

Major Phytochemicals in Apple Cultivars: Contribution to Peroxyl Radical Trapping Efficiency

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Forty-one samples of apples (peel plus pulp), obtained from eight cultivars, were examined for concentration of some important phytochemicals and for antioxidant activity expressed as peroxyl radical trapping efficiency. Five major polyphenolic groups plus ascorbate were identified and quantified by HPLC in the apple varieties. Oligomeric and polymeric proanthocyanidins were found to be about two-thirds of total polyphenols. The antioxidant efficiency of the apple extracts and of representative pure compounds for each group of phytochemicals was measured in a micellar system mimicking lipid peroxidation in human plasma. Although the amount of polyphenols measured by HPLC is similar to that measured by standard methods, the antioxidant efficiency calculated on the basis of the contribution of the pure compounds was lower than the antioxidant efficiency of the apple extracts. The higher efficiency of apples appears to be strictly related to the overwhelming presence of oligomeric proanthocyanidins.

KEYWORDS: Apple; antioxidant; lipid peroxidation; proanthocyanidins; polyphenols

INTRODUCTION

Very reactive free radicals are major species that cause human diseases such as cancer and cardiovascular disease. Therefore, much attention has recently been paid to the possible health benefits of dietary phenolics, which are characterized by a high radical scavenging activity.

Polyphenolic compounds of apple may play an important role in this context because apples are a very significant part of the diet and epidemiological studies have shown an inverse correlation between the consumption of apple and various diseases. Apple intake has been negatively associated with lung cancer incidence (1, 2) and with cardiovascular disease, coronary, and total mortality (3), risk of thrombotic stroke (4), symptoms of chronic obstructive pulmonary disease (5), and proliferation activities (6, 7).

Five major polyphenolic groups have been found in apple: flavanols, hydroxycinnamates, dihydrochalcones, flavonols, and anthocyanins (8, 9). The presence in apples of ascorbic acid (10) also contributes to the antioxidant activity of polyphenols (11). Because polyphenols are effective antioxidants *in vitro*, the antioxidant activity of apple (6, 12), apple pomace (13), and apple peels (14) has been evaluated. In particular, the

contribution of major phenolics to the total antioxidant capacity of apples was determined, and the results obtained indicate that flavonoids such as quercetin, (–)-epicatechin, and proanthocyanidin B2 rather than vitamin C contribute significantly to the total antioxidant activity of apples (15).

Previous studies (16, 17) reported that the calculated antioxidant activity in apples or whole apple juices accounts for only 45–48% of the measured activity. However, in these studies proanthocyanidins were not quantified and therefore not taken into account. Also, in a recent study, based on the titration of H-donating compounds by 1,1-diphenyl-2-picrylhydrazyl (DPPH) (18), in which proanthocyanidins were partially taken into account, it was reported that the calculated total antioxidant capacity (TAC) represents only 42% of the measured TAC.

In this paper we report on the peroxyl radical trapping efficiency (PRTE) of eight apple cultivars and on the contribution of the various classes of phytochemicals to this efficiency in a micellar system mimicking lipid peroxidation in plasma. The higher efficiency of apple extracts with respect to that calculated on the basis of the contribution of the various groups of phytochemicals using pure compounds as reference appears to be related to the presence of oligomeric proanthocyanidins, which, on average, represent >70% of the total polyphenols (19).

MATERIALS AND METHODS

Reagents. The reagents were purchased from Fluka (Buchs, Switzerland) and were of the highest available quality. 2,2'-Azobis[2-

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Table 1. Antioxidant Composition of Some Apple Cultivars^a

