JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

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Varietal Differences among the Phenolic Profiles and Antioxidant Activities of Seven Table Grape Cultivars Grown in the South of Italy Based on Chemometrics

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ABSTRACT: Seven table grape cultivars grown in Apulia region were considered: Italia, Baresana, Pizzutello, Red Globe, Michele Palieri, Crimson Seedless, and Thompson Seedless. Seeds, skins and pulps were extracted and analyzed for their phenolic profiles and antioxidant activities. The hierarchy in the phenolic contents was seeds, skins, and pulps. These results indicate that the intake of the whole berries (seeds included) must be strongly recommended. The highest phenolic contents were detected on Italia and Michele Palieri cv., respectively within the white and the red/black table grapes. Seeds gave a high contribution to the berry antioxidant activity, as they had higher phenolic content than skins and contained high quantities of proanthocyanidines, but the strongest antioxidant activity was shown by the pulp juices due to their content in hydroxycinnamyl acids. The principal component analysis applied to the phenolic composition and antioxidant activity of skins, pulps, and seeds allowed a good separation of Italia and Michele Palieri cultivars. According to the cluster analysis, cultivars were grouped into two clusters, one including Michele Palieri and the other one including Italia, Baresana, Pizzutello, and Thompson Seedless.

KEYWORDS: antioxidant activity, phenolic compounds, pulp, seed, skin, table grape

INTRODUCTION

Table grape is one of the most consumed fruits in the world, and phenols represent the third most abundant constituent in grapes and wines after carbohydrates and fruit acids.[1] Phenolic compounds are distributed differently among skin, pulp and seeds. The total extractable phenolics are present at only about 10% or less in pulp, 60−70% in the seeds (5−8% of weight), and 28−35% in the skin.[2] Among nonflavonoid compounds (low molecular weight), hydroxybenzoic acids are mainly located in the skin whereas hydroxycinnamic acids are located primarily in the pulp. Flavonoids are found in skins and seeds mainly as glycosides. Anthocyanins and, in minor measures, their aglycons anthocyanidins are accumulated in the skins of red grapes. Within flavanols, monomers such as catechin and epicatechin and their oligomers procyanidins B1, B2, and B3 are mainly located in seeds and the reminder in the skin. Flavonols are localized in the grape skins. Seedlessness is a highly desirable commercial quality in table grape. In fact, the most common variety of grapes consumed in the United States is Thompson Seedless.[3] Nevertheless, an offset to the improved eating quality of seedlessness is the partial loss of potential health benefits provided by the grape seeds.[2]

In a different measure, all the classes of phenolic compounds contribute to the antioxidant activities of berries, thus indicating a different contribution of the various parts of the berry to this important function. Natella et al.[4] highlighted the higher antioxidant activity of hydroxycinnamic acids compared to that of the corresponding hydroxybenzoic ones. Flavonoids possess excellent antioxidant properties related to their ability to interfere with the formation and propagation reaction of free radicals, chelate the transition metals, and inhibit the enzymes involved in the initiation reaction. Anthocyanins strongly prevent oxidation of low density lipoprotein.[5] However, in in vitro studies, the correlations between antioxidant activity and monomeric anthocyanin concentration were very low,[6] depending on assay condition, and non-anthocyanin flavonoids were found to be the main phenolic class exerting antioxidant activity on red wines produced in Brazil.[7] Flavanols and flavonols are known to be the most effective flavonoids in prevention of oxidation.[5] In a recent study performed by Granato et al.,[8] quercetin, kaempferol, rutin, ferulic acid, catechin and myricetin were the main phenolic compounds exerting antioxidant activity of South American red wines as measured by the ORAC and DPPH assays.

Phenolic content and composition are affected by ripening time, climate, soil and location of growth, but they greatly depend on grape cultivar. A study performed by Revilla et al.[9] on more than twenty table grape cultivars showed that it is possible to classify the different cultivars according to their phenolic composition and, prior to them, Fernández de Simón et al.[10] found that variations in low-molecular-weight phenolic compounds in different parts (must, skin, and seeds) of berries of Cencibel variety during ripening were chiefly quantitative.

On the other hand, the importance of phenolic compounds is mainly related to their contribution to human health through their multiple biological effects such as antioxidant activity, antimutagenic and/or anticarcinogenic activities, and anti-inflammatory action.[11,12] Phenolic compounds act as reducing agents by trapping free radicals, acting as chelators, donating hydrogen, and quenching singlet oxygen. These highly reactive species are present in biological systems and may oxidize lipids,

Received: March 14, 2011
Accepted: August 24, 2011
Revised: August 24, 2011
Published: August 24, 2011

Table 1. Phenolic, Anthocyanin, Flavonoid, Flavan Reactive with Vanillin, Proanthocyanidins, and Hydroxycinnamoyl Tartaric Acid Contents of the Various Parts of Table Grape Berries[a]

