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Polyphenol Levels and Free Radical Scavenging Activities of Four Apple Cultivars from Integrated and Organic Farming in Different Italian Areas

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This paper investigates the influence of cultivar (Annurca, Golden Delicious, Red Chief, and Stayman Neepling), rural practice (integrated and organic), and growing region (different Italian regions) on polyphenol composition and antiradical activity of the pulp and skin of apples, as presented to the consumer at the market. Antiradical activity of fruit was strongly related with the total polyphenolic content, determined both by the spectrophotometric Folin−Ciocalteu method (R^2 = 0.90; P < 0.01) and by HPLC (R^2 = 0.85; P < 0.01). Considering the edible portion of the fruit, polyphenolics contribute toward explaining approximately 90% of the overall antiradical activity, thus highlighting their important role in human health protection. Therefore, the data indirectly indicated that ascorbic acid and other antiradical molecules differing from polyphenols play a much less important role in explaining the health-protecting properties of apples. Cultivar effect was by far the most important, and Annurca and Golden Delicious were respectively the best and the worst apples from the point of view of the health-protecting attributes.

KEYWORDS: Apple; DPPH; flavonoids; polyphenols; free radical scavenging activity; HPLC; organic; integrated

INTRODUCTION

Polyphenolic compounds represent a large group of secondary plant metabolites, which include flavonoids and hydroxycinnamic acids that occur ubiquitously in fruits and vegetables, and are often associated with sugar moieties (*1*). Many of these polyphenolic substances have shown strong antioxidant properties as oxygen scavengers, peroxide decomposers, metal-chelating agents, and free radical inhibitors (*2–5*).

The apple is one of the most extensively produced and consumed fruits worldwide [2005 global production was >62 million metric tons; (*6*)]. It is available on the market for the whole year and, due to its polyphenol content and dietary databases, represents the major source of intake of these compounds in the European and U.S. diet (*7, 8*). Epidemiological investigations have revealed an inverse correlation between the consumption of apples and many chronic human diseases (*9, 10*). Moreover, as reported in in vitro studies, apple extracts exhibit inhibition effects on the proliferation of tumor cells (*11*), show a significant potential to protect DNA from γ-radiation-induced damage (*12*), and prevent oxidative stress injuries to human gastric epithelial cells (*13*).

The health-protecting properties of apples have been attributed to the presence of polyphenolics, rather than to ascorbic acid (*11, 14*). In fact, although ascorbic acid has been found to be the most abundant vitamin in apples, its occurrence is about 10−200 times lower than that usually reported for polyphenolics (*15*). Hence, a thorough evaluation of apple antioxidant properties should be carried out to accurately investigate the polyphenolic content and composition of fruit extracts and their radical scavenging activity.

On the basis of data in the literature, qualitative and quantitative polyphenol occurrence in fresh apples seems to depend on many parameters such as variety (*5, 16–19*), stage of maturity (*5, 20*), growing region (*18, 21*), and rural practices (*22*).

Although several studies investigated the differences in polyphenolic composition and/or radical scavenging activities occurring in various apple cultivars, numerous investigations

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have shown deficiencies concerning the experimental design and/or the lack of a comprehensive statistical approach. In some papers in fact, the various cultivars were collected in different areas, even though in the same geographic region, introducing a confusing variable into the study of the cultivar effect (*15, 17*). Other studies investigated flesh or peel only or considered the whole edible fraction without making any distinction between pulp and skin (*17–19*).

A "growing region effect" was observed by McGhie et al. (*18*) by studying the polyphenol composition of 10 apple cultivars from three regions of New Zealand, but no evaluation of the antioxidant activity was performed in that investigation.

Moreover, the rural practice effect is scarcely investigated, although integrated and organic farming practices have been regulated in Europe for many years (*23, 24*). The only research published on this subject showed that the integrated production method (also called "sustainable") led to fruits richer in polyphenols and with a higher antioxidant activity than the organic method (*22*). However, this paper investigated only one cultivar (Golden Delicious), and its conclusions should be further confirmed by a study involving a greater number of varieties.

Furthermore, in almost all of the studies reported in the literature (*5, 15, 16, 19*), the experimental design was not consistent with the assessment of variability among single fruits, which is necessary information for evaluating the statistical significance of the differences observed among fruit groups that are compared within each investigated parameter.

On the basis of the above-mentioned considerations, the aim of this study was to investigate the influence of cultivar, rural practice, and growing region on polyphenol composition and antiradical activity of pulp and skin of apples, as presented to the consumer at the market.

MATERIALS AND METHODS

Reagents, Solvents, and Materials. Polyphenol standards were supplied as follows: (+)-catechin, (−)-epicatechin, quercetin-3-rhamnoglucoside, phloridzin, and chlorogenic acid by Aldrich (Milan, Italy); procyanidin B1, procyanidin B2, phloretin, quercetin-3-glucoside, quercetin-3-galactoside, and quercetin-3-rhamnoside by Extrasynthese (Genay, France); quercetin-3′-xyloside and gallic acid by Fluka (Milan, Italy); quercetin-3-arabinoside by Apin Chemicals (Oxon, U.K.); and cyanidin-3-galactoside by Carbomer Inc. (San Diego, CA). HPLC grade methanol and water were purchased from J. T. Baker (Deventer, The Netherlands). Formic acid, Folin−Ciocalteu reagent, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical were obtained from Merck (Darmstadt, Germany). Ultrapure water was taken from a Milli-Q system supplied by Millipore (Billerica, MA).

Polytetrafluoroethylene (PTFE) membranes (porosity = 0.2 μm) for the filtration of the apple extracts before HPLC analysis were obtained from Sartorius (Goettingen, Germany).

The glassware was cleaned before use by repeatedly washings with chromic and concentrated sulfuric acid hot mixture and purified water and finally dried at 150 °C.

Study Design and Fruit Sampling. Four popular apple varieties in Italy (Golden Delicious, Annurca, Red Chief, and Stayman Neepling) were selected. To evaluate the "cultivar effect", a production farm was chosen where all of the cultivars were grown with the same cultivation method and under the same environmental conditions. In addition, apples belonging to the same variety and cultivated under the same environmental conditions, by both integrated and organic productions, were analyzed to investigate the "rural practice effect". Finally, apples from the same cultivar produced under the same growth conditions but in different Italian areas were evaluated to obtain information about the "growing region effect"; for this purpose, within each cultivar, Tuscan apples were compared with fruits grown in the region historically renowned for their production. The growing regions were also chosen on the basis of the willingness of the orchard owners, the availability of cultivars, and the presence of organic and integrated cultivation methods.

Table 1. Varieties, Rural Practices, Growing Regions, and Abbreviations of the Apple Types Investigated

cultivar	rural practice	growing region	abbreviation
Annurca	integrated	Civitella in Val di Chiana (Tuscany)	AIT
	integrated	Mignano Monte Lungo (Campania)	AIC
	organic	Sessa Aurunca (Campania)	AOC
Golden Delicious	integrated	Civitella in Val di Chiana (Tuscany)	GDIT
	integrated	Ponte in Valtellina (Lombardy)	GDIL
	organic	Siena (Tuscany)	GDOT
Red Chief	integrated	Civitella in Val di Chiana (Tuscany)	RCIT
	integrated	Faenza (Emilia)	RCIE
Stayman Neepling	integrated	Civitella in Val di Chiana (Tuscany)	SNIT
	integrated	Nalles (Alto Adige)	SNIAA

The apple cultivars, rural practices, growing regions, and abbreviations used in the present study are given in **Table 1**.

On the basis of the available apple cultivars, their growing regions, and types of rural practices, it was possible to compare the following samples:

• GDIT, AIT, RCIT, and SNIT, to emphasize the differences among cultivars grown with the same rural practice and under the same environmental conditions (*cultivar effect*).

• GDIT versus GDOT and AIC versus AOC, to highlight the differences occurring within the same cultivar between two samples grown under the same environmental conditions, but with different rural practices (*rural practice effect*).

• GDIL versus GDIT, AIC versus AIT, RCIE versus RCIT, and SNIAA versus SNIT, to point out differences within the same cultivar grown under the same rural practices, but in different environmental conditions (*growing region effect*).

For each lot, a fruit box containing 30−35 apples (the result of a random sampling from the commercial harvest) was supplied by the farms before distribution to the market. Some physicochemical measures were performed on 20 fruits free of defects, and the results of these analyses are listed in **Table 2**. To evaluate the statistical significance of the differences evidenced among apple lots, chemical analyses were performed separately on eight fruits.

