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Radical Scavenging Activities of Peels and Pulps from cv. Golden Delicious Apples as Related to Their Phenolic Composition

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The relationship between phenolic composition and radical scavenging activity of apple peel and pulp was investigated in fruit produced according to both organic and integrated agricultural methods. Apple tissue extracts were subjected to high-performance liquid chromatography separation, which showed that as compared with pulps, peels are richer in almost all of the quantified phenolics. Flavonols, flavanols, procyanidins, dihydrochalcones, and hydroxycinnamates were the identified phenolic classes in peel tissue, and the most abundant compounds were epicatechin, procyanidin B2, and phloridzin. Pulps were poorer in phytochemicals. Their major phenolics were procyanidins and hydroxycinnamates. Flavonols in amounts <20 mg kg^{-1} fresh weight (fw) were also found. In both peels and pulps, integrated production samples were richer in polyphenols. Among the 14 compounds identified, only phloridzin had a tendency to appear higher in organic peels. The total antioxidant capacities (TAC) of extracts were evaluated using the 1,1-diphenyl-2-picrylhydrazyl radical assay and were expressed as Trolox equivalents. Integrated peels gave the highest TAC (18.56 mM kg^{-1} fw), followed by organic peels (TAC = 14.96), integrated pulps (TAC = 7.12), and organic pulps (TAC = 6.28). In peels, the top contributors to the antioxidant activity were found to be flavonols, flavanols, and procyanidins, which accounted for about 90% of the total calculated activity whereas in pulps, the TAC was primarily derived from flavanols (monomers and polymers) together with hydroxycinnamates. A good correlation between the sum of polyphenols and the radical scavenging activities was found. Among the single classes of compounds, procyanidins (in peels and pulps) and flavonols (in peels) were statistically correlated to the TAC.

KEYWORDS: DPPH; HPLC; organic apple; phenolics; antioxidant activity

INTRODUCTION

There is increasing evidence that the consumption of vegetables and fruits is associated with a reduced risk of degenerative diseases such as cancer, cardiovascular diseases, and cataracts (*1*−*4*). This "protective" property of vegetables and fruits is thought to depend on their contents of bioactive antioxidant compounds that exert a scavenging activity toward free radicals, which are thought to be responsible for many age-related diseases (*5*).

Naturally occurring radical scavengers include, mainly, phenolics, ascorbic acid, carotenoids, tocopherols, and glucosinolates (*6*). Phenolics in particular have received a great deal of attention because of their wide and abundant presence in fresh produce and their strong antioxidant activity.

In an epidemiological study, Hertog et al. (*7*) found a positive correlation between intake of flavonoids and reduction of coronary heart disease while Knekt et al. (*8*) showed an inverse correlation between cancer and flavonoid consumption.

In numerous national diets, apples represent an important source of bioavailable flavonoids. In Finland and the United States, they were found to be the top phenolics contributor (*8*, *9*) while in Holland and Denmark only onions and tea were calculated to give a larger percentage of these compounds (*10*).

The consumption of apples has been linked to the prevention of degenerative diseases. Wolfe et al. (*11*) reported that peels from various apple cultivars greatly inhibited the growth of liver tumor cells in vitro.

The aim of this work was, hence, to evaluate the antiradical activity of the peel and pulp from both organic and integrated Golden Delicious apples in relation to their polyphenolic content as determined by an improved high-performance liquid chromatography (HPLC) method. To test the scavenging activity of apple extracts, we used the widely known 1,1-diphenyl-2-

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Table 1. Agricultural Conditions and Localization of the Sampled Orchards

agricultural method	location	altitude (m on sea)	soil type	crop age (years)	irrigation
organic	Pergine Valsugana (Trent)	485	clay, loam	7	yes (creek)
integrated	Pergine Valsugana (Trent)	480	clay, loam	8	yes (creek)