	Renetta	Red Delicious	Granny Smith	Morgenduft	Golden Delicious	Royal Gala	Braeburn	Fuji
1 cropping areas (N)	6	7	3	4	9	7	2	3
2 TP _{HPLC} ^b	8.19 ± 0.94	4.28 ± 0.59	3.73 ± 0.32	4.04 ± 0.23	2.84 ± 0.31	2.75 ± 0.51	2.35 ± 0.20	2.34 ± 0.60
3 TP _{FC} ^c	7.31 ± 0.70	4.52 ± 0.48	4.17 ± 0.24	3.65 ± 0.28	2.98 ± 0.26	2.89 ± 0.42	2.60 ± 0.01	2.28 ± 0.45
4 TP _{HPLC} /TP _{FC}	1.17	0.98	0.94	1.16	0.99	0.98	0.94	1.03
5 catechin ^d	0.75 ± 0.10	0.44 ± 0.07	0.40 ± 0.15	0.40 ± 0.08	0.19 ± 0.04	0.23 ± 0.06	0.201 ± 0.001	0.24 ± 0.04
6 procyanidin B2 ^e	0.34 ± 0.06	0.14 ± 0.03	0.19 ± 0.05	0.19 ± 0.01	0.12 ± 0.02	0.10 ± 0.03	0.10 ± 0.01	0.12 ± 0.03
7 proanthocyanidin ^d oligomers	5.59 ± 0.74	3.15 ± 0.58	2.86 ± 0.19	2.67 ± 0.17	2.01 ± 0.25	1.91 ± 0.41	1.63 ± 0.14	1.33 ± 0.49
8 hydroxycinnamates ^f	1.09 ± 0.23	0.28 ± 0.03	0.13 ± 0.02	0.53 ± 0.10	0.30 ± 0.05	0.32 ± 0.04	0.18 ± 0.001	0.38 ± 0.13
9 dihydrochalcones ^g	0.35 ± 0.06	0.094 ± 0.018	0.046 ± 0.008	0.057 ± 0.012	0.063 ± 0.011	0.044 ± 0.006	0.047 ± 0.003	0.046 ± 0.010
10 flavonols ^h	0.073 ± 0.023	0.126 ± 0.065	0.106 ± 0.035	0.117 ± 0.035	0.154 ± 0.044	0.118 ± 0.053	0.179 ± 0.013	0.103 ± 0.043
11 anthocyanins ⁱ	0	0.052 ± 0.026	0	0.076 ± 0.057	0	0.021 ± 0.008	0.011 ± 0.005	0.008 ± 0.002
12 ascorbic acid	0.307 ± 0.153	0.04 ± 0.028	0.153 ± 0.017	0.318 ± 0.153	0.437 ± 0.160	0.023 ± 0.017	0.46 ± 0.017	0.119 ± 0.051

^a Composition data are expressed as mmol/kg of fresh weight fruit. ^b Total polyphenols from HPLC measurement as a sum of the value of rows 5–11. ^c Total polyphenols from Folin–Ciocalteu method. In this case (–)-epicatechin was used for calibration. ^d Sum of (+)-catechin and (–)-epicatechin, expressed as (–)-epicatechin. ^e Expressed as Procyanidin B2. ^f Expressed as chlorogenic acid. ^g Expressed as phloridzin. ^h Expressed as quercetin 3-glucoside. ⁱ Expressed as cyanidin 3-glucoside.

(2-imidazolyl)propane] (ABIP) was a kind gift of Wako Chemicals. Aqueous solutions were prepared with doubly distilled water and when necessary were passed through a column of Chelex-100 (Bio-Rad, Richmond, CA) to minimize the concentration of heavy metal ions. (–)-Epicatechin, (+)-catechin, phloridzin, and chlorogenic acid (5'-caffeoylquinic acid) were purchased from Carl Roth. Quercetin 3-glucoside was purchased from Indofine Chemical Co. (Hillsborough, NJ), whereas cyanidin 3-glucoside was from Polyphenols Laboratories (Sandes, Norway) and procyanidin B2 was from LCG Promochem (Sesto S. Giovanni, Italy).

Sampling and Extraction. Apples (≥3 kg for each lot) were sampled in Trentino, Italy, between August and November 2001. Samples of the cultivar Renetta were collected in six orchards covering the area included in the Protected Designation of Origin "Val di Non". All other varieties were sampled to cover the whole cropping area in Trentino: Valsugana, Arco, Bleggio Superiore, Sarche, Vigolana, and Trento South and North.

A total of 90 g of the edible part of the fresh fruit (including both pulp and skin) was radially cut from three apples (three pieces from each fruit, sampled from opposite parts) and homogenized in 375 mL of a mixture of acetone/water (70:30 w/w) for the extraction of total polyphenols, including polymeric proanthocyanidins. An aliquot of 100 g of the edible part of the frozen fruit, sampled with the same technique from three apples, was homogenized in 1 L of a solution of metaphosphoric acid (6%) in water containing sodium metabisulfite (1 g/L) for the extraction of ascorbic acid. The extracts were stored at –80 °C until activity measurements.

The HPLC analyses were done according to the method of Vrhovsek et al. (19), where a detailed description is reported.

Measurement of Peroxyl Radical Trapping Efficiency. The PRTE of apple extracts and of some representative antioxidants found in this fruit was measured according to the method of Wayner et al. (20) with the following modifications (21). This method reproduces in vitro the peroxidation of linoleic acid (LH), one of the major unsaturated fatty acids present in low-density lipoprotein (LDL). The peroxidation of LH, induced by peroxyl radicals generated by thermal decomposition of ABIP, was carried out in sodium dodecyl sulfate (SDS) micelles. The standard assay solution contains 2.5 mM linoleic acid, 8 mM ABIP, and 50 mM SDS in 50 mM phosphate at pH 7.4 and was equilibrated with atmospheric oxygen at 37 °C.

Typical kinetic runs, obtained by an oxygraphic apparatus, show that the O₂ consumption rate (α₀) in the system LH–SDS–ABIP is inhibited by the presence of antioxidants, both apple extracts and/or polyphenols, to a much lower rate (α₁).