Sample Treatment. For each lot, eight apples free of defects were carefully peeled with a potato peeler, and the flesh residues were scraped off the skin to obtain samples of peel only. The peeled fruits were cut into quarters, and seeds and core were removed. For each apple, flesh and peel were frozen separately in liquid nitrogen and kept at −80 °C until the analysis was performed.

Polyphenol Extraction. The solvents most widely used for the recovery of polyphenols from apples are methanol or water/methanol mixtures with high percentages (70−95%) of alcohol (*16, 22, 25, 26*). In this study the extraction was carried out according to the method of Tomas-Barberan et al. (*27*), with some modifications: the frozen flesh or peel of each fruit was minced separately to obtain a fine powder, and a weighted amount (about 5 g of flesh or 1 g of peel) was homogenized in an ice bath under magnetic stirring with 10 mL of a methanol/water solution 8:2 v/v containing 2 mM NaF to inactivate polyphenol oxidase; the mixture was centrifuged at 4000 rpm for 7 min and the supernatant recovered.

This procedure was repeated twice, and the extracts were combined and filtered through a Büchner funnel under vacuum. The resulting solution was evaporated to 10 mL by a Rotavapor.

Total Polyphenol Determination. Total polyphenols were spectrophotometrically determined, both in peels and in pulps, with the Folin−Ciocalteau (F−C) method, using (+)-catechin as a reference standard. Eight hundred microliters of the extract (suitably diluted to obtain an absorbance value included in the calibration line) and 200 μL of F−C reagent were mixed. After 3 min, 400 μL of an aqueous solution saturated with sodium carbonate was added, and the mixture obtained was made up to 10 mL with ultrapure water. The solution

Table 2. Physicochemical Parameters of the Apple Samples[a]

apple type	weight (g)	width (mm)	height (mm)	firmness (kg/cm^2)	refractometric solid residue (°Brix)	acidity (mequiv/100 mL)
GDIT	182.6 ± 7.4	77 ± 2	70 ± 2	6.7 ± 0.6	15.1 ± 1.0	3.07 ± 0.79
GDIL	218.6 ± 7.5	80 ± 2	76 ± 3	6.6 ± 0.3	14.5 ± 0.7	2.98 ± 0.40
GDOT	214.9 ± 8.3	81 ± 2	74 ± 3	4.9 ± 0.4	13.4 ± 1.3	2.86 ± 0.47
AIT	134.8 ± 8.0	70 ± 4	57 ± 4	9.2 ± 0.6	16.0 ± 1.1	5.90 ± 0.74
AIC	145.6 ± 9.4	71 ± 2	60 ± 2	7.5 ± 0.7	14.4 ± 1.2	4.40 ± 0.68
AOC	134.8 ± 8.0	69 ± 2	56 ± 2	8.2 ± 1.0	15.2 ± 1.2	3.86 ± 0.22
RCIT	205.6 ± 13.4	79 ± 3	71 ± 3	6.9 ± 0.7	14.2 ± 0.8	2.83 ± 0.29
RCIE	213.5 ± 12.5	79 ± 4	74 ± 4	6.3 ± 0.8	14.3 ± 0.6	3.07 ± 0.21
SNIT	178.0 ± 7.9	77 ± 2	63 ± 3	7.1 ± 0.4	12.0 ± 0.6	3.65 ± 0.78
SNIAA	185.0 ± 13.4	76 ± 3	63 ± 2	6.5 ± 0.6	13.0 ± 0.8	4.30 ± 0.80

[a] Data are mean ± standard deviation ($n = 20$).

was dark incubated for 1 h, and afterward the absorbance was measured at 740 nm. The results were expressed as milligrams of (+)-catechin per 100 g of fresh weight (FW).

HPLC-DAD Analysis of Polyphenols. Chromatographic analysis was performed on the extracts, after their filtration at 0.2 μm, using a Shimadzu (Kyoto, Japan) system consisting of two solvent delivery pumps LC-10AD VP, an autoinjector SIL-10AD VP, a diode array detector (DAD) SPD-M10A VP, and a system controller CBM-20AD VP. The column was a Kromasil 100 C_{18} (15 cm × 0.46 cm i.d.; particle size = 5 μm) equipped with a guard column containing the same stationary phase. Chromatograms were acquired and processed with Shimadzu Class-VP 5.032 software.

The elution solvents were water/formic acid 95:5 v/v (A) and methanol/formic acid 95:5 v/v (B). Elution was carried out according to the method of Tomas-Barberan et al. (*27*) with some modifications. The following gradient (at room temperature) was used: 0−10 min, linear gradient 5−12% B; 10−25 min, linear gradient 12−14% B; 25−60 min, linear gradient 14−70% B; 60−70 min, 70% B isocratic. The flow rate was 1 mL/min, and the injection volume was 20 μL.

Analyte identification was carried out by comparing their retention times and ultraviolet−visible (UV−vis) spectra with those of standards. Unknown DAD chromatographic peaks were tentatively identified from their spectral features and from data in the literature. Structure assignment was afterward confirmed by HPLC-MS/MS analysis.

The quantification of the analytes for which standards were available was performed with external calibration curves, whereas that for analytes for which standard were lacking was performed by reporting the measured chromatographic area in the calibration equation of the reference standards (cyanidin-3-galactoside and quercetin-3-glucoside for unknown anthocyanidins and quercetin-glycosides, respectively).

In **Figure 1** the HPLC-DAD chromatograms of Annurca peel and flesh extracts (AOC type) are reported as an example. The chromatograms showed a good resolution of most of the major flavonoids, especially when specific wavelengths were used: flavanols, procyanidins, and dihydrochalcones were detected at 280 nm, quercetin-glycosides at 258 nm, chlorogenic acid at 325 nm, and cyanidin-glycosides at 529 nm. UV−vis spectra were acquired in the range of 190−800 nm. According to Tomas-Barberan et al. (*27*), quercetin-3-rhamnoglucoside and quercetin-3-glucoside, which gave rise to incomplete resolution, were quantified by expressing their sum as quercetin-3-glucoside equivalent, because the glucoside was found to be considerably more abundant than the rhamnoglucoside (*28*).

As shown in **Figure 1**, procyanidin B2 and chlorogenic acid overlapped; therefore, the quantification of the latter was performed at 325 nm (where procyanidin B2 had no absorbance).

HPLC-MS/MS Analysis. To identify and quantitatively determine procyanidin oligomers and confirm the identity of several quercetin and cyanidin glycosides, the standards of which were not commercially available, HPLC-MS/MS analysis was performed on the extracts after filtration at 0.2 μm using a Prominence HPLC system (Shimadzu) consisting of two solvent delivery pumps LC-20AD Ultra Fast Liquid Chromatography (UFLC), an autoinjector SIL-20A HT, a column thermostat CTO/20A, and a system controller CBM-20A UFLC.

Two Xterra RP_{18} columns (10 cm × 0.21 cm i.d., particle size = 3.5 μm; and 15 cm × 0.21 cm i.d., particle size = 3.5 μm) (Waters, Milford, MA) were used in series. Chromatograms were processed with the Analyst version 1.4.2 software.

The elution solvents were water/acetic acid 99:1 v/v (A) and methanol/acetic acid/2-propanol 74.25:0.75:25 v/v/v (B). Elution was carried out at 40 °C, according to the following gradient: 0−2 min, isocratic 5% B; 2−11 min, linear gradient 5−22% B; 11−15 min, linear gradient 22−30% B; 15−28 min, linear gradient 30−45% B; 28−35 min, linear gradient 45−80% B; 35−36 min, linear gradient 80−100% B; 36−38 min, isocratic 100% B; 38−55 min, linear gradient 100−5% B. The flow rate was 0.15 mL/min and the injection volume, 5 μL.

The HPLC system was interfaced with a 3200QTrap mass detector (Applied Biosystem, Foster City, CA) by a Turbo V interface equipped with a heated nebulizer and turbo ion spray (TIS) probes. The following conditions were adopted in TIS mode: curtain gas (N_2) at 30 psi; ion source gas (GS1) at 50 psi; turbo gas (GS2) at 25 psi; desolvatation temperature (TEM), 500 °C; collision activated dissociation gas (CAD), 10.0 au (arbitrary units). Ion spray voltage (IS) was set at +3.5 and −3.5 kV for cyanidin and quercetin/procyanidin analyses, respectively. Identification of target compounds was performed by a screening loop consisting of a survey scan (full scan between 270 and 1450 amu), an enhanced resolution analysis, and an MS-MS experiment. The 3200QTrap was used in multiple reaction monitoring (MRM) negative ion mode as the quantitative method for quantifying the procyanidins according to the transitions defined with the above-mentioned analysis loop (see **Table 3**). Unit mass resolution was established and maintained in each mass-resolving quadrupole by keeping a full width at half-maximum of about 0.7 amu and a dwell time of 100 ms for each monitored transition. **Figure 2** illustrates an example of the total ion current (TIC) chromatogram of procyanidin oligomers ($n = 3-6$) found in a Golden Delicious peel extract, the corresponding extracted ion chromatogram (XIC), mass spectra, and the product ion mass spectra.