		white grapes				red/black grapes		
		Baresana	Italia	Pizzutello	Thompson Seedless	Crimson Seedless	Michele Palieri	Red Globe
TPC	skins	39.6 ± 6.7 c (18%)	49.3 ± 10.3 d (22%)	29.4 ± 4.2 b (15%)	22.7 ± 4.6 a (20%)	62.9 ± 13.6 c (20%)	44.6 ± 6.7 b (16%)	33.1 ± 6.9 a (21%)
	seeds	116 ± 23 a (21%)	183 ± 20 b (10%)	154 ± 21 c (15%)			189 ± 38 b (21%)	111 ± 12 a (11%)
	pulp juices	0.395 ± 0.025 a (7%)	0.745 ± 0.102 c (15%)	0.503 ± 0.059 b (15%)	0.823 ± 0.080 d (10%)	0.617 ± 0.057 c (10%)	0.449 ± 0.036 b (10%)	0.349 ± 0.049 a (15%)
TA	skins					8.30 ± 1.88 a (24%)	13.3 ± 1.8 c (15%)	10.1 ± 1.1 b (12%)
TF	skins	25.9 ± 3.1 c (14%)	24.9 ± 2.2 c (9%)	19.3 ± 1.9 b (10%)	15.8 ± 2.911 a (18%)	37.2 ± 5.5 b (15%)	35.6 ± 1.6 b (5%)	25.7 ± 4.5 a (18%)
	seeds	55.7 ± 12.5 a (23%)	110 ± 11 b (10%)	62.3 ± 3.9 a (6%)			37.9 ± 5.1 b (14%)	26.3 ± 1.9 a (10%)
F	skins	13.5 ± 2.8 b (20%)	17.5 ± 3.3 c (20%)	12.3 ± 1.4 b (12%)	4.28 ± 0.45 a (13%)	22.1 ± 3.4 b (16%)	21.7 ± 2.3 b (12%)	9.59 ± 1.91 a (20%)
	seeds	71.3 ± 11.8 a (18%)	105 ± 13 b (14%)	70.8 ± 12.2 a (18%)			120 ± 22 b (20%)	69.2 ± 8.7 a (13%)
P	skins	3.45 ± 0.64 c (20%)	2.50 ± 0.46 b (20%)	2.39 ± 0.24 b (10%)	1.24 ± 0.22 a (18%)	3.96 ± 0.67 c (18%)	3.10 ± 0.47 b (15%)	2.30 ± 0.37 a (18%)
	seeds	8.31 ± 0.68 a (10%)	12.8 ± 0.9 b (8%)	7.68 ± 0.90 a (13%)			10.7 ± 1.9 b (20%)	7.86 ± 0.43 a (5%)
HCTA	pulp juices	0.013 ± 0.001 a (34%)	0.022 ± 0.003 c (14%)	0.016 ± 0.002 a,b (13%)	0.019 ± 0.001 b (6%)	0.023 ± 0.003 b (14%)	0.040 ± 0.005 c (14%)	0.011 ± 0.001 a (10%)

[a] Phenolic: TPC, g of gallic acid/kg of dry skins or seeds and g of gallic acid/L of pulp juices. Anthocyanin: TA, g of gallic acid/kg of dry skins. Flavonoid: TF, g of (+)-catechin/kg of dry matter. Flavan reactive with vanillin: F, g of (+)-catechin/kg of dry matter. Proanthocyanidins: P, g of cyanidin chloride/kg of dry matter. Hydroxycinnamoyl tartaric acid: HCTA, g of caffeic acid/L of pulp juice. Within each of the two groups (white and red grapes), different letters indicate significant differences at $p < 0.05$. Coefficients of variation reported within parentheses are referred to the variability within batches of the same cultivar (not within replicates).

proteins, and nucleic acids. Phenolic compounds also contribute to the sensory quality of foods (color, astringency, and bitterness).[13]

The main aim of this study was to investigate and contribute to knowledge of the phenolic composition and antioxidant activities of skins, seeds, and pulps of the following seven table grape cultivars grown in Apulia, a region placed in the south of Italy: Thompson Seedless, Italia, Baresana, Pizzutello, Crimson Seedless, Red Globe, and Michele Palieri. The choice of cultivars Thompson Seedless (the name given in the U.S. to Sultana grape, in honor of William Thompson Seedless, one of the first growers in California) and Crimson Seedless (established at the University of California), was due to the marked preference of world consumers for fresh seedless table grapes. Red Globe (established at the University of California) and Italia (a vine from the crossbreed of Bicane and Moscato of Amburgo, established in 1911) are among the most cultivated ones. Michele Palieri, whose name stems from Michele Palieri who manufactures it by interbreeding the varieties Alphonse Lavallée and Red Malaga, is known as "the grape of diabetics" since it has lower sugar content compared to that of the other cultivars. Baresana has eastern origin and is mainly cultivated in the Apulia region (Italy) whereas Pizzutello is also known as Teta de Vacca and Dedos de Doncella in Spain, Nab el Djemel in Algeria, Lady Finger in Argentina, and Cornichon blanc in France. The possibility to classify the different cultivars according to their phenolic composition was also checked using a chemometric approach.

MATERIALS AND METHODS

Samples. Four white table grapes (Thompson Seedless, Italia, Baresana, and Pizzutello) and 3 red/black table grapes (Crimson Seedless, Red Globe, and Michele Palieri) were purchased from local fruit markets. Five batches, differing for suppliers and places of origin and consisting of approximately 5 kg of clusters, were withdrawn for each of the considered cultivars.

For each batch, three 100-berry samples were selected.

