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Polyphenolic Composition, Antioxidant Activity, and Polyphenol Oxidase (PPO) Activity of Quince (*Cydonia oblonga* Miller) Varieties

Aneta Wojdyło,*[,†] Jan Oszmiański,[†] and Paweł Bielicki[§]

[†]Department of Fruit and Vegetable Processing, Wroclaw University of Environmental and Life Science, 37/41 Chełmońskiego Street, 51-630 Wroclaw, Poland

[§]Research Institute of Horticulture, Department of Pomology, Gene Resources and Nurseries, Konstytucji 3 Maja 1/3 Street, 96-100 Skierniewice, Poland

ABSTRACT: Phytochemical profiles (phenolic compounds, L-ascorbic acid, antioxidant and PPO activities) of 13 different quince varieties and 5 genotypes were studied. Polyphenols were identified by LC-PDA-QTof/MS and quantified by UPLC-PDA and UPLC-FL. A total of 26 polyphenolic compounds found in quince tissues were identified and presented: 9 flavan-3-ols ((−)-epicatechin, procyanidin B2, 3 procyanidin dimers and trimers, and 1 tetramer); 8 hydroxycinnamates, derivatives of caffeoylquinic and coumaroylquinic acid; and 9 kaempferol and quercetin derivatives. The content of total polyphenols was between 1709.43 (genotype 'S1') and 3436.56 mg/100 g dry weight ('Leskovač'). Flavan-3-ols, which are the major class of quince polyphenols, represented between 78 and 94% of the total polyphenolic compounds. The activity of PPO enzyme ranged from 709.85 to 1284.59 ΔU/min, and that of L-ascorbic acid ranged from 5.86 to 26.42 mg/100 g. Some quince varieties and their products characterized by a higher content of phenolic compounds may be selected to promote their positive effect on health.

KEYWORDS: *polyphenols, polymeric procyanidins, mDP, LC-MS-QTof, L-ascorbic acid, antioxidant activity, bioactive compounds, PPO*

INTRODUCTION

Edible fruits constitute a source of nutrients such as carbohydrates, vitamins, and minerals as well as non-nutrients, especially polyphenols, which display various health-promoting properties. Phytochemicals of fruits and vegetables have been considered of crucial nutritional importance in the prevention of chronic diseases such as cancer and cardiovascular and neurodegenerative diseases. This may be related to their antioxidant activity as well their ability to regulate cellular activities of inflammation-related cells (mast cells, macrophages, lymphocytes, and neutrophils).[1,2]

Less popular edible fruits are being sought to increase the availability of potentially biologically active phytochemicals. Fruits that display a particularly high antioxidative potential owing to such phytochemicals as proanthocyanidins, anthocyanins, and stilbenes, which are known for their multiple biological activities, have recently gained increasing appreciation.

Quince (*Cydonia oblonga* Mill.) belongs to the Maloideae subfamily of the Rosaceae family, which includes commercially important fruits such as apples and pears. Because of its hardness, acidity, and astringency, it is not edible unprocessed; nevertheless, it is often used to prepare jam, jelly, liqueur, and marmalade, as well as applied in canning and for aromatic distillation.[3]

In the past decade, several studies on *C. oblonga* Miller fruit and its derivatives have been performed by a research group from Portugal. A few analytical methods were developed to determine phenolic compounds, organic acids, and free amino acids in quince fruit and jam.[4−6] Among these chemical parameters, phenolic profile determination seemed to be the most useful in the discrimination of different parts of quince fruit (pulp, peel, and seed),[7,8] in the evaluation of the genuineness of quince products (puree, jam, and jelly),[9,10] in the production of fiber-rich powders from quince waste,[11] and in drying.[12] The influence of jam processing upon the contents of phenolics, organic acids, and free amino acids in quince fruit was evaluated.[13] Studies were also addressed the antioxidant activity of the methanolic extracts of quince pulp, peel, seed, and jam[9] as well as methods and conditions of quince fruit preservation.[14] Recently, some authors have described antioxidant, antimicrobial (antibacterial and anti-influenza viral), antihemolytic, antiulcerative, and antiproliferative properties of quince fruit phenolics.[15−21]

However, the available information about quince fruit is still related only to the comparison of the value of the bioactive compounds among the varieties. Rop et al.[22] described the basic chemical characteristics of 22 quince genotypes and varieties, that is, dry matter content, soluble solid content, organic acids, pectins, and mineral elements. Bayazit et al.[23] reported on different genetic relationships among 13 quince accessions selected from different parts of Turkey. Investigations conducted by Silva et al.[5,24] addressed fruit of quince growing in different parts of Portugal, but with no variety-specific correlations. Compared to a previous paper about apples, the study clearly showed very high biodiversity in antioxidant contents between varieties.[25−28] Therefore, it is the aim of this paper to report a comprehensive study of the phytochemical profiles (phenolic compounds, L-ascorbic acid, and antioxidant and polyphenol oxidase (PPO) activity) of 13 different quince varieties and 5 genotypes. The identities of polyphenolics were confirmed using LC-MS.

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MATERIALS AND METHODS

Chemicals. 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ), acetic acid, phloroglucinol, and methanol were purchased from Sigma-Aldrich (Steinheim, Germany). (−)-Epicatechin, (+)-catechin, quercetin, kaempferol-3-*O*-glucoside, and procyanidins B1 and B2 were purchased from Extrasynthese (Lyon, France). Chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, and 3,5-dicaffeoylquinic acid were purchased from TRANS MIT GmbH (Giessen, Germany). Acetonitrile for UPLC (Gradien grade) and ascorbic acid were from Merck (Darmstadt, Germany). UPLC grade water, prepared by using an HLP SMART 1000s system (Hydrolab, Gdańsk, Poland), was additionally filtered through a 0.22 μm membrane filter immediately before use.

Plant Material. Thirteen different quince (*C. obolonga* Miller) varieties ('Akademiczeskaja', 'Bereczki', 'Cezar', 'Darunok Onuk', 'Kaszczenko', 'Konstantynopeler', 'Leskovač', 'Marija', 'Portugesicka', 'Późna Rejmana', 'Ronda', 'Uspiech', 'Wołgogradzkaja Aromatnaja') and five genotypes ('S1K', 'S3', 'SDK', 'BA29', 'S1') obtained from 10-year-old trees were hand-harvested at optimum ripeness (the degree of ripeness was determined on the basis of fruit coloring, separability, and change of fruit peel from a lanuginous to waxy condition) in October 2010. The fruit were harvested at the Nursery Farm Radwan-Pytlewski Piotr from Miedniewice (52° 09′ N, 20° 30′ E), and genotypes were from the Research Institute of Horticulture in Skierniewice (51° 99′ N, 20° 16′ E). In the course of the measurements, 6 replications (6 randomly chosen fruits) from 3 trees, that is, 18 replications per variety, were established.

For chemical analyses, the whole fruits without the core were cut directly in liquid nitrogen and freeze-dried (24 h; Alpha 1-4 LSC; Martin Christ GmbH, Osterode am Harz, Germany). The homogeneous powder was obtained by crushing the dried tissues with the use of a closed laboratory mill to avoid hydration (IKA 11A; Staufen, Germany). The samples were subsequently ground in a pestle and mortar to a fine powder and stored in a freezer (−70 °C; Frilabo; Lyon, France) until analysis.

Identification of Polyphenols by the LC-PDA-MS Method. The extract of polyphenols for analysis was prepared as described previously by Wojdyło et al.[25] Identification and quantification of polyphenols of quince extracts was carried out using an Acquity ultra-performance LC system equipped with a photodiode detector (PDA; UPLC)) with binary solvent manager (Waters Corp., Milford, MA, USA) series with a mass detector G2 QTof Micro mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization (ESI) source operating in negative and positive modes. Separations of polyphenols were carried out using a UPLC BEH C18 column (1.7 μm, 2.1 × 50 mm; Waters Corp., Milford, MA, USA) at 30 °C. Samples (5 μL) were injected, and elution was completed within 15 min using a sequence of elution modes: linear gradients and isocratic. The flow rate was 0.45 mL/min. The mobile phase was composed of solvent A (4.5% formic acid) and solvent B (100% of acetonitrile). The program began with isocratic elution with 99% A (0−1 min), and then a linear gradient was used until 12 min, lowering A to 0%; from 12.5 to 13.5 min, returned to the initial composition (99% A); and then held constant to re-equilibrate the column. Analysis was carried out using full scan, data-dependent MS scanning from m/z 100 to 1500. The mass tolerance was 0.001 Da, and the resolution was 5.000. Leucine enkephalin was used as the mass reference compound at a concentration of 500 pg/μL at a flow rate of 2 μL/min, and the $[M - H]^-$ ion at 554.2615 Da was detected over 15 min of analysis during ESI-MS accurate mass experiments, which was permanently introduced via the LockSpray channel using a Hamilton pump. The lock mass correction was ±1.000 for Mass Window. The mass spectrometer was operated in a negative ion mode and set to the base peak intensity (BPI) chromatograms and scaled to 12400 counts per second (cps) (=100%). The optimized MS conditions were as follows: capillary voltage of 2500 V, cone voltage of 30 V, source temperature of 100 °C, desolvation temperature of 300 °C, and desolvation gas (nitrogen) flow rate of 300 L/h. Collision-induced fragmentation experiments were performed using argon as collision gas, with voltage ramping cycles from 0.3 to 2 V. The characterization of the single components was carried out via the retention time and the accurate molecular masses. Each compound was optimized to its estimated molecular mass $[M - H]^-$ in the negative mode before and after fragmentation. The data obtained from LC-MS were subsequently entered into MassLynx 4.0 ChromaLynx Application Manager software. On the basis of these data, the software is able to scan different samples for the characterized substances.