Evaluation of the Recovery Efficiency. The polyphenol recovery of the extraction procedure was evaluated in two different manners. (1) Four sequential extractions, each with 10 mL aliquots of methanol/water solution 8:2 v/v containing 2 mM NaF, were performed on five test samples of peel and flesh, and the F−C spectrophotometric response of each extract was determined. The results showed that the fourth extraction accounted for 5.4 ± 1.2% (peel) and 3.1 ± 0.7% (pulp) of the sum of the others. This finding indicated that the last extraction step did not contribute significantly to the total polyphenol content. (2) Known amounts of pure standards (gallic acid and phloretin) were added to five test samples of flesh and peel, and afterward extraction and HPLC analysis were conducted as described above. The mean recoveries and standard deviations were 92.7 ± 5.1 and 91.1 ± 3.0 (peel) and 92.3 ± 6.4 and 94.2 ± 2.2 (pulp) for gallic acid and phloretin, respectively, confirming the good recovery efficiency of the procedure adopted.

DPPH Antiradical Activity. Antiradical activity was spectrophotometrically tested using the free radical DPPH, which has been widely adopted for assessing antioxidant properties in different types of fruits (*22, 29*). The free radical scavenging activity of peel and pulp extracts was determined according to the method of Brand-Williams

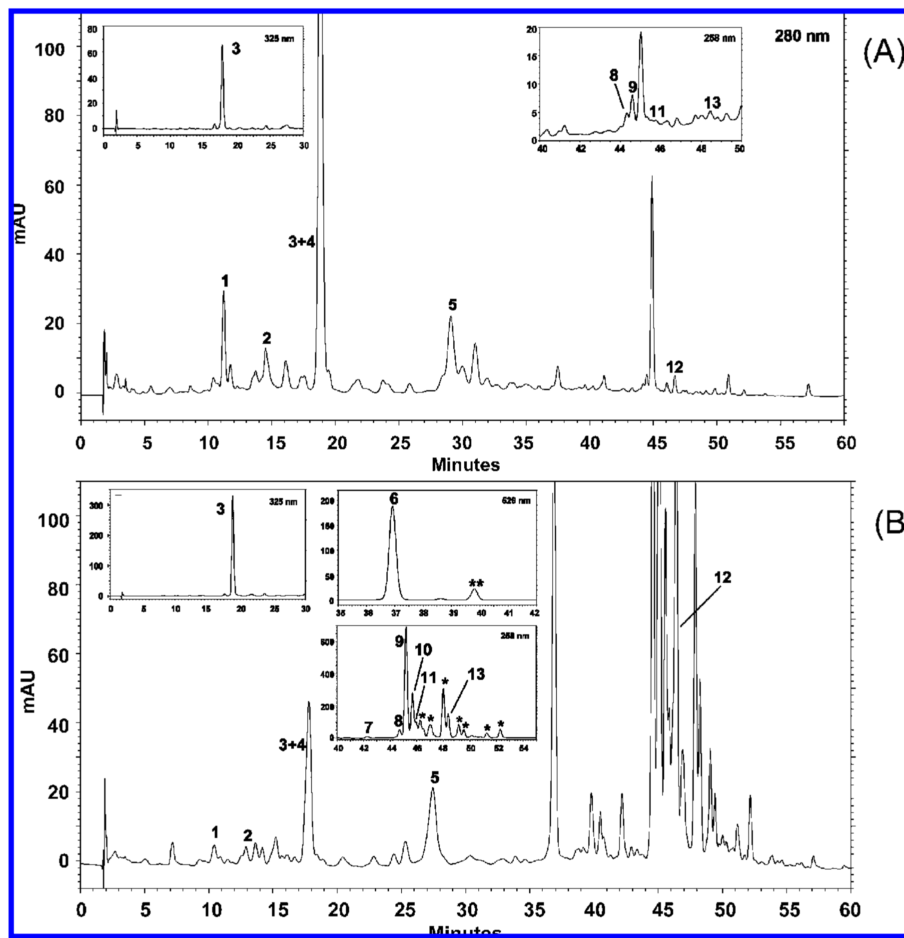

Figure 1. HPLC-DAD (λ = 280 nm) chromatograms of Annurca flesh (**A**) and peel (**B**) extracts and zoomed portions of the corresponding profiles at λ = 258, 325, and 529 nm. Peaks: (1) procyanidin B1; (2) (+)-catechin; (3) chlorogenic acid; (4) procyanidin B2; (5) (−)-epicatechin; (6) cyanidin-3-galactoside; (7) quercetin-3-xyloside; (8) quercetin-3-arabinoside; (9) quercetin-3-galactoside; (10) quercetin-3-glucoside; (11) quercetin-3-rhamnoglucoside; (12) phloridzin; (13) quercetin-3-rhamnoside; (∗) unknown quercetin hexosides; (∗∗) unknown cyanidin pentoside.

Table 3. Monitored Ions for Quantitative Determination of Procyanidins

	precursor ions		fragment ions
oligomer	*m/z*	charge state	*m/z*
monomer	289.0	$(M - H)^-$	125.0
dimer	577.1	$(M - H)^-$	125.0
trimer	865.2	$(M - H)^-$	125.0
tetramer	576.1	$(M - 2H)^{-2}/2$	125.0
pentamer	720.2	$(M - 2H)^{-2}/2$	125.0
esamer	864.2	$(M - 2H)^{-2}/2$	125.0

et al. (*30*), with little modification. Five different amounts of each extract were added to 5 mL of a 0.1 mM DPPH solution. For each extract, a blank was performed by using 250 μL of ultrapure water. The decrease in absorbance at 517 nm was monitored at 0, 1, 5 min, every 5 min until 30 min, every 10 min after 30 min and until 60 min, and every 15 min after 60 min and until the reaction reached a plateau. For each concentration tested, the reaction kinetic was plotted. The percentage of DPPH neutralized at the steady state (%DPPH) was determined by using the equation

$$\%\mathrm{DPPH} = 100 \times \frac{(A_0^{\mathrm{E}} - A_{\mathrm{SS}}^{\mathrm{E}}) - (A_0^{\mathrm{B}} - A_{\mathrm{SS}}^{\mathrm{B}})}{A_0^{\mathrm{E}}} \quad (1)$$

where A_0^{E} is the absorbance of the extract at time zero, $A_{\mathrm{SS}}^{\mathrm{E}}$ is the absorbance of the extract at the steady state, A_0^{B} is the absorbance of the blank at time zero, and $A_{\mathrm{SS}}^{\mathrm{B}}$ is the absorbance of the blank at the steady state.

The DPPH inhibition percentages obtained were plotted as a function of the corresponding concentration of the extract, and the best equation fitting the experimental points was calculated by the least-squares method. IC_{50} was defined as the concentration of the apple sample (mg of fruit, FW) in 1 mL of reaction mixture, necessary for decreasing the initial DPPH concentration (which was kept constant in all of the experiments) by 50%; therefore, higher IC_{50} values correspond to lower radical scavenging activities.

Radical Scavenging Activity. With the aim of investigating the contribution of polyphenolics determined in apple extracts to the actual antiradical activity of the samples, it is possible to calculate a radical scavenging activity (RSA_{calcd}) on the basis of the concentrations of the individual polyphenols found in each apple and their IC_{50} values, according to the equation (*31*)

$$\mathrm{RSA}_{\mathrm{calcd}} = \sum_i \frac{c_i}{(\mathrm{IC}_{50})_i} \quad (2)$$

where c_i is the concentration of polyphenol i (mg/100 g of FW) and $(IC_{50})_i$ is the IC_{50} value of polyphenol i (mg/L DPPH solution).

IC_{50} values of each polyphenol identified in apple extracts were determined on standard solutions. These values are reported in **Table 4** and comply well with those reported in the literature (*14*).

To quantify the contribution to RSA_{calcd} of oligomeric procyanidins, the standards of which were not available, the IC_{50} value of epicatechin was used as reference. In fact, as reported by Lu and Foo (*14*), the molar IC_{50} of oligomers was inversely proportional to the number of epicatechin units contained in the molecule. This behavior was confirmed from results obtained in this study for epicatechin and procyanidin B2, which exhibit quite similar IC_{50} values (see **Table 4**).