Extraction Procedures. Extraction of the phenolic fraction from skin, pulp and seeds has been made according to Di Stefano and Cravero.[14]

From the 100-berry samples, lots of 10 berries were peeled and the seeds were separated from the pulps. In order to avoid undesired browning, pulps were immediately combined with 50 mg of $K_2S_2O_5$; and, with the aim of protecting them from exposure to air, after peeling and addition of metabisulfite, pulps were immediately separated from seeds, crushed and centrifuged. Peeling and separation of seeds from pulps can be performed only manually with the assistance of stainless steel tweezers and, besides the reducing the working time and limiting the contact with oxygen, all the operations were performed under a laminar flow cabinet near a Bunsen type flame. Skins and seeds were immediately submitted to the extraction procedures, whereas pulps were crushed, the recovered juices were centrifuged at 6500*g* for 15 min, and the supernatants were filtered through common filter paper, weighed, and combined with concentrated H_2SO_4 10 N in order to avoid tartaric precipitation (juices:H_2SO_4 10 N, 9:1). The acidified juices were filtered and immediately analyzed or stored at −18 °C. The skins from lots were weighed and combined with 25 mL of a solution of ethanol:water: hydrogen chloride 37% (70:30:1). After 24 h under dark conditions, the mixtures were filtered and immediately analyzed or stored at −18 °C.

The seeds from lots of 10 berries were weighed and ground, and to the mixture was added 100 mL of a tartaric buffer (1 L is made of 500 mL of distilled water, 5 g of tartaric acid, 22 mL of NaOH 1 N, 600 mg of

$Na_2S_2O_5$, 500 mg of a mixture of pectinase and cellulase, 120 mL of ethanol, and distilled water) at pH 3.2. After 48 h at 37 °C, the mixtures were filtered and immediately analyzed or stored at −18 °C.

Skin extracts were analyzed for phenolic, anthocyanin, flavonoid, flavan, and proanthocyanidin contents, phenolic profiles, and antioxidant activities. Seed extracts were analyzed for phenolic, flavonoid, flavan, and proanthocyanidin contents, phenolic profiles, and antioxidant activities. Juices were analyzed for their contents in hydroxycinnamoyl tartaric acids and their antioxidant activities.

Total Phenolic Content. The total phenolic content (TPC) was measured at 765 nm through a UV−visible spectrophotometer (Varian Cary 50 SCAN, Palo Alto, CA, USA) according to the Folin−Ciocalteu method as reported by Singleton and Rossi.[15] Results were expressed as gallic acid equivalents (mg/kg of dry matter). A calibration line was built on the basis of solutions at known and increasing concentrations of gallic acid (ExtraSynthese, Genay, France).

Anthocyanin, Flavonoids, Flavans, Proanthocyanidins, and Hydroxycinnamoyl Tartaric Acids. They were determined according to the methods of Di Stefano et al.[16] and Di Stefano and Cravero.[14] When necessary, extracts were opportunely diluted with aliquots of the extraction solution. An absorbance spectrum between 230 and 700 nm was recorded.

The total anthocyanin contents (TA) were measured at 540 nm and expressed as mg per kg of dry matter.

The total flavonoids (TF) were calculated on the basis of their absorbance at 280 nm and the results expressed as mg of catechin/kg of dry matter.

Flavans (F), expressed as mg of catechin/kg of dry matter, were determined using the vanillin assay at 500 nm. The test is specific for flavan-3-ols, proanthocyanins, and dihydrochalcones having a single bond at the 2,3-position and free metahydroxy groups on the B-ring.

Proanthocyanidins (P) are tannins derived from polymerization of elementary molecules of 3-flavonols (catechins) and of 3,4-flavandiols (leucoanthocyanidins). They were measured at 532 nm after acid hydrolysis at high temperature and expressed as mg of cyanidin chloride/kg of dry matter.

The hydroxycinnamoyl tartaric acids, expressed as mg of caffeic acid/L of pulp juice, were determined on the pulp juices acidified with sulfuric acid on the basis of their absorbance at 320 nm.

Phenolic Profiles of Skins, Seeds, and Pulp Juices. The HPLC-DAD analyses were performed in an apparatus consisting of a degasser model G1322A, a binary pump model G1312A, an autosampler model G1329A equipped with a 20 μL loop, and a diode array detector model G1315D (Agilent, Santa Clara, CA, USA). Data were collected and processed through a 2DChemstation G2175BA Rev. B 04 02 (Agilent, Santa Clara, CA, USA). Chromatographic separation was carried out according to the method of Revilla and Ryan[17] opportunely modified. The extracts and the pulp juices were previously filtered, injected into a Zorbax SB C18 (100 × 4.6 mm, 1.8 μm, Agilent, Santa Clara, CA, USA) column protected by a guard column, and eluted at flow rate of 0.5 mL/min. Solvent A was represented by water−acetonitrile (95:5) adjusted to pH 1.8 with perchloric acid whereas solvent B was water−acetonitrile (50:50) adjusted to pH 1.8 with perchloric acid. The gradient program of solvent A was as follows: 0 min 95%, 4.8 min 90%, 16.8 min 80%, 21.6 min 70%, 31.2 min 60%, 40.8 min 55%, 48 min 0%, 58 min 0%, 60 min 95%, 80 min 95%. Detection was performed at 520 nm for anthocyanins; 280 nm for gallic acid, procyanidin B1, procyanidin B2, catechin, epicatechin, epigallocatechin, epicatechingallates, and epigallocatechingallates; 313 nm for *trans*-caffeoyltartaric, *trans*-coumaroyltartaric, and caffeic acid; 350 nm for rutin and quercetin. Quantification of phenolic compounds was made on the basis of standard calibration curves.