The plot of the ratio α₁/α₀ versus the initial concentration *C* of antioxidant in the oxygraphic cell is fitted (*r* > 0.98) by an exponential function according to the equation

$$\frac{\alpha_1}{\alpha_0} = A + B \times \exp\left(-\frac{C}{C_{1/2}} \times \ln 2\right) \quad (1)$$

where *C*_{1/2} is the concentration that halves the rate of oxygen consumption due to peroxidation of LH and *A* and *B* are constants obtained from the fitting procedure. The constant *A* represents the value of the α₁/α₀ ratio when LH peroxidation is fully inhibited by antioxidants. Under these conditions the observed O₂ consumption is almost completely due to the formation of peroxyl radicals from primary ABIP radicals. The constant *B* is the value of (α₁/α₀ – *A*) extrapolated at [antioxidant] = 0, and usually it is *B* = 1 – *A*. According to eq 1 we defined as PRTE the value (C_{1/2})^{–1}.

In the presence of two or more antioxidants, as in the case of apple extracts, and when the antioxidant effects are additive, eq 1 can be rewritten as

$$\frac{\alpha_1}{\alpha_0} = A + B \times \exp\left(-\ln 2 \times \sum \frac{C_i}{C_{i/2}}\right) \quad (2)$$

where *C*_{*i*} and *C*_{*i*/2} refer to the *i* antioxidant and the ratio *C*_{*i*}/*C*_{*i*/2} indicates the contribution of the *i*th antioxidant to the scavenging of peroxyl radicals.

RESULTS AND DISCUSSION

Composition and Quantification of Major Phytochemicals of Eight Apple Cultivars. Forty-one samples of apples with skin, from eight apple cultivars, were examined. For each cultivar, from a minimum of 2 to a maximum of 8 samples for cropping area, characterized by different sun exposure, microclimate, soil, etc., were analyzed. The major classes of phytochemicals of eight apple cultivars were identified and quantified by HPLC (19), and the results obtained are presented on a molar basis in **Table 1** as the average of the calculated values for the various samples of apples examined for each cultivar.

Polyphenols were classified in five groups: flavanols, hydroxycinnamates, dihydrochalcones, flavonols, and anthocyanins. In the case of flavanols, which on a molar basis account for ~80% of total polyphenols, the three main components were considered, that is, the monomers [(–)-epicatechin and (+)-catechin], the procyanidin B2, and the proanthocyanidin oligomers with *n* ≥ 3. The presence of ascorbic acid was also taken into account. In general, the influence of the cropping conditions was usually small. In fact, only in a few cases was the standard deviation, which also takes into account the cropping conditions, >15% of the average value.

The total amount of polyphenols (TP) measured by HPLC and by the Folin–Ciocalteu (FC) method are reported in **Table 1**, rows 2 and 3, respectively. Moreover, the ratio between these quantities is reported in row 4, and its average value, taking into account the eight cultivars, was 1.08 ± 0.11. From **Table**

Table 2. Average $C_{1/2}$ Value and Relative Standard Deviation of Some Apple Cultivars and Contribution of the Major Phytochemicals to Total Antioxidant Efficiency of Various Apple Cultivars

cultivar	cropping areas (N)	$C_{1/2}$ (g/L)	PRTE $(C_{1/2})^{-1}$ (L/g)	$(C/C_{1/2})\%$								$\sum_i C/C_{1/2}\%$
				catechin ^a	procyanidin B2	proanthocyanidin oligomers ^a	hydroxycinnamates	dihydrochalcones	flavonols	anthocyanins	ascorbate	
Renetta	6	0.61 ± 0.06	1.64	3.9	5.1	28.2	6.9	0.50	2.7	0.0	4.1	51.4
Red Delicious	7	1.07 ± 0.13	0.93	3.9	3.7	27.9	3.1	0.23	8.1	2.2	0.9	50.0
Granny Smith	3	1.07 ± 0.10	0.93	3.5	5.0	25.3	1.4	0.12	6.8	0.0	3.6	45.7
Morgenduft	4	1.04 ± 0.08	0.96	3.4	4.8	23.0	5.8	0.14	7.2	3.2	7.2	54.7
Golden Delicious	9	1.34 ± 0.13	0.75	2.1	3.9	22.2	4.2	0.20	12.3	0.0	12.8	57.7
Royal Gala	7	1.44 ± 0.23	0.69	2.7	3.5	22.7	4.8	0.15	10.1	1.2	0.7	45.9
Braeburn	2	1.89 ± 0.26	0.53	3.3	4.6	25.5	3.6	0.21	20.1	0.8	19.0	77.1
Fuji	3	1.48 ± 0.20	0.68	2.9	4.3	16.3	5.8	0.16	9.1	0.5	3.9	43.0

^a Calculated as (-)-epicatechin.