According to Van der Sluis et al. (*31*), the radical scavenging activity of fruit samples (RSA_{sample}) can be derived from IC_{50} values (mg of FW/mL DPPH solution) determined on apple extracts ($IC_{50,sample}$) by

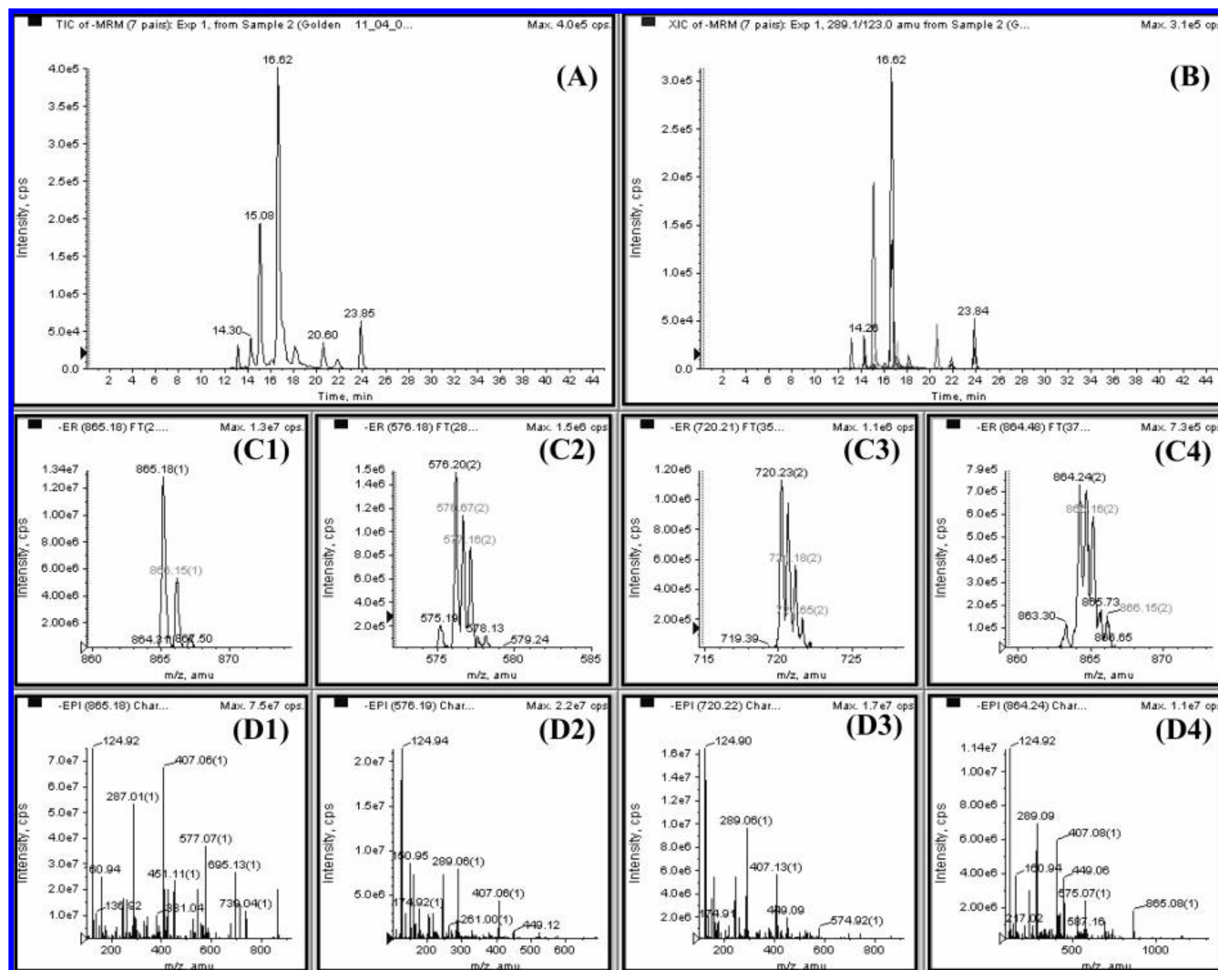

Figure 2. Total ion current (**A**) and extracted ion (**B**) chromatograms, mass spectra (**C**), and product ion mass spectra (**D**) of procyanidin trimers (1), tetramers (2), pentamers (3), and hexamers (4) found in a Golden Delicious peel extract.

Table 4. IC_{50} Values of the Polyphenols Investigated

compound	IC_{50} (mg/L DPPH solution)	compound	IC_{50} (mg/L DPPH solution)
procyanidin B1	1.576	cyanidin-3-galactoside	3.047
(−)-epicatechin	1.729	quercetin-3-glucoside	4.656
procyanidin B2	1.844	chlorogenic acid	7.211
(+)-catechin	2.545	phloridzin	116.2

using eq 3, where the factor 100 refers to the measurement unit of polyphenol concentration in the fruits, allowing for comparing RSA_{sample} with RSA_{calcd}.

$$RSA_{sample} = \frac{100}{IC_{50,sample}} \qquad (3)$$

The percentage of RSA_{sample} explained by individual polyphenol composition was calculated as indicated by eq 4:

$$\%\ \text{of explained}\ RSA_{sample} = \frac{RSA_{calcd} \times 100}{RSA_{sample}} \qquad (4)$$

Statistical Analysis. Linear univariate correlations between variables were investigated by the least-squares method, using Microsoft Excel.

One-way analysis of variance (ANOVA) and the Tamhane test were performed on the original data, at a 0.05 probability level, by using the statistical package SPSS, version 13.0, for Windows.

RESULTS AND DISCUSSION

Polyphenol Composition. Tables 5 and **6** illustrate the mean phenolic compositions determined by HPLC, the total phenolic contents measured with the F−C method, and the RSA values and percentage of RSA_{sample} explained by RSA_{calcd} for apple peel and pulp, respectively.

Depending on the lot and parameter investigated, different degrees of variability were observed. In particular, the variability coefficient of the total HPLC polyphenols was in the ranges of 9−28% (mean = 15%) and 11−26% (mean = 17%) for peels and pulps, respectively. The variability of F−C and RSA_{sample} was higher in peel (up to maxima of 43 and 51%, respectively) than in pulp (23 and 36%, respectively).

The results evidenced several chemical characteristics that are common to all investigated apple types, apart from the variety, the rural practice, and the geographical provenience. As reported by several authors (*5, 16, 22, 25, 32*), peel was richer than pulp in total polyphenols, as well as in the majority of the single polyphenolic compounds, in accordance with its defensive role against pathogenic pressure, which mainly acts on the skin. The only exception was chlorogenic acid, which was significantly more abundant in the pulp than in the peel of Red Chief and Stayman Neepling, whereas similar concentrations were found for Annurca and Golden Delicious. A prevalence of chlorogenic acid in flesh was observed by Tsao et al. (*16*) for Red Delicious, a cultivar from which Red Chief derives via bud mutation. Moreover, similar or equal amounts of this organic acid have been found by several authors in the two matrices of Golden Delicious (*5, 16, 22, 25*).