Table 2. Agricultural Practices and Chemical Inputs for Organic and Integrated Orchards

agricultural method	pest management		fertilization	
	molecule	treatment nos.	formulation (description)	rate
organic	copper oxichloride	2	cow manure	62.5 tons/Ha
	copper hydroxide	2	Kieserite (K)	0.25 tons/Ha
	calcium polisulfide	9		
	mineral oil	4		
	neem oil	2		
	pyretrum	1		
	potassium soap	2		
integrated	tirham	1	N-P-K-Mg (12 + 12 + 17 + 2)	0.375 tons/Ha
	esaconazole	8	Fruvax (lees fertilizer)	2.64 kg/Ha
	fusilazole	7		
	mancozeb	2		
	kresomix-methyl	3		
	dodine	2		
	pirimicarb	1		
	chlorpyrifos-methyl	1		
	imidaclopyr	1		
	acephate	1		

picrylhydrazyl (DPPH) method and expressed the results as 6-hydroxy-2,5,7,8,tetramethylchroman-2-carboxylic acid (Trolox) equivalents.

MATERIALS AND METHODS

Reagents and Solvents. The solvents were from Merck and of analytical or HPLC grade. DPPH, flavanol, hydroxycinnamic acids, and dihydrochalcones were from Sigma (St. Louis, MO), Trolox was from Aldrich (Germany) while procyanidin B2, hyperin (quercetin-3-galactoside), rutin (quercetin-3-rutinoside), quercitrin (quercetin-3-rhamnoside), avicularin (quercetin-3-arabinofuranoside), and isoquercitrin (quercetin-3-glucoside) were purchased from Extrasynthese (Lyon, France). In all cases, water was of HPLC quality and purified in a Simplicity system (Millipore, Bedford, MA).

Samples. Both organic and integrated Golden Delicious apples were used. A description of the orchards' agricultural conditions and the chemical inputs, the latter according to Reg. CEE 2092/91 (*12*) and Reg. CEE 2078/92 (*13*) for the organic and integrated production methods, respectively, is presented in **Tables 1** and **2**. After harvest, the apples were stored for 15 days at 4 °C to provide a comparable degree of maturation as assessed by the starch iodine test (*14*). For each agricultural protocol (organic and integrated), eight trees were sampled and six apples per tree were used to obtain a representative starting material. The peels were separated from the pulps using a manual device for a reproducible peel thickness of 0.5 cm. For each apple, separate portions of pulp slices and peels of about 2.5 g were exactly weighted, freeze-dried, and stored at −18 °C until extraction.

Extraction Procedure. The freeze-dried samples (each representing six apples from the same tree) were extracted at room temperature using an ultrasonic bath. The samples were extracted three times with aqueous 95% methanol as follows: 20 mL of solvent for 1 h, 10 mL for 1 h, and 10 mL for 30 min. The three extracts were combined, brought to a final volume of 50 mL with aqueous 95% methanol, and stored at −18 °C until analyzed. All of the samples were filtered through a PTFE membrane filter (0.22 μm) before HPLC runs.

HPLC−Diode Array Detection. HPLC phenolics separation was carried out according to a method previously devised by our group (*15*) and was conducted using a Jasco apparatus (Tokyo, Japan) equipped with a binary pump (PU 1580), a 20 μL loop, a Rheodyne valve (Cotati, United States), a photodiode array detector (PU MD 910), and a column oven. The column was a Chromolith Performance RP-18e (100 mm long × 4.6 mm i.d.) (Merck, Darmstadt, Germany). All runs were acquired and processed using Borwin 5.0 software (JMBS Developments, Grenoble, France). Detection was performed at 280 nm for flavanols and dihydrochalcones, at 320 nm for hydroxycinnamic acids, and at 350 nm for flavonol glycosides. The spectra were acquired from 200 to 400 nm. The elution solvents were 0.5% methanol in aqueous 0.01 M phosphoric acid (solvent A) and 100% acetonitrile (solvent B). The linear gradient elution was as follows: from 96.5% A to 90.0% A in 11 min, then 80% A at 22.5 min, followed by reequilibration at the initial conditions (5 min). The column was thermostated at 25 °C with a constant flow rate of 2.5 mL/min.

