a]
'Alwa'	sower 'Macoun' variety	2005	old
'Amulet'	nd	2006	old
'Arlet'	'Golden Delicious' × 'Idared'	2005	new
'Beforest'	nd	2006	old
'Bramley's Seeding'	nd	2006	old
'Cortland'	'McIntosh' × 'Ben Davis'	2005	old
'Dalijo'	mutant 'Jonagold' variety	2005	new
'Delbarestivale'	'Stark Jongrimes' × 'Golden Delicious'	2006	new
'Discovery'	'Worcester Pearmain' × 'Beauty of Bath'	2006	new
'Ecolette'	'Elstar' × 'Prima'.	2005	new
'Elise'	'Septer' × 'Koksa Pomarańczowa'	2005	new
'Elstar'	'Golden Delicious' × 'Ingrid Marie'	2005	old
'Fameuse'	sower 'McIntosh' variety	2006	old
'Fantazja'	'McIntosh' × 'Linda'	2006	old
'Fiałka'	nd	2006	old
'Fireside Red'	mutant 'Fireside' variety	2005	old
'Florina'	hybrid 612-1 × 'Jonathan'	2005	new
'Freedom'	(Malus floribunda 821 × 'Rome Beauty') × 'Golden Delicious' × 'McIntosh' × 'Jersey Black' × 'Macoun' × 'Antonówka'	2005	new
'Fuji'	'Ralls Janet' × 'Red Delicious'	2005	old
'Geneva Early'	'Quinte' × 'Julyred'	2006	old
'George Cave'	nd	2006	old
'Getmanskoje'	nd	2006	old
'Golden Delicious'	nd	2005	old
'Idared'	'Jonatan' × 'Wagener'	2005	old
'Jester'	'Worcester Pearmain' × 'Stark Spur Golden Delicious'	2005	new
'Jonafree'	(PRI 2016-100 = PRI 855-102 × NJ. 31)	2005	new
'Jonagold'	'Jonatan' × 'Golden Delicious'	2006	old
'Jonathan'	nd	2005	old
'Juga'	'Melrose' × 'Idared'	2006	new
'Julyred'	('Petrel' × 'Early McIntosh') × ('Williams' × 'Starr')	2006	new
'Jupiter'	'Cox's orange' × 'Starking'	2005	new
'Katja'	'James Grieve' × 'Worcester Pearmain'	2006	old
'Cox's orange'	sower 'Pepina Ribstona' variety	2005	old
'Kosztela'	nd	2006	old
'Ligol'	'Linda' × 'Golden Delicious'	2006	new
'Lodel'	'Lobo' × 'Redspur Delicious'	2005	new
'Macfree'	'McIntosh' × hybrid 48-177	2005	new
'Meris'	nd	2006	old
'Mutsu'	'Golden Delicious' × 'Indo'	2005	old
'Nela'	'Prima' × 'Krasava'	2006	new
'New Jonagold'	mutant 'Jonagold' variety	2005	new
'Nova Easygro'	'Spartan' × hybrid Prog 565 × hybrid 27-435	2006	new
'Novamac'	'McIntosh' × hybrid 1018-3	2006	new
'Odra'	Primula' × 'Bankroft'	2005	new
'Oliwka Zŏłta'	nd	2006	old
'Ozark Gold'	'Golden Delicious' × ('Conard' × 'Red Delicious')	2005	new
'Paulared'	nd	2006	old
'Herrnhut Beauty'	nd	2006	old
'Pinova'	'Clivia' × 'Golden Delicious'	2005	new
'Priam'	'Jonathan' × hybrid 14-126	2006	new
'Priscilla'	'Starking' × hybrid 610-2 (Malus floribunda 821 × 'Rome Beauty')	2005	new
'Rajka'	'Szampion' × 'Katja'	2005	new
'Redfree'	(PRI 2175-7 = 'Raritan' × PRI 1018-101)	2006	new
'Reneta Sudecka'	'Reneta Ananasowa' × 'Reneta Kanadyjska'	2006	old
'Rubin'	'Lord Lambourne' × 'Golden Delicious'.	2005	new
'Rubinola'	'Rubin' × 'Prima'	2006	new
'Sawa'	'Fantazja' × 'Primula'	2006	new
'Starkrimson'	mutant 'Red Delicious' variety	2005	old
'Sunrise'	'McIntosh' × 'Golden Delicious'	2006	new
'Shampion Arno'	mutant 'Shampion' variety	2006	new
'Shampion'	'Golden Delicious' × 'Koksa Pomarańczowa'	2006	new
'Teremok'	nd	2006	old
'Titówka'	nd	2006	old
'Topaz'	'Rubin' × 'Vanda'	2005	new
'Waleria'	sower 'Primula' variety	2006	new
'Witos'	'Fantazja' × 'Primula'	2006	new
'Zimnieje Limonnoje'	'Reneta Simirienko' × 'Antonówka Gold Monarch'	2005	old

[a] New and old varieties and the origin of apple classified on data from refs *24–31; nd, no data.*

were added to each well and mixed thoroughly. The absorbance was taken at 593 nm after 10 min. Standard curve was prepared using different concentrations of Trolox. All solutions were used on the day of preparation. The results were corrected for dilution and expressed in micromolar Trolox per 100 g of dry weight (dw). All determinations were performed in triplicates.

Free-Radical-Scavenging Ability by the Use of a Stable DPPH Radical. The DPPH radical scavenging activity was determined using the method proposed by Yen et al. (*22*). DPPH (100 μM) was dissolved in pure ethanol (96%). The radical stock solution was prepared fresh daily. The DPPH solution (1 mL) was added to 1 mL of polyphenol extracts with 3 mL of ethanol. The mixture was shaken vigorously

Table 2. Characterization of Phenolic Compounds of Apple Fruits by Using Their Spectral Characteristic in LC−DAD (Retention Time, λ_{max}) and Negative Ions in LC−MS

group of polyphenols	R_t (min)	λ_{max} (nm)	compound	$[M - H]^-$	MW
	19.9	320	5-*O*-caffeoylquinic acid	353	354
	21.5	305	p-coumarylquinic acid	337	338
flavanol and procyanidins	16.8	275	procyanidin B1	577	578
	19.5	280	(+)-catechin	289	290
	21.8	275	procyanidin B2	577	578
	22.4	280	(−)-epicatechin	289	290
	24.5	280	procyanidin C1	867	868
dihydrochalcones	34.0	285	phloretin-2′-*O*-xyloglucoside	567	568
	36.8	285	phloretin-2′-*O*-glucoside	435	436
flavonols	31.3	345	quercetin-3-*O*-rhamnoside	447	448
	31.6	350	quercetin-3-*O*-rutinoside	609	610
	32.4	355	quercetin-3-*O*-galactoside	463	464
	32.9	350	quercetin-3-*O*-glucoside	463	464
	34.3	355	quercitin-3-*O*-arabinoside	433	434
	35.5	350	quercitin-3-*O*-xyloside	433	434
anthocyanins	18.1	520	cyanidin 3-glucoside	$450 + [H]^-$	499
	19.2	525	cyanidin 3-galactoside	$450 + [H]^-$	499

and allowed to stand at room temperature in the dark for 10 min. The decrease in absorbance of the resulting solution was monitored at 517 nm at 10 min. The results were corrected for dilution and expressed in micromolar Trolox per 100 g of dry weight (dw). All determinations were performed in triplicates.

Free-Radical-Scavenging Ability by the Use of a Stable ABTS Radical Cation. The free-radical-scavenging activity was determined by ABTS radical cation decolorization assai described by Re et al. (*23*). ABTS was dissolved in water to a 7 μM concentration. ABTS radical cation ($ABTS^{\bullet +}$) was produced by reacting ABTS stock solution with 2.45 μM potassium persulfate (final concentration) and kept in the dark at room temperature for 12−16 h before use. The radical was stable in this form for more than 2 days when stored in the dark at room temperature. For the study of infusion, the samples containing the $ABTS^{\bullet +}$ solution were diluted with redistilled water to an absorbance of 0.700 (±0.02) at 734 nm and equilibrated at 30 °C. After the addition of 3.0 mL of diluted $ABTS^{\bullet +}$ solution ($A_{734\ nm} = 0.700 \pm 0.02$) to 30 μL of polyphenolic extracts, the absorbance reading was exactly 6 min after initial mixing. The results were corrected for dilution and expressed in micromolar Trolox per 100 g of dry weight (dw). All determinations were performed in triplicates.

Statistical Analysis. Principal component analysis (PCA) were performed using XLSTAT (Addinisoft, France) on mean values of 68 samples and 8 variables. The PCA is a multilinear modeling method given an interpretable overview of the main information in a multidimensional data table.

RESULTS AND DISCUSSION

Qualitative Analysis. The names of 67 varieties chosen for the study included the 36 "old" and the 31 "new" cultivars, together with the origin of apple, were presented in **Table 1**. As an initial step, apple samples were analyzed by LC−MS and HPLC−DAD systems. Qualitative analysis obtained by LC−MS methods and quantitative analysis obtained by HPLC (quantified using DAD and fluorescence detection) are summarized in **Tables 2–4**. A total of 18 kinds of polyphenolic compounds found in apple tissues were identified and presented.