Determination of Polyphenols by UPLC Coupled to PDA and FL Detector. The analysis of polyphenolic compounds was carried out on a UPLC system Acquity (Waters Corp., Milford, MA, USA) consisting of a binary solvent manager, sample manager, PDA (model λe), and fluorescence detector (FL). Empower 3 software was used for chromatographic data collection and integration of chromatograms. The UPLC analyses were performed on a BEH Shield C18 analytical column (2.1 mm × 5 mm; 1.7 μm). The flow rate was 0.45 mL/min. A partial loop injection mode with a needle overfill was set up, enabling 5 μL injection volumes when a 10 μL injection loop was used. Acetonitrile (100%) was used as a strong wash solvent and acetonitrile−water (10%) as a weak wash solvent. All incubations were done in triplicate.

Analysis of Polyphenol Compounds. Five milliliters of the resultant extract were centrifuged for 10 min at 15000*g* at 4 °C. The analytical column was kept at 30 °C by column oven, wheress the samples were kept at 4 °C. The mobile phase was composed of solvent A (4.5% formic acid) and solvent B (acetonitrile). Elution was as follows: 0−5 min, linear gradient from 1 to 25% B; 5.0−6.5 min, linear gradient from 25 to 100%; 6.5−7.5 min, column washing; and reconditioning for 0.5 min. PDA spectra were measured over the wavelength range of 200−600 nm in steps of 2 nm. The runs were monitored at the following wavelengths: flavan-3-ols at 280 nm, hydroxycinnamates at 320 nm, and flavonol glycosides at 360 nm. Retention times (t_R) and spectra were compared with those of pure standards. Calibration curves at concentrations ranging from 0.05 to 5 mg/mL ($r^2 \leq 0.9998$) were made from (−)-epicatechin, (+)-catechin, procyanidins B2 and C1, chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, quercetin, and kaempferol 3-*O*-glucoside as standards. *p*-Coumarylquinic acid was expressed as *p*-coumaric acid, and quercetin and kampferol derivatives were expressed as quercetin and kaempferol-3-*O*-glucoside, respectively. The results was expressed as milligrams per 100 g dry weight (dw).

Analysis of Proanthocyanidins by Phloroglucinolysis Method. Direct phloroglucinolysis of freeze-dried quince varieties was performed as described by Kennedy et al.[29] Portions (0.05 g) of powder were precisely measured into 2 mL Eppendorf vials, then 0.8 mL of the methanolic solution of phloroglucinol (75 g/L) and ascorbic acid (15 g/L) was added. After the addition of 0.4 mL of methanolic HCl (0.3 mol/L), the vials were closed and incubated for 30 min at 50 °C with continuous vortexing using a thermo shaker (TS-100; BIOSAN, Lithuania). The reaction was stopped by placing the vials in an ice bath with drawing 0.5 mL of the reaction medium and diluting with 0.5 mL of 0.2 mol/L sodium acetate buffer. Next the vials were cooled in ice water and centrifuged immediately at 20000*g* for 10 min at 4 °C. The analytical column was kept at 15 °C by column oven, whereas the samples were kept at 4 °C. The mobile phase was composed of solvent A (2.5% acetic acid) and solvent B (acetonitrile). Elution was as follows: 0−0.6 min, isocratic 2% B; 0.6−2.17 min, linear gradient from 2 to 3% B; 2.17−3.22 min, linear gradient from 3 to 10% B; 3.22−5.00 min, linear gradient from 10 to 15% B; 5.00−6.00 min, column washing; and reconditioning for 1.50 min. The fluorescence detection was recorded at an excitation wavelength of 278 nm and an emission wavelength of 360 nm. The calibration curves, which were based on peak area, were established using (+)-catechin, (−)-epicatechin, and procyanidin B1 after phloroglucinol reaction as (+)-catechin− and (−)-epicatechin−phloroglucinol adduct standards. The average degree of polymerization was calculated as the molar ratio of all the flavan-3-ol units (phloroglucinol adducts + terminal units) to

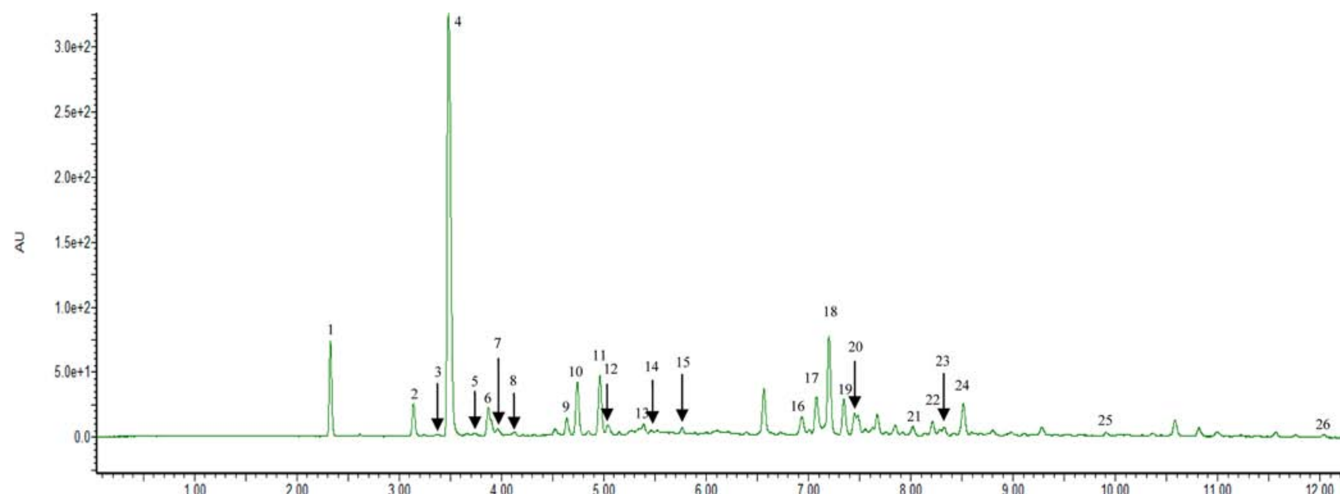

Figure 1. LC-QTof/MS phenolic profile of the quince fruit (at optimal BPI chromatogram) 'Leskovač' variety. For peak numbering, see Table 1.

(−)-epicatechin and (+)-catechin, which correspond to terminal units. The results were expressed as milligrams per 100 g dw.

Analysis of Antioxidant Activity. The solvent for analysis was prepared as described previously by Wojdyło et al.[25] The DPPH radical scavenging activity of the sample was determined according to the method of Yen et al.[30] The $ABTS^{\bullet+}$ activity of the sample was determined according to the method of Re et al.[31] The total antioxidant potential of the sample was determined using a ferric reducing ability of plasma (FRAP) assay by Benzie et al.[32] as a measure of antioxidant power. For all analyses, a standard curve was prepared using different concentrations of Trolox. All determinations were performed in triplicate using a Shimadzu UV-2401 PC spectrophotometer (Kyoto, Japan). The results were corrected for dilution and expressed in micromoles Trolox per gram dw.

L-Ascorbic Acid Analysis. L-Ascorbic acid was analyzed according to the method described previously by Oszmiański et al.[33] Fresh fruits (3−4 g) were mixed with 50 mL of 0.1 M phosphoric acid and centrifuged at 20000*g* for 10 min. The estimation of L-ascorbic acid was carried out using a Waters liquid chromatograph with a tunable absorbance detector (Waters 486) and a quaternary pump with a Waters 600 Controller apparatus (Waters Associates). A 20 μL sample was injected into a Chromolith Performance RP-18e column (100 × 4.6 mm) (Merck, Darmstadt, Germany). The elution was carried out using 0.1 M phosphoric acid at the flow rate of 1.0 mL/min. The absorbance was monitored at 254 nm. L-Ascorbic acid was identified by comparison with the standard. The calibration curve was prepared by plotting different concentrations of the standard versus the area measurements in HPLC.

Enzyme Extraction Procedure for Determination of PPO Activity. Enzymatic extracts were prepared following the method previously described by Gonzalez et al.[34] In all assays, 10 g of pulverized liquid nitrogen frozen fruits were homogenized and mixed with 25 mL of sodium phosphate buffer (pH 7.0) and 2% of poly(vinylpyrrolidone) (PVP) for 2 h at 4 °C in the dark. The homogenates were centrifuged for 10 min at 18000*g* and 4 °C until assayed for PPO activity. The PPO activity was determined by measuring the initial rate of increase in absorbance after reaction at 420 nm. Unless otherwise stated, the activity was assayed in 3 mL of the reaction mixture, consisting of 2.7 mL of 0.1 M catechol in 0.2 M sodium phosphate buffer (pH 5.5) plus 0.3 mL of prepared enzyme, with a Shimadzu UV-2401 PC spectrophotometer (Tokyo, Japan). The enzyme activity was determined by measuring the slope of the reaction curve, at zero time (initial rate) and after 2 min of reaction. The enzyme activity unit was defined as the difference in absorbance per minute and gram of tissue.

Statistical Analysis. Results are given as the mean ± standard deviation of three independent determinations. All statistical analyses were performed with Statistica version 10 (StatSoft, Krakow, Poland). One-way analysis of variance (ANOVA) by Duncan's test was used to compare the means. Differences were considered to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

Qualitative Analysis. As an initial step, quince varieties were analyzed by LC-MS-QToF systems. Typical UPLC traces are illustrated in Figure 1, whereas the LC-MS and mass spectral data obtained are summarized in Table 1. Results of the quantitative analysis made by UPLC-PDA and UPLC-FL are summarized in Table 2. In total, 26 polyphenolic compounds found in quince tissues were identified and presented. The phenolic compounds were identified by comparing the UV−vis spectra, λ_{max}, MS spectra, and retention times to those of available standards.