1 it also appears that the total amount of polyphenols changes by a factor of 3 in the examined cultivars (the lowest content being in Fuji and Braeburn and the highest in Renetta). With regard to the distribution of polyphenols among the various classes, see **Table 1**, rows 5–11; flavanols, that is (-)-epicatechin and its polymers, are by far the dominant class, representing 77–93% of TP on a molar basis. The second largest class is represented by hydroxycinnamates, which range from ~3.5% (Granny Smith) to ~17% (Fuji), whereas flavonols, dihydrochalcones, glycosides, and anthocyanins, when present, are only a few percent of TP. It should be remarked that proanthocyanidins with $n \geq 3$ are on average ~80% of flavanols in all of the examined cultivars, and therefore polymeric proanthocyanidins are about two-thirds of TP, on average.

The amount of ascorbic acid, measured according to the method of Vrhovsek et al. (19), reported in **Table 1**, row 12, was <19% of the total amount of phytochemicals on a molar basis, in all eight cultivars.

Peroxyl Radical Scavenging Efficiency of Apples and of Their Most Representative Antioxidants. The calculated $C_{1/2}$ values for the eight cultivars, expressed as the amount of fresh apple (grams of fresh apple per liter of peroxidation system) which halves the rate of oxygen consumption due to peroxidation of LH, are reported in **Table 2**, column 3, together with the standard deviation, which contains also the influence of cropping area. The correspondent PRTE values are also reported in this table.

The $C_{1/2}$ values range from a minimum value of 0.61 g/L, found for Renetta (the cultivar with the highest efficiency in trapping ROO^{\bullet} radicals), to a maximum value of 1.89 g/L, found for Braeburn (the cultivar with the lowest efficiency in trapping ROO^{\bullet} radicals); that is, the $C_{1/2}$ values change by a factor of 3 as in the case of TP. The average values of the fitting constants A and B (eq 1) calculated for the eight cultivars were 0.26 and 0.74, respectively, the standard deviation being <6%. Examples of the fitting of α_1/α_0 experimental values, measured in the presence of various amounts of apple extracts, to eq 1, in the case of Golden and Red Delicious apples, are reported in panels **A** and **B**, respectively, **Figure 1**. The solid lines clearly indicate that the fitting of the experimental results to eq 1 is satisfactory.

The $C_{1/2}$ values of representative compounds of the major classes of antioxidants found in the examined apple cultivars were also measured and are reported in **Table 3**. The $C_{1/2}$ values of pure antioxidant range from a minimum value of 1.68 μM in the case of quercetin to a maximum of ~43 μM in the case of phloridzin. In **Figure 2**, panels **A** and **B**, respectively, the fitting of α_1/α_0 experimental values in the cases of (-)-epicatechin and quercetin, to eq 1 are shown. Also, in the case of pure antioxidants the A value was 0.27 ± 0.02 , whereas the

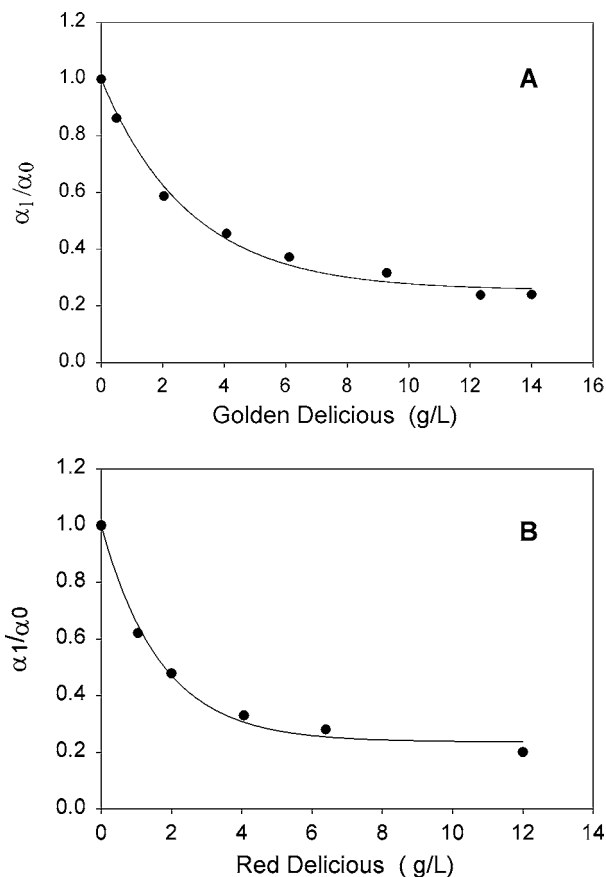


Figure 1. Inhibition of LH peroxidation by apple extracts: Golden Delicious (**A**); Red Delicious (**B**); solid line, fitting of experimental data to eq 1 ($r = 0.995$, **A**; $r = 0.996$, **B**).