Two hydroxycinnamates were detected, *p*-coumaroylquinic acid and chlorogenic acid (5-*O*-caffeoylquinic acid). The compound that had a $[M - H]^-$ at *m/z* 337 and $\lambda_{max} = 305$ nm was identified as *p*-coumaroylquinic acid. 5-*O*-Caffeoylquinic acid had a characteristic mass spectral data as is produced on $[M - H]^-$ at *m/z* 353. Five flavan-3-ols were detected, (+)-catechin, (−)-epicatechin, and procyanidin B1, B2, and C1. In the presence retention time at 19.5 min, λ_{max} for 270 nm was identified as (+)-catechin with the fragmentation of the negatively charged molecular ion ($[M - H]^-$) at *m/z* 289. Procyanidin B1 and B2 ($\lambda_{max} = 275$ nm) had a $[M - H]^-$ at *m/z* 577, but the retention time for procyanidins B1 was ($R_t = 16.8$ min) and for B2 was ($R_t = 21.8$ min). The compound with ($R_t = 24.5$ min, $\lambda_{max} = 280$ nm) that had the highest MW, with a $[M - H]^-$ at *m/z* 867 is procyanidin C1. The compound that had the $R_t = 22.4$ min and $\lambda_{max} = 275$ nm was identified as (−)-epicatechin.

Dihydrochalcones were detected: phloretin-2′-*O*-xyloglucoside and phloretin-2′-*O*-glucoside. The peak with a $[M - H]^-$ at *m/z* 567 that had a retention time and λ_{max} ($R_t = 34.0$ min, $\lambda_{max} = 285$ nm) is phloretin-2′-*O*-xyloglucoside. The peak ($R_t = 36.8$ min, $\lambda_{max} = 285$ nm) produced a $[M - H]^-$ at *m/z* 435, and the loss of 163 amu indicates the loss of a hexose moiety, which is other dihydrochalcones. This is the fragmentation pattern phloretin-2′-*O*-glucoside.

Flavonols were detected: quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-rhamnoside, quercetin-3-*O*-rutinoside, quercetin-3-*O*-arabinoside, and quercetin-3-*O*-xyloside. Peaks with ($R_t = 32.4$ and 32.9 min) had λ_{max} values of 355 and 350 nm, respectively. Both had a $[M - H]^-$ at *m/z* 463, and fragmentation yielded a quercetin ion at *m/z* 301. The loss of 162 amu indicates the cleavage of a hexose group. This fragmentation pattern demonstrates that this peak is quercetin-3-*O*-galactoside and quercetin-3-*O*-glucoside, respectively. A similar situation was found for quercetin-3-xyloside and quercetin-3-arabinoside that brought the same *m/z* 433 but different retention times (R_t) 35.5 and 34.3 min, respectively.

The compound ($R_t = 31.3$ min, $\lambda_{max} = 345$ nm) that produced a $[M - H]^-$ at *m/z* 447 and a fragment at *m/z* 301 was identified as quercetin-3-*O*-rhamnoside. The loss of 146 amu equates to the loss of the rhamnosyl group. The peak with a retention time on $R_t = 31.6$ min and $\lambda_{max} = 370$ nm suggested the presence of quercetin-3-*O*-rutinoside, and this identification was confirmed by the mass spectral data. These data are agreement with data obtained by Mullen et al. (*24*) and Tsao et al. (*25*).

Peak in 18.1 min was identified as cyanidin 3-galactoside and, in 19.2 min, was identified as cyanidin 3-glucoside. The identification of this antocyanin was based on the retention time and molar mass of the authentic standard (**Table 2**).

Quantitative Analysis. The major polyphenolic groups were hydroxycinnamic acids, flavan-3-ols/procyanidins, flavonols, dihydrochalcones, and anthocyanins. Types of polyphenolic compounds detected in these apple cultivars studies were similar to previous studies (*10, 14, 19*). The content of total polyphenols lies between 5230.2 and 27239.6 mg/kg dm. The average content

Table 3. Proanthocyanidin and Hydroxycinnamic Acid Content of Apple Varieties [Data Expressed as mg/kg ± Standard Error ($n = 3$)][a]