Compounds Identification and Quantification. Identification of the compounds was carried out by comparing their retention times and spectra with those of standards when available. The identified peaks were then confirmed by spiking samples with standard mixtures. Unknown chromatographic peaks were tentatively identified via their spectral features and by literature data.

Quantification was performed by the external standard method. For compounds lacking standards, quantification was carried out using similar compounds as standards. Thus, phloretin-2-xyloglucoside was quantified as phloridzin, *p*-coumaroylquinic acid was quantified as coumaric acid, and reynoutrin (quercetin-3-xyloside) was quantified as hyperin. For quercitrin and isoquercitrin, the standard amounts at our disposal were too small to obtain suitable calibration curves thus these glycosides were quantified as hyperin. Procyanidins, which in our extracts were found to be represented by dimers and trimers (*15*), were quantified as procyanidins B2.

DPPH Radical Scavenging Activity. The antioxidant activities of fruits and standard compounds were evaluated according to Brand-Williams et al. (*16*) with some modifications. All extracts were diluted 1:5 with aqueous methanol (5:95). An aliquot of 100 μL of diluted samples or standard compounds was added to 2.9 mL of DPPH solution (0.1 mM in methanol). Absorbances at 517 were read at 0 time and at 10 min intervals until the reaction reached a plateau. The DPPH methanolic solution was used as a blank to correct absorbances at the end of the reaction. The decreased absorbances of DPPH remaining at the steady state were calculated and expressed as Trolox equivalents [mM kg^{-1} fresh weight (fw)] using standard curves.

Statistics. Statistical data elaboration was carried out using the software STATISTICA version 6, (StatSoft Italia srl, Vigonza, Padova). *p* values less than 0.05 were considered significant.

RESULTS

Phenolic Composition of Apple Extracts. The mean phenolic composition of apple peels and pulps in both organic and integrated samples is presented in **Table 3**. The peels were richer than pulps in almost all of the phenolic classes quantified. In the peels, procyanidins and quercetin glycosides proved to be the main phenolics, followed by flavanols, dihydrochalcones, and cinnamic acids. Epicatechin and its 4-8 dimer, procyanidin B2, were the principal single compounds, accounting for 28.1 and 29.4% of total phenolics in integrated and organic peels, respectively. Quercetin arabinoside was the most abundant flavonol glycoside, followed by quercetin galactoside and quercetin rhamnoside. Rutin was found in very low amounts (<5 mg kg^{-1} fw). The peels from organic fruit had a lower total polyphenolic content than those from integrated fruit ($p = 0.0014$). Statistically significant differences were found for procyanidins ($p = 0.0009$), flavonols ($p = 0.015$), and hydroxycinnamates ($p = 0.013$), while phloridzin was the only compound for which the concentration had a tendency to be higher in organic peels. Inferences about the causes of such differences are beyond the scope of this work and will be the subject of a separate communication, when data from further harvests, together with information on climatic conditions and

Table 3. Average (± SD) Phenolic Composition (mg/Kg^{-1} f.w.) of Golden Delicious Peels and Pulps