Nine compounds belonging to flavan-3-ols were detected in quince fruits. (−)-Epicatechin, peak 9 (t_R = 4.61 min, λ_{max} = 278 nm) had an $[M - H]^-$ at m/z 289 and an MS/MS fragment at m/z 245. Peak 5 (t_R = 3.73 min, λ_{max} = 278 nm) was identified as procyanidin B2 using the fragmentation pattern of $[M - H]^-$ at m/z 577; the MS/MS fragmentation of this peak was at m/z 289. Cochromatography with a standard was used to confirm the identity of this compound. Besides these compounds, three procyanidin dimers (compounds **3**, **15**, and **19**) at t_R 3.41, 5.56, and 7.36 min with m/z 577; three procyanidin trimers (compounds **8**, **13**, and **14**) at t_R 4.14, 5.07, and 5.48 min with m/z 865; and one procyanidin tetramer (compound **7**) at t_R 3.95 min with m/z 1155 were identified in quince fruits. All detected procyanidins had the characteristic fragmentation pattern of a negatively charged molecular ion $[M - H]^-$ at m/z 577 and/or 289. However, they produced the MS/MS base peak at m/z 407 by the loss of an RDA fragment (168 Da) from the top ring of the dimer followed by the loss of water molecule (18 Da); a secondary peak at m/z 289 originated from a QM fragment and m/z at 425 ($[M - H^+ - 168\ Da]^-$) from RDA fragment.[35]

Eight hydroxycinnamates, six derivatives of caffeoylquinic acid and two of coumaroylquinic acid, were detected as well. Peaks 1, 4, 6, 11, and 22 with λ_{max} ~325 nm possessed characteristic mass spectral data of $[M - H]^-$ at m/z 353 and MS/MS fragmentation at m/z 191. Those compounds at (t_R) 2.31, 3.48, and 3.86 min were identified as 3-, 4-, and 5-*O*-caffeoylquinic acid (neochlorogenic, cryptochlorogenic, and chlorogenic acid, respectively) after comparison with corresponding standards.

Table 1. LC-MS-QTof Analysis of Main Phenolic Compounds Exclusive of Polymeric Procyanidins in Quince Fruits

peak[a]	name	t_R (min)	λ_{max} (nm)	MS $[M-H]^-$ (m/z)	MS/MS $[M-H]^-$ (m/z)
1	3-*O*-caffeoylquinic acid (neochlorogenic acid)	2.31	325	353	191
2	4-*O*-*p*-coumaroylquinic acid	3.13	309	337	173/136
3	procyanidin dimer	3.41	278	577	289
4	5-*O*-caffeoylquinic acid (chlorogenic acid)	3.48	325	353	191
5	procyanidin B2	3.73	278	577	289
6	4-*O*-caffeoylquinic acid (cryptochlorogenic acid)	3.86	325	353	173
7	procyanidin tetramer	3.95	278	1153	577/289
8	procyanidin trimer	4.14	278	865	577/289
9	(−)-epicatechin	4.61	278	289	245
10	*p*-coumaroylquinic acid derivatives	4.73	310	337	136
11	caffeoylquinic acid	4.96	324	353	136
12	caffeoylshikimic acid	5.01	323	335	179
13	procyanidin trimer	5.07	278	865	577
14	procyanidin trimer	5.48	278	865	577/289
15	procyanidin dimer	5.56	278	577	289
16	quercetin-3-*O*-robinoside	6.94	244; 352	609	300
17	quercetin-3-*O*-galactoside	7.12	243; 352	463	300
18	quercetin-3-*O*-rutinoside	7.24	243; 352	609	301
19	procyanidin dimer	7.36	278	577	289
20	quercetin-3-*O*-glucoside	7.38	243; 352	463	300
21	kaempferol-3-*O*-galactoside	8.04	264; 345	447	284
22	3,5-dicaffeoylquinic acid	8.21	326	515	353/136/182
23	kaempferol-3-*O*-rutinoside	8.35	264; 345	593	285
24	kaempferol-3-*O*-glucoside	8.54	264; 345	447	285
25	quercetin glucoside acylated by *p*-coumaric acid	10.83	242; 352	609	463/301/136
26	kaempferol glucoside acylated by *p*-coumaric acid	12.08	265, 345	593	285

[a]Peak numbers refer to Figure 1.

Peak 2 (t_R = 3.13 min, λ_{max} = 309 nm) was identified as 3-*O*-*p*-coumaroylquinic acid on the basis of mass spectral data. It had an $[M-H]^-$ at m/z 337, which fragmented on MS/MS to yield a major ion at m/z 173 and a minor peak at m/z 163. This fragmentation pattern has been shown to be characteristic of the 4-isomer of *p*-coumaroylquinic acid.[36] This identification of 4-*O*-*p*-coumaroylquinic acid supports the reporting of this compound in apples in a previous study.[37] Peak 22 with t_R= 8.21 with λ_{max} = 326 nm gave a characteristic $[M-H]^-$ at m/z 515, which fragmented on MS/MS to pa recursor ion at m/z 353, confirmed the 3-regiochemistry of the caffeoyl substituent,[37] and was identified as 3,5-dicaffeoylquinic acid. Additionally, compounds **11** and **12** with λ_{max} = 310 and 323 nm had $[M-H]^-$ at m/z 353 and 335, respectively. The fragmentation of these compounds was $[M-H]^-$ at m/z 136, which was typical of caffeic acid. The loss of 156 amu corresponded to the molecular weight of shikimic acid. Therefore, compound **11** was identified as caffeoyl quinate, and compound **12** was identified as caffeoylshikimic acid isomer. This compound has been implicated as a possible intermediate of the phenylpropanoid pathway.[38] Interestingly, the regioisomeric shikimic acid esters showed reproducible differences in their MS/MS spectra, allowing their identification as previously described by Jaiswal.[38]

Quercetin and kaempferol derivatives are the flavonols found in quince fruits[7,8,15] and leaves.[39] They exhibit UV−vis absorption maxima at about 352 nm for quercetin and at 345 nm for kaempferol derivatives (Table 1). Peaks 16−18, 20, and 25 had a characteristic MS/MS fragment at m/z 301. Peak 16 (t_R = 6.94 min, λ_{max} = 352 nm) was identified as a quercetin derivative on the basis of an $[M-H]^-$ at m/z 609 and an MS/MS fragment at m/z 301. Peak 17 and 20 with t_R = 7.12 and 7.38 min had a characteristic mass at m/z 463 and an MS/MS fragment 301. The loss of 162 amu equates to the loss of a hexose sugar. These peaks compared with retention time of standard were identified as quercetin-3-*O*-galactoside and -3-*O*-glucoside, respectively. Peak 18 (t_R = 7.24 min, λ_{max} = 352 nm) was identified as quercetin-3-*O*-rutinoside on the basis of an $[M-H]^-$ at m/z 609 and an MS/MS fragment at m/z 301. Three peaks were identified as kaempferol derivatives according to their UV spectra and MS fragmentation leading to the kaempferol aglycone at m/z 285 in a negative mode. Peak 21 was identified as kaempferol-3-*O*-galactoside with m/z 447 and an MS/MS fragment at 284 obtained after the loss of 162 amu (galactose moiety). Peak 24 had $[M-H]^-$ at m/z 447 with a fragment at m/z 285 (loss of 162 amu: hexose moiety) and was identified as kaempferol-3-*O*-glucoside. Peak 23 had $[M-H]^-$ ion at m/z 593 in the negative mode, which is consistent with kaempferol-3-*O*-rutinoside. The molecular ion fragmentation yielded fragment ions corresponding to kaempferol after losing a hexose moiety (−162 amu). Peaks 25 and 26 were also identified as quercetin and kaempferol derivatives according to the mass spectrum with a distinctive fragment at m/z 463 and 301, respectively. However, these compounds had a maximum absorption at shorter wavelength (314 nm), which on the other hand indicated acylation of the sugar moiety on this flavonol with hydroxycinnamic acid.[40] An analogous relationship was observed for compound **26**. MS in the negative mode gave the base peak at m/z 593 and an MS/MS main fragment at m/z 285 (loss m/z 308 − *p*-coumaroylglucoside moiety), which corresponds to kaempferol.