	flavan-3-ols							hydroxycinnamic acid		
apple variety	CAT	EC	P B2	PB1	PC1	PO	DPn	CQAy	PCQ	CQAy/PCQ
Alwa	10.7 ± 0.2	440.5 ± 2.3	628.5 ± 2.6	94.4 ± 0.6	293.3 ± 3.4	5213.1 ± 11	4.1	732.4 ± 1.2	34.4 ± 1.2	21.3
Amulet	60.9 ± 0.4	766.9 ± 3.6	836.4 ± 3.4	150.2 ± 1.3	387.6 ± 2.5	10036.1 ± 12	3.6	106.9 ± 1.3	23.8 ± 2.4	4.5
Arlet	14.2 ± 0.9	292.7 ± 1.7	425.0 ± 2.6	104.5 ± 0.9	248.3 ± 1.1	5888.9 ± 17	5.6	782.3 ± 1.8	77.0 ± 1.0	10.2
Beforest	43.1 ± 1.0	243.9 ± 1.1	334.8 ± 1.9	89.1 ± 0.5	332.3 ± 1.9	4750.5 ± 9	5.3	760.0 ± 0.4	35.3 ± 0.7	21.5
Bramleys Seeding	180.3 ± 1.2	609.7 ± 1.9	836.8 ± 4.2	454.3 ± 1.7	350.2 ± 2.6	11687.1 ± 21	3.8	212.1 ± 1.6	144.8 ± 1.3	1.5
Cortland	66.3 ± 1.3	283.8 ± 2.0	380.8 ± 1.8	95.0 ± 1.1	377.9 ± 3.0	6910.7 ± 13	6.2	455.7 ± 2.1	79.7 ± 1.11	5.7
Dalijo	16.5 ± 0.7	297.4 ± 1.2	543.4 ± 1.9	66.2 ± 0.3	335.3 ± 0.8	7847.0 ± 25	5.7	734.0 ± 2.4	47.6 ± 0.5	15.4
Delbarestivale	51.1 ± 0.6	385.5 ± 1.6	490.3 ± 2.0	134.6 ± 1.5	254.5 ± 1.1	10040.1 ± 36	3.3	590.1 ± 2.2	52.0 ± 1.1	11.4
Discovery	466.6 ± 2.8	948.4 ± 3.9	389.7 ± 1.6	262.9 ± 1.9	575.7 ± 1.9	7569.6 ± 12	4.9	510.6 ± 1.5	61.1 ± 0.8	8.4
Ecolette	71.4 ± 1.2	77.4 ± 0.5	72.4 ± 2.0	23.8 ± 0.2	71.9 ± 1.4	9727.7 ± 23	28.7	1086.6 ± 2.7	57.5 ± 1.1	18.9
Elise	21.7 ± 0.3	65.9 ± 1.1	68.7 ± 1.1	21.5 ± 0.5	58.4 ± 2.8	5869.5 ± 17	17.8	441.1 ± 1.1	08.5 ± 0.2	51.9
Elstar	30.4 ± 0.8	282.4 ± 0.4	378.9 ± 1.0	103.1 ± 1.7	183.0 ± 1.7	4968.2 ± 11	4.8	256.3 ± 0.9	31.8 ± 0.6	8.1
Fameuse	91.3 ± 1.1	671.1 ± 1.9	536.3 ± 2.9	52.9 ± 0.2	278.7 ± 2.8	7438.3 ± 21	6.0	51.3 ± 1.1	24.7 ± 0.9	2.1
Fantazja	48.8 ± 0.4	323.3 ± 1.8	571.0 ± 3.0	141.9 ± 0.7	317.1 ± 1.2	12608.4 ± 10	5.4	14.5 ± 1.9	39.2 ± 0.2	0.4
Fiałka	197.9 ± 1.4	1889.0 ± 4.9	2002.0 ± 2.4	568.1 ± 1.5	973.7 ± 1.9	19849.6 ± 9	3.4	62.0 ± 0.7	49.1 ± 0.7	1.3
Fireside Red	14.6 ± 0.2	287.9 ± 1.9	345.8 ± 1.6	41.5 ± 0.4	231.5 ± 2.8	9359.7 ± 2	4.2	740.6 ± 2.1	10.1 ± 0.0	73.3
Florina	98.9 ± 1.5	536.4 ± 2.4	674.2 ± 2.9	128.4 ± 1.5	345.5 ± 2.1	8117.3 ± 11	4.6	234.7 ± 1.4	03.6 ± 0.1	65.2
Freedom	10.1 ± 0.0	349.3 ± 1.0	568.6 ± 3.1	71.4 ± 0.8	295.7 ± 2.9	6905.5 ± 15	5.1	974.9 ± 2.0	40.2 ± 0.6	24.3
Fuji	124.9 ± 1.3	237.2 ± 0.9	522.5 ± 2.0	126.3 ± 1.3	156.0 ± 1.4	8621.2 ± 20	4.2	724.2 ± 2.1	20.4 ± 0.7	35.5
Geneva Early	326.3 ± 1.6	673.4 ± 1.1	680.0 ± 1.6	313.1 ± 1.0	299.1 ± 2.0	4237.4 ± 31	8.0	322.8 ± 1.1	258.2 ± 1.3	1.3
George Cave	124.0 ± 1.3	679.3 ± 2.5	1068.0 ± 1.4	159.2 ± 2.0	257.5 ± 0.5	3417.9 ± 11	4.7	337.5 ± 2.1	156.1 ± 1.6	2.2
Getmanskoje	723.2 ± 2.5	1432.0 ± 1.9	1220.0 ± 0.3	17.1 ± 0.4	620.3 ± 1.8	11899.3 ± 17	3.4	96.2 ± 1.9	74.8 ± 0.1	1.3
Golden Delicious	92.8 ± 0.5	167.8 ± 1.9	267.2 ± 2.5	93.9 ± 1.8	101.9 ± 1.1	5251.6 ± 12	4.9	475.1 ± 2.7	09.5 ± 0.0	50.0
Idared	24.2 ± 0.2	322.6 ± 2.8	524.5 ± 1.6	146.0 ± 1.6	520.5 ± 1.7	9964.7 ± 10	5.4	983.9 ± 3.0	54.9 ± 0.7	17.9
Jester	105.2 ± 1.6	889.8 ± 3.7	988.8 ± 2.7	270.2 ± 1.6	520.7 ± 2.3	10995.3 ± 11	3.7	935.0 ± 1.0	24.6 ± 0.7	38.0
Jonafree	22.0 ± 0.4	406.3 ± 1.9	706.8 ± 3.0	137.0 ± 2.0	354.5 ± 3.1	7076.7 ± 17	4.4	1684.4 ± 2.7	18.6 ± 0.3	90.6
Jonagold	16.3 ± 0.1	275.9 ± 1.9	382.4 ± 3.1	88.7 ± 0.5	215.6 ± 2.7	5753.6 ± 14	5.2	667.7 ± 4.8	56.7 ± 0.7	11.8
Jonathan	35.7 ± 0.3	503.9 ± 2.4	724.0 ± 2.6	141.7 ± 1.1	414.6 ± 2.1	12046.2 ± 21	4.8	230.7 ± 2.8	32.6 ± 0.3	7.1
Juga	93.2 ± 1.0	616.3 ± 0.7	563.7 ± 2.4	115.1 ± 1.6	321.0 ± 1.0	10338.4 ± 17	5.1	43.7 ± 2.2	14.8 ± 0.7	2.9
Julyred	70.2 ± 1.3	1199.0 ± 4.2	1027.0 ± 2.8	113.2 ± 1.4	440.0 ± 2.0	10111.0 ± 24	3.4	69.1 ± 1.1	42.8 ± 0.3	1.6
Jupiter	44.1 ± 0.6	411.2 ± 3.9	634.7 ± 1.0	136.0 ± 1.9	312.3 ± 2.6	6688.0 ± 31	4.1	297.7 ± 2.6	74.6 ± 0.9	4.0
Katja	165.0 ± 1.9	776.6 ± 1.3	148.5 ± 2.1	81.4 ± 1.0	471.1 ± 3.1	3997.8 ± 11	5.1	191.8 ± 1.1	52.2 ± 1.0	3.7
Cox's orange	66.2 ± 0.2	365.8 ± 1.9	506.1 ± 2.6	129.4 ± 1.1	502.3 ± 2.7	6481.1 ± 21	5.4	149.1 ± 4.7	32.9 ± 0.0	4.5
Kosztela	150.4 ± 1.6	1092.0 ± 2.9	1210.0 ± 1.6	342.4 ± 2.4	651.3 ± 2.3	13012.4 ± 25	3.6	2960.0 ± 4.8	40.4 ± 1.1	73.3
Ligol	119.5 ± 2.4	115.0 ± 1.3	646.9 ± 3.6	37.3 ± 2.5	282.6 ± 2.2	6095.8 ± 28	4.1	88.7 ± 15	10.7 ± 0.3	8.2
Lodel	45.7 ± 0.2	378.3 ± 1.9	624.6 ± 1.7	82.9 ± 1.7	318.6 ± 1.3	7739.2 ± 31	4.7	800.7 ± 1.6	60.6 ± 0.5	13.2
Macfree	10.6 ± 0.3	254.7 ± 2.0	389.4 ± 1.2	103.4 ± 1.9	242.2 ± 2.5	9136.0 ± 14	6.8	408.9 ± 2.9	11.2 ± 1.1	36.5
Meris	55.8 ± 0.3	449.2 ± 1.1	604.6 ± 0.3	183.8 ± 2.6	315.4 ± 1.1	10090.7 ± 17	4.5	40.3 ± 3.1	9.5 ± 0.4	4.2
Mutsu	33.3 ± 0.1	250.8 ± 1.9	389.1 ± 2.6	59.9 ± 1.1	212.0 ± 1.4	6444.4 ± 21	8.3	668.4 ± 2.4	37.6 ± 1.1	17.8
Nela	218.2 ± 2.4	876.1 ± 1.6	263.3 ± 1.6	156.8 ± 1.3	612.7 ± 1.8	7547.7 ± 20	5.2	277.7 ± 1.5	71.4 ± 0.6	3.9
New Jonagold	37.7 ± 0.2	336.6 ± 2.0	540.2 ± 2.9	86.6 ± 1.9	305.1 ± 2.7	8658.9 ± 15	6.2	908.3 ± 1.6	54.3 ± 0.8	16.7
Nova Easygro	26.9 ± 0.1	334.9 ± 3.1	439.7 ± 3.8	135.4 ± 2.6	260.2 ± 2.7	7634.5 ± 10	5.6	401.9 ± 2.7	18.1 ± 0.1	22.2
Novamac	74.9 ± 1.3	124.6 ± 2.9	153.9 ± 1.1	46.6 ± 1.5	190.2 ± 2.9	3287.3 ± 15	7.3	126.6 ± 1.5	40.8 ± 0.6	3.1
Odra	68.4 ± 1.5	695.2 ± 4.0	884.5 ± 2.9	188.5 ± 1.8	877.8 ± 3.6	7892.4 ± 7	3.4	889.1 ± 2.8	48.9 ± 0.9	18.2
Oliwka Zǒłta	353.3 ± 2.6	1108.0 ± 1.4	1040.0 ± 2.9	171.6 ± 2.6	464.4 ± 3.1	5662.8 ± 14	10.6	91.3 ± 2.5	12.8 ± 0.5	7.1
Ozark Gold	148.2 ± 1.5	518.4 ± 2.0	785.0 ± 4.8	312.0 ± 1.9	494.6 ± 2.7	17685.6 ± 24	4.6	331.5 ± 2.1	95.8 ± 1.1	3.5
Paulared	145.6 ± 2.9	2756.0 ± 2.4	1323.0 ± 2.4	74.6 ± 1.5	630.6 ± 2.7	6264.7 ± 21	4.0	210.9 ± 2.8	241.0 ± 2.0	0.9
Herrnhut Beauty	188.3 ± 0.2	554.0 ± 1.9	813.4 ± 1.8	396.4 ± 0.8	377.6 ± 4.1	12442.3 ± 25	4.1	228.9 ± 1.1	90.7 ± 0.6	2.5
Pinova	23.0 ± 0.4	543.0 ± 2.6	878.9 ± 0.3	140.7 ± 1.4	445.9 ± 2.8	8303.5 ± 12	4.3	1112.4 ± 3.4	9.7 ± 0.1	114.7
Priam	220.3 ± 2.4	741.2 ± 2.9	311.1 ± 2.7	80.9 ± 1.7	330.2 ± 0.9	7390.3 ± 11	5.3	540.1 ± 1.5	10.0 ± 0.2	54.0
Priscilla	41.3 ± 1.0	381.1 ± 1.4	565.9 ± 1.8	147.4 ± 0.4	284.1 ± 1.5	8022.5 ± 6	3.9	328.2 ± 2.4	8.5 ± 0.1	38.6
Rajka	44.8 ± 0.5	297.1 ± 0.3	422.9 ± 4.1	82.3 ± 1.4	238.9 ± 2.9	4902.4 ± 15	5.1	250.4 ± 2.9	55.2 ± 0.8	4.5
Redfree	211.2 ± 1.5	763.6 ± 2.8	641.6 ± 3.0	610.6 ± 2.7	294.7 ± 1.1	2871.1 ± 17	6.7	217.8 ± 3.1	206.7 ± 1.5	1.1
Reneta Sudecka	111.9 ± 1.2	485.4 ± 1.9	536.2 ± 1.6	192.1 ± 2.1	253.1 ± 1.4	5531.7 ± 21	4.0	1220.5 ± 4.9	42.3 ± 0.2	28.9
Rubin	80.2 ± 0.9	165.5 ± 3.1	255.1 ± 2.8	46.0 ± 1.6	138.1 ± 2.6	4913.3 ± 26	6.8	332.2 ± 2.8	3.6 ± 0.3	92.3
Rubinola	48.9 ± 1.3	492.1 ± 2.5	555.9 ± 1.0	139.3 ± 1.9	270.1 ± 3.6	6824.0 ± 18	4.7	108.0 ± 2.1	4.6 ± 0.3	23.3
Sawa	190.8 ± 1.7	752.7 ± 3.9	266.4 ± 0.9	46.9 ± 1.5	426.7 ± 2.0	2412.3 ± 11	14.6	28.2 ± 2.6	15.1 ± 0.7	1.9
Starkrimson	70.5 ± 0.4	655.8 ± 1.3	640.8 ± 1.4	151.3 ± 2.3	396.1 ± 1.4	10184.8 ± 10	4.4	760.8 ± 1.9	68.6 ± 0.9	11.1
Sunrise	383.5 ± 1.9	703.4 ± 1.0	211.1 ± 2.9	129.4 ± 1.1	460.7 ± 1.7	6573.1 ± 17	5.9	121.1 ± 2.0	31.6 ± 1.0	3.8
Shampion Arno	64.2 ± 1.03	710.6 ± 1.9	880.8 ± 1.1	158.3 ± 1.5	424.3 ± 2.3	8697.7 ± 24	4.1	40.5 ± 1.0	57.5 ± 1.1	0.7
Shampion	150.5 ± 1.1	533.5 ± 1.3	716.6 ± 2.0	143.2 ± 2.9	406.1 ± 3.9	11169.2 ± 31	4.5	23.9 ± 0.9	54.4 ± 0.7	0.4
Teremok	68.7 ± 0.9	979.3 ± 2.0	921.3 ± 1.6	106.4 ± 1.1	419.4 ± 1.1	14153.6 ± 16	4.7	115.9 ± 1.7	42.2 ± 0.3	2.7
Titówka	717.8 ± 1.8	1549.0 ± 1.7	683.8 ± 1.9	110.8 ± 2.6	694.8 ± 2.1	8492.9 ± 25	3.2	309.2 ± 2.0	67.5 ± 0.8	4.6
Topaz	21.0 ± 0.4	303.7 ± 3.4	446.8 ± 2.1	70.6 ± 1.0	203.6 ± 1.8	3576.4 ± 37	4.1	285.9 ± 1.1	4.0 ± 0.1	71.5
Waleria	33.9 ± 0.9	687.4 ± 2.6	417.0 ± 3.0	33.0 ± 0.5	523.5 ± 1.4	1374.0 ± 11	4.2	146.0 ± 2.3	87.7 ± 0.7	1.7
Witos	57.0 ± 1.3	570.1 ± 1.9	221.6 ± 4.1	30.8 ± 0.6	250.3 ± 2.0	4242.2 ± 18	12.5	26.0 ± 1.0	45.4 ± 0.3	0.6
Zimnieje Limonnoje	71.6 ± 2.0	357.5 ± 2.0	719.3 ± 2.7	87.9 ± 1.6	371.1 ± 1.6	9522.2 ± 16	5.7	1043.8 ± 1.1	32.9 ± 0.0	31.7