	peels		pulps	
	organic ($n = 8$)	integrated ($n = 8$)	organic ($n = 8$)	integrated ($n = 8$)
catechin	0.62 ± 0.59	0.60 ± 0.53	0.67 ± 0.19	0.68 ± 0.28
epicatechin	222 ± 26.3	233 ± 31.8	45.4 ± 9.32	70.4 ± 12.2
total flavanols	**223 ± 26.4**	**234 ± 32.2**	**46.1 ± 9.22**	**71.1 ± 12.2**
procyanidin B2	132 ± 17.8	153 ± 19.0	33.7 ± 5.85	58.6 ± 8.83
other procyanidins[a]	304 ± 37.2	344 ± 22.7	56.1 ± 3.92	88.7 ± 5.49
total procyanidins	**436 ± 56.2**	**497 ± 28.9**	**89.8 ± 2.61**	**147.3 ± 19.0**
quercetin 3-galactoside	69.1 ± 19.8	94.6 ± 35.9	1.65 ± 1.30	0.89 ± 0.35
quercetin 3-rutinoside	3.17 ± 1.47	4.80 ± 2.88	ND[b]	ND
quercetin 3-glucoside[c]	24.4 ± 6.75	31.2 ± 13.3	1.07 ± 0.67	1.36 ± 0.75
quercetin 3-xyloside[c]	36.31 ± 5.00	46.1 ± 11.7	2.01 ± 0.58	2.01 ± 0.19
quercetin 3-arabinoside	84.4 ± 8.77	105 ± 23.9	3.30 ± 1.67	2.92 ± 0.36
quercetin 3-rhamnoside[c]	64.10 ± 8.35	94.3 ± 18.4	5.70 ± 3.87	9.05 ± 0.76
total flavonols	**282 ± 46.6**	**376 ± 105**	**13.7 ± 7.74**	**16.2 ± 3.05**
phloretin 2′-xyloglucose[d]	40.8 ± 13.1	45.3 ± 6.32	8.59 ± 1.62	9.45 ± 1.18
phloridzin	125 ± 30.1	103 ± 32.4	11.3 ± 1.54	13.3 ± 1.38
total dihydrochalcones	**166 ± 31.7**	**149 ± 37.8**	**19.9 ± 2.61**	**22.8 ± 2.47**
chlorogenic acid	92.6 ± 6.18	111 ± 13.8	67.2 ± 8.64	114 ± 15.9
p-coumaroylquinic acid[e]	4.44 ± 0.45	7.19 ± 0.75	4.75 ± 0.73	7.63 ± 1.74
total hydroxycinnamic acids	**97 ± 6.1**	**118 ± 14.5**	**72.0 ± 9.61**	**122 ± 17.3**
total polyphenolics (HPLC)	**1204 ± 76.2**	**1374 ± 162**	**241 ± 30.2**	**379 ± 45.0**

[a] Tentatively identified. Quantified as procyanidin B2. [b] Not detectable. [c] Tentatively identified. Quantified as quercetin 3-galactoside. [d] Tentatively identified. Quantified as phloridzin. [e] Tentatively identified. Quantified as chlorogenic acid.

nutritional and pest control management, will be obtained. The higher polyphenolic content in sustainable vs organically produced crops was reported by Asami et al. (*17*) who suggested that this was attributable to the balance between the adequate nutrition of sustainable crops and the pathogenic pressure that led to the synthesis of polyphenolics. Moreover, the pathogenic pressure (apple scab particularly) and moderate nitrogen fertilization have been reported to directly influence the metabolism of phloridzin in apples (*18*, *19*), offering a possible explanation for the higher content of this dihydrochalcone in the pesticide-free organic apples. Overall and irrespective of the orchards' agricultural practices, the values that we found largely agree with previous findings for Golden Delicious peels (*20*, *21*).

The polyphenolic composition of the flesh was characterized by the principal contribution of procyanidins (which represented about 37% of total phenolics) and hydroxycinnamics acids, the latter being composed almost exclusively of chlorogenic acid. Hydroxycinnamates in pulps, moreover, were statistically not different than in peels. Five quercetin glycosides were also found (in very low amounts). Tsao et al. (*20*), studying eight different apple cultivars, reported similar amounts of quercetin rhamnoside in Red and Golden Delicious flesh (3.7 and 6.4 mg kg^{-1} fw, respectively). As determined for peels, the integrated pulps were richer than organic pulps in phenolics, the main differences being for procyanidins ($p = 0.002$) and hydroxycinnamates ($p = 0.0001$).