Quantitative Analysis. The major polyphenolic groups were flavan-3-ols > hydroxycinnamic acids > flavonols. Compared to apples, quince fruits do not contain dihydrochalcones and anthocyanins. Types of polyphenolic compounds detected

Table 2. Main Phenolic Composition of Quince Varieties and Genotypes (Milligrams per 100 g Dry Weight)[a]

	flavan-3-ols			phenolic acids				flavonols				
variety	E	PB2	PP	Nha	CA	ChA	3.5-CA	QR	QG	KR	KG	Σ TP
Uspiech	21.8 ± 5.2e	41.0 ± 2.5 cd	1964.2 ± 56.2f	12.3 ± 1.7a	12.5 ± 2.4c	235.7 ± 2.4c	13.1 ± 1.3e	5.5 ± 1.1d	39.5 ± 1.0c	9.0 ± 0.9e	8.6 ± 0.9i	2363.2
Akademiczeskaja	21.6 ± 5.1e	31.5 ± 3.0d	1586.3 ± 85.4j	5.5 ± 2.7f	8.1 ± 0.5.8e	319.6 ± 12.3a	10.4 ± 2.4f	5.5 ± 1.0d	53.1 ± 2.0b	19.8 ± 2.4c	19.2 ± 1.1c	2080.6
Kaszczenko	40.2 ± 8.2b	47.9 ± 3.6c	1369.9 ± 23.1l	6.5 ± 1.3e	7.1 ± 0.6.5f	298.6 ± 21.2b	2.0 ± 0.7j	3.4 ± 0.5	27.8 ± 2.4d	6.5 ± 1.0f	6.9 ± 0.6j	1816.6
Ronda	26.3 ± 3.3d	41.2 ± 3.1 cd	1424.5 ± 89.2k	13.7 ± 1.6a	9.8 ± 1.2 cd	274.4 ± 2.3b	14.4 ± 1.3d	4.6 ± 0.3de	46.6 ± 2.2b	18.2 ± 2.0c	26.9 ± 1.3a	1900.2
Portugesicka	34.1 ± 1.9c	49.8 ± 3.5c	1736.7 ± 67.4h	11.3 ± 1.6b	7.8 ± 1.6f	290.9 ± 15.3b	18.3 ± 3.2bc	5.4 ± 0.5d	44.2 ± 0.6b	11.4 ± 2.4d	16.4 ± 1.2e	2226.3
Marija	50.3 ± 5.3a	67.1 ± 2.7a	2119.7 ± 10.1e	11.8 ± 1.5b	9.3 ± 1.6d	200.7 ± 11.0e	14.3 ± 2.2d	3.7 ± 0.9e	32.3 ± 1.4c	10.7 ± 1.0d	12.0 ± 11.6	2531.9
Bereczki	40.0 ± 2.4b	52.2 ± 1.6b	1615.2 ± 36.0i	12.6 ± 1.7ab	16.7 ± 2.5b	249.5 ± 9.6c	22.3 ± 1.1b	5.2 ± 1.0d	33.7 ± 2.0c	9.4 ± 1.6e	13.3 ± 2.4f	2070.0
Cezar	29.6 ± 1.3c	57.6 ± 3.5b	2412.7 ± 47.3d	11.4 ± 3.5b	8.6 ± 2.1e	171.2 ± 10.4fg	27.7 ± 1.0a	2.6 ± 0.2g	32.2 ± 0.6c	6.4 ± 1.3f	9.4 ± 3.5h	2769.4
Leskovač	33.0 ± 3.2c	55.2 ± 2.7b	3074.1 ± 32.5a	9.3 ± 2.4c	8.7 ± 2.2e	189.4 ± 10.4ef	12.9 ± 2.5e	6.4 ± 0.4c	31.6 ± 3.0c	8.2 ± 1.1ef	7.9 ± 0.6i	3436.6
Darunok Onuk	31.7 ± 2.2c	55.0 ± 2.6b	2754.7 ± 45.1c	5.2 ± 0.6g	4.2 ± 1.3g	194.1 ± 2.7e	5.5 ± 2.5i	7.7 ± 0.1b	62.8 ± 2.6a	35.2 ± 2.0a	22.7 ± 1.3b	3178.8
Późna Rejmana	23.1 ± 2.5d	45.7 ± 2.7c	2750.1 ± 62.6c	9.9 ± 1.1c	8.0 ± 1.3ef	180.3 ± 12.4f	8.6 ± 0.9g	8.5 ± 0.1a	36.7 ± 4.3c	9.3 ± 1.1e	11.9 ± 1.4g	3092.0
Konstantynopeler	43.0 ± 1.2b	61.8 ± 1.9ab	2883.7 ± 84.4b	13.4 ± 2.5a	8.6 ± 2.3e	199.5 ± 14.3e	15.7 ± 2.4d	7.6 ± 0.4b	51.2 ± 1.6ab	23.1 ± 2.3b	15.2 ± 1.1e	3322.9
Wołgogradzkaja Aromatnaja	41.8 ± 4.1b	69.0 ± 2.0a	2834.4 ± 78.1b	12.1 ± 2.3ab	8.2 ± 1.2e	181.1 ± 18.9f	11.9 ± 3.2f	3.7 ± 1.0e	20.8 ± 2.2e	3.2 ± 1.7g	3.4 ± 0.6k	3189.5
S1K	24.6 ± 2.2d	35.6 ± 2.4d	1839.7 ± 530.4fg	4.5 ± 4.1g	7.7 ± 0.7f	227.9 ± 23.3 cd	7.4 ± 1.1h	5.5 ± 0.4d	42.0 ± 1.7b	14.3 ± 3.6 cd	18.6 ± 1.3 cd	2227.7
S3	33.7 ± 2.0c	55.1 ± 3.5b	2661.0 ± 69.0c	4.9 ± 1.5g	8.2 ± 0.5e	239.7 ± 12.3c	8.6 ± 3.5g	8.3 ± 0.2a	58.2 ± 1.4a	17.62.4c	19.9 ± 1.1c	3115.1
SDK	42.0 ± 1.6b	55.0 ± 2.9b	1926.7 ± 28.1f	6.5 ± 1.5e	9.7 ± 0.2d	171.9 ± 12.3fg	7.1 ± 1.2h	5.2 ± 0.4d	36.0 ± 2.7c	8.2 ± 3.5e	12.2 ± 1.3f	2280.5
BA29	33.4 ± 2.4c	56.6 ± 2.5b	1692.5 ± 58.4h	5.1 ± 2.6f	10.1 ± 1.1d	178.7 ± 12.1fg	7.8 ± 1.0h	6.5 ± 0.7c	33.7 ± 3.5c	8.8 ± 1.1e	10.9 ± 1.3gh	2044.2
S1	33.9 ± 3.5c	53.0 ± 1.4b	1351.0 ± 46.5l	8.4 ± 1.9d	20.3 ± 1.1a	175.0 ± 9.7fg	7.3 ± 0.9h	4.0 ± 0.1e	32.9 ± 3.4c	10.8 ± 1.1d	12.7 ± 1.7f	1709.5

[a]Values are the mean ± standard deviation, $n = 3$; in columns, mean values with different letters (a, b, c, ...) are significantly different at $P < 0.05$. E, (−)-epicatechin; PB2, procyanidin B2; PP, polymeric procyanidins; NhA, neochlorogenic acid; CA, cryptochlorogenic acid; ChA, chlorogenic acid; 3,5-CA, 3,5-dicaffeoylquinic acid; QG, quercetin-3-*O*-galactoside; QR, quercetin-3-*O*-rutinoside; KR, kaempferol-3-*O*-rutinoside; KG, quercetin-3-*O*-rhamnoside; Σ TP, sum of the determined phenolics.

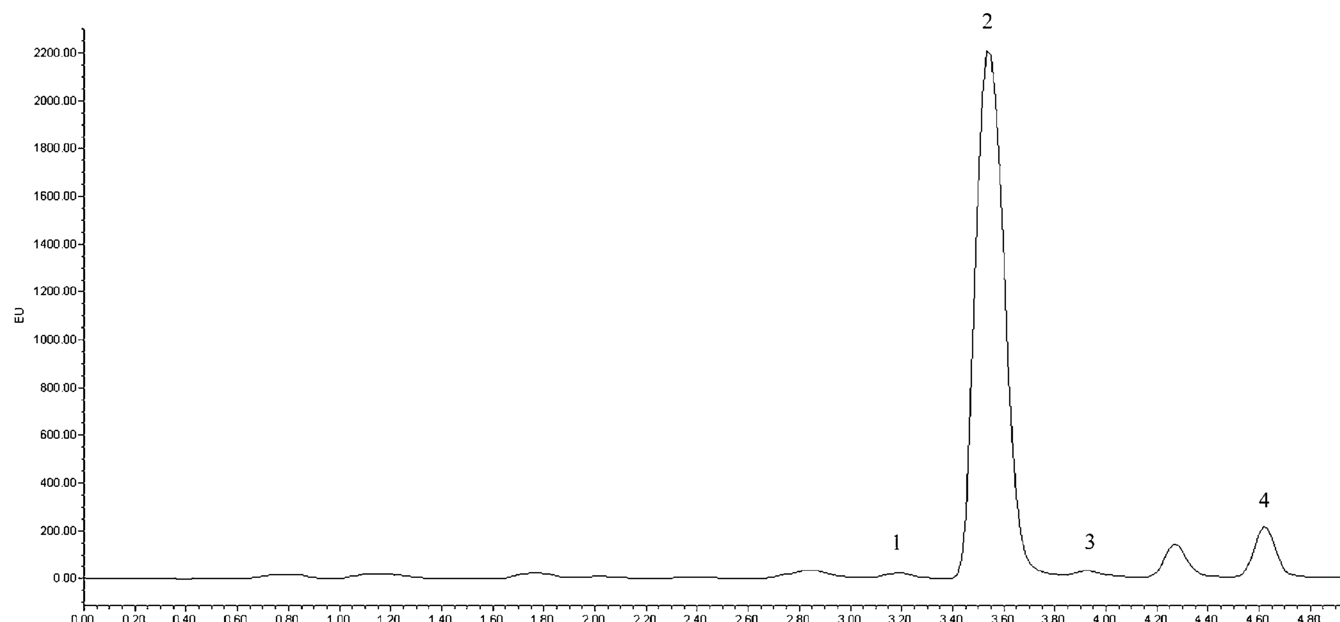

Figure 2. UPLC-FL chromatograms of quince fruit ('Leskovač') after phloroglucinol analysis. Peaks: 1, (+)-catechin−phloroglucinol; 2, (−)-epicatechin−phloroglucinol; 3, (+)-catechin; 4, (−)-epicatechin.