The qualitative composition of peel polyphenols was analogous to that observed for flesh, with the exception of

Table 5. Mean Concentrations and Standard Deviations (n = 8) of Individual and Total Polyphenolics Determined by HPLC (Milligrams per 100 g of FW) and F−C Method [Milligrams of (+)-Catechin per 100 g of FW], Experimental and Calculated RSA Values (Milliliters of DPPH Solution per 100 mg of FW), and Percentage of Explained RSA in Peel Samples

	AIT	GDIT	RCIT	SNIT	AIC	GDIL	RCIE	SNIAA	AOC	GDOT
chlorogenic acid	35.7 ± 7.2	10.3 ± 3.8	2.2 ± 0.6	4.4 ± 0.7	31 ± 13	10.8 ± 4.4	3.3 ± 1.6	5.5 ± 2.5	24.4 ± 3.3	16.3 ± 5.1
(+)-catechin	10.1 ± 3.8	5.8 ± 0.9	10.0 ± 0.4	12.2 ± 1.7	13.4 ± 2.3	7.7 ± 1.4	6.9 ± 1.9	12.1 ± 1.9	10.7 ± 0.9	6.6 ± 1.2
(−)-epicatechin	53 ± 10	52 ± 20	153 ± 29	85 ± 13	78 ± 25	58 ± 18	53 ± 13	93 ± 45	62 ± 26	59 ± 24
total flavanols	**63.5 ± 7.3**	**58 ± 20**	**163 ± 29**	**97 ± 14**	**91 ± 26**	**66 ± 18**	**60 ± 12**	**105 ± 47**	**73 ± 27**	**66 ± 24**
procyanidin B1	22.0 ± 4.7	5.9 ± 0.8	17.1 ± 2.0	30.4 ± 6.9	15.3 ± 2.5	8.5 ± 1.5	14.0 ± 2.5	25.6 ± 4.2	19.4 ± 2.4	6.0 ± 0.9
procyanidin B2	84 ± 12	50.7 ± 8.1	87 ± 10	84 ± 21	48.6 ± 6.8	78 ± 13	51 ± 14	62 ± 12	55 ± 8	48.6 ± 8.7
other procyanidins	102 ± 17	48.8 ± 6.9	124 ± 18	115 ± 17	78 ± 14	63 ± 10	73 ± 12	81 ± 13	83 ± 10	60 ± 10
total procyanidins	**208 ± 34**	**105 ± 16**	**228 ± 29**	**229 ± 40**	**142 ± 22**	**150 ± 24**	**138 ± 28**	**169 ± 29**	**157 ± 18**	**115 ± 19**
cyanidin-3-galactoside	21 ± 10	Bdl[d]	35 ± 14	10.7 ± 7.1	47.5 ± 9.2	Bdl	15.7 ± 8.7	10.9 ± 6.0	27.3 ± 5.8	Bdl
other cyanidin-glycosides[a]	1.7 ± 0.8	Bdl	5.1 ± 2.1	1.2 ± 0.4	5.6 ± 1.6	Bdl	4.3 ± 2.3	2.6 ± 1.8	3.2 ± 0.5	Bdl
total anthocyanins	**23 ± 11**	**Bdl**	**40 ± 16**	**12 ± 7**	**53 ± 9**	**Bdl**	**20 ± 9**	**13 ± 7**	**30 ± 6**	**Bdl**
quercetin-3-xyloside	0.6 ± 0.3	0.3 ± 0.1	0.4 ± 0.1	0.1 ± 0.1	0.8 ± 0.3	0.6 ± 0.2	0.2 ± 0.0	0.2 ± 0.1	2.2 ± 1.0	0.4 ± 0.1
quercetin-3-arabinoside	0.4 ± 0.3	Bdl	1.1 ± 0.6	0.3 ± 0.1	0.9 ± 0.2	1.0 ± 0.4	0.4 ± 0.2	Bql	3.4 ± 3.2	1.4 ± 0.4
quercetin-3-galactoside	16.1 ± 2.2	17.1 ± 4.3	35 ± 16	34 ± 15	73 ± 22	76 ± 38	43 ± 16	11.0 ± 5.1	119 ± 39	24 ± 10
quercetin-3-glucoside[b]	5.6 ± 2.7	2.3 ± 0.5	2.2 ± 0.9	3.8 ± 1.2	15.5 ± 6.1	10.6 ± 6.4	4.6 ± 2.5	1.6 ± 0.8	35 ± 12	3.3 ± 1.2
quercetin-3-rhamnoside	7.4 ± 2.3	11.7 ± 3.9	11.2 ± 3.9	10.6 ± 3.1	11.6 ± 1.7	34.0 ± 8.0	1.0 ± 0.2	10.1 ± 7.4	25.6 ± 2.4	14.6 ± 4.3
other quercetin-glycosides[c]	20.9 ± 7.7	18 ± 10	29 ± 10	27.7 ± 8.7	42 ± 14	44 ± 17	41.5 ± 5.7	25 ± 20	81 ± 32	12.8 ± 3.6
total flavonols	**51 ± 13**	**49 ± 16**	**79 ± 23**	**77 ± 24**	**144 ± 30**	**166 ± 54**	**90 ± 21**	**48 ± 30**	**266 ± 68**	**56 ± 15**
phloridzin	25.8 ± 8.7	9.1 ± 3.3	21.7 ± 5.2	11.5 ± 2.3	26.1 ± 9.2	9.4 ± 4.0	16.4 ± 3.7	9.3 ± 3.0	24.7 ± 0.9	8.7 ± 2.3
HPLC total polyphenols	**407 ± 44**	**232 ± 49**	**533 ± 47**	**431 ± 43**	**487 ± 76**	**403 ± 66**	**328 ± 45**	**351 ± 98**	**576 ± 91**	**262 ± 40**
F−C total polyphenols	**565 ± 73**	**396 ± 69**	**644 ± 161**	**576 ± 114**	**609 ± 86**	**528 ± 99**	**574 ± 78**	**545 ± 232**	**736 ± 178**	**449 ± 142**
RSA_{sample}	**170 ± 23**	**104 ± 11**	**288 ± 89**	**238 ± 53**	**172 ± 27**	**166 ± 47**	**164 ± 18**	**196 ± 99**	**228 ± 57**	**132 ± 61**
RSA_{calcd}	**177 ± 19**	**104 ± 21**	**252 ± 19**	**206 ± 23**	**184 ± 32**	**158 ± 22**	**139 ± 20**	**171 ± 46**	**201 ± 32**	**116 ± 18**
explained RSA (%)	**105**	**99**	**94**	**90**	**107**	**99**	**84**	**96**	**91**	**100**

[a] Tentatively identified; quantified as cyanidin-3-galactoside. [b] Sum of quercetin-3-glucoside and quercetin-3-rhamnoglucoside. [c] Tentatively identified; quantified as quercetin-3-glucoside. [d] Bdl, below the detection limit.

Table 6. Mean Concentrations and Standard Deviations (n = 8) of Individual and Total Phenolic Compounds Determined by HPLC (Milligrams per 100 g of FW) and F−C Method [Milligrams of (+)-Catechin per 100 g of FW], Experimental and Calculated RSA Values (Milliliters of DPPH Solution per 100 mg of FW), and Percentage of Explained RSA in Flesh Samples