Radical Scavenging Activities. The measurement of the scavenging ability of antioxidants toward radical DPPH$^{\bullet}$ is considered a valid and easy assay to evaluate and to compare different antioxidants (*22*). In the presence of a hydrogen donor, the free radical DPPH$^{\bullet}$ is reduced to the corresponding hydrazine, depletion of the radical being photometrically evaluated by the decrease in absorbance at 515−528 nm. To obtain comparable sets of data, scavenging activities can be correlated, in a dose−response curve, with standard antioxidants such as ascorbic acid and trolox (a water soluble analogue of vitamin E), allowing the calculation of AEAC (ascorbic acid equivalent antioxidant capacity) and TEAC (Trolox equivalent antioxidant capacity), respectively (*23*).

Table 4. Trolox Equivalent Antioxidant Activity (TEAC) of Standard Compounds

compound	TEAC[a]	*n*	SD
epicatechin	4.88	4	0.07
procyanidin B2	5.50	4	0.06
quercetin galactoside	6.59	4	0.06
quercetin arabinoside	5.99	4	0.08
chlorogenic acid	2.75	4	0.03
phloridzin	1.10	4	0.02

[a] TEAC, millimolar concentration of a Trolox solution having the antioxidant capacity equivalent to 1.0 mM solution of the assayed substance.

In our experiments, the total antioxidant capacity (TAC) of the pulp and peel extracts was expressed as Trolox equivalents (mM kg^{-1} fw), which appeared a more descriptive expression as compared with methodologies that calculate the percent decrease in absorbance or in DPPH moles. Moreover, this approach enables direct comparison with TAC values derived from other assays using the same antioxidant as a reference.

To evaluate the contribution of the individual phenolics to the total antioxidant activity of apple extracts, the antioxidant capacity of 1 mM solutions of each standard compound was calculated and expressed as TEAC (**Table 4**). Among the compounds tested, quercetin glycosides showed the highest TEAC, followed by procyanidin B2, epicatechin, chlorogenic acid, and phloridzin. The DPPH scavenging rank was consistent with the results reported by Lu and Yeap Foo (*32*) (obtained using the DPPH assay), who, however, expressed the antiradical activity as EC_{50}.

For peel and pulp extracts, the TAC values that we found are reported in **Table 5**. Peels furnished TAC values about 2.5 times higher than their respective pulps. Integrated peels had a TAC significantly higher than organic ones ($p = 0.018$) whereas for pulps no statistical differences were found between organic and integrated extracts. The differences in TACs between

Table 5. Contribution of the Quantified Antioxidant Compounds to the TAC of Apple Extracts