in this study in quince varieties were similar to those reported in previous studies.[8,15] Previous research showed that hydroxycinnamic acid was mainly from the polyphenol group, but the content of flavan-3-ols, especially polymeric procyanidins, has not been previously described. Hamauzu et al.[16] described that not only the total phenolic content but also the phenolic profiles were different for different fruits, that is, Chinese quince and apple fruits. As such, quince fruit was characterized by the presence of a significant amount of hydroxycinnamic derivatives (mainly 3-*O*-caffeoylquinic acid and 5-*O*-caffeoylquinic acid) and a large amount of polymeric procyanidins (PP). However, Chinese quince fruit contained PP as a major component and a small amount of 3-*O*-caffeoylquinic acid. Apple fruit did not contain 3-*O*-caffeoylquinic acid and a noticeable amount of PP, but it was characterized by the presence of 5-*O*-caffeoylquinic acid, (+)-catechin, (−)-epicatechin, procyanidin dimers (B1, B2, and C1), and oligomeric and dihydrochalcone derivatives (phloretin glycoside and phloridzin).[16]

The content of total polyphenols was between 1709.4 mg/100 g dw (genotype 'S1') and 3436.6 mg/100 g dw ('Leskovač'). The average content of total polyphenols found in the apple evaluated with the HPLC method was 2−5 g/kg of dw, with significant differences depending upon the apple variety.[27,41−44] The 'Leskovač' (3436.6 mg/100 g dw), 'Konstantynopeler' (3322.9 mg/100 g dw), 'Wołgogradzkaja Aromatnaja' (3189.5 mg/100 g dw), and 'Darunok Onuk' (3178.8 mg/100 g dw) varieties were characterized by above average contents of polyphenols, whereas 'Kaszczenko' (1816.0 mg/100 g dw), 'Ronda' (1900.2 mg/100 g dw), and 'Bereczki' (2070.0 mg/100 g dw) varieties and genotype 'S1' had the lowest contents of total polyphenols (Table 2). Only the 'S3' genotype was characterized by a similarly high content of polyphenols compared to the other varieties (3115.1 mg/100 g dw). Other genotypes contained <2300 mg/100 g dw of polyphenols. Karedeniz et al.[42] reported that the content of total polyphenolics determined with the Folin−Ciocalteu method in quince was higher than in apple, grape, or pear but comparable to that in pomegranate. Fruits with high contents of polyphenols are very important for human health. The World Health Organization has recommended fruit consumption at least 5 times a day or at the level of 400 g of fruits and vegetables per day per capita to reduce the risk of chronic diseases.[45] Therefore, it seems reasonable to believe that an increased consumption of products with quince polyphenolics may be beneficial for health and well-being.

The 26 polyphenolic compounds were identified with the LC-MS method (Table 1; Figure 1) but only major compounds were quantified using UPLC-PDA detection and UPLC-FL (Figure 2). Fluorescence was used to quantify polymeric proanthocyanidins, which were minor peaks with the PDA detector operating at 280 nm. These are additional procyanidins and will be the subject of a subsequent publication reporting on analyses based on normal phase HPLC-MS^2 and thiolysis or phloroglucinolysis.[29,43,44] Flavan-3-ols (monomer, dimer, and polymeric proanthocyanidins), which are the major class of quince polyphenols (Table 2), represented between 78 and 94% of the total polyphenolic compounds in quince. These data are concurrent for apple (71−90%).[41,44] The high concentrations of procyanidins in quince fruit may explain its slight astringency and bitterness, typical of cider apples. The main flavan-3-ol compounds were polymeric procyanidins (94−98%), followed by dimer (2−4%) and monomer (1−3%) procyanidins. (−)-Epicatechin concentrations ranged from 50.3 mg in 'Marija' to 21.6 mg/100 g dw in 'Akademiczeskaja'. In turn, procyanidin B2 concentrations ranged from 69.0 mg/100 g dw in 'Wołgogradzkaja Aromatnaja' and 'Marija' to 31.5 mg/100 g dw in 'Akademiczeskaja'. The average concentrations for dessert apple monomers and dimers (procyanidins B2) ranged between 11.6 and 209.5 mg and between 38.8 and 162.2 mg/100 g, respectively, whereas the presence of (+)-catechin was lower (0.5−3.4 mg/100 g).[27]

Polymeric proanthocyanidins were determined using phloroglucinol methods, which provide more detailed information on quince proanthocyanidin fractions. The analysis of these compounds is not possible without phloroglucinol or thiolysis methods. After phloroglucinol depolymerization, these compounds

were converted into monomer units. Figure 2 shows phloroglucinol products of quince phenolics, indicating that phenolics in quince consist of polymers of (−)-epicatechin and a small amount of (+)-catechin as terminal units. The profile of polymeric flavan-3-ols in quince was similar to that in Chinese quince and apple fruit, although the percentages of (+)-catechin as terminal units differed among the fruits. The percentage of (+)-catechin as terminal units was the highest in Chinese quince (31.3%) and in apple (25.8%), compared to quince (3.6%).[16] Polymeric proanthocyanidin present in the analyzed varieties and genotypes of quince had concentrations ranging from 1369.9 mg/100 g dw in 'Kaszczenko' and 1351.0 mg/100 g dw in genotype 'S1' to 3074.1 mg/100 g dw in 'Leskovač' (Table 2). These results might be due to the fact that the quince fruit contained a large amount of polymeric procyanidins in comparison to apples: dessert and cider.[25,41,43,44] However, cider apples contained a large amount of polymeric procyanidins when compared to dessert apples.

The degree of polymerization (mDP; number of flavan-3-ol units) modulates the physicochemical properties of procyanidins.[16,43,44,46] Reserve-phase HPLC following the phloroglucynolysis 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.[41,43,44,46] The mDP of the polymeric fraction for the quince fruit was from 8.3 to 11.2; thus, on average it accounted for 9.7. The highest mDP was determined in the following varieties: 'Kaszczenko', 'Ronda', 'Konstantynopeler', and 'Marija'. The degree of polymerization of cranberry has been previously determined by Gu et al.[47] to reach 15 and 9 for lingonberry.[48] In considering the Polish apple variety, mDP varied from 3.2 ('Titówka') to 28.7 ('Ecolette'), with the average values ranging from 3.8 to 6.2.[25] The mean mDP of flavan-3-ols for Chinese quince was 25.[16] These high mDP values for Chinese quince and quince procyanidins were comparable with those of a variety of Portuguese pear[49] or some cider apple varieties (4.2−50.3).[44,50]

Hydroxycinnamic acid was the second major group of quince polyphenols. The concentration of these compounds ranged from 195.2 to 343.5 mg/100 g dw. The predominating phenolic acid in quince fruit was chlorogenic acid > neochlorogenic acid > cryptochlorogenic acid ≥ 3,5-dicaffeoylquinic acid. Chlorogenic acid concentrations ranged from 319.6 mg ('Akademiczeskaja') to 171.2 mg/100 g dw ('Cezar'), those of neochlorogenic acid ranged from 45.1 mg (genotype 'S1K') to 13.4 mg/100 g dw ('Konstantynopeler'), those of cryptochlorogenic acid ranged from 4.2 mg ('Darunok Onuk') to 20.3 mg/100 g dw (genotype 'S1'), and finally those of 3,5-dicaffeoylquinic acid ranged from 2.0 mg ('Kaszczenko') to 27.7 mg/100 g dw ('Cezar') (Table 2). We assumed that the major hydroxycinnamic acid in different quince varieties was the chlorogenic acid. The results described by Silva et al.[8,24] show that in pulps and seeds of quince, the most abundant phenolic compound was also chlorogenic acid. Wojdyło et al.,[25] Tsao et al.,[28] Marks et al.,[40] and Mullen et al.[28] clearly show that the concentration of phenolic acid was strongly dependent on variety. Alonso-Salces et al.[37] have also observed that the concentrations of phenolic acid in cider apples are considerably greater than those found in dessert apples. Marks et al.[40] described that the concentrations of chlorogenic acid in cider apple ranged from 30 to 1163 mg/100 g. The concentration of phenolic acid, especially chlorogenic acid, may be important when fruits are processed into food, because these compounds are considered to be a preferential natural substrate of the catecholase activity of PPO. 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 flavor when their concentrations are low.[41,44] Aside from enzymatic oxidation, the product of chlorogenic acid (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 chlorogenic acid contents but also the flavan-3-ols/hydroxycinnamic acid ratio. Hence, those varieties with a high content of flavan-3-ols and hydroxycinnamic acids, including chlorogenic acid, contribute to a low stability of the finished (processed) food products. In this sense, the varieties 'Akademiczeskaja', 'Kaszczenko', 'Ronda', 'Portugesicka', and 'Bereczki' and genotype 'S1' would be the least suitable.