Table 3. $C_{1/2}$ Value of Some Apple Antioxidants

compound	chemical class	$C_{1/2}$ (μM)	PRTE $(C_{1/2})^{-1}$ (μM^{-1})
(-)-epicatechin	flavanols	12.1 ± 1.4	0.08
procyanidin B2	flavanols	4.1 ± 1.2	0.24
chlorogenic acid	hydroxycinnamates	9.6 ± 1.4	0.10
phloridzin	dihydrochalcones	42.8 ± 8.3	0.02
quercetin	flavonols	1.68 ± 0.39	0.60
cyanidin 3-glucoside	anthocyanins	2.50 ± 0.41	0.40
ascorbate		4.58 ± 0.02	0.22

B values only in the cases of (-)-epicatechin and chlorogenic acid were slightly lower than $1 - A$.

To verify the effect of the apple extracts on the behavior of the antioxidants, (-)-epicatechin and chlorogenic acid, as

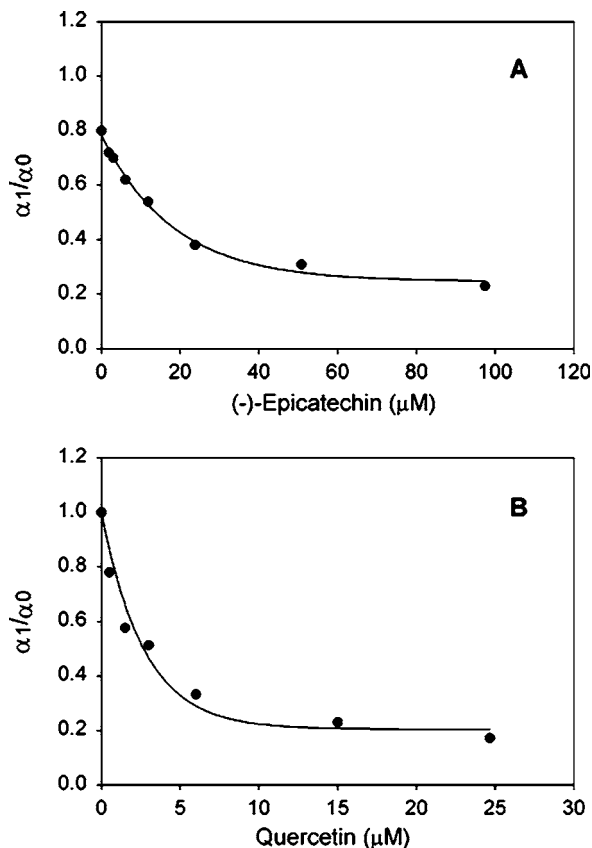


Figure 2. Inhibition of LH peroxidation by apple antioxidants: (–)-epicatechin (A); quercetin (B); solid line, fitting of experimental data to eq 1 ($r = 0.996$, A; $r = 0.980$, B).

representatives of the two major classes of antioxidants present in apples, were added to a fixed amount of apple extract. According to eq 2, the $C_{1/2}$ values obtained for these two antioxidants in the presence of apple extract were similar, within the experimental errors, to those measured in the absence of apple extract (see **Figure 3**). This behavior indicates additive contribution to PRTE of these two antioxidants when added into the oxygraphic cell together with apple extract. Similar results were also obtained in the case of quercetin and cyanidin.

In the case of flavanols, the class of polyphenols with the highest concentration, the PRTE of procyanidin B2 ($C_{1/2} = 4.1 \mu\text{M}$) was found to be ~ 3 times higher than the PRTE of (–)-epicatechin ($C_{1/2} = 12.1 \mu\text{M}$). This result is in strong contrast with the data reported by Chinnici et al. (18), which show that the amount of DPPH reduced by procyanidin B2 is similar to that reduced by (–)-epicatechin. This similar behavior is not in accord with the molecular structure of procyanidin B2, which consists of two (–)-epicatechin moieties bound through a C4–C8 interflavanic bond, or with the data reported by Butkovich et al. (22) and by Goupy et al. (23), using the DPPH method. In particular, the latter authors found that the total hydrogen atom donating capacity of procyanidin B2 is twice that of epicatechin.