The identification of phenolic components was carried out by comparing spectra and peak retention times of the 26 pure standards with those of the samples and by injection of samples and standards in a HPLC−MS apparatus consisting of a degasser model G1379A, a binary pump model G1376A solvent delivery, an auto sampler model G1377A, a DAD model G1315C, and an XCT-trap Plus mass detector model G2447A (Agilent, Santa Clara, CA, USA) coupled with a pneumatic

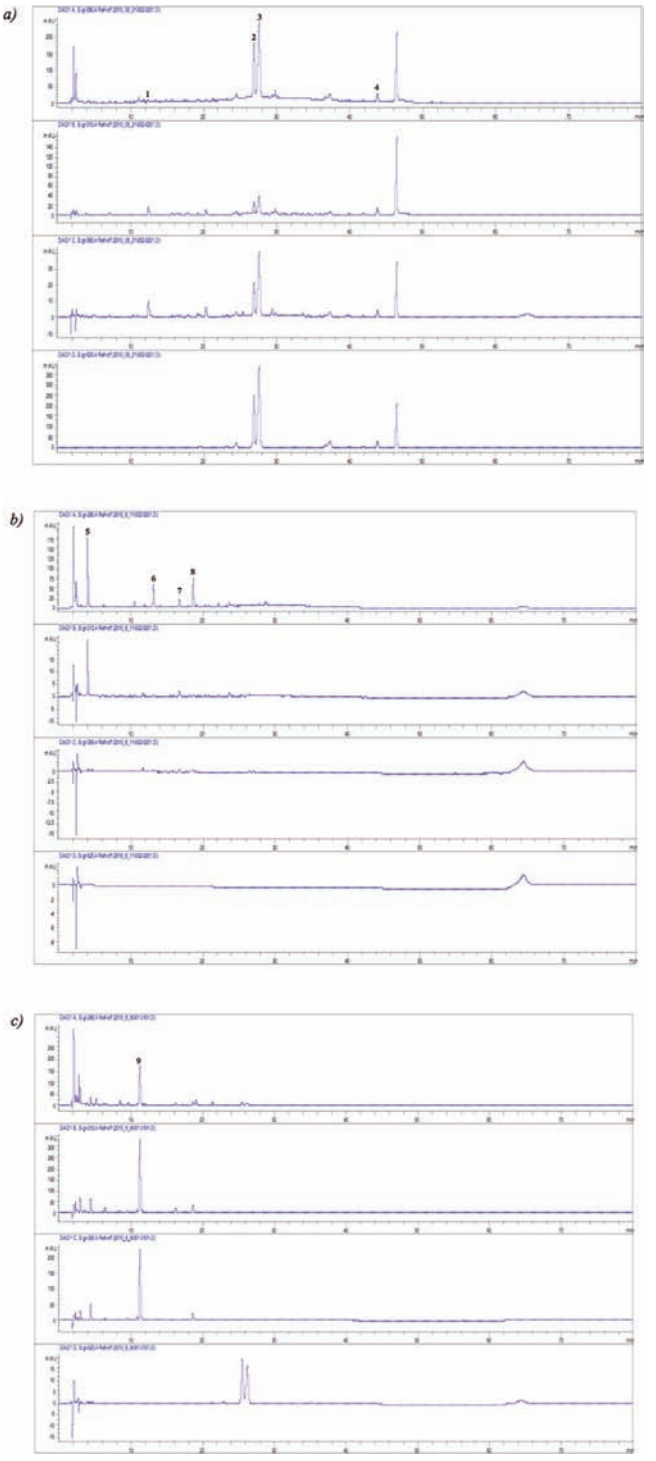

Figure 1. HPLC-DAD chromatograms of (a) skins, (b) seeds, and (c) pulps of *Michele Palieri* grape. Identification of the main peaks: (1) chorogenic acid, (2) peonidin-3-*O*-glucoside, (3) malvidin-3-*O*-glucoside, (4) *trans*-cinnamic acid, (5) gallic acid, (6) catechin, (7) procyanidin B2, (8) epicatechin, (9) protocatechualdehyde.

Table 2. Phenolic Compounds Identified in Grape Skins, Seeds (mg gallic acid equivalents/kg dry matter), and Pulp Juices (mg gallic acid equivalents/L)