	peels				pulps			
	organic		integrated		organic		integrated	
compound	mM/Kg^{-1}	TAC[a]	mM/kg	TAC	mM/Kg^{-1}	TAC	mM/kg	TAC
catechin[b]	0.002	0.01	0.002	0.01	0.002	0.01	0.002	0.01
epicatechin	0.793	3.88	0.832	4.06	0.162	0.79	0.251	1.22
total flavanols	**0.795**	**3.89**	**0.835**	**4.07**	**0.164**	**0.80**	**0.253**	**1.23**
procyanidin B2	0.237	1.29	0.274	1.51	0.060	0.33	0.105	0.58
other procyanidins[c]	0.545	3.00	0.616	3.39	0.100	0.55	0.159	0.87
total procyanidins	**0.782**	**4.29**	**0.890**	**4.90**	**0.160**	**0.88**	**0.264**	**1.45**
quercetin 3-galactoside	0.149	0.98	0.204	1.34	0.004	0.03	0.002	0.01
quercetin 3-rutinoside[d]	0.005	0.03	0.007	0.04				
quercetin 3-glucoside[d]	0.053	0.35	0.067	0.44	0.002	0.01	0.003	0.02
quercetin 3-xyloside[e]	0.084	0.50	0.106	0.63	0.005	0.03	0.005	0.03
quercetin 3-arabinoside[e]	0.194	1.16	0.241	1.44	0.008	0.05	0.007	0.04
quercetin 3-rhamnoside[e]	0.143	0.86	0.210	1.26	0.013	0.08	0.020	0.12
total flavonols	**0.628**	**3.88**	**0.835**	**5.15**	**0.032**	**0.20**	**0.069**	**0.42**
phloretin 2′-xyloglucose[f]	0.077	0.08	0.085	0.09	0.016	0.02	0.018	0.02
phloridzin	0.291	0.32	0.239	0.26	0.026	0.03	0.031	0.03
total dihydrochalcones	**0.368**	**0.40**	**0.324**	**0.35**	**0.042**	**0.05**	**0.091**	**0.05**
chlorogenic acid	0.261	0.72	0.314	0.86	0.258	0.71	0.322	0.89
p-coumaroylquinic acid[g]	0.013	0.04	0.021	0.06	0.014	0.04	0.022	0.06
total hydroxycinnamic acids	**0.274**	**0.76**	**0.609**	**0.92**	**0.272**	**0.75**	**0.344**	**0.95**
calculated TAC		13.22		15.39		2.68		4.10
measured TAC		14.96 (±2.36)		18.56 (±2.92)		6.28 (±2.72)		7.12 (±1.60)
unaccounted TAC		1.74 (11.6%)		3.17 (17.1%)		3.60 (57.3%)		3.02 (42.4%)

[a] Relative TAC based on TEAC × concentration (mM Kg^{-1}) of each quantified substance. [b] Assuming the TEAC of epicatechin. [c] Assuming the TEAC of procyanidin B2. [d] Assuming the TEAC of quercetin 3-galactoside. [e] Assuming the TEAC of quercetin 3-arabinoside. [f] Assuming the TEAC of phloridzin. [g] Assuming the TEAC of chlorogenic acid.

samples could be preliminarily attributed to their different contents of polyphenols (**Table 3**).

We are not aware of any published data on antioxidant capacity of organic vs integrated apples. Hence, the present work appears to be the first comparative study published on this subject.

The TAC values that we found in pulps are the same order of magnitude as the values reported by Leong and Shui (*24*) for peeled apple extracts (AEAC, 789 mg kg^{-1} fw, which is equivalent to 4.48 mM kg^{-1} fw). In their work, the ABTS$^{\bullet+}$ method was used, which they found to be comparable with the DPPH$^{\bullet}$ method for determination of TAC of apples and 26 other fruits from the Singapore market. Two moles of both ABTS and DPPH radicals were found to be scavenged by 1 mole of trolox or ascorbic acid; the two assays hence showed the same stoichiometry toward these latter standard antioxidants (*25*−*27*). Some differences between the two methods have been also reported due to sample interferences at 517 nm or differences in the kinetics of individual phenolics (i.e., pyrogallol) (*28*).

Kim et al. (*29*), using the DPPH assay, found AEAC values of 1360 mg kg^{-1} fw (i.e., 7.72 mM kg^{-1}) for whole Gala apple extracts, with an understimation of about 36% as compared with ABTS values. To the best of our knowledge, for apple peels, no TACs measured by the DPPH method and expressed as Trolox equivalents have been reported in the literature.

Wolfe et al. (*11*) studied the antioxidant activity of apple peels using a modified total oxyradical scavenging assay in which a peroxyradical oxidizes α-cheto-γ-methiolbutyric acid to form ethylene. With this test, the authors found a value of 0.1114 mM vitamin C equivalents g^{-1} of peel from Golden Delicious apples.