[a] CAT, (+)-catechin; EC, (−)-epicatechin; PB2, procyanidin B2; PB1, procyanidin B1; PC1, procyanidin C1; PO, oligomeric procyanidins; DPn, mean degree of polymerization; QAy, chlorogenic acid; PCQ, *p*-coumaroyloquinic acid.

of total polyphenols found in the apple evaluated by HPLC analysis was 11 151.9 mg/kg of dry weight (dw), with significant differences depending upon the apple cultivar, and was similar to data obtained by Vrhovsek et al. (*19*). The Kosztela, Ozark

Table 4. Dihydrochalcones, Flavonol, and Anthocyanins Content of Apple Varieties (Data Expressed as mg/kg ± Standard Error (n=3)[a]

	dihydrochalcones		flavonol						anthocyanins		
apple variety	PLXG	PLG	QRUT	QGAL	QGLU	QARA	QXYL	QRHM	CGAL	CGLU	total of polyphenols
Alwa	19.9.1 ± 0.1	19.57 ± 1.2	0.0 ± 0.0	32.6 ± 0.3	13.2 ± 0.4	18.2 ± 0.8	37.2 ± 0.2	20.8 ± 0.2	29.5 ± 0.3	48.1 ± 0.3	808.3
Amulet	9.7.6 ± 1.1	7.68 ± 1.4	0.0 ± 0.0	43.6 ± 0.7	21.5 ± 0.7	34.6 ± 0.6	63.9 ± 1.2	75.3 ± 1.1	0.0 ± 0.0	400.2 ± 2.4	1321.8
Arlet	19.9.0 ± 2.3	16.42 ± 1.6	59.5 ± 0.2	535.8 ± 1.8	98.7 ± 1.1	103.0 ± 1.1	236.9 ± 2.3	100.0 ± 1.2	153.7 ± 2.1	12.6 ± 0.4	955.2
Beforest	3.4.3 ± 0.9	7.36 ± 2.1	19.6 ± 0.2	260.7 ± 0.4	19.5 ± 0.2	43.4 ± 0.9	104.7 ± 2.1	100.2 ± 1.3	63.9 ± 0.5	27.5 ± 0.3	739.0
Bramleys Seeding	9.1.4 ± 1.5	7.90 ± 1.9	2.9 ± 0.0	42.6 ± 0.3	22.8 ± 0.3	16.8 ± 0.4	37.2 ± 0.4	21.1 ± 0.6	300.0 ± 1.2	900.0 ± 2.3	1602.7
Cortland	13.2.9 ± 1.3	8.75 ± 2.1	94.0 ± 1.4	335.1 ± 1.6	127.4 ± 0.7	77.5 ± 1.2	188.0 ± 2.2	68.0 ± 0.3	202.8 ± 1.1	80.5 ± 1.4	1010.6
Dalijo	11.2.8 ± 1.1	15.91 ± 1.7	12.3 ± 0.4	151.7 ± 1.9	23.2 ± 0.9	48.5 ± 0.9	95.6 ± 1.3	134.2 ± 1.2	24.2 ± 0.3	15.8 ± 0.2	1072.2
Delbarestivale	4.0.1 ± 0.3	3.05 ± 0.9	0.0 ± 0.0	40.3 ± 0.3	10.0 ± 0.1	22.6 ± 0.3	53.2 ± 1.1	82.3 ± 0.3	0.0 ± 0.0	20.1 ± 0.9	1233.0
Discovery	14.6.4 ± 1.4	14.92 ± 1.7	0.0 ± 0.0	288.9 ± 2.1	38.6 ± 0.2	89.9 ± 1.1	183.1 ± 2.3	75.2 ± 0.5	1100.0 ± 4.3	800.0 ± 2.5	1370.5
Ecolette	3.9.3 ± 1.1	5.14 ± 0.4	08.1 ± 0.3	92.8 ± 1.1	18.8 ± 0.4	31.0 ± 0.4	53.4 ± 0.8	223.3 ± 2.1	75.1 ± 1.1	26.7 ± 0.2	1209.5
Elise	5.2.6 ± 1.0	4.29 ± 1.1	11.1 ± 0.6	155.0 ± 1.3	14.1 ± 0.3	51.2 ± 0.5	106.4 ± 1.1	46.9 ± 1.1	149.1 ± 1.9	6.8 ± 0.1	737.0
Elstar	6.4.6 ± 0.5	14.36 ± 1.0	51.2 ± 0.8	372.4 ± 1.8	96.2 ± 1.2	54.1 ± 1.1	111.3 ± 1.4	45.6 ± 1.0	67.8 ± 0.8	10.3 ± 0.3	729.9
Fameuse	7.7.5 ± 0.9	5.59 ± 1.1	0.0 ± 0.0	77.2 ± 1.3	21.4 ± 1.4	26.4 ± 1.0	43.7 ± 0.3	25.4 ± 0.8	100.0 ± 0.2	400.1 ± 2.1	1003.2
Fantazja	4.3.7 ± 1.0	10.73 ± 1.6	0.0 ± 0.0	59.0 ± 0.7	50.8 ± 1.1	49.9 ± 0.6	97.3 ± 1.1	54.0 ± 0.5	100.0 ± 1.4	600.2 ± 1.6	1528.1
Fiałka	12.8.9 ± 0.2	13.20 ± 1.4	0.0 ± 0.0	188.6 ± 1.1	34.0 ± 0.4	92.7 ± 1.4	159.2 ± 2.1	78.1 ± 0.9	100.1 ± 1.6	700.0 ± 2.5	2724.0
Fireside Red	13.1.4 ± 1.3	9.94 ± 1.5	57.3 ± 0.3	343.9 ± 1.8	42.1 ± 0.4	92.9 ± 1.7	165.0 ± 2.6	135.6 ± 1.1	96.7 ± 0.7	25.9 ± 0.2	1226.4
Florina	3.8.2 ± 0.7	9.40 ± 1.1	04.8 ± 0.0	147.6 ± 0.8	12.3 ± 0.3	63.4 ± 1.4	96.4 ± 1.2	223.9 ± 1.3	72.3 ± 1.3	253.8 ± 2.1	1119.2
Freedom	5.8.9 ± 0.9	10.36 ± 1.0	04.7 ± 0.0	63.9 ± 1.5	06.7 ± 0.1	17.7 ± 0.3	33.3 ± 1.2	160.3 ± 1.2	22.8 ± 0.1	23.0 ± 0.1	976.2
Fuji	6.2.0 ± 1.1	20.42 ± 2.9	32.6 ± 0.6	239.5 ± 1.0	38.0 ± 0.7	66.4 ± 0.5	134.8 ± 2.3	95.6 ± 1.5	26.7 ± 0.1	03.6 ± 0.1	1147.9
Geneva Early	14.9.4 ± 1.4	13.52 ± 1.3	1.4 ± 0.0	147.4 ± 1.0	97.4 ± 0.2	89.6 ± 1.3	233.6 ± 3.1	109.7 ± 1.0	500.0 ± 2.4	3500.0 ± 3.6	1215.5
George Cave	14.0.6 ± 1.3	93.9 ± 2.6	0.0 ± 0.0	62.4 ± 0.3	18.1 ± 0.5	31.5 ± 0.7	76.3 ± 1.2	20.7 ± 0.7	800.0 ± 3.7	200.1 ± 1.1	769.1
Getmanskoje	9.9.8 ± 0.2	89.3 ± 1.0	0.0 ± 0.0	98.0 ± 1.2	20.3 ± 0.2	36.5 ± 0.9	76.6 ± 1.5	30.4 ± 0.9	200.0 ± 1.2	300.0 ± 2.1	1706.9
Golden Delicious	7.3.0 ± 0.7	146.8 ± 1.5	7.0 ± 0.2	92.4 ± 1.3	13.2 ± 0.1	18.2 ± 0.1	27.3 ± 0.7	170.1 ± 1.7	1.1 ± 0.1	12.8 ± 0.2	707.1
Idared	1.7.2 ± 1.1	114.3 ± 1.4	5.3 ± 0.4	165.9 ± 1.4	18.0 ± 0.5	70.5 ± 0.5	151.9 ± 1.2	90.8 ± 1.2	90.4 ± 1.3	23.5 ± 0.3	1334.3
Jester	7.9.4 ± 1.5	166.9 ± 1.5	11.5 ± 0.2	183.3 ± 1.7	23.9 ± 0.2	73.6 ± 0.1	149.5 ± 2.1	99.6 ± 1.0	114.9 ± 0.3	04.2 ± 0.1	1567.4
Jonafree	9.4.0 ± 1.1	68.1 ± 0.3	29.2 ± 0.4	100.6 ± 0.9	22.8 ± 0.1	20.1 ± 0.1	40.0 ± 0.6	126.1 ± 1.6	85.0 ± 1.1	12.5 ± 0.2	1104.8
Jonagold	7.7.7 ± 1.7	1354 ± 1.3	4.6 ± 0.0	72.1 ± 1.1	08.9 ± 0.0	27.5 ± 0.5	52.3 ± 1.1	62.3 ± 1.0	36.9 ± 0.2	17.0 ± 0.4	800.4
Jonathan	14.2.7 ± 1.5	179.4 ± 1.8	4.9 ± 0.2	260.5 ± 2.1	26.9 ± 0.8	92.6 ± 1.6	195.8 ± 1.9	96.8 ± 2.9	200.7 ± 1.5	22.7 ± 0.1	1540.0
Juga	4.2.2 ± 1.0	130.7 ± 1.9	0.0 ± 0.0	148.5 ± 2.8	31.7 ± 0.8	84.6 ± 0.4	116.3 ± 1.4	69.3 ± 1.1	0.0 ± 0.0	400.1 ± 1.7	1318.1
Julyred	8.1.8 ± 0.7	53.2 ± 0.9	0.0 ± 0.0	85.1 ± 1.5	33.0 ± 0.3	46.6 ± 0.2	107.7 ± 1.4	45.9 ± 0.5	100.0 ± 1.2	1500.0 ± 2.9	1516.1