Experimental versus Calculated Antioxidant Activity of Apples. On the basis of the concentration of the various classes of polyphenols in the apple cultivars (**Table 1**) and of their antioxidant activity as pure (+)-catechin, chlorogenic acid, phloridzin, quercetin, cyanidin, and ascorbate (**Table 3**), we calculated the contribution to the total antioxidant efficiency of each class of phytochemicals, that is, the values of $C_i/C_{i/2}$ %. The sum of these values, $\sum_i(C_i/C_{i/2})$ % (see **Table 2**, last column), indicated that the phytochemicals we have considered

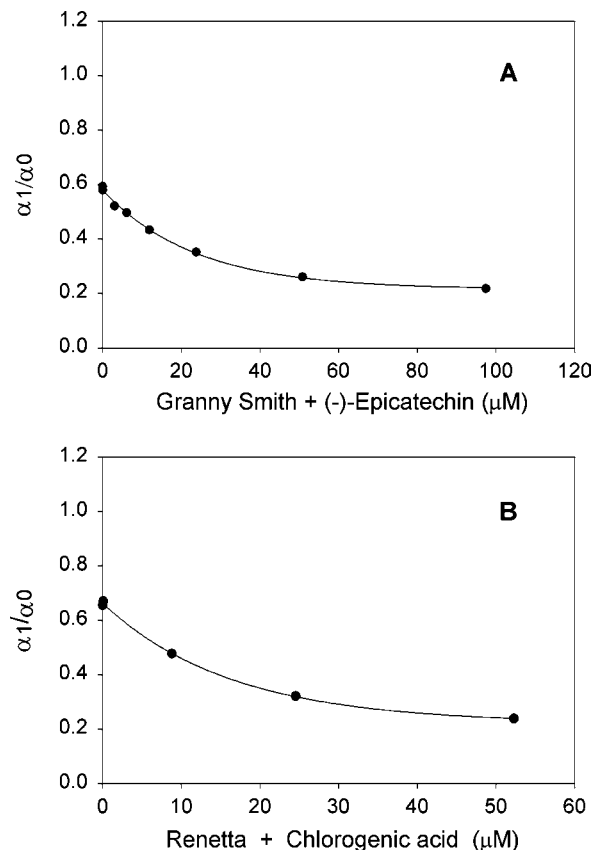


Figure 3. Inhibition of LH peroxidation by apple antioxidants in the presence of apple extract: to a fixed amount (1 g/L) of Granny Smith extract ($\alpha_1/\alpha_0 = 0.60$) were added progressive amounts of (–)-epicatechin (A); to a fixed amount (0.5 g/L) of Renetta extract ($\alpha_1/\alpha_0 = 0.65$) were added progressive amounts of chlorogenic acid (B); solid line, fitting to eq 1 of experimental data ($r = 0.998$, A; $r = 0.999$, B).

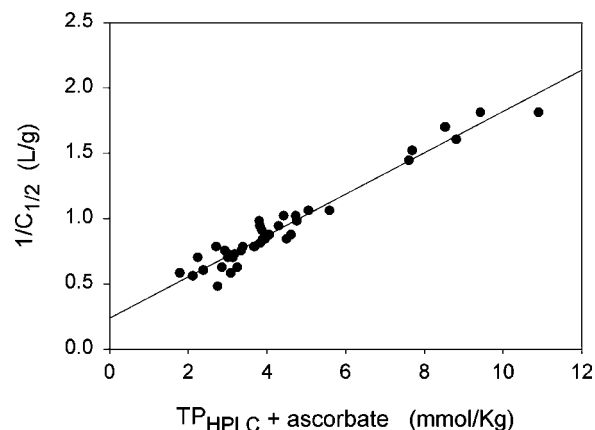


Figure 4. Dependence of apple PRTE on the phytochemical content (total polyphenols plus ascorbate) in the 41 samples of apple examined.

contributed from 47% (Granny Smith) to 78% (Braeburn), with an average value of 54% to the total antioxidant efficiency experimentally measured in the apple extracts. From **Table 2** it also appears that the major contribution to $\sum_i(C_i/C_{i/2})$ values is given by flavanols, whereas the contribution of ascorbate alone was on average 10.8%. This last datum is in good agreement with the relative contribution of ascorbate to the total antioxidant activity of apples reported by Lee et al. (15).

From **Figure 4**, where the efficiency of each sample of apple, expressed as $(C_{1/2})^{-1}$, was plotted versus the amount of TP_{HPLC} plus ascorbic acid, expressed as millimoles per kilogram of fresh

weight (FW), there appears to be a good linear relationship ($r > 0.97$) between these two quantities. However, the Y -axis intercept higher than zero may indicate the presence of an additional antioxidant efficiency. By prolonging the fitting line on the negative part of the X -axis, it was possible to quantify the average unexplained additional antioxidant efficiency, which corresponds to ~ 1.6 mmol of antioxidant/kg of FW.

The results obtained clearly show the superior antioxidant activity of apple extracts with respect to the sum of the antioxidant activity of pure compounds. This higher activity of apples can be due to (i) an underestimation of the amount of antioxidants found by HPLC, (ii) a synergistic effect among the various antioxidants present in apples as observed for the catechin–malvidin couple (24), and (iii) an underestimation of the $C_{1/2}$ values assigned to the various classes of antioxidants found in apples (see **Table 3**).