	AIT	GDIT	RCIT	SNIT	AIC	GDIL	RCIE	SNIAA	AOC	GDOT
chlorogenic acid	28.0 ± 5.8	16.8 ± 3.4	13.5 ± 2.9	9.3 ± 1.7	33.7 ± 6.5	14.3 ± 3.2	10.7 ± 2.7	14.6 ± 5.2	30.8 ± 4.8	18.5 ± 7.8
(+)-catechin	4.2 ± 1.2	2.0 ± 0.4	2.9 ± 0.5	4.0 ± 0.7	6.7 ± 1.4	1.7 ± 0.4	3.3 ± 0.5	4.5 ± 0.4	6.3 ± 0.9	1.6 ± 0.4
(−)-epicatechin	14.9 ± 3.4	8.2 ± 4.2	12.6 ± 5.1	9.8 ± 1.9	20.9 ± 6.5	8.3 ± 2.2	9.8 ± 3.3	16.6 ± 2.8	14.5 ± 4.8	8.7 ± 1.2
total flavanols	**19.2 ± 4.0**	**10.1 ± 4.4**	**15.5 ± 5.1**	**13.8 ± 2.4**	**27.7 ± 7.0**	**9.9 ± 2.4**	**13.0 ± 3.3**	**21.1 ± 3.0**	**20.8 ± 5.4**	**10.2 ± 1.3**
procyanidin B1	12.1 ± 1.4	6.4 ± 1.2	6.6 ± 0.8	8.6 ± 2.0	12.7 ± 2.2	4.7 ± 1.0	7.0 ± 1.0	7.3 ± 1.7	13.9 ± 3.3	5.1 ± 0.9
procyanidin B2	18.8 ± 2.7	9.0 ± 1.5	8.3 ± 0.8	13.2 ± 2.3	13.1 ± 2.1	6.8 ± 1.0	8.7 ± 1.2	11.2 ± 2.2	17.5 ± 4.0	6.1 ± 1.6
other procyanidins	17.7 ± 2.0	9.3 ± 1.3	10.6 ± 1.3	12.7 ± 2.1	16.9 ± 3.7	6.6 ± 1.2	10.4 ± 1.5	12.9 ± 1.9	20.8 ± 4.3	5.4 ± 1.3
total procyanidins	**48.5 ± 4.5**	**24.7 ± 4.0**	**25.5 ± 2.8**	**34.6 ± 6.0**	**42.7 ± 7.8**	**18.1 ± 3.1**	**26.1 ± 3.5**	**31.5 ± 4.3**	**52 ± 11**	**16.5 ± 3.5**
quercetin-3-xyloside	0.1 ± 0.0	Bdl[c]	Bdl	Bql[d]	Bdl	Bdl	Bdl	Bql	Bql	Bdl
quercetin-3-arabinoside	Bdl	0.1 ± 0.0	Bql	0.1 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
quercetin-3-galactoside	0.2 ± 0.1	Bdl	0.2 ± 0.0	Bql	0.1 ± 0.1	Bdl	Bdl	0.1 ± 0.0	1.1 ± 0.2	Bdl
quercetin-3-glucoside[a]	0.1 ± 0.0	0.1 ± 0.0	Bql	Bql	0.1 ± 0.0	0.1 ± 0.0	Bdl	0.1 ± 0.0	Bql	Bql
quercetin-3-rhamnoside	0.2 ± 0.1	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.5 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.5 ± 0.3
other quercetin-glycosides[b]	0.5 ± 0.2	0.2 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.6 ± 0.5	0.3 ± 0.1	0.2 ± 0.0	0.4 ± 0.2	0.4 ± 0.3	0.4 ± 0.4
total flavonols	**1.1 ± 0.3**	**0.7 ± 0.1**	**0.5 ± 0.1**	**0.6 ± 0.1**	**1.3 ± 0.5**	**1.0 ± 0.1**	**0.3 ± 0.0**	**0.8 ± 0.2**	**1.7 ± 0.4**	**1.0 ± 0.6**
phloridzin	0.3 ± 0.1	0.5 ± 0.1	1.1 ± 0.4	0.8 ± 0.2	0.3 ± 0.1	0.5 ± 0.2	1.1 ± 0.4	0.8 ± 0.1	0.3 ± 0.1	0.6 ± 0.3
HPLC total polyphenols	**97 ± 11**	**53 ± 10**	**56.2 ± 9.5**	**59.1 ± 8.6**	**106 ± 19**	**43.8 ± 7.0**	**51.3 ± 5.6**	**69 ± 11**	**106 ± 18**	**47 ± 12**
F−C total polyphenols	**126 ± 19**	**92 ± 12**	**115 ± 23**	**104 ± 24**	**142 ± 23**	**85 ± 12**	**118 ± 9**	**94 ± 11**	**133 ± 14**	**77 ± 14**
RSA_{sample}	**43.7 ± 6.9**	**31.8 ± 7.6**	**33.8 ± 8.2**	**37.9 ± 8.9**	**42.2 ± 4.8**	**26.2 ± 5.5**	**33.8 ± 7.2**	**30.6 ± 3.5**	**42.8 ± 8.3**	**28 ± 10**
RSA_{calcd}	**37.5 ± 4.2**	**22.3 ± 4.7**	**25.2 ± 4.4**	**28.7 ± 4.6**	**44.6 ± 8.6**	**18.1 ± 2.8**	**23.7 ± 2.9**	**31.8 ± 4.0**	**45.9 ± 8.4**	**18.0 ± 3.3**
explained RSA (%)	**98**	**71**	**76**	**78**	**106**	**70**	**73**	**105**	**108**	**69**

[a] Sum of quercetin-3-glucoside and quercetin-3-rhamnoglucoside. [b] Tentatively identified; quantified as quercetin-3-glucoside. [c] Bdl, below the detection limit. [d] Bql, below the quantification limit.

cyanidin-glycosides, which were present only in red apple peel, in agreement with the results of other researchers (*16, 22, 25*).

On the basis of polyphenols determined in this study, procyanidins, flavanols, and flavonols were generally predominant in peel, representing 77−95% of the whole HPLC polyphenolic content, and accounting for 27−53, 13−31, and 13−46%, respectively. Lower concentrations were found for hydroxycinnamics, anthocyanins, and dihydrochalcones, even though different relative contributions were observed in relation to the samples investigated.

HPLC-MS results evidenced that total procyanidins in skin consisted mainly of dimers (56−64%), trimers (13−18%), and

tetramers (9−12%), whereas pentamers and hexamers together accounted for only 10−15%. Higher molecular weight oligomers (up to dodecamers) were identified but not taken into consideration from a quantitative point of view, because they were present at negligible levels.

On the basis of the mean concentrations determined in skin for single polyphenols, (−)-epicatechin and procyanidin B2 were predominant in almost all samples analyzed, accounting, respectively, for 11−29 and 10−22% of the total HPLC phenolic concentration.

Among quercetin-glycosides, the most abundant individual compound was quercetin-3-galactoside, which was found in a few cases (AIC, AOC, and GDIL) at mean concentrations similar to or higher than those determined for (−)-epicatechin. Similar results were observed elsewhere for Golden Delicious and Red Delicious (*16, 22, 25*).

As reported by several authors (*16, 17, 22*), polyphenols in flesh were almost completely represented (96−99%) by procyanidins, flavanols, and hydroxycinnamics, which accounted for 35−59, 19−31, and 16−39%, respectively. Similarly to findings for peel, the procyanidolic fraction of pulp consisted mainly of dimers (67−76%), trimers (12−18%), and tetramers (6−8%), whereas pentamers and hexamers together represented 5−11%. Negligible concentrations were determined for higher molecular weight procyanidins. The trend observed for oligomeric procyanidins in skin and flesh was in accordance with data reported by Lazarus et al. (*33*) for whole apples. In contrast to what was observed in peel, flavonols were determined at very low concentrations and, together with dihydrochalcones, can be considered as negligible. Among individual polyphenols identified in flesh, chlorogenic acid was found at the highest mean concentrations (9.3−33.7 mg/100 g of FW) in almost all of the samples investigated and represented 16−39% of the total HPLC polyphenolics. As reported for skin, (−)-epicatechin and procyanidin B2 were also present at high concentrations, and together accounted for 30−40% of the whole; lower contributions were observed for procyanidin B1 and (+)-catechin.

F−C polyphenol concentrations, expressed as (+)-catechin, ranged between 77 and 142 mg/100 g of FW and between 396 and 736 mg/100 g of FW for flesh and peel, respectively; these values are in agreement with those reported in the literature (*5, 15, 16*).

For all samples, total polyphenolic concentrations, measured by HPLC, were lower than those found using the F−C method. This result can be partially explained on the basis of the lower spectrophotometric response of (+)-catechin (the reference standard used for F−C method) than procyanidins, which represented an intensely abundant polyphenolic class. Moreover, the F−C method is not specific for polyphenols, also leading to a positive response for other reducing molecules (*34*). However, when F−C data and total HPLC flavonoids found in all of the single peels and pulps were plotted, a significant and strong linear relation was observed ($R^2 = 0.92$; $P < 0.01$).

Radical Scavenging Activity. In line with the higher abundance of polyphenols in peel than in flesh, the former showed higher RSA_{sample} values (see **Tables 5** and **6**). When RSA_{sample} values of skin and pulp were plotted as a function of HPLC total polyphenols and F−C results, strong linear relations were observed ($R^2 = 0.85$, $P < 0.01$; and $R^2 = 0.90$, $P < 0.01$), indicating that both of these parameters are indicative of the antiradical activity evaluation.

Comparison between RSA_{calcd} and RSA_{sample} results showed high values of explained RSA in all cases; these values were included in the ranges of 84−107 and 69−108% for peel and pulp, respectively.

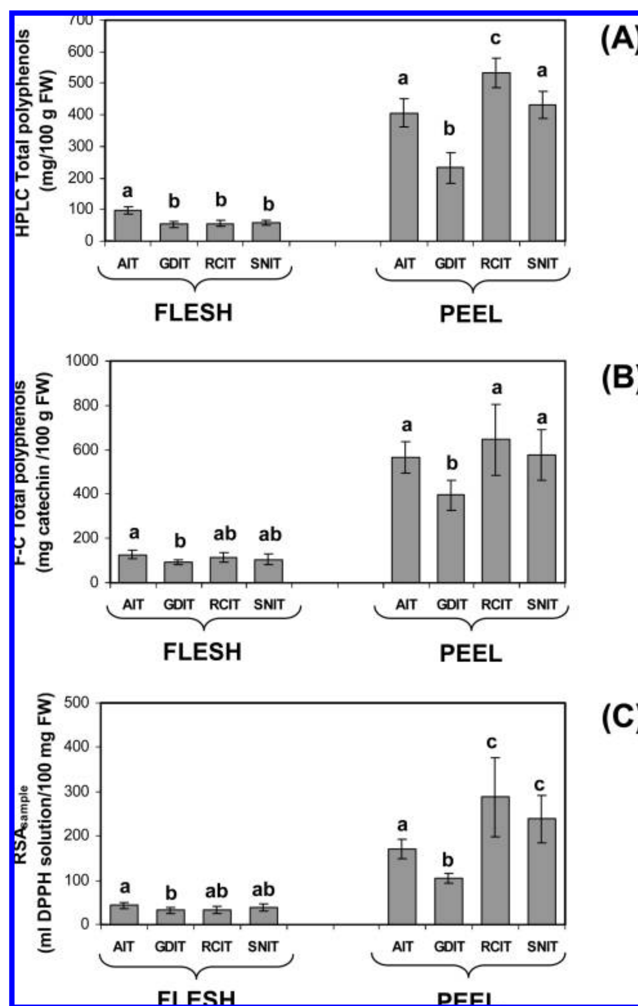

Figure 3. Mean concentrations of total polyphenols as determined by HPLC (**A**) and F−C method (**B**) and mean RSA_{sample} values (**C**) of flesh and peel of the four apple varieties cultivated with the same rural practice and in the same growing region (Tuscany). Values with the same letter were not significantly different at the 5% significance level according to the Tamhane test.