Significant differences ($P < 0.05$) were found in the phenolic profiles of quince from different variety origins, in terms of flavonol derivatives. As described previously,[8] the main flavonols present in quince are a mixture of different glycosylated quercetin and kaempferol. For all examined varieties and genotypes of quince, the content of flavonols ranged from 1 to 5% of total polyphenols, but quercetin-3-*O*-rutinoside was the most abundant quercetin glycoside in all quince fruit, and its content ranged from 49 to 63% of total flavonols. Also, the quercetin-3-*O*-rutinoside in quince fruit was identified as quercetin-3-*O*-galactoside, kaempferol-3-*O*-rutinoside, and 3-*O*-glucoside (Table 2). Generally, considering the content of these compounds, quince varieties had significantly more quercetin-type (∼45.2 mg/100 g dw) than kaempferol-type derivatives (∼26.3 mg/100 g dw). A high content of flavonol derivatives was found in 'Darunok Onuk' (128.4 mg/100 g dw), 'Akademiczeskaja' (97.7 mg/100 g dw), and genotype 'S3' (104.0 mg/100 g dw), whereas the lowest content was in 'Kaszczenko' and 'Wołgogradzkaja Aromatnaja' (44.6 and 31.1 mg/100 g dw, respectively). This was consistent with findings described by Silva et al.[8] and Oliveira et al.[39]

Comparing this qualitative profile with that of quince fruit parts (pulps, peels, and seeds), usually, quince peel contained higher levels of quercetin and kaempferol derivatives. These compounds were reported as the major phenolic compounds in skin.[15,51] The levels of these glycoside derivatives of flavonol were higher in the leaves than in the skin of quince fruit.[39] As such, quince leaves presented the highest relative contents of kaempferol derivatives, especially of kaempferol-3-*O*-rutinoside, which represented 12.5% of the total phenolic content (against ca. 4% in peels and an absence in pulps and seeds). In peel we found additional quercetin and kaempferol glycosides acylated with *p*-coumaric acid, but these compounds were present in very small amounts in comparison to the whole fruit. The content of these compounds has been suggested previously by Silva et al.[4,9,24] Quercetin derivatives are not the major polyphenolic components of fruit, but they are very important for human health. Quercetin was found to inhibit human prostate and lung cancer cell growth[52] and to reduce the incidence of cardiovascular diseases.[53]

L-Ascorbic Acid. Smaller but significant differences were found in the contents of L-ascorbic acid (Figure 3). The values measured with the HPLC method ranged from 5.9 mg/100 g to as much as 26.4 mg/100 g. For contents of L-ascorbic acid in fruit, quince fruit can be divided into three groups: low content ('Późna Rejmana', 'S1K', 'S3', 'S1'), average content ('Uspiech',

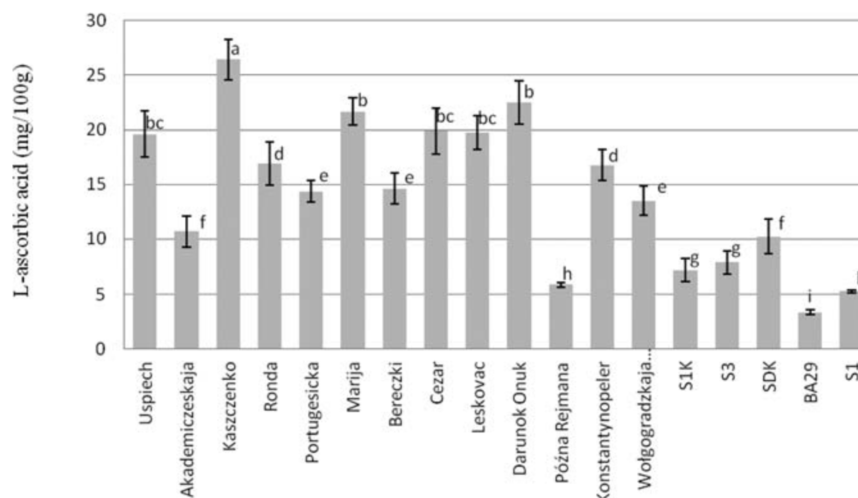

Figure 3. L-Ascorbic acid (mg/100 g fw) concentrations in different quince fruit varieties. Different lower or upper case letters (a, b, c, ...) denote significant differences ($P < 0.05$) between variety, $n = 3$.

'Akademiczeskaja', 'Ronda', 'Portugesicka', 'Bereczki', 'Konstantynopeler', 'Wołgogradzkaja Aromatnaja', 'SDK'), and high content of L-ascorbic acid ('Kaszczenko', 'Marija', 'Cezar', 'Leskovač', 'Darunok Onuk'). The 'BA29' genotype is characterized by the lowest content of L-ascorbic acid, <5 mg/100 g. Rop et al.[22] analyzed the content of L-ascorbic acid in different quince genotypes and varieties. In comparison to the analyzed fruit, the content of L-ascorbic acid in these 22 quince fruit was also higher, from 41.1 to 79.3 mg/100 g fw. According to Szeto et al.[54] the maximum value of L-ascorbic acid in quince fruit was comparable to that in apple, whereas in pears its content did not exceed half of this value.[55,56] Compared to other fruits, the range of ascorbic acid contents (mg/100 g) in quince fruit was higher than in nectarines (4.8–13.2), peaches (3.6–12.6), litchi (13.8), and plums (2.5–10.2)[57] but lower than in mango (60.5) and papaya (92.9).[58]

PPO Activity. PPOs are very important enzymes in the food industry because of their involvement in enzymatic browning. They are degraded to undesirable brown, red, or black pigments. Studies on PPO from quince in research literature are scarce.[59−61] The activity of this enzyme ranged from 709.9 to 1284.6 ΔU/min. The higher PPO activity was found in 'BA29' > 'Konstantynopeler' > 'Kaszczenko' > 'Wołgogradzkaja Aromatnaja' ≥ 'Darunok Onuk'. Podsędek et al.[62] clearly show that the PPO activity differed significantly between apple varieties, from 270 U/g ('Shampion') to 3120 U/g ('Warta'). Browning of damaged tissues of fresh fruits and vegetables occurs mainly due to the oxidation of phenolic compounds and contributes significantly to the diminished quality.[63] Enzymatic browning results in a change of not only color but also the level of biologically active compounds, known to be good PPO substrates.

Antioxidant Activity. The antioxidant activity of the quince varieties determined by ABTS, DPPH, and FRAP value (μmol Trolox equiv/g dw) is shown in Table 3. Significant variation was found in the antioxidant activity among the studied varieties. The effects of quince variety on the antioxidant activity measured by $ABTS^{\bullet+}$ ranged from 2.4 to 0.9; for DPPH they ranged from 2.5 to 0.9; and for FRAP they ranged from 1.5 to 0.4 TEAC. 'Kaszczenko', 'Uspech', 'Ronda', and 'Portugesica' varieties were characterized by the highest activity measured with all methods, whereas the 'Późna Rejmana' variety and 'S1', 'S1K', 'SDK', and

Table 3. Antioxidant Activity of Quince Varieties and Genotypes (Micromoles Trolox Equivalents per Gram Dry Weight)[a]

variety	ABTS	DPPH	FRAP
Uspiech	2.3 ± 0.9a	2.5 ± 0.5a	1.5 ± 1.0a
Akademiczeskaja	1.9 ± 0.6b	2.2 ± 0.3c	1.0 ± 0.1d
Kaszczenko	2.0 ± 0.2b	2.2 ± 0.6c	1.3 ± 0.5b
Ronda	2.0 ± 0.5b	2.2 ± 0.3c	1.3 ± 0.1b
Portugesicka	2.4 ± 1.0a	2.4 ± 0.3b	1.6 ± 0.3a
Marija	1.1 ± 0.6e	1.1 ± 0.2e	0.7 ± 0.1ef
Bereczki	2.0 ± 1.0ab	2.1 ± 0.5c	1.3 ± 0.1b
Cezar	1.4 ± 0.2d	1.8 ± 0.2d	0.7 ± 0.2e
Leskovač	1.8 ± 0.1b	1.8 ± 0.1d	0.9 ± 0.1de
Darunok Onuk	1.4 ± 0.4d	1.7 ± 0.5d	0.7 ± 0.1e
Późna Rejmana	0.9 ± 0.1g	1.1 ± 0.0e	0.5 ± 0.1f
Konstantynopeler	1.5 ± 0.2c	1.9 ± 0.1d	0.9 ± 0.2d
Wołgogradzkaja Aromatnaja	1.0 ± 0.2f	1.1 ± 0.2e	0.4 ± 0.0g
S1K	1.0 ± 0.1fg	1.1 ± 0.1e	0.8 ± 0.3e
S3	1.1 ± 0.1f	1.0 ± 0.1ef	1.1 ± 0.2c
SDK	0.9 ± 0.0fg	1.0 ± 0.1e	0.7 ± 0.2e
BA29	0.9 ± 0.1g	1.2 ± 0.1e	0.6 ± 0.0f
S1	1.0 ± 0.1f	0.9 ± 0.1f	0.6 ± 0.1f

[a]Values are the means ± standard deviation, $n = 3$; in columns, mean values with different letters (a, b, c, ...) are significantly different at $P < 0.05$.

'BA29' genotypes had the lowest TEAC values. The differences in TEAC between quince varieties could be preliminarily attributed to their different contents of polyphenols. Bandoniene et al.[64] tested different antioxidant kinetic behaviors of standard compounds present in apple and apple extract with the HPLC-DPPH method and showed that polyphenolic compounds had the greatest impact on the antioxidant activity from the group of flavan-3-ols such as (+)-catechin and (−)-epicatechin, as well as their oligomers such as procyanidins B1, B2, and C. Compounds belonging to the group of phenolic acids, quercetin glycosides, and dihydrochalcones affect the antioxidant activity of apples to a lesser extent. Lee et al.[65] have proved that (−)-epicatechin and procyanidins B2 had a 40% relative contribution to the total antioxidant activity of apples measured with the $ABTS^{\bullet+}$ method. (−)-Epicatechin and procyanidin B2

contribution was the highest among major phytochemicals, followed by quercetin glycosides (34.7%), whereas chlorogenic acid (7.6%) had little contribution to the total antioxidant activity.

Relationship between Phytochemical Content and Total Antioxidant Activity. A point worth mentioning is the contribution of each bioactive compound to the antioxidant activity assays. When the contents of polyphenolic compounds were individually correlated with ABTS, DPPH, FRAP, and even L-ascorbic acid, it was clear that phenolic acid content was highly correlated with all of these assays, whereas no significant correlation was noted with the total flavan-3-ols (Table 4).