The use of DPPH was reported by Kondo et al. (*30*) for the examination of two Japanese apple cultivars (Fuji and Oorin) and one cider cultivar (Redfield) during fruit development. However, the results were expressed as IC_{50} (e.g., the sample concentration that produces a 50% decrease in the DPPH mixture absorbance, expressed as mg fw mL^{-1} of DPPH solution), and consequently, direct comparison with our data is difficult. Likewise, using a lipid peroxidation test, Van der Sluis et al. (*31*) determined the antioxidant activities of apple pomace and expressed their results as the dilution factor that gives 50% inhibition of lipid peroxidation (mL mg^{-1} fw).

In peels, quercetin glycosides, procyanidins, and flavanols, due to their high radical scavenging activities and high concentrations, proved to be the major contributors to the calculated antioxidant activity (**Table 5**). A very low contribution was calculated for hydroxycinnamates and dihydrochalcones. Lee et al. (*33*) already reported such an order of contribution in whole extracts from six apple cultivars.

In pulps, flavanols (monomers and polymers) accounted for about 65% of the calculated activity; procyanidins were the top contributors. As in peels, hydroxycinnamates and dihydrochalcones contributed to the antioxidant activity of apple pulp to a lesser extent. Even if present in very low amounts, quercetin glycosides in pulps accounted for up to 10% of TAC (**Table 5**).

In both organic and integrated peels, 11.6 and 17.1% of the measured TAC, respectively, was unaccounted for while in pulps, unexplained TAC was 57.3 and 42.4%. The actual unaccounted for activity ranged from 1.74 (for organic peels) to 3.60 mM kg^{-1} fw (for organic pulps).

Previous similar studies reported even higher percentages of unexplained antiradical activity. In whole apple juices, Miller et al. (*23*) found a 48.6% unaccounted for total antioxidant activity. Similarly, Van der Sluis et al. (*31*) reported that the calculated activity accounted for only 35, 38, and 45% of the measured activity in apple pulps, pomace, and raw juice, respectively. To explain these extra activities, these authors, who, unlike us, did not quantify procyanidins, postulated the

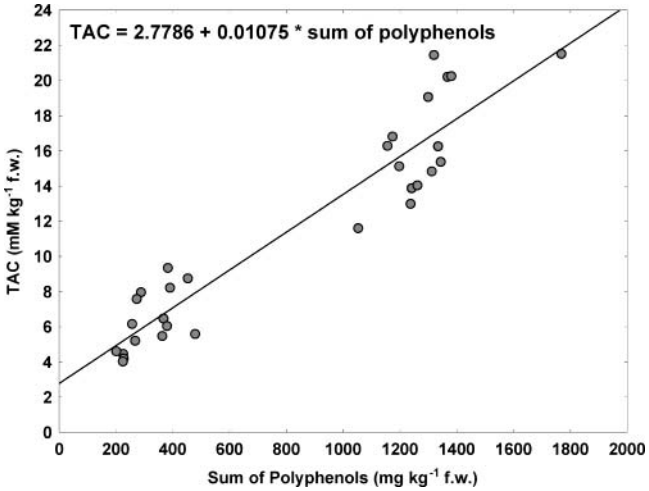

Figure 1. Correlation between the sum of polyphenols and the TAC values as obtained considering the entire population of samples ($n = 32$).

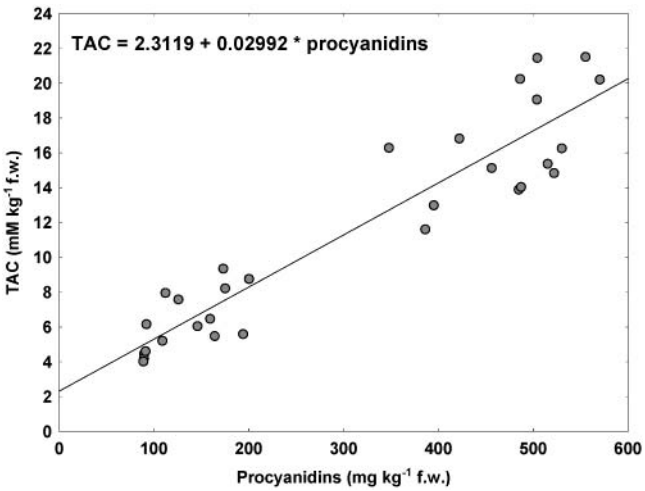

Figure 2. Correlation between procyanidins and TAC values as obtained considering the entire population of samples ($n = 32$).

existence of synergistic effects between phenolics, together with the contribution of unmeasured compounds, procyanidins especially.