Cultivar Effect. Figure 3 illustrates the mean values and standard deviations of HPLC and F−C total polyphenols and RSA_{sample}, as well as the results of the Tamhane test obtained for cultivars grown in the same area with integrated rural practice. According to HPLC data, RCIT had the highest peel polyphenolic concentration (533 ± 47), followed by SNIT (431 ± 43), AIT (407 ± 44), and GDIT (232 ± 49) (see **Table 5**). In particular, the mean polyphenolic concentration found for Golden Delicious was remarkably low and statistically different from those determined for the other varieties. This finding was a consequence of a general minor abundance of all the individual polyphenol classes. The difference in polyphenol concentration observed between Red Chief and the others was statistically significant as well ($P < 0.01$); this result was mainly due to the large abundance of (−)-epicatechin in Red Chief skin, the concentration of which was 2 or 3 times higher than that found in the other varieties. Conversely, Stayman Neepling and Annurca contained statistically similar total HPLC polyphenol amounts.

Chlorogenic acid was the only individual compound that showed statistically different peel concentrations for all of the investigated varieties ($P < 0.02$). On the other hand, it was observed that such similar individual polyphenol levels and such high standard deviations make most differences among the

cultivars studied insignificant. F−C mean values showed the same trend observed for HPLC; however, when the cultivars were compared according to the Tamhane test, a picture differing from the one obtained from the HPLC data was observed (see **Figure 3**). In fact, in this case, the differences between RCIT and AIT or SNIT were lower than those observed with the chromatographic analysis and, as a result, Golden Delicious was the only cultivar differing significantly from the others ($P < 0.05$). Mean RSA_{sample} values followed the same trend observed for F−C and HPLC results; Golden Delicious and Red Chief were respectively the cultivars with the lowest and highest experimental radical scavenging activity, and they were statistically different from each other. The antiradical activity of SNIT was comparable to that observed for RCIT and significantly higher than that determined for AIT and GDIT ($P < 0.05$).

When HPLC results of flesh were considered, it was evident that the pulp of AIT, RCIT, and SNIT reacted differently to skins; Golden Delicious confirmed the lowest mean polyphenolic content, although its concentration was comparable with that of Red Chief and Stayman Neepling. Annurca showed the highest total polyphenol concentration and was the only cultivar differing statistically from the others. This finding was due to a general higher concentration of the individual compounds and, above all, chlorogenic acid and procyanidins. Our results were in agreement with data obtained by Napolitano et al. (*17*) on Annurca and Golden Delicious. According to the F−C results, the significant difference between Annurca and Golden was confirmed ($P < 0.01$), whereas RCIT and SNIT were not significantly different from each other or from the other varieties.

The comparison of RSA_{sample} values evidenced the same significant differences observed for F−C measures. According to this pattern, RSA_{calcd} mean values followed the trend of RSA_{sample} results and explained 71−98% of the entire antiradical activity for each variety.

Rural Practice Effect. When HPLC, F−C, and RSA_{sample} results obtained for peel and pulp from integrated and organic rural practices were compared according to the Tamhane test, it was clear that for both Annurca and Golden Delicious, the production method did not significantly contribute to the polyphenol content or to the antiradical activity of the fruit ($P > 0.05$) (see **Figure 4**).

However, it should be noted that, on average, when organic practice was considered, the peels of both cultivars showed an antiradical activity about 25% higher than their corresponding integrated farmed equivalents. With regard to Golden Delicious, the higher RSA_{sample} may be interpreted by the different polyphenol composition, as confirmed by RSA_{calcd}. On the contrary, Annurca samples showed more similar RSA_{calcd} values; as a result, significantly higher percentages of explained RSA were found in AIC peels than in AOC ones ($P < 0.01$). A trend similar to that observed for antiradical activity was observed for HPLC and F−C total polyphenols as well, even though with minor differences. These findings are in agreement with the physiological role of skin to provide a physical and chemical barrier against pathogen attacks and could be attributed to the fact that integrated fruits are protected by synthetic pesticides and do not need to rely so much on their own defense mechanisms, including polyphenol production.

A different behavior was observed in pulp, where more similar radical scavenging properties were found.

With regard to individual polyphenol classes, the only significant differences were evidenced between GDIT and GDOT pulps for total procyanidins ($P < 0.01$) and between AIC and AOC peels, the latter being poorer in anthocyanins ($P < 0.01$) and richer in flavonols ($P < 0.01$).

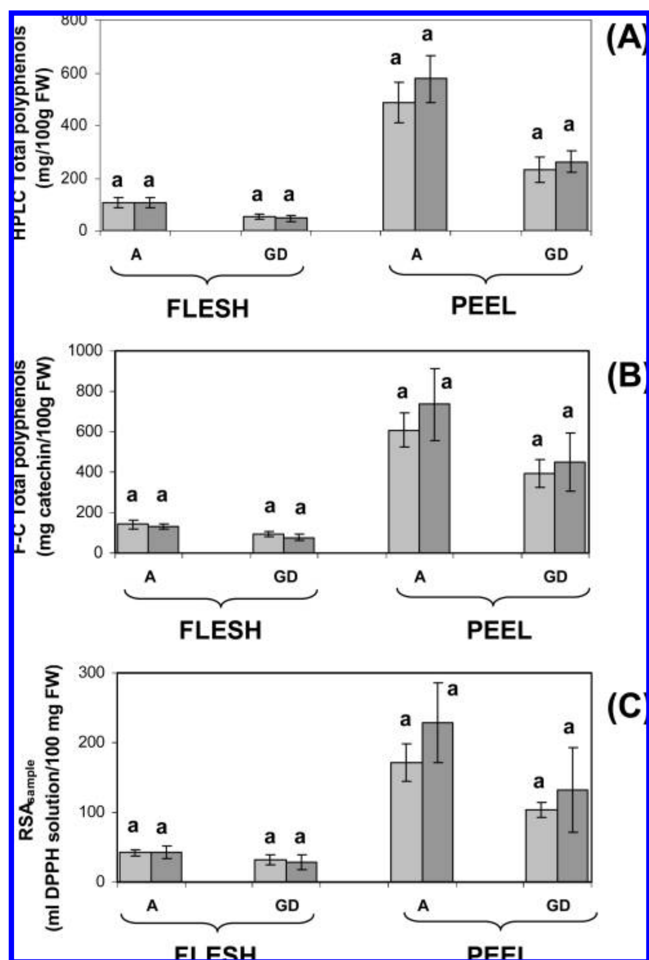

Figure 4. Mean concentrations of total polyphenols as determined by HPLC (**A**) and F−C method (**B**) and mean RSA_{sample} values (**C**) of flesh and peel of Annurca and Golden Delicious apples from integrated and organic productions. Values with the same letter were not significantly different at the 5% significance level according to the Tamhane test.

Growing Region Effect. The results indicated that the growing region mostly affected peel attributes, whereas for the flesh, no significant differences were observed (see **Figure 5**). More specifically, HPLC total polyphenols, F−C results, and antiradical activity did not vary significantly in Golden Delicious flesh, whereas strong differences were observed in all of the parameters investigated for peels. In particular, besides having a much higher concentration of the main flavonols, apples grown in Valtellina also showed a total polyphenolic content and an overall antioxidant activity significantly higher than those of the corresponding samples from Tuscany ($P \leq 0.025$).

Red Chief did not show any significant difference in polyphenolic flesh composition or radical scavenging properties, whereas HPLC total polyphenols, most individual flavonoids, and antiradical activity of Tuscan peels were significantly higher than those found in the fruit from Emilia-Romagna. F−C total polyphenols were also prevalent in the former, but the difference was not significant owing to the very high variability within this group.