Jupiter	5.1.4 ± 0.1	74.8 ± 0.3	14.4 ± 0.2	220.1 ± 2.0	30.1 ± 0.1	53.1 ± 0.6	116.1 ± 1.0	49.5 ± 1.1	78.5 ± 1.1	11.2 ± 0.3	933.9
Katja	9.0.9 ± 0.5	60.1 ± 1.1	0.0 ± 0.0	54.1 ± 2.5	9.1 ± 0.1	34.5 ± 0.1	86.9 ± 1.2	21.1 ± 0.5	400.1 ± 2.3	400.0 ± 2.9	709.2
Cox's orange	4.1.5 ± 0.1	33.1 ± 0.1	5.9 ± 0.2	121.4 ± 1.1	20.3 ± 0.4	34.3 ± 0.1	77.5 ± 1.4	34.3 ± 0.9	54.4 ± 0.2	7.1 ± 0.1	871.6
Kosztela	5.0.3 ± 0.3	100.0 ± 1.4	0.0 ± 0.0	123.0 ± 1.3	120.2 ± 0.5	20.5 ± 0.6	62.1 ± 1.1	110.5 ± 1.1	10.0 ± 0.1	60.2 ± 0.2	2011.3
Ligol	42.9 ± 1.1	53.2 ± 1.4	0.0 ± 0.0	76.4 ± 2.0	16.9 ± 0.2	28.4 ± 0.3	58.5 ± 0.9	46.4 ± 0.3	0.0 ± 0.0	100.0 ± 1.1	786.0
Lodel	52.7 ± 1.0	86.9 ± 1.6	5.1 ± 0.1	115.9 ± 1.1	10.3 ± 0.1	37.8 ± 0.1	75.2 ± 0.5	52.3 ± 0.5	17.8 ± 1.1	25.0 ± 0.9	1057.7
Macfree	203.0 ± 2.1	231.3 ± 2.6	12.9 ± 0.4	53.9 ± 1.6	48.6 ± 1.1	34.7 ± 1.0	70.2 ± 1.3	494.9 ± 3.1	26.5 ± 1.2	42.7 ± 0.1	1184.3
Meris	72.4 ± 1.1	186.2 ± 1.1	34.3 ± 0.9	141.2 ± 1.9	106.0 ± 1.6	56.7 ± 0.6	113.2 ± 1.1	31.6 ± 1.3	0.0 ± 0.0	500.0 ± 1.2	1303.6
Mutsu	57.6 ± 1.3	99.3 ± 1.9	18.0 ± 0.4	289.7 ± 2.3	48.0 ± 0.4	73.2 ± 1.5	119.7 ± 1.0	154.7 ± 1.2	1.1 ± 0.0	10.9 ± 1.2	905.0
Nela	134.5 ± 2.9	135.6 ± 2.1	0.0 ± 0.0	456.0 ± 3.0	42.2 ± 0.1	130.0 ± 1.2	341.3 ± 2.1	84.8 ± 1.9	700.0 ± 3.1	100.0 ± 0.2	1220.1
New Jonagold	153.2 ± 1.4	175.8 ± 1.5	7.4 ± 0.1	136.8 ± 1.2	16.8 ± 0.7	58.0 ± 0.5	112.9 ± 1.4	171.5 ± 2.1	15.9 ± 0.4	12.1 ± 0.1	1185.1
Nova Easygro	141.3 ± 1.8	202.9 ± 1.1	13.4 ± 0.4	70.2 ± 1.4	55.9 ± 1.1	41.0 ± 0.1	88.6 ± 0.9	219.6 ± 2.3	70.8 ± 0.9	40.6 ± 0.2	1025.2
Novamac	147.7 ± 1.9	177.9 ± 1.1	0.0 ± 0.0	268.1 ± 1.2	193.3 ± 2.3	137.9 ± 2.4	255.5 ± 2.3	512.6 ± 3.1	100.0 ± 1.4	200.0 ± 2.1	611.1
Odra	49.4 ± 0.9	73.3 ± 1.6	1.3 ± 0.1	102.8 ± 15	10.8 ± 0.4	30.0 ± 0.7	50.3 ± 1.1	231.2 ± 1.2	54.5 ± 0.2	35.8 ± 0.1	1222.8
Oliwka Żółlta	108.8 ± 1.1	78.2 ± 1.8	1.3 ± 0.0	134.1 ± 2.1	18.3 ± 0.7	82.5 ± 0.4	197.5 ± 1.7	114.6 ± 1.9	100.0 ± 1.1	600.0 ± 2.3	1044.8
Ozark Gold	140.6 ± 1.0	226.1 ± 2.0	13.9 ± 0.4	237.3 ± 2.9	30.6 ± 0.9	57.4 ± 1.1	134.0 ± 1.4	47.6 ± 0.9	2.0 ± 0.0	19.5 ± 0.2	2132.6
Paulared	78.9 ± 1.2	60.0 ± 1.0	0.0 ± 0.0	225.9 ± 1.1	84.1 ± 0.3	113.4 ± 1.7	200.4 ± 2.0	37.7 ± 0.4	100.0 ± 1.3	1000.0 ± 2.9	1358.9
Herrnhut Beauty	130.2 ± 1.7	136.6 ± 1.6	1.7 ± 0.0	42.7 ± 1.4	21.3 ± 0.9	36.8 ± 0.5	60.2 ± 1.0	51.0 ± 0.4	200.0 ± 1.1	600.0 ± 3.1	1641.3
Pinova	89.7 ± 1.4	120.6 ± 1.8	24.8 ± 0.7	440.1 ± 2.5	87.3 ± 1.2	102.5 ± 1.3	207.4 ± 3.4	117.1 ± 1.1	36.8 ± 0.2	10.2 ± 0.2	1273.7
Priam	73.2 ± 2.0	71.0 ± 1.1	0.0 ± 0.0	80.4 ± 1.0	83.3 ± 2.6	11.1 ± 0.1	30.3 ± 0.4	60.4 ± 0.3	0.0 ± 0.0	61.1 ± 1.1	1014.8
Priscilla	91.7 ± 1.1	130.4 ± 1.8	2.9 ± 0.0	52.9 ± 1.1	10.6 ± 0.1	22.6 ± 0.9	44.7 ± 1.1	33.5 ± 1.2	25.5 ± 0.2	525.4 ± 3.5	1075.8
Rajka	34.5 ± 1.1	34.3 ± 1.4	5.7 ± 0.1	88.1 ± 0.3	13.0 ± 0.2	29.4 ± 0.7	52.7 ± 0.9	157.3 ± 0.9	82.2 ± 1.1	49.9 ± 1.0	689.2
Redfree	123.9 ± 1.4	107.8 ± 1.3	1.4 ± 0.0	402.7 ± 1.8	37.3 ± 1.5	189.9 ± 1.2	342.1 ± 2.4	692.2 ± 3.4	0.0 ± 0.0	1000.0 ± 3.1	878.3
Reneta Sudecka	40.5 ± 1.9	42.8 ± 1.7	0.0 ± 0.0	43.2 ± 0.9	47.3 ± 0.2	20.4 ± 0.6	23.1 ± 0.9	50.2 ± 1.1	11.1 ± 0.8	62.0 ± 1.2	875.4
Rubin	29.4 ± 1.0	46.7 ± 1.1	3.4 ± 0.2	96.1 ± 1.3	154 ± 0.2	19.1 ± 0.8	41.7 ± 0.7	32.2 ± 0.9	39.4 ± 0.9	3.7 ± 0.0	632.9
Rubinola	39.0 ± 0.3	66.1 ± 1.4	0.7 ± 0.0	8.4 ± 0.0	2.4 ± 0.0	09.6 ± 0.7	14.8 ± 0.8	51.5 ± 1.2	100.0 ± 1.1	100.0 ± 1.1	888.2
Sawa	105.6 ± 1.1	132.6 ± 1.7	0.0 ± 0.0	417.0 ± 2.6	98.9 ± 1.2	72.1 ± 1.1	163.9 ± 1.1	400.7 ± 1.2	0.0 ± 0.0	1300.0 ± 4.1	697.7
Starkrimson	62.2 ± 1.9	303.3 ± 2.3	3.0 ± 0.0	113.1 ± 1.1	6.4 ± 0.2	52.2 ± 0.3	94.4 ± 1.2	38.1 ± 0.4	151.3 ± 2.3	23.1 ± 0.7	1382.0
Sunrise	181.1 ± 2.3	169.7 ± 2.7	0.0 ± 0.0	295.5 ± 2.8	201.1 ± 1.4	109.8 ± 0.5	245.4 ± 2.1	254.7 ± 2.4	0.0 ± 0.0	1000.0 ± 2.9	1113.1
Shampion Arno	72.2 ± 1.5	50.6 ± 1.0	0.0 ± 0.0	21.1 ± 1.4	8.6 ± 0.1	16.6 ± 0.9	29.2 ± 0.9	32.7 ± 0.9	0.0 ± 0.0	100.0 ± 1.5	1140.6
Shampion	82.8 ± 1.3	107.4 ± 1.5	0.0 ± 0.0	268.6 ± 1.1	60.2 ± 0.5	77.8 ± 0.4	174.2 ± 1.1	106.8 ± 1.1	0.0 ± 0.0	00.0 ± 0.0	1412.0
Teremok	57.8 ± 1.4	88.6 ± 1.6	18.9 ± 0.9	517.3 ± 2.9	206.7 ± 1.8	130.3 ± 0.8	326.3 ± 1.2	180.9 ± 1.9	200.0 ± 3.1	400.0 ± 1.2	1898.1
Titówka	44.0 ± 1.1	138.8 ± 1.8	0.0 ± 0.0	42.0 ± 1.0	3.4 ± 0.1	17.9 ± 0.6	47.5 ± 0.0	24.4 ± 0.7	0.0 ± 0.0	400.0 ± 2.1	1337.7
Topaz	20.4 ± 0.6	28.8 ± 0.3	1.2 ± 0.0	39.2 ± 0.9	6.5 ± 0.2	15.2 ± 0.4	30.6 ± 0.9	107.3 ± 1.0	22.9 ± 0.7	05.1 ± 0.1	523.0
Waleria	141.9 ± 1.7	149.3 ± 1.3	0.0 ± 0.0	109.8 ± 1.1	51.1 ± 0.7	36.2 ± 0.9	72.6 ± 0.5	148.2 ± 1.1	0.0 ± 0.0	1400.0 ± 2.8	545.4
Witos	45.2 ± 1.6	61.9 ± 1.9	0.0 ± 0.0	129.4 ± 1.3	29.3 ± 0.9	51.2 ± 0.1	94.1 ± 1.1	134.1 ± 2.1	0.0 ± 0.0	700.0 ± 3.4	681.4
Zimnieje Limonnoje	61.9 ± 1.9	77.6 ± 0.7	3.1 ± 0.1	527.7 ± 2.4	86.1 ± 1.1	130.2 ± 1.2	242.5 ± 2.1	280.5 ± 1.1	1.0 ± 0.0	37.7 ± 1.0	1371.2