Table 4. Pearson's Correlation Coefficients

	flavan-3-ols	phenolic acids	flavonols	L-ascorbic acid
ABTS	−0.301	0.773	0.076	0.555
DPPH	−0.263	0.704	0.105	0.570
FRAP	−0.389	0.819	0.248	0.364
PPO	0.635	0.823	−0.067	−0.191

With regard to L-ascorbic acid, they were correlated with the antioxidant activity, except for the FRAP assay in which only a low relationship was observed. Different studies carried out with fruits have reported differences relating to the polyphenolic compound contribution to the antioxidant capacity assays,[66] which results from their nature. Our data indicated that polymeric procyanidins did not participate in the antioxidant activity for fruit, as was corroborated by Oszmiański et al.;[66] however, procyanidins have displayed important biological activities and were implicated in most of the beneficial health properties of fruit.[48] The obtained results suggested different mechanisms of radical scavenging of polyphenols. At the beginning, the reaction proceeds very quickly. It is likely that the effective, low molecular weight antioxidants (chlorogenic acid, (−)-epicatechin) participate in the first stage of the reaction with the radical. The polymerized procyanidins are mainly responsible for the antioxidant capacity when the measurements are done after 10 min or later.

Polyphenolics are the most susceptible compounds to oxidation by PPO (Table 4), but according to the literature,[62] ascorbic acid is more susceptible to being oxidized by PPO than polyphenols. When the correlation between PPO and phenolic compounds was analyzed, a strong correlation was found between flavan-3-ol and phenolic acid contents and PPO than between the rest of the polyphenol class, L-ascorbic, and PPO. This result confirmed that quince fruit had a very active PPO enzyme and phenolic compounds, which ought to be protected during food product preparation. However, in research by Podsędek et al.,[62] no correlation was found between specific phenolic groups or between PPO activity and phenolic or ascorbic acid contents.

To conclude, the presented data clearly demonstrate that compounds of the analyzed varieties of quince were characterized by a high biological activity, especially compounds belonging to flavan-3-ols. The analysis of the phenolic profile clearly indicated that derivatives of quercetin and kaempferol were the minor phenolic components of quince, whereas polymers of procyanidins and chlorogenic acid derivatives constituted the majority of the polyphenolics. The content of flavonols is relatively consistent in different varieties. The concentration of procyanidins/flavan-3-ols is the most important contributor to the antioxidant activity. Quince varieties and their products that have a higher content of phenolic compounds may be selected to promote their positive effect on health.

AUTHOR INFORMATION

Corresponding Author

*Phone: (+48) 71-3205 7706. Fax: (+48) 71-3205 7706. E-mail: aneta.wojdylo@up.wroc.pl.

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REFERENCES

(1) Paredes-Lopez, O.; Cervantes-Ceja, M. L.; Vigna-Perez, M.; Hernandez-Perez, T. Berries: improving human health and healthy aging, and promoting quality life − a review. *Plant Foods Hum. Nutr.* **2010**, *65*, 299−308.

(2) Seeram, N. P. Berry fruits: compositional elements, biochemical activities, and the impact of their intake on human health, performance, and disease. *J. Agric. Food Chem.* **2008**, *56*, 627−629.

(3) Naf, R.; Velluz, A.; Decorzant, R.; Naf, F. Structure and synthesis of 2 novel ionone-type compounds identified in quince brandy (*Cydonia oblonga* Mill.). *Tetrahedron Lett.* **1991**, *32*, 753−756.

(4) Silva, B. M.; Andrade, P. B.; Ferreres, F.; Domingues, A. L.; Seabra, R. M.; Ferreira, M. A. Phenolic profile of quince fruit (*Cydonia oblonga* Miller) (pulp and peel). *J. Agric. Food Chem.* **2002**, *50*, 4615−4618.

(5) Silva, B. M.; Andrade, P. B.; Seabra, R. M.; Ferreira, M. A. Determination of selected phenolic compounds in quince jams by solid-phase extraction and HPLC. *J. Liq. Chromatogr. Relat. Technol.* **2001**, *24*, 2861−2872.

(6) Silva, B. M.; Casal, S.; Andrade, P. B.; Seabra, R. M.; Oliveira, M. B.; Ferreira, M. A. Development and evaluation of a GC/FID method for the analysis of free amino acids in quince fruit and jam. *Anal. Sci.* **2003**, *19*, 1285−1290.

(7) Fiorentino, A.; D'abrosca, B.; Pacifico, S.; Mastellone, C.; Piscopo, V.; Caputo, R.; Monaco, P. Isolation and structure elucidation of antioxidant polyphenols from quince (*Cydonia vulgaris*) peels. *J. Agric. Food Chem.* **2008**, *56*, 2660−2667.

(8) Silva, B. M.; Andrade, P. B.; Seabra, R. M.; Oliveira, M. B. P. P.; Ferreira, M. A.; Ferreres, F. Composition of quince (*Cydonia oblonga* Miller) seeds: phenolics, organic acids and free amino acids. *Nat. Prod. Res.* **2005**, *19*, 275−281.

(9) Silva, B. M.; Andrade, P. B.; Valentão, P.; Ferreres, F.; Seabra, R. M.; Ferreira, M. A. Quince (*Cydonia oblonga* Miller) fruit (pulp, peel, and seed) and jam: antioxidant activity. *J. Agric. Food Chem.* **2004**, *52*, 4405−4712.

(10) Silva, B. M.; Andrade, P. B.; Mendes, G. C.; Seabra, R. M.; Ferreira, M. A. Study of the organic acids composition of quince (*Cydonia oblonga* Miller) fruit and jam. *J. Agric. Food Chem.* **2002**, *50*, 2313−2317.

(11) de Escalada Pla, M. F.; Uribe, M.; Fissore, E. N.; Gerschenson, L. N.; Rojas, A. M. Influence of the isolation procedure on the characteristics of fiber-rich products obtained from quince wastes. *J. Food Eng.* **2010**, *96*, 239−248.

(12) Kaya, A.; Aydin, O.; Demirtas, C.; Akgun, M. An experimental study on the drying kinetics of quince. *Desalination* **2007**, *212*, 328−343.

(13) Silva, B. M.; Andrade, P. B.; Gonçalves, A. C.; Seabra, R. M.; Oliveira, M. B.; Ferreira, M. A. Influence of jam processing upon the contents of phenolics, organic acids and free amino acids in quince