We can exclude point i because of the good agreement between TP values measured by HPLC and by Folin–Ciocalteu method (see **Table 1**) and point ii because the measurements we have carried out with pure compounds, or by addition of these compounds to apple extracts, indicate an additive effect and not a synergistic effect of these compounds on the antioxidant efficiency of apples (see **Figure 3**).

With regard to point iii it should be remarked that, to calculate the contribution $C_i/C_{1/2}$ % of the various classes of phytochemicals, the $C_{1/2}$ value of (–)-epicatechin was also assigned to oligomeric proanthocyanidins with $n \geq 3$ [the molar concentration of which was calculated on the basis of the (–)-epicatechin unit], representing on average two-thirds of TP. However, there is experimental evidence clearly indicating that the oligomeric proanthocyanidins are characterized by a stronger antioxidant activity than their monomeric repeating units. First of all, we found that the PRTE of procyanidin B2, when considered in terms of (–)-epicatechin units, appears to be $\sim 50\%$ higher than that of the constituent monomer. Second, the proanthocyanidin fractions isolated from chocolate show a relative monomer efficiency higher than that of the constituent monomer (–)-epicatechin (25, 26). According to these findings, the strong discrepancy we have found between the calculated and experimental values of antioxidant efficiency of apples (the efficiency is $\sim 53\%$ on average for the eight varieties of apples we have studied, see **Table 2**) can be reasonably attributed to the higher efficiency of polymeric proanthocyanidins in scavenging peroxy radicals. In fact, if we assign to oligomeric proanthocyanidins the PRTE of procyanidin B2 on a monomer basis, the calculated PRTE represents $65 \pm 11\%$ of the measured PRTE. The activity can further increase to unitary value if the monomeric efficiency of the oligomers increases with the degree of polymerization, as demonstrated in the case of proanthocyanidins from cocoa seeds (26). This conclusion is in contrast with those of Chinnici et al. (18), who, to explain the unaccounted for capacity in reducing DPPH in apple pulp ($\sim 50\%$ in average), attributed the unaccounted for activity to synergistic interactions of phenolics with no phenolics compounds such as ascorbic acid.

In conclusion, the analysis of the data we have obtained strongly supports the superior activity of polymeric proanthocyanidins of apples to inhibit lipid peroxidation. In consideration of the overwhelming amount of proanthocyanidins in apples and of the supposed impact of this fruit on human health, the results we have obtained call for investigation of the putative mechanisms of protection that are mediated by apple proanthocyanidins. In this regard it has been shown that polymeric proanthocyanidins afford an effective antioxidant protection directly in the digestive tract (27) and are hydrolyzed to a

mixture of (–)-epicatechin monomer and dimer, in an acidic environment, as found in the gastric milieu, thus enhancing the potential for their absorption in the small intestine (28).

ABBREVIATIONS USED

PRTE, peroxy radical trapping efficiency; LH, linoleic acid; ABIP, 2,2'-azobis[2-(2-imidazolin-2-yl)propane]; TP, total polyphenols; FC, Folin–Ciocalteu.