[a] PLXG, phloretin 2′-xyloglucose; PLG, phloridzin; QRUT, quercetin 3-rutinoside; QGAL, quercetin 3-galactoside; QGLU, quercetin 3-glucoside; QARA, quercetin 3-arabinoside; QXYL, quercetin 3-xyloside; QRHM, quercetin 3-rhamnoside; CGAL, cyanidin 3-galactoside; CGLU, cyanidin 3-glucoside. Total of polyphenols = sum of the determined phenolic compound from **Tables 2** and **3**.

Gold, Pinova, Jester, Jonathan, Zimnieje Limonnoje, Odra, Idared, Fireside Red, Starkrimson, Titovka, Discovery, Nela, Julyred, Paulared, Witos, Novamac, Sawa, Waleria, Getmanskoje, Fantazja, Fiałka, Bramley's Seeding, Meris, Teremok, Amulet, Juga, Shampion Arno, and Delbarestivale varieties had more polyphenol compounds than the average amount, but Topaz and Waleria had significantly the lowest content of polyphenols (**Table 4**). It has been observed that, among researched varieties, old varieties more often had more polyphenols than new varieties. Lee et al. (*35*) demonstrated that Fuji, McIntosh, Red Delicious, Granny Smith, and Liberty apples have over 100 mg of total phenolics per 100 g, Golden Delicious apples have 82.2 mg/100 g, but Empire apples have only 50.9 mg phenolics per 100 g of apple (data do not include polymeric procyanidins content). Raw apple material contains high concentrations of phenolics and has beneficial effects on health because it reduces the risk of cancer and cardiovascular heart disease (*6*).

These data confirm the fact that regular consumption of apples enriches the diet in an important amount of polyphenolic compounds. The World Health Organization has recommended that people should consume fruits at least 5 times a day or 400 g of fruit and vegetables per day to reduce the risk of civilization diseases (*36*). Therefore, it seems reasonable to believe that an increased consumption of apple polyphenolics may be beneficial for health and well-being.

Procyanidins were the most predominant phenolic group found in apples and constituted more than 80% of the total polyphenolic compounds. The content of procyanidins in investigated apples depended upon the apple variety. The total procyanidins ranged from 4622.1 to 2548.0 mg/kg dw (**Table 3**). These data are in agreement with the findings of other authors (*16, 19, 20*). The Kosztela, Fiałka, Getmanskoje, Teremok, and Ozark Gold (new variety) had the highest concentrations of procyanidins, whereas Topaz, Elise, Elstar, Rajka, Rubin, Golden Delicous, Redfree, Katja, and George Cave had the lowest concentrations of procyanidins. Among procyanidins, monomers and dimers, the most important compounds are procyanidins B2 (68.7−2000.0 mg/kg dw), procyanidins C1 (58.4−970.0 mg/kg dw), and (−)-epicatechin (65.9−2760.0 mg/kg dw), while the content of (+)-catechin is the lowest (10.1−720.0 mg/kg dw). Terminal (−)-epicatechin units are always predominant, with the exception of the Ligol variety. However, some varieties (Getmanskoje, Discovery, Sunrise, Titovka, Ecolette, Rubin, Fuji, and Golden Delicious) showed relatively high proportions of terminal (+)-catechin (31.7−48.0% of total units). The Renette variety has been found to have the highest amount of polyphenols and procyanidin compounds (*14, 18, 32*). However, investigated in this study, Reneta Sudecka had a lower content of polyphenols and procyanidins than other apple varieties. Khanizadeh et al. (*14*) found that new apple lines (SJCA38R6A74) had a small amount of procyanidins and that they were present only in the peel, whereas Chinnici et al. (*37*) clearly show that the type of production (integrated production was better than organic production) had the main influence on the content of procyanidins and hydroxycinnamates in apple peels and pulps.

Oligomeric procyanidins represent the major fraction inside this class. The average concentrations for the single variety range between 1374.0 mg/kg dw in a new variety, Waleria, up to 19849.6 mg/kg dw in an old variety, Fiałka. In the Fiałka variety, polymeric procyanidins represented 77.6% of total compounds from flavan-3-ol groups. Concentrations of PO were in good agreement with those previously published by Vrhovsek et al. (*19*) for Fuji variety (53%) and for Granny Smith (71%). It is difficult to estimate the oligomeric procyanidins content of foods because procyanidins have a wide range of structures and molecular weights. Most often, the only available data concern dimers and trimers, which are as abundant as the catechins themselves (*38*).

Reserve-phase HPLC following the thiolysis reaction allows for the determination of the nature and proportions of procyanidins constitutive units and makes the distinction between terminal and extension units, thus allowing for the calculation of the average degree of polymerization (*39*). Dependent upon the variety, DP varied from 3.2 (Titówka) to 28.7 (Ecolette), with the average values ranging from 3.8 to 6.2 (**Table 3**). In cider apples, the mean degree of polymerization ranges from 4.2 to 50.3 (*16*).

Hydroxycinnamic acids corresponded to the second polyphenol class in apples, and they accounted for 1.2−31.2% depending upon the variety. The highest concentration has been observed in 22 varieties of apple, with a value close to 1000−3500 mg/kg dw (**Table 3**). However, hydroxycinnamic acid was more dominant in the flesh (40.1%) than in the apple peel (9.3%) (*10*). 5-Caffeoylquinic acid (CQA) is the most abundant hydoxycinnamic acid in all apple varieties. CQA less than 90.0% was found only in a few apple varieties: Ozark Gold (new variety), Jupiter, Elstar, Rajka, Jonathan, Cortland, Cox's Orange, Paulared, Witos, Fantazja, Shampion, and Shampion Arno (new variety). The second hydroxycinnamic acid was *p*-coumaroylquinic acid (PCQ). The PCQ concentration ranged from 3.6 mg/kg dw for Florina and Rubin to 260.0 mg/kg dw for Geneva Early. Bitter varieties generally contain higher concentrations of hydroxycinnamic acids than nonbitter ones. Ligol, Sawa, Witos, and Juga are the representatives of new apple varieties, which contain not only the lowest hydroxycinnamic acid but also a high extract (12−15 °Bx, data not shown). Alonso-Salces et al. (*41*) have also observed that the concentrations of phenolic acid in cider apples are considerably greater than those found in dessert apples. The 5-caffeoylquinic acid/*p*-coumaroylquinic acid ratio varied widely according to apple variety: between 34.6 and 1146.8 mg/kg dw. This ratio may be important when apple fruits are processed into juices and ciders, because CQA is considered to be a preferential natural substrate of the catecholase activity of polyphenol oxidase (PPO), whereas PCQ seems to be a competitive inhibitor of enzyme activity (*42*). Therefore, the relative concentrations of these compounds could influence the oxidation processes and color development during the technological process. Moreover, these compounds are the precursors of cider flavor when their concentration is low. Besides, the enzymatic oxidation product of CQA (their *o*-quinones) can co-oxidize other substances, such as flavan-3-ols, generating colored products. Thus, the browning degree depends upon not only the CQA contents but also the flavan-3-ols/hydroxycinnamic acids ratio. Hence, those varieties with balanced compositions of flavan-3-ols and hydroxycinnamic acids, low CQA contents, and small CQA/PCQ ratio would be the most appropriate for apple juice production to minimize the enzymatic browning and control the stability of the final product. In this sense, the varieties Geneva Early, Redfree, Titovka, Sunrise, Katja, George Cave, Discovery, Nela, Paulared, Waleria, and Getmanskoje would be the least suitable.