	white grapes				red/black grapes		
compd (retention time, min)	Baresana	Italia	Pizzutello	Thompson Seedless	Crimson Seedless	Red Globe	Michele Palieri
			Skins				
procyanidin b1 (10.4)	58 ± 6 (10%)[a]						
protocatechualdehyde (10.7)						112 ± 28 (25%)	
4-hydroxybenzoic acid (11.8)	143 ± 9 (7%)	211 ± 91 (45%)	37 ± 9 (25%)				
chlorogenic acid (13.2)							49 ± 9 (20%)
2,5-dihydroxybenzoic acid (15.0)						31 ± 2 (8%)	
caffeic acid (15.3)	103 ± 3 (5%)						
syringic acid (16.9)	62 ± 9 (15%)	39 ± 1 (5%)					
peonidin-3-*O*-glucoside (26.7)					1825 ± 101 (5%)	2309 ± 453 (20%)	731 ± 213 (30%)
cyanidin-3-*O*-glucoside (27.2)					246 ± 18 (7%)		
malvidin-3-*O*-glucoside (27.5)							1511 ± 349 (23%)
quercetin-3-*O*-glucopyranoside (28.5)							2528 ± 211 (10%)
resveratrol (39.5)						61 ± 17 (29%)	
trans-cinnamic acid (44.6)						141 ± 39 (29%)	68 ± 8 (15%)
other phenolics	3	10	3	2	3	4	10
			Seeds				
gallic acid (3.9)	889 ± 28 (5%)	874 ± 244 (29%)	990 ± 433 (45%)			584 ± 70 (12%)	2359 ± 448 (20%)
procyanidin b1 (10.3)		307 ± 56 (20%)				179 ± 30 (18%)	
4-hydroxybenzoic acid (11.8)		277 ± 56 (20%)				144 ± 11 (8%)	
catechin (12.9)	406 ± 182 (46%)	281 ± 89 (32%)	1716 ± 586 (35%)			264 ± 56 (20%)	830 ± 275 (34%)
procyanidin b2 (16.5)		280 ± 69 (26%)	267 ± 39 (20%)			339 ± 64 (20%)	208 ± 30 (15%)
epicatechin (18.6)	530 ± 156 (30%)	213 ± 69 (32%)	1346 ± 358 (27%)			683 ± 177 (27%)	353 ± 49 (15%)
other phenolics	1	10				4	2
			Pulp Juices				
gallic acid (3.9)		3 ± 1 (35%)					
3,4-dihydroxyphenylacetic acid (8.9)			4 ± 1 (25%)			4 ± 0 (0%)	
protocatechualdehyde (10.7)	1 ± 0 (0%)	2 ± 1 (55%)					21 ± 4 (20%)
4-hydroxybenzoic acid (11.8)		19 ± 9 (48%)				1 ± 0 (0%)	
caffeic acid (15.6)		4 ± 1 (26%)	14 ± 3 (21%)				
syringic acid (16.9)			3 ± 0 (0%)				
other phenolics	3	12	7	5	12	6	12

[a] Coefficients of variation reported within parentheses refer to the variability within batches of the same cultivar (not within replicates).

nebulizer assisted electrospray LC−MS interface. Positive electrospray mode was used for the ionization of molecules with capillary voltage at −3500 V and skimmer voltage at 40 V. The nebulizer pressure was 40 psi, and the nitrogen flow rate was 10 L/min. Temperature of drying gas was 350 °C. In the full scan mode, the monitored mass range was from m/z 100 to 800. Column and operative conditions were the same as used in the HPLC-DAD analysis. The injection volume was 8 μL.

Results were expressed as mg per kilogram of dry matter (skins and seeds) and per liter of pulp juices.

Antioxidant Activity. The evaluation of the antioxidant activity of pulp juices and skin and seed extracts was made according to the DPPH and ABTS methods.

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) method[18] measures the free radical scavenging capacity of pulp juices and skin and seed extracts. Increasing aliquots (12.5, 25, 50, 100, 200, 400, and 800 μL) of juices and extracts were mixed with 4 mL of a 6×10^{-5} M methanolic solution of the stable organic radical DPPH (DPPH$^{\bullet}$). The absorbance at 515 nm was read at regular intervals of time until the end of the reaction, with respect to a reference solution represented by 9 mL of methanolic solution of DPPH$^{\bullet}$ + 100 μL of methanol. The percent inhibition of the DPPH$^{\bullet}$ by each dilution of samples was calculated considering the percentage of the steady DPPH$^{\bullet}$ in solution after reaction. Results were expressed as the amount of dry matter (for seeds and skins) or the volume of pulp juice that gives rise to a 50% reduction in DPPH$^{\bullet}$).

The 2,2-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS$^{+\bullet}$)−metmyoglobin method[19] measures the absorbance of the chromophore ABTS radical cation at 734 nm. Antioxidant activity was expressed as percentage of inhibition of the ABTS radical cation formation. It was necessary to opportunely dilute the extracts in order to keep their absorbance in the scale between the maximum value of the control and