In the present work, apple procyanidins were quantified together with their contribution to the total antioxidant activity of apple tissue extracts. Our data, hence, furnish a confirmation of that latter supposition and, for peels, raise the explained measured activity up to 85%.

In pulps, when compared with peels, larger percentages of activity, besides synergistic interactions, seem to be attributable to substances other than the quantified phenolics. Ascorbic acid, for instance, has been reported to give contributions ranging from 1 to 10% (*23*, *24*, *33*). Carotenoids, on the other hand, are thought to contribute in very small proportions (*34*).

Our group is currently evaluating a further hypothesis based on the possible interaction between oxidized phenolics and ascorbic acid to regenerate the active radical scavenging hydroxyl group. A regression analysis, carried out by grouping each identified compound into their respective phenolic class, confirmed the correlation between the TAC and the phenolic content of apples extracts. This relationship has been reported previously (*31*, *35*), but another study found only a weak correlation between phenolic composition of fruits and their antioxidant capacity (*36*). Taking into account the entire population of samples ($n = 32$), irrespective of the matrix (pulp or peel) or the agricultural practices (organic or integrated), the strongest correlation with TAC was found for the sum of polyphenols and for procyanidins ($r = 0.944$ and $r = 0.936$, respectively) (**Figures 1** and **2**).

In **Figure 1**, the TAC value at the *Y*-intercept, e.g., the term "*a*" in the ($a + bx$) equation, represents the mean antioxidant activity that cannot be explained by the presence of phenolics in apple extracts. This value (2.78 mM kg^{-1} fw) is nearly the same of the average unexplained activity that we found in pulps and peels (2.88 mM kg^{-1} fw) and could offer a confirmation of our experimental findings.

Quercetin glycosides and flavanols had weaker but still statistically significant correlations, notwithstanding that the former ones were almost absent in pulps (**Table 3**). In **Table 6**, the correlations between TAC and each phenolic class identified in peels and pulps are presented separately. In peels, among the classes, quercetin glycosides gave the highest correlation together with procyanidins. Lower correlations were found for hydroxycinnamates, flavanols, and dihydrochalcones. In pulps, the only significant correlation ($p < 0.05$) was found for procyanidins, confirming, for these compounds, the results obtained from peels.

Table 6. Correlation Coefficient (*r*) and Relative Significance (*p*) between TAC and the Identified Classes of Phenolics in Apple Peels and Pulps

	peels ($n = 16$)		pulps ($n = 16$)	
	r	*p*	*r*	*p*
total flavanols	0.329	0.230	0.412	0.127
total procyanidins	0.553	0.032	0.635	0.011
total flavonols	0.583	0.023	0.421	0.120
total dihydrochalcones	0.048	0.864	0.489	0.064
total hydroxycinnamics	0.498	0.063	0.389	0.152
total polyphenolics (HPLC)	0.678	0.005	0.578	0.024

In conclusion, our results show that the scavenging activity of apple extracts depends on their phenolic composition in a qualitative and quantitative way. Peels, because of their high content in phytochemicals (especially quercetin glycosides and procyanidins), possess a TAC more than 2-fold higher than pulps and could represent a valuable source of healthy, beneficial compounds.

Overall, integrated apples were found to be richer in phenols and to have more antioxidant activity than organic apples. On the other hand, unpeeled pesticide-free organic apples could provide a higher intake of phytochemicals as compared with peeled integrated apples. Moreover, further benefits from organic agriculture, such as low environmental impact and reduced cancer risks for farm operators, should be taken into account.

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