For all varieties, the content of dihydrochalcones ranged from 0.5−4.9% of total polyphenols. The Macfree variety showed the highest content of dihydrochalcones (434.3 mg/kg dw), whereas the lowest level was observed for Topaz variety (49.2 mg/kg dw). Dihydrochalcones content observed for the Golden Delicious variety amounted to about 219.1 mg/kg dw, which was in good agreement with previous data obtained for this variety by Sanoner et al. (*16*), Burda et al. (*43*), and Amiot et al. (*44*). Phloretin 2′-*O*-glucoside (PLG) and phloretin 2′-*O*-xyloglucoside (PLXG) were the two major dihydrochalcones reported for apple (*45–47*). Other phloretin derivatives, such as 3-hydroxyphloridzin and phloretin, have occasionally been found in apple at trace amounts (*48, 49*). Although they are present at low concentrations in raw material, dihydrochalcones might contribute significantly to apple juice and cider quality (*16*). When they are present in apple juice together with (−)-epicatechin, PLG may be involved in the formation of orange oxidation products, which account for about one-half of the juice color (*50*). Moreover, PLG and particularly some of its oxidation-derived products may contribute to the antioxidant potential of apple products (*51*). Khanizadeh et al. (*14*) observed that in all investigated varieties the amount of PLG was higher than that of PLXG. However, in this study, we have found larger values of PLXG than PLG, but some varieties had the same quantity of dihydrochalcones content (**Table 4**).

Flavonols had the lowest concentration in apple and were constituted fundamentally by qercetin-3-galactoside > 3-rhamnoside > 3-xyloside > 3-arabinoside > 3-glucoside > 3-rutinose. Dependent upon the genotypes, the proportion of total flavonols varied from 1660.0 to 80.0 mg/kg dw, with Redfree having the highest and Rubinola having the lowest concentrations of flavonols (both are new varieties). In general, genotypes with the highest phenolic concentration had a simultaneously high content of flavonols (**Table 4**). In this study, we found a higher amount of quercetin glycoside in a whole apple than Lee et al. (*35*) (13.2 mg/100 g dw) and Burda et al. (*43*) (21−200 mg/kg dw). Commonly, glycosylated quercetin is essentially located in the apple peel (*37, 43*); therefore, it was suggested that consumption of apple together with its skins may have beneficial effects on consumers' health. Apple peels are often discarded in the production of processed apple products. Wastes such as apple peels left after applesauce and canned apple production should be regarded as a valuable product (*13*) because they possess high levels of antioxidant and bioactive compounds. Quercetin derivatives are not the major polyphenolic components of apple, but they are very important for human health. Quercetin was found to inhibit human prostate and lung cancer cell growth (*52, 53*) and to reduce the incident of cardiovascular diseases (*5*). Anthocyanins are essentially located in apple peel and represent less than 1% of total polyphenols. The concentration varies a lot also within each variety. Apple anthocyanins are a mix of two different cyanidin glycosides, of which cyanidin-3-galactoside is more common than cyanidin-3-glucoside. Vrhovsek et al. (*19*) described about four different cyanidin glycosides, additionally about 3-arabinoside and 3-galactoside; however, these cyanidins were present in minor amounts in some red varieties. The content of cyanidin-3-galactoside and 3-glucoside ranged from 10.0 to 550.9 mg/kg dw, with the highest concentration being observed in Priscilla, Geneva Early, Florina, Cortland, and Jonathan. The results are consistent with what was observed in other studies (*10*). These apple varieties had red or partially dark red peels. Therefore, Łata (*54*) demonstrated that the richest sources of anthocyanins were Idared, Gloster, and Starking Delicious. However Priscilla, which had a red color of skin, showed trace amounts of anthocyanins, much below the average concentration. Ozark Gold, Topaz, Mutsu, Golden Delicious, Fuji, Ligol, Shampion, Rubinola, and Delbarestivale have a typically green skin and the lowest anthocyanin content (10−30 mg/kg dw). These results were consistent with our previous study performed for Golden Delicious and Fuji (*13, 19, 34*). The reasons for this difference in anthocyanin concentrations in apple skins are often attributed to the combination of low overnight temperatures and high level of sunshine hours during ripening (*55*), as well as to the genotype of apple. In Shampion Arno variety, which is a new variety, the content of anthocyanin was 0.0 mg/kg dw because this apple had typically green skin and probably the growing season in our climatic conditions is too short for it to develop a red color of skin (**Table 4**).

Antioxidant Activity and Correlation Coefficient. In this study, the results of the improved ABTS, DPPH, and FRAP methods were expressed in the same unit, i.e., Trolox equivalent antioxidant capacity (TEAC) (millimolar), to directly compare the tested results of these three methods. For apple extracts, the TEAC values that we found are reported in **Table 5**. The effects of apple cultivars on antioxidant capacity measured by $ABTS^{\bullet +}$ ranged from 36.0 to 770.49; for DPPH, they ranged from 10.11 to 129.09; and for FRAP, they ranged from 13.46 to 127.98 TEAC. Kosztela, Fiałka, and Ozark Gold varieties possessed the highest and greatest activity measured by all methods, whereas the Topaz variety possessed the lowest TEAC measured by DPPH and FRAP methods and Jonafree for $ABTS^{\bullet +}$ values. This result was consistent with the total polyphenolic concentration in this variety. The differences in TEAC between apples varieties could be preliminarily attributed to their different contents of polyphenols (**Tables 3** and **4**). Therefore, we observed that Ozark Gold and Fiałka varieties have more total polyphenols (2132.6 and 2724.0 mg/kg dw, respectively) than Kosztela variety (20152.4 mg/kg dw) but lower antioxidant activity (**Table 3–5**). The Kosztela variety had the highest antioxidant activity because it had more (+)-catechin, (−)-epicatechin, chlorogenic acid, and anthocyanins than the Ozark Gold variety. Lee et al. (*35*) and Sun et al. (*56*) have proven that (−)-epicatechin and procyanidins B2 had 40.0% of relative contribution to the total antioxidant activity of apples measured by the $ABTS^{\bullet +}$ method. (−)-Epicatechin and procyanidins B2 contribution is the highest among major phytochemicals, followed by quercetin glycosides (34.7%), whereas chlorogenic acid (7.6%) and phloretin (9.11) had little participation in the total antioxidant activity. Tsao et al. (*10*) indicate that the antioxidant activity (measured by the FRAP method) of the five polyphenolic groups found in apple occurred in the following decreasing order: cyanidin-3-galactoside > procyanidins > quercetin glycosides > chlorogenic acid > phloridzin. This study clearly demonstrated that apple variety whose average content of total polyphenols lies between 6261.1 and 27040.0 mg/kg dw has a higher antioxidant activity than others (**Tables 3** and **4**). Kosztela is one of old and typical Polish varieties grown in the 17th century (*24*).

The best correlation was found for the total polyphenols and ABTS method, with a lower correlation for FRAP and DPPH method (r = 0.871, 0.839, and 0.804, respectively). A good correlation was observed between the antioxidant potentials determined by ABTS, DPPH, FRAP, and procyanidins and hydroxycinnamic acid (**Table 6**). The obtained result showed that flavan-3-ols, including monomers, dimers, and oligomers were the most important compounds for antioxidant activity of apples. Other polyphenols showed lower correlation coefficients, but for quercetin glycosides and anthocyanins, a weak correlation was observed. Quantitatively, anthocyanins, flavonols, and dihydrochalcons are minor phenolic components of apple, whereas procyanidins and chlorogenic acid constitute the majority of the polyphenolics. Tsao et al. (*51*) demonstrated that anthocyanins had the highest antioxidant activity among all tested standards. However, it accounted only for 1% of the total polyphenolics and its concentration did not correlate with the antioxidant activity (**Table 6**).

In the literature, the relationship between antioxidant activity and food concentration of phenolic compounds is highly disputed. Some studies did not find any correlation between the

Table 5. Antioxidant Activity of Apple Varieties Expressed as TEAC[a]