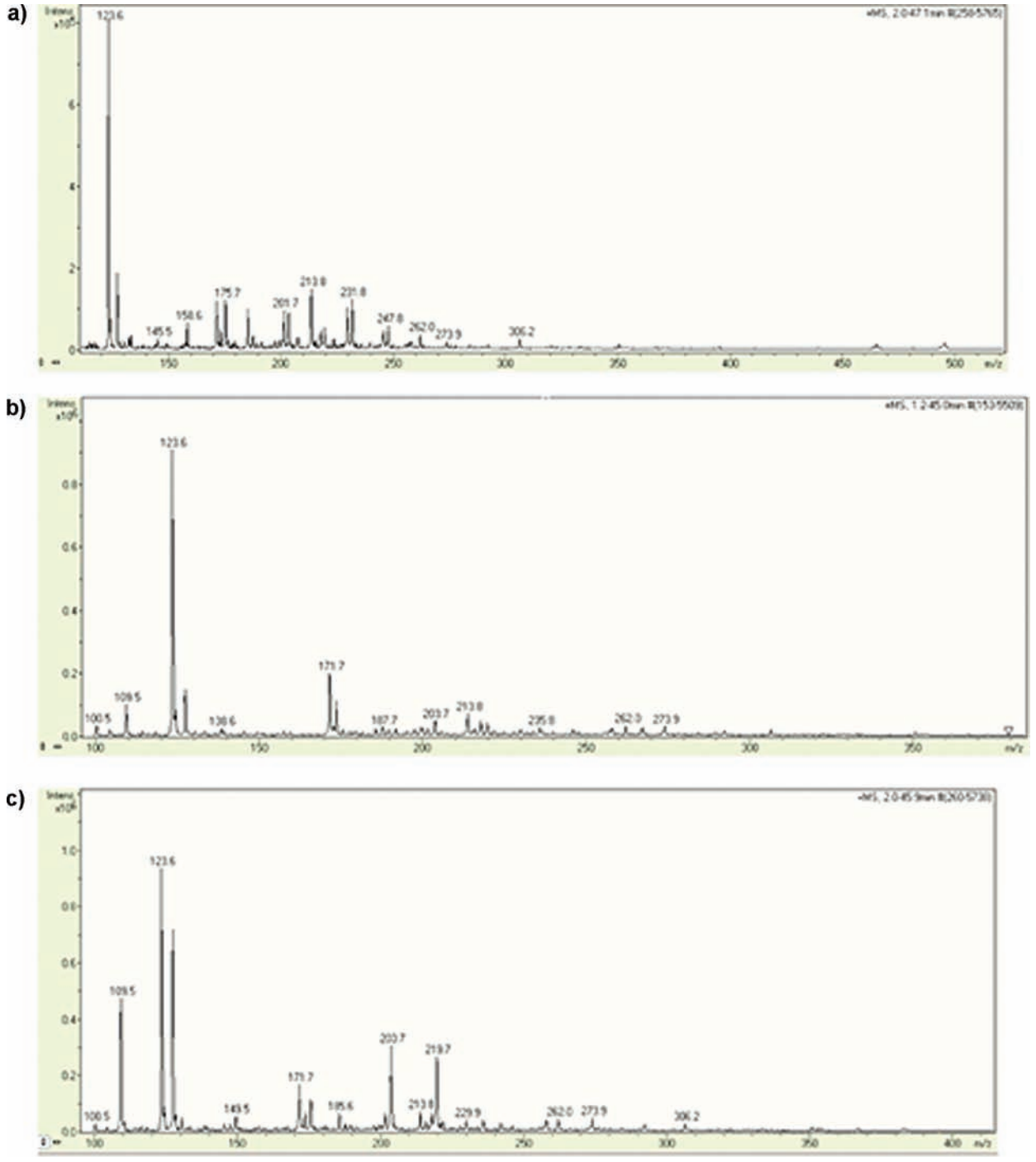

Figure 2. HPLC−MS chromatograms of (a) skins, (b) seeds, and (c) pulps of Michele Palieri grape.

the minimum value represented by the Trolox in the extraction solution. Results were expressed as the amount of dry matter (for seeds and skins) or the volume of pulp juice that gives rise to an antioxidant activity equal to 50% of the antioxidant activity of a solution of Trolox 2.5 mM.

Statistical Analysis. The averages and the standard deviations were calculated using Excel software ver. 11.5.1 (Microsoft, Redmond, WA). The analysis of variance (one-way ANOVA) followed by Duncan's test was applied to highlighted significant differences among samples. In order to highlight relationships between phenolic content and antioxidant activity, linear regression analyses were performed between the TPC (or each class of phenolic compounds) and the antioxidant activity measured according to the DPPH and ABTS methods. The relative determination coefficients (R^2 adjusted) and correlation coefficients (R) were reported. A multiple regression model incorporating all the classes of phenolic compounds was also applied. The best subgroups of tested variables were obtained on the basis of the highest multiple determination coefficient (R^2 adjusted). Analysis of variance, simple correlations, and multiple regressions were performed at $p < 0.05$. PCA was applied to separate the cultivars according to phenolic composition and antioxidant activity of skins, seeds, and pulps. Among the eigenvalues, those showing absolute values higher than 0.1 were adopted to explain the projection of the samples on the factor-plane. The data were autoscaled before

Table 3. Antioxidant Activities of Skins, Seeds, and Pulp Juices Measured Using DPPH[a] and ABTS[b]

	white grapes				red/black grapes		
	Baresana	Italia	Pizzutello	Thompson Seedless	Crimson Seedless	Michele Palieri	Red Globe
				Skins			
DPPH	61.5 ± 3.5 c[c] (7%)[d]	58.2 ± 6.4 c (12%)	84.9 ± 10.6 b (13%)	153.8 ± 19.3 a (12%)	54.5 ± 11.4 c (21%)	61.3 ± 3.0 b (5%)	66.9 ± 11.3 a (18%)
ABTS	53.3 ± 8.5 c (17%)	64.5 ± 11.6 b (19%)	199.4 ± 3.4 a (13%)	205.4 ± 23.0 a (12%)	49.6 ± 8.8 c (19%)	95.1 ± 14.4 b (16%)	132.7 ± 16.4 a (13%)
				Seeds			
DPPH	21.3 ± 3.6 a (18%)	15.3 ± 0.9 c (7%)	19.3 ± 2.5 b (14%)			14.6 ± 1.1 b (8%)	20.6 ± 1.6 a (9%)
ABTS	32.8 ± 5.6 a (18%)	17.3 ± 1.4 c (8%)	25.2 ± 2.6 b (11%)			20.3 ± 2.2 a (11%)	11.7 ± 0.6 b (6%)
				Pulp Juices			
DPPH	3.27 ± 0.1 c (0%)	6.54 ± 1.08 a (18%)	5.45 ± 1.10 b (21%)	5.45 ± 0.2 b (0%)	4.3 ± 0.1 c (0%)	7.64 ± 1.08 b (15%)	8.71 ± 1.09 a (13%)

[a] μg of dry skins/seeds or μL of pulp juice that gives rise to a 50% reduction in 2 mL of a 6×10^{-5} M DPPH$^{\bullet}$ methanolic solution. [b] μg of dry skins/seeds or μL of pulp juice that gives rise to an antioxidant activity equal to 50% of the antioxidant activity of a solution of Trolox 2.5 mM. [c] In a line, within each of the two groups (white and red grapes), different letters indicate significant differences at $p < 0.05$. [d] Coefficients of variation reported within parentheses refer to the variability within batches of the same cultivar (not within replicates).