fruit (*Cydonia oblonga* Miller). *Eur. Food Res. Technol.* **2004**, *218*, 385−389.
(14) Yurdug, S. Preservation of quinces by the combination of an edible coating material, Semperfresh, ascorbic acid and cold storage. *Eur. Food Res. Technol* **2000**, *220*, 579−586.
(15) Fattouch, S.; Caboni, P.; Coroneo, V.; Tuberoso, C. I. G.; Angioni, A.; Dessi, S.; Marzouki, N.; Cabras, P. Antimicrobial activity of Tunisian quince (*Cydonia oblonga* Miller) pulp and peel polyphenolic extracts. *J. Agric. Food Chem.* **2007**, *55*, 963−969.
(16) Hamauzu, Y.; Yasui, H.; Inno, T.; Kume, C.; Omanyuda, M. Phenolic profile, antioxidant property, and anti-influenza viral activity of Chinese quince (*Pseudocydonia sinensis* Schneid.), quince (*Cydonia oblonga* Mill.), and apple (*Malus domestica* Mill.) fruits. *J. Agric. Food Chem.* **2005**, *53*, 928−934.
(17) Hamauzu, Y.; Inno, T.; Kume, C.; Irie, M.; Hiramatsu, K. Antioxidant and antiulcerative properties of phenolics from Chinese quince, quince, and apple fruits. *J. Agric. Food Chem.* **2006**, *54*, 765−772.
(18) Wang, X.; Jia, W.; Zhao, A.; Wang, X. Anti-influenza agents from plants and traditional Chinese medicine. *Phytother. Res.* **2006**, *20*, 335−341.
(19) Costa, R. M.; Magalhães, A. S.; Pereira, J. A.; Andrade, P. B.; Valentão, P.; Carvalho, M.; Silva, B. M. Evaluation of free radical-scavenging and antihemolytic activities of quince (*Cydonia oblonga*) leaf: a comparative study with green tea (*Camellia sinensis*). *Food Chem. Toxicol.* **2009**, *47*, 860−865.
(20) Carvalho, M.; Silva, B. M.; Silva, R.; Valentao, P.; Andrade, P. B.; Bastos, M. L. First report on *Cydonia oblonga* Miller anticancer potential: differential antiproliferative effect against human kidney and colon cancer cells. *J. Agric. Food Chem.* **2010**, *58*, 3366−3370.
(21) Magalhães, A. S.; Silva, B. M.; Pereira, J. A.; Andrade, P. B.; Valentão, P.; Carvalho, M. Protective effect of quince (*Cydonia oblonga* Miller) fruit against oxidative hemolysis of human erythrocytes. *Food Chem. Toxicol.* **2009**, *47*, 1372−1377.
(22) Rop, O.; Balík, J.; Řezníček, V.; Juríková, T.; Škardová, P.; Salaš, P.; Sochor, J.; Mlček, J.; Kramářová, D. Chemical characteristics of fruits of some selected quince (*Cydonia oblonga* Mill.) cultivars. *Czech J. Food Sci.* **2011**, *29* (1), 65−73.
(23) Bayazit, S.; Imrak, B.; Küden, A.; Kemal Güngör, M. RAPD analysis of genetic relatedness among selected quince (*Cydonia oblonga* Mill.) accessions from different parts of Turkey. *Hortic. Sci. (Prague)* **2011**, *38*, 134−141.
(24) Silva, B. M.; Andrade, P. B.; Martins, R. C.; Valentão, P.; Ferreres, F.; Seabra, R. M.; Ferreira, M. A. Quince (*Cydonia oblonga* Miller) fruit characterization using principal component analysis. *J. Agric. Food Chem.* **2005**, *53*, 111−122.
(25) Wojdyło, A.; Oszmiański, J.; Laskowski, P. The polyphenolic compounds and antioxidant activity of new and old apple varieties. *J. Agric. Food Chem.* **2008**, *56*, 6520−6530.
(26) Łata, B. Relationship between apple peel and the whole fruit antioxidant content: year and cultivar variation. *J. Agric. Food Chem.* **2007**, *55*, 663−671.
(27) Vrhovsek, U.; Rigo, A.; Tonon, D.; Mattivi, F. Quantitation of polyphenols in different apple varieties. *J. Agric. Food Chem.* **2004**, *52*, 6532−6538.
(28) Tsao, R.; Yang, R.; Young, J. C.; Zhu, H. Polyphenolic profiles in eight apple cultivars using high-performance liquid chromatography (HPLC). *J. Agric. Food Chem.* **2003**, *51*, 6347−6353.
(29) Kennedy, J. A.; Jones, G. P. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.* **2001**, *49*, 1740−1746.
(30) Yen, G. C.; Chen, H. Y. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem.* **1995**, *43*, 27−32.
(31) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26* (9/10), 1231−1237.
(32) Benzie, I. F. F.; Strain, J. J. Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Anal. Biochem.* **1996**, *239*, 70−76.
(33) Oszmiański, J.; Wojdyło, A. Effects of blackcurrant and apple pulp blended on phenolics, antioxidant capacity and colour of juices. *J. Czech Food Technol.* **2009**, *27*, 338−351.
(34) Gonzalez, E. M.; de Ancos, B.; Cano, M. P. Partial characterization of polyphenol oxidase activity in raspberry fruits. *J. Agric. Food Chem.* **1999**, *47*, 4068−4072.
(35) Jaiswal, R.; Jayasinghe, L.; Kuhnert, N. Identification and characterization of proanthocyanidins of 16 members of the *Rhododendron* genus (Ericaceae) by tandem LC-MS. *J. Mass Spectrom.* **2012**, *47*, 502−515.
(36) Clifford, M. N.; Johnston, K. L.; Knight, S.; Kuhnert, N. Hierarchical scheme for LC−MSn identification of chlorogenic acids. *J. Agric. Food Chem.* **2003**, *51*, 2900−2911.
(37) Alonso-Salces, R. M.; Barranco, A.; Abad, B.; Berrueta, L. A.; Gallo, B.; Vicente, F. Polyphenolic profiles of Basque cider apple cultivars and their technological properties. *J. Agric. Food Chem.* **2004**, *52*, 2938−2952.
(38) Jaiswal, R.; Kuhnert, N. Identification and characterization of two new derivatives of chlorogenic acids in arnica (*Arnica montana* L.) flowers by high-performance liquid chromatography/tandem mass spectrometry. *J. Agric. Food Chem.* **2010**, *58*, 5471−5484.
(39) Oliveira, A. P.; Pereira, J. A.; Andrade, P. B.; Valentão, P.; Seabra, R. M.; Silva, B. M. Phenolic profile of *Cydonia oblonga* Miller leaves. *J. Agric. Food Chem.* **2007**, *55*, 7926−7930.
(40) Marks, S. C.; Mullen, W.; Crozier, A. Flavonoid and chlorogenic acid profiles of English cider apples. *J. Sci. Food Agric.* **2007**, *87*, 719−728.
(41) Guyot, S.; Marnet, N.; Sanoner, P. Variability of the polyphenolic composition of cider apple (*Malus domestica*) fruits and juices. *J. Agric. Food Chem.* **2003**, *51*, 7522−7728.
(42) Karadeniz, F.; Burdurlu, H. S.; Koca, N.; Soyer, Y. Antioxidant activity of selected fruits and vegetables grown in Turkey. *Turk. J. Agric.* **2005**, *29*, 297−303.
(43) Guyot, S.; Le Bourvellee, C.; Marnet, N.; Drilleau, J. F. Procyanidins are the most abundant polyphenols in dessert apples at maturity. *Lebensm.-Wiss. Technol.* **2002**, *35*, 289−291.
(44) Guyot, S.; Marnet, M.; Laraba, D.; Sanoner, 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.
(45) WHO. *Diet, Nutrition, and the Prevention of Chronic Diseases*; Geneva, Switzerland, 2003.
(46) Le Bourvellec, C.; Picot, M.; Renard, C. M. G. C. Size exclusion chromatography of procyanidins: comparison between apple and grape procyanidins and application to the characterization of fractions of high degrees of polymerization. *Anal. Chim. Acta* **2006**, *563*, 33−43.
(47) Gu, L.; Kelm, M.; Hammerstone, J. F.; Beecher, G.; Cunningham, D.; Vannozzi, S.; Prior, R. L. Fractionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected foods with an optimized normal-phase HPLC-MS fluorescent detection method. *J. Agric. Food Chem.* **2002**, *50*, 4852−4860.
(48) Kylli, P.; Nohynek, L.; Puupponen-Pimica, R.; Westerlund-Wikstrcom, B.; Leppcanen, T.; Welling, J.; Moilanen, E.; Heinonen, M. Lingonberry (*Vaccinium vitisidaea*) and European cranberry (*Vaccinium microcarpon*) proanthocyanidins: isolation, identification, and bioactivities. *J. Agric. Food Chem.* **2012**, *59*, 3373−3384.
(49) Ferreira, D.; Guyot, S.; Marnet, N.; Delgadillo, I.; Renard, C. M. G. C.; Coimbra, M. A. Composition of phenolic compounds in a Portuguese pear (*Pyrus communis* L. var. S. Bartolomeu) and changes after sun-drying. *J. Agric. Food Chem.* **2002**, *50*, 4537−4544.
(50) Sanoner, P.; Guyot, S.; Marnet, N.; Molle, D.; Drilleau, J.-F. Polyphenol profiles of French cider apple varieties (*Malus domestica* sp.). *J. Agric. Food Chem.* **1999**, *47*, 4847−4853.

(51) McGhie, T. K.; Hunt, M.; Barnett, L. E. Cultivar and growing region determine the antioxidant polyphenolic concentration and composition of apples grown in New Zealand. *J. Agric. Food Chem.* **2005**, *53*, 3065−3070.
(52) 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.
(53) Knekt, P.; Isotupa, S.; Rissanen, H.; Heliovaara, M.; Jarvinen, R.; Hakkinen, S.; Aroma, A.; Reunanen, A. Quercetin intake and the incidence of cerebrovascular disease. *Eur. J. Clin. Nutr.* **2000**, *54*, 415−417.
(54) Szeto, Y. T.; Tomlinson, B.; Benzie, I. F. F. Total antioxidant and ascorbic acid content of fresh fruits and vegetables: implications for dietary planning and food preservation. *Br. J. Nutr.* **2002**, *87*, 55−59.
(55) Ozturk, I.; Ercisli, S.; Kalkan, F.; Demir, B. Some chemical and physico-mechanical properties of pear cultivars. *Afr. J. Biotechnol.* **2009**, *8* (4), 687−693.
(56) Sanchez, A. C. G.; Gil-Izquierdo, A.; Gil, M. I. Comparative study of six pear cultivars in terms of their phenolic and vitamin C contents and antioxidant capacity. *J. Sci. Food Agric.* **2003**, *83*, 995−1003.
(57) Gil, M. I.; Tomas-Barberan, F. A.; Hess-Pierce, B.; Kader, A. A. Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. *J. Agric. Food Chem.* **2002**, *50*, 4976−4982.
(58) Luximon-Ramma, A.; Bahorun, T.; Crozier, A. Antioxidant actions and phenolic and vitamin C contents of common Mauritian exotic fruits. *J. Sci. Food Agric.* **2003**, *83*, 496−502.
(59) Orenes-Pinero, E.; Garcia-Caromana, F.; Sanchezferrer, A. A kinetic study of *p*-cresol oxidation by quince fruit polyphenol oxidase. *J. Agric. Food Chem.* **2005**, *53*, 1196−1200.
(60) Orenes-Pinero, E.; Garcia-Caromana, F.; Sanchezferrer, A. Latent polyphenol oxidase from quince fruit pulp (*Cydonia oblonga*): purification, activation and some properties. *J. Sci. Food Agric.* **2006**, *86*, 2172−2178.
(61) Ümit Ünal, M.; Sener, A.; Bozdogan, A. A comparative study of polyphenol oxidase from two varieties of quince (*Cydonia oblonga*). *J. Food Biochem.* **2010**, *34* (2), 356−367.
(62) Podsędek, A.; Wilska-Jeszka, J.; Anders, B.; Markowski, J. Compositional characterisation of some apple varieties. *Eur. Food Res. Technol.* **2000**, *210*, 268−272.
(63) Le Bourvellec, C.; Le Queäreä, J. M.; Sanoner, P.; Drilleau, J. F.; Guyot, S. Inhibition of apple polyphenol oxidase activity by procyanidins and polyphenol oxidation products. *J. Agric. Food Chem.* **2004**, *52*, 122−130.
(64) Bandoniene, D.; Murkovic, M. On-line HPLC-DPPH screening method for evaluation of radical scavenging phenols extracted from apples (*Malus domestica* L.). *J. Agric. Food Chem.* **2002**, *50*, 2482−2487.
(65) Lee, C. Y.; Smith, N. L. Apples: an important source of antioxidants in the American diet. *N. Y. Fruit Q.* **2000**, *8*, 15−17.
(66) Oszmiański, J.; Wolniak, M.; Wojdyło, A.; Wawer, I. Comparative study of polyphenolic content and antiradical activity of cloudy and clear apple juices. *J. Food Agric. Sci.* **2007**, *87*, 573−579.