With regard to Stayman Neepling, higher mean values were generally found in the Tuscan apples; however, the differences between Tuscan and Alto Adige valley products were not statistically significant, either in peel or in flesh, owing to the

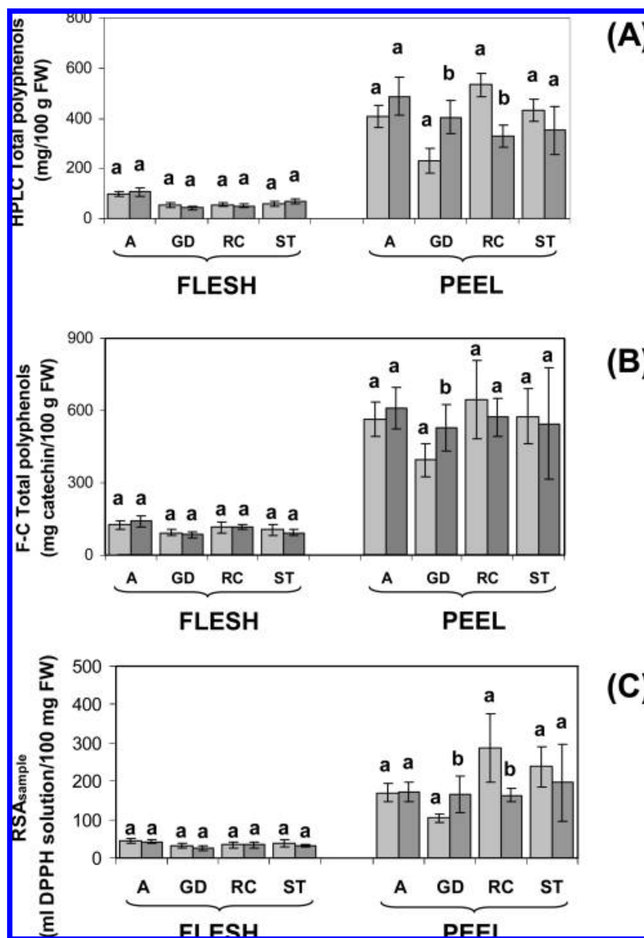

Figure 5. Mean concentrations of total polyphenols as determined by HPLC (**A**) and F−C method (**B**) and mean RSA_{sample} values (**C**) of flesh and peel of the four apple varieties from integrated production, cultivated in different growing regions: (light gray bars) Tuscany; (dark gray bars) regions historically renowned for the cultivation of each cultivar. Values with the same letter were not significantly different at the 5% significance level according to the Tamhane test.

high variance associated with these data, especially when peel was considered.

The skin of the two Annurca lots showed similar antiradical activities and total polyphenol concentrations. However, when individual polyphenol classes were compared, it was clear that a significant difference occurred between AIT and AIC composition, the latter being richer in anthocyanins ($P < 0.01$) and flavonols ($P < 0.01$) and poorer in procyanidins ($P < 0.01$).

The lower content of tannins in apples from Campania could be related to the more advanced ripening level of AIC fruits with respect to the AIT ones, as demonstrated by the firmness and acidity data shown in **Table 2**. In accordance with the above-mentioned hypothesis, a similar behavior was also observed in flesh, even though to a lesser extent. The more advanced ripening level of apples from Campania is probably attributable to the harvest and postharvest strategies, as well as to the climatic conditions that in southern Italy are more suitable for growing this fruit variety.

Edible Portion. Because apples are usually consumed as a whole fruit, the evaluation of the different lots on the basis of the edible portion is of great interest. **Figure 6** illustrates mean concentration and standard deviation of HPLC and F−C total polyphenols, together with RSA_{sample} values for individual lots and lots aggregated on the basis of genotype.

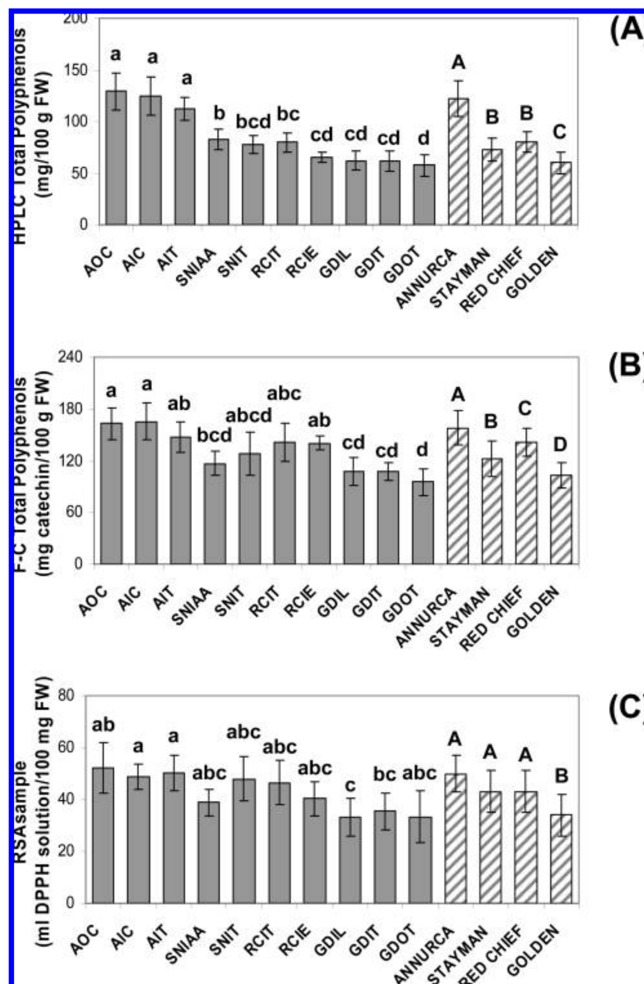

Figure 6. Mean concentrations of total polyphenols as determined by HPLC (**A**) and F−C method (**B**) and mean RSA_{sample} values (**C**) of the edible portion of the lots investigated: (dark gray bars) individual lots; (slashed bars) lots aggregated on the basis of genotype. Values with the same letter were not significantly different at the 5% significance level according to the Tamhane test.

Comparison of the four cultivars grown in the same region (Tuscany) and produced by the integrated method showed that on average, Annurca (AIT) had the highest polyphenol content and radical scavenging activity. This evaluation also indicated a statistically significant cultivar effect between Annurca and Golden Delicious ($P < 0.04$) for HPLC and F−C total polyphenol content, as well as for DPPH antiradical activity. Conversely, rural practice did not have any significant effect on polyphenol content or radical scavenging properties of either Annurca (AOC vs AIC) or Golden Delicious (GDOT vs GDIT) varieties. Our data also indicated that, for all cultivars investigated, the growing region did not influence any parameters, even though Red Chief and Stayman Neepling cultivated in Tuscany showed a mean antiradical activity appreciably higher than that determined in the other lots. Among the three effects studied, the genotype was therefore the most relevant. Comparison among the genotype-aggregated lots highlighted the superiority of Annurca, which exhibited the highest concentrations of HPLC and F−C total polyphenols, as well as the maximum RSA_{sample} value. Conversely, Golden Delicious showed the lowest values, and the differences observed with Annurca were statistically significant for all the parameters investi-

gated. Finally, Red Chief and Stayman Neepling showed intermediate and similar polyphenol contents and antiradical activities.

Concluding Remarks. The results obtained indicate that the radical scavenging activity of apples is significantly related to their polyphenol content. Considering the edible portion of the fruit, these phytochemicals contribute toward explaining approximately 90% of the overall antiradical activity, thus highlighting their important role in human health protection. Therefore, our data indirectly confirm that ascorbic acid and other antiradical molecules differing from polyphenols play a much less important role in explaining the health-protecting properties of apples.

Genotype, rural practice, and growing region all exert a differentiated influence on polyphenol concentration and antiradical activity of fruits, and peel is more easily affected than pulp, due to the role of the former toward pathogenic pressure, which mainly acts on the skin.

On the basis of data referring to the edible portion of apples, the cultivar effect is by far the most effective in determining their qualitative−quantitative polyphenolic composition and radical scavenging properties and, therefore, the consumer should mainly pay attention to this aspect when choosing fruit at the market. Annurca and Golden Delicious are respectively the best and the worst apples from the point of view of health-protecting attributes, and therefore the old adage "an apple a day keeps the doctor away" is much more valid for the former than for the latter.

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