LITERATURE CITED

- (1) Knekt, P.; Jarvinen, R.; Seppanen, R.; Heliövaara, M.; Teppo, L.; Pukkala, E.; Aromaa, A. Dietary flavonoids and risk of lung cancer and other malignant neoplasms. *Am. J. Epidemiol.* **1997**, *146*, 223–230.
- (2) Le Marchand, L.; Murphy, S. P.; Hankin, J. H.; Wilkens, L. R.; Kolonel, L. N. Intake of flavonoids and lung cancer. *J. Natl. Cancer Inst.* **2000**, *92*, 154–160.
- (3) Knekt, P.; Jarvinen, R.; Reunanen, A.; Maatela, J. Flavonoid intake and coronary mortality in Finland: a cohort study. *Br. Med. J.* **1996**, *312*, 478–481.
- (4) Knekt, P.; Isotupa, S.; Rissanen, H.; Heliövaara, M.; Jarvinen, R.; Hakkinen, S. H.; Aromaa, A.; Reunanen, A. Quercetin intake and the incidence of cerebrovascular disease. *Eur. J. Clin. Nutr.* **2000**, *54*, 415–417.
- (5) Tabak, C.; Arts, I. C. W.; Smit, H. A.; Heederik, D.; Kromhout, D. Chronic obstructive pulmonary disease and intake of catechins, flavanols, and flavones: The MORGEN Study. *Am. J. Respir. Crit. Care Med.* **2001**, *164*, 61–64.
- (6) Eberhardt, M. V.; Lee, C. Y.; Liu, R. H. Antioxidant activity of fresh apples. *Nature* **2000**, *405*, 903–904.
- (7) Sun, J.; Chu, Y. F.; Wu, X. Z.; Liu, R. H. Antioxidant and antiproliferative activities of common fruits. *J. Agric. Food Chem.* **2002**, *50*, 7449–7454.
- (8) Chinnici, F.; Gaiani, A.; Natali, N.; Riponi, C.; Galassi, S. Improved HPLC determination of phenolic compounds in Cv. Golden Delicious apples using a monolithic column. *J. Agric. Food Chem.* **2004**, *52*, 3–7.
- (9) 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.
- (10) USDA Nutrient Database for Standard Reference, 1998.
- (11) Saucier, C. T.; Waterhouse, A. L. Synergetic activity of catechin and other antioxidants. *J. Agric. Food Chem.* **1999**, *47*, 4491–4494.
- (12) Imeh, U.; Khokhar, S. Distribution of conjugated and free phenols in fruits: Antioxidant activity and cultivar variations. *J. Agric. Food Chem.* **2002**, *50*, 6301–6306.
- (13) Lu, Y.; Foo, L. Y. Antioxidant and radical scavenging activities of polyphenols from apple pomace. *Food Chem.* **2000**, *68*, 81–85.
- (14) Wolfe, K.; Wu, X. Z.; Liu, R. H. Antioxidant activity of apple peels. *J. Agric. Food Chem.* **2003**, *51*, 609–614.
- (15) Lee, K. W.; Kim, Y. J.; Kim, D. O.; Lee, H. J.; Lee, C. Y. Major phenolics in apple and their contribution to the total antioxidant capacity. *J. Agric. Food Chem.* **2003**, *51*, 6516–6520.
- (16) Miller, N. J.; Diplock, A. T.; Rice-Evans, C. A. Evaluation of the total antioxidant activity as a marker of the deterioration of apple juice on storage. *J. Agric. Food Chem.* **1995**, *43*, 1794–1801.
- (17) Van der Sluis, A.; Dekker, M.; Skrede, G.; Jongen, W. M. F. Activity and concentration of polyphenolic antioxidants in apple juice. I. Effect of existing production methods. *J. Agric. Food Chem.* **2002**, *50*, 7211–7219.
- (18) Chinnici, F.; Bendini, A.; Gaiani, A.; Riponi, C. Radical scavenging activities of peels and pulps from cv. Golden delicious apples as related to their phenolic composition. *J. Agric. Food Chem.* **2004**, *52*, 4684–4689.
- (19) Vrhovsek, U.; Rigo, A.; Tonon, D.; Mattivi, F. Quantitation of polyphenols in different apple varieties. *J. Agric. Food Chem.* **2004**, *52*, 6532–6538.

- (20) Wayner, D.; Burton, G.; Ingold, K.; Locke, S. Quantitative measurement of the total peroxy radical-trapping antioxidant capability of human blood plasma by controlled peroxidation. The important contribution made by plasma proteins. *FEBS Lett.* **1985**, *187*, 33–37.
- (21) Rigo, A.; Vianello, F.; Clementi, G.; Rossetto, M.; Scarpa, M.; Vrhovsek, U.; Mattivi, F. Contribution of proanthocyanidins to the peroxy radical scavenging capacity of some Italian red wines. *J. Agric. Food Chem.* **2000**, *48*, 1996–2002.
- (22) Butkovic, V.; Klasinc, L.; Bors, W. Kinetic study of flavonoid reactions with stable radicals. *J. Agric. Food Chem.* **2004**, *52*, 2816–2820.
- (23) Goupy, P.; Dufour, C.; Loonis, M.; Dangles, O. Quantitative kinetic analysis of hydrogen transfer reactions from dietary polyphenols to the DPPH radical. *J. Agric. Food Chem.* **2003**, *51*, 615–622.
- (24) Rossetto, M.; Vanzani, P.; Mattivi, F.; Lunelli, M.; Scarpa, M.; Rigo, A. Synergistic antioxidant effect of catechin and malvidin 3-glucoside on free radical-initiated peroxidation of linoleic acid in micelles. *Arch. Biochem. Biophys.* **2002**, *408*, 239–245.
- (25) Arteel, G. E.; Sies, H. Protection against peroxynitrite by cocoa polyphenol oligomers. *FEBS Lett.* **1999**, *462*, 167–170.
- (26) Counet, C.; Collin, S. Effect of the number of flavanol units on the antioxidant activity of procyanidin fractions isolated from chocolate. *J. Agric. Food Chem.* **2003**, *51*, 6816–6822.
- (27) Ursini, F.; Sevanian, A. Postprandial oxidative stress. *Biol. Chem.* **2002**, *383*, 599–605.
- (28) Spencer, J. P. E.; Chaudry, F.; Pannala, A. S.; Srai, S. K.; Debnam, E.; Rice-Evans, C. Decomposition of cocoa proanthocyanidins in the gastric milieu. *Biochem. Biophys. Res. Commun.* **2000**, *272*, 236–241.

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