apple variety	antioxidant activity ABTS	DPPH	FRAP
Alwa	86.7 ± 2.0	14.9 ± 0.4	25.1 ± 1.5
Amulet	283.2 ± 0.3	28.0 ± 2.2	37.4 ± 0.8
Arlet	217.7 ± 0.6	20.7 ± 1.7	35.3 ± 0.9
Beforest	88.2 ± 1.6	17.1 ± 3.1	25.1 ± 1.7
Bramleys Seeding	456.8 ± 2.4	52.5 ± 2.8	89.8 ± 0.3
Cortland	196.3 ± 0.6	17.4 ± 3.7	28.1 ± 0.6
Dalijo	217.0 ± 2.2	18.2 ± 2.4	30.0 ± 0.5
Delbarestivale	218.9 ± 2.7	25.9 ± 4.9	39.2 ± 7.6
Discovery	532.5 ± 1.6	63.5 ± 1.6	92.0 ± 0.3
Ecolette	70.4 ± 1.1	16.0 ± 2.4	19.5 ± 0.6
Elise	102.9 ± 1.8	11.1 ± 3.6	18.4 ± 1.6
Elstar	80.9 ± 1.2	13.6 ± 9.7	23.4 ± 4.0
Fameuse	165.0 ± 1.5	25.3 ± 0.9	30.7 ± 7.0
Fantazja	364.2 ± 1.1	39. ± 2.6	60.3 ± 7.0
Fiałka	760.8 ± 0.8	74.5 ± 5.8	125.9 ± 6.0
Fireside Red	186.0 ± 2.5	19.0 ± 3.8	27.9 ± 3.0
Florina	85.9 ± 2.8	15.4 ± 2.4	21.1 ± 5.0
Freedom	223.2 ± 0.8	20.4 ± 3.6	32.6 ± 9.0
Fuji	199.1 ± 0.1	17.7 ± 4.9	29.7 ± 6.0
Geneva Early	449.1 ± 0.9	48.2 ± 4.0	76.5 ± 1.0
George Cave	277.8 ± 0.6	38.2 ± 5.6	45.6 ± 1.0
Getmanskoje	346.9 ± 4.7	35.5 ± 8.2	56.7 ± 1.0
Golden Delicious	88.6 ± 6.7	13.7 ± 3.7	23.2 ± 7.0
Idared	242.4 ± 2.7	24.1 ± 5.6	36.4 ± 0.6
Jester	178.7 ± 6.1	19.9 ± 1.6	29.3 ± 0.2
Jonafree	36.0 ± 4.5	18.2 ± 2.5	29.9 ± 1.9
Jonagold	181.9 ± 0.9	19.8 ± 1.7	26.4 ± 6.3
Jonathan	272.1 ± 1.3	21.5 ± 2.4	42.0 ± 1.7
Juga	289.5 ± 0.2	45.8 ± 8.3	56.8 ± 4.2
Julyred	415.9 ± 0.7	43.5 ± 3.1	66.2 ± 0.4
Jupiter	191.7 ± 2.4	20.3 ± 4.2	33.3 ± 5.2
Katja	373.0 ± 0.8	39.8 ± 3.7	58.8 ± 9.4
Cox's orange	92.2 ± 0.9	16.1 ± 2.6	26.0 ± 1.0
Kosztela	770.5 ± 7.7	129.0 ± 5.5	128.0 ± 3.2
Ligol	218.5 ± 4.5	26.9 ± 5.3	38.0 ± 1.1
Lodel	222.4 ± 1.9	17.4 ± 0.1	30.4 ± 0.9
Macfree	125.7 ± 6.1	21.6 ± 0.9	37.2 ± 4.3
Meris	419.1 ± 1.1	64.7 ± 0.6	97.0 ± 4.3
Mutsu	87.6 ± 1.2	17.5 ± 4.7	24.4 ± 5.4
Nela	373.8 ± 4.9	46.2 ± 1.7	63.1 ± 2.1
New Jonagold	196.4 ± 2.9	18.0 ± 2.4	27.7 ± 4.3
Nova Easygro	170.8 ± 8.3	15.9 ± 8.3	25.3 ± 2.1
Novamac	364.1 ± 3.1	38.1 ± 3.1	60.9 ± 4.3
Odra	224.3 ± 3.5	17.9 ± 1.6	32.3 ± 2.1
Oliwka Zõłlta	380.0 ± 1.6	32.6 ± 2.4	62.9 ± 2.9
Ozark Gold	781.2 ± 2.4	79.4 ± 0.6	96.4 ± 4.4
Paulared	288.2 ± 0.6	29.6 ± 2.2	46.6 ± 3.5
Piêkna z Herrnhut	274.9 ± 2.2	63.8 ± 5.1	120.1 ± 3.2
Pinova	98.0 ± 5.1	17.0 ± 1.2	27.8 ± 4.3
Priam	238.6 ± 1.2	36.5 ± 3.4	49.2 ± 3.2
Priscilla	368.0 ± 3.4	29.1 ± 1.2	55.3 ± 3.2
Rajka	220.60 ± 1.9	19.4 ± 0.9	32.3 ± 1.0
Redfree	377.1 ± 0.9	46.3 ± 1.1	67.7 ± 4.3
Reneta Sudecka	185.1 ± 0.7	32.2 ± 3.7	45.3 ± 0.7
Rubin	101.9 ± 2.4	11.3 ± 2.4	16.1 ± 8.6
Rubinola	173.9 ± 2.4	49.2 ± 4.9	80.2 ± 1.0
Sawa	657.5 ± 4.9	52.9 ± 1.6	97.8 ± 3.2
Starkrimson	247.2 ± 1.6	25.6 ± 2.4	35.4 ± 0.0
Sunrise	375.5 ± 2.4	38.6 ± 4.1	61.5 ± 0.0
Shampion Arno	345.9 ± 1.0	21.7 ± 1.3	32.0 ± 6.4
Shampion	325.7 ± 1.5	37.4 ± 1.7	43.7 ± 4.3
Teremok	379.5 ± 1.6	36.2 ± 1.5	46.8 ± 4.3
Titówka	482.1 ± 7.5	49.8 ± 5.9	76.3 ± 6.4
Topaz	49.9 ± 1.6	10.1 ± 1.7	13.5 ± 1.0
Waleria	467.7 ± 1.4	67.9 ± 0.1	68.4 ± 4.3
Witos	278.0 ± 1.5	30.6 ± 1.5	50.9 ± 4.3
Zimnieje Limonnoje	225.5. ± 5.1	18.2 ± 1.5	34.5 ± 5.4

[a] Values are expressed as means of three determinations ± standard deviation.

antioxidant activity and the concentration of phenol constituents in apple extracts (*57, 58*), while others found a strong correlation between antioxidant activity and total phenols (*14, 35, 56*).

PCA Analysis. New varieties, when grown, were based on old varieties, such as Renette, Red Delicious, Granny Smith, and Golden Delicious. Some of these new varieties had more

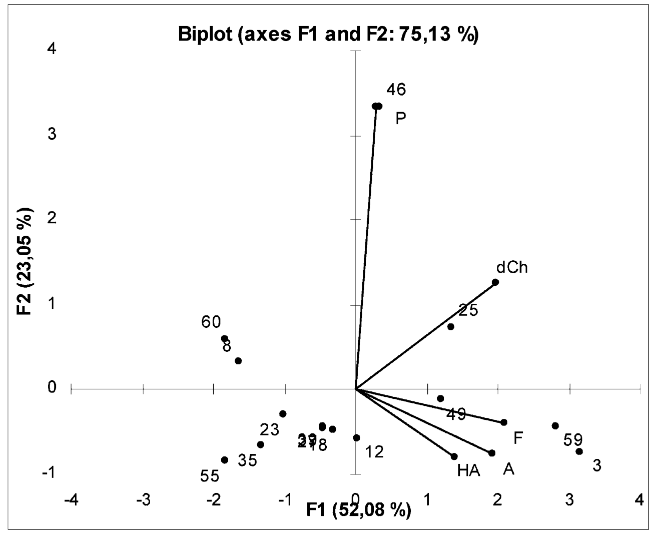

Figure 1. PCA of new apple cultivar polyphenols [procyanidins (P), dihydrochalcones (Ph), hydroxycinnamic acid (CA), flavonols (F), and anthocyanins (A)] grown for Golden Delicious.

Table 6. Positive Correlation between Phenolic Compounds and Antioxidant Activity

variables	ABTS	DPPH	FRAP
total of polyphenols	0.871	0.839	0.804
dihydrochalcones	0.260	0.198	0.256
flavonols	0.279	0.143	0.175
anthocyanins	0.146	0.035	0.095
procyanidins	0.690	0.591	0.633
hydroxycinnamic acid	0.542	0.636	0.549

bioactive compounds than old varieties, but some of them had less. In this study, we have researched 12 varieties grown from one old variety, Golden Delicious (**Table 1**). In Golden Delicious, the concentration of total polyphenolic compounds was 7021.7 mg/kg dw, but in all crossed varieties, the concentration ranged from 6261.1 to 21280.1 mg/kg dw. Therefore, PCA was performed to obtain further information on investigated varieties, namely, which varieties were similar to the Golden Delicious variety with respect to polyphenol content. The PCA analysis was based on five variables selected to limit the redundancy in the data set: the sum of flavan-3-ol and procyanidins and the sum of dihydrochalcones, anthocyanins, quercetin glycosides, and hydroxycinnamic acid. The cumulative percentage of the total variance explained by the first two factors was 75.13% (**Figure 1**). Elstar, Freedom, Jonagold, Ligol, Mutsu, Delbarestivale, and Shampion apples were confined in the lower left and upper left portions of the figure with the Golden Delicious variety. These varieties are unbalanced against the rest of the observation and cannot be distinguished, because they have the same or lower value of total polyphenols. The Ozark Gold variety had 3.0 times more total polyphenol compounds, especially procyandins, than Golden Delicious. The Ozark Gold with the highest concentration of procyanidins and Jester with dihydrochalcones had the lowest content of hydroxycinnanic acid, antocyanins, and flavonols. In the right portion of the figure, Pinova, Sunrise, and Arlet cultivars with a higher content of anthocyanins, flavonols, and hydroxycinnamic acids than Golden Delicious were displayed. This investigation clearly demonstrated that not all of new varieties grown from old varieties (for example, Golden Delicious) had a lower content of polyphenol compounds. Probably, the second variety used for cross had a higher content of polyphenol compounds, and these genes were well-preserved. The genotype is one of factors that markedly determine the content of individual phenolics, and it is necessary to focus on this aspect in the future and analyze the second variety used for growing new varieties.

To conclude, the presented data clearly demonstrated that new varieties of apple had the same or higher value of bioactive compounds in comparison to the old varieties. Analysis of phenolic profile content clearly indicated that anthocyanins and phloridzin are minor phenolic components of apple, whereas procyanidins, flavan-3-ols, and chlorogenic acid constitute the majority of the polyphenolics. The amount of flavonols is relatively consistent in different cultivars, but the proportion of the total changes substantially some new varieties contained the highest levels of phenolics, whereas some old varieties contained the lowest. The concentration of procyanidins/flavan-3-ols is the most important contributor to the in vitro antioxidant activity. Apple cultivars that have a higher content of phenol compounds can be selected to promote their positive effect on health because "an apple a day keeps the doctor away".

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