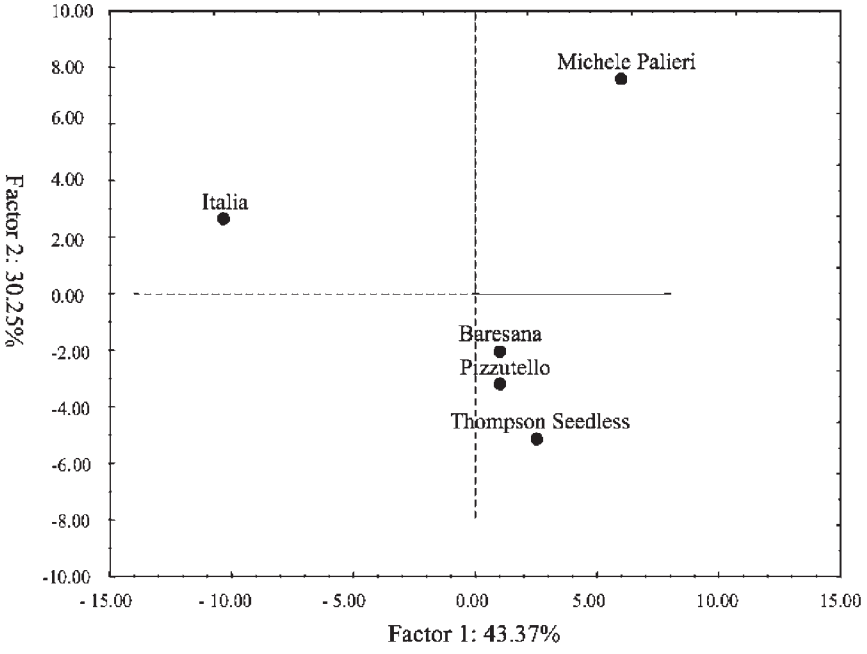

Figure 3. PCA of phenolic classes, phenolic profiles, and antioxidant activity of skins, seeds, and pulps of the seven table grape cultivars: projection of the samples on the factor plane.

analysis. A uniform hierarchical cluster analysis (HCA) methodology was also applied. HCA performed using the tree clustering method and Euclidean distances generated a dendrogram for samples whereas the *k*-means clustering was used to highlight the number of clusters in which cultivars could be grouped.

All the statistical analyses were made by the software Winstat ver. 5.1 (Statsoft, Tulsa, OK).

RESULTS AND DISCUSSION

Phenolic Composition of Skins, Seeds, and Pulp Juices. Table 1 shows the total phenolic contents and the amounts of the different classes of phenolics in skins, seeds, and pulp juices of table grapes. The amounts of each class of phenolics, expressed as mg per kg of dry matter, were always higher in seeds than in skins.[20] Within the white cultivars, their ratio ranged from 0.19 (Pizzutello) to 0.34 (Baresana) for the total phenolic content, from 0.23 (Italia) to 0.46 (Baresana) for the total flavonoid content, from 0.17 (Italia and Pizzutello) to 0.19 (Baresana) for flavans, and from 0.20 (Italia) to 0.41 (Baresana) for proanthocyanidins. This means that, for each class of phenolic compounds, the skins of Baresana had the highest relative-to-seeds concentrations among the considered cultivars. Concerning the absolute concentrations of phenolics in skins, Italia cv. showed the highest total phenolics, total flavonoids (together with Baresana), and flavans whereas Baresana had the highest proanthocyanidin content. Among seeds, Pizzutello cv. was the richest in total phenolics, and Italia cv. had the highest total flavonoids, flavans, and proanthocyanidins. Among pulp juices, the highest total phenolic content was detected in Thompson Seedless whereas Italia cv. had the highest concentration of hydroxycinnamoyl tartaric acids.

Within the red/black grapes, their ratio ranged from 0.24 (Michele Palieri) to 0.30 (Red Globe) for the total phenolic content, from 0.94 (Michele Palieri) to 0.98 (Red Globe) for the total flavonoid content, and from 0.18 (Michele Palieri) to 0.14 (Red Globe) for flavonoids, and it was equal to 0.29 both in Michele Palieri and Red Globe for proanthocyanidins. Concerning the absolute concentrations of phenolics in skins, Crimson Seedless cv. showed the highest total phenolics, total flavonoids (together with Michele Palieri), flavans (together with Michele Palieri) and proanthocyanidins whereas Michele Palieri had the highest total anthocyanin content. Among seeds, the highest amounts of all the phenolic classes were detected in Michele Palieri cv. Among pulp juices, the highest total phenolic content was detected in Crimson Seedless whereas Michele Palieri cv. showed the highest concentration of hydroxycinnamoyl tartaric acids. With the exception of the total flavonoids, the lowest amounts of each class of phenolic compounds were detected on Red Globe cv.

Generally, the total phenolic of red grape skins is greatly higher than that of white grapes due to the ability to produce anthocyanins. However, the experimental data described above showed that the phenolic content of different grapes depends mainly on the varietal differences, not on grape skin color, thus confirming the results of Yang et al.[21] Another important finding is that the above-reported results are confirmation of the varietal dependence of content of total polyphenols and also individual phenolic subgroups, as already pointed out by other authors.[22]

The total phenolic content of pulp juices as determined by the Folin−Ciocalteau method could be altered by a series of interferences. Because the color formation of the Folin−Ciocalteau reaction is based on chemical reduction of the reagent, this reaction suffered for interference from a number of sources. In grape juice, the principal interfering compounds are sulfur dioxide and sugars. Sugars indirectly enhance the readings of other analytes whereas, concerning the interference of SO_2, presumably, the phenols are oxidized by the Folin−Ciocalteau reagent and then reduced by the sulfur dioxide, creating an interfering response by a type of catalytic cycle. The magnitude of the interference is not constant,[23] though approximate mass correction factors of 0.1 to 0.2 have been suggested (thus, 10 mg/L sulfur dioxide would yield a response of 1 to 2 mg/L in the Folin−Ciocalteau assay).[24] This means that probably the real concentrations of phenolics in grape pulps were slightly different (few mg per liter) from those calculated but the differences were not significant. Nevertheless, the literature included several examples of addition of $K_2S_2O_5$ to grapes prior to phenolic extraction.[25−27]

For each class of phenolic compounds, both in white and in red/black grapes, seeds showed contents higher than skins. According to these results, the fresh consumption of the whole berries and the incorporation of seeds into preparation such as juices and purée are strongly recommended.

The variability over the batches of each cultivar, expressed as the percent coefficients of variation (ratio between standard deviations and mean values multiplied by 100), was very low, including, for each cultivar, between 6 and 24%, except for HCTA that, in the case of Baresana, was equal to 34%. These results demonstrated that the main source of variance is attributable to differences among grape cultivars and did not come from the variability of the method.

Phenolic Profiles of Skins, Seeds, and Pulp Juices. Examples of chromatograms of skin, seeds, and pulp juices are shown in Figure 1 whereas the phenolic compounds identified in skins, seeds, and pulps are presented in Table 2. Although the phenolic profiles of table grapes are less complex than those of wine grapes, large differences in the phenolic composition and amounts were found among the investigated grape cultivars. Not all the phenolics were identified, and the structure of some of them is the object of further investigation due to the similarity of their structure and the consequent little differences in their spectra. Nevertheless, the unidentified compounds were clearly distinguishable on the basis of their specific retention times.

Among white grapes, the skins of Italia cv. had the highest number of peaks (12, sum of identified and unknown compounds) whereas those of Thompson Seedless cv. showed the simplest phenolic profile with only 2 peaks. The phenolics identified were mainly procyanidin B1 (in Baresana), benzoic (4-hydroxybenzoic, in Baresana, Italia, and Pizzutello, and syringic in Baresana) and cinnamic (caffeic, in Baresana) acids. Any one of the phenolics was detected in all the cultivars with the exception of an unidentified compound having a retention time of 28.7 min. Concerning the red grapes, the highest[15] and the lowest[5] number of phenolic compounds were detected on skins of Michele Palieri and Crimson Seedless cv., respectively. The compounds detected mainly belong to the class of anthocyanins (peonidin-3-*O*-glucoside in all the cultivars, cyanidin-3-*O*-glucoside in Crimson, malvidin-3-*O*-glucoside in Michele Palieri) and a flavan-3-ol (quercetin-3-*O*-glucoside in Michele Palieri). Small amounts of protocatechualdehyde, resveratrol, and 2,5-dihydroxybenzoic acid were found in Red Globe samples whereas chlorogenic acid was detected in Michele Palieri and *trans*-cinnamic acid in Red Globe and Michele Palieri.

Concerning seeds, Italia was the white cultivar in which the highest number of phenolic compounds[16] were detected whereas only 4 peaks were found in the chromatograms of Baresana. Among the red grapes, the most complex phenolic profile was detected on Red Globe seeds[10] whereas only 6 peaks were present in the chromatograms of Michele Palieri seeds. The main phenolics identified were benzoic acids (gallic and 4-hydroxybenzoic), catechin and epicatechin, found in all white and red cultivars, and procyanidins B1 (in Italia and Red Globe) and B2 (in Italia, Pizzutello, Red Globe, and Michele Palieri).

Among white grapes, the pulps of Italia cv. had the highest number of peaks[16] whereas those of Thompson Seedless cv. showed the simplest phenolic profile with only 4 peaks. The phenolics identified were gallic, 3,4-dihydroxyphenylacetic, and 4-hydroxybenzoic acids and protocatechualdehyde. Anyone of the phenolics was detected in all the cultivars. Among the red cultivars, the pulps of Michele Palieri and Red Globe showed the phenolic profiles having the highest[13] and the lowest[8] number of peaks, respectively. Three unidentified compounds (retention times 2.5, 2.7, and 3 min) were detected in all the red cultivars. The low level of caffeic and gallic acids (*o*-diphenols) could be due to an initial oxidation during the sample preparation (peeling) that gave rise to quinone compounds. Also, the 4-hydroxybenzoic acid is a potent antioxidant compound and probably rapidly oxidized during grape peeling.

The variability over the batches of each cultivar, expressed as the percent coefficient of variation, was generally below 30%, with some exception concerning compounds such as catechin, procyanidin B2, epicatechin, gallic acid, protocatechualdehyde, and 4-hydroxybenzoic acid. Also in this case, it was highlighted that cultivars represented the main source of variance, even in the presence of very low concentrations of phenolic compounds that affected the coefficients of variation.

Table 4. Eigenvectors of the Included Variables in Principal Component Analysis of Figure 3 on the Two Principal Components (Factors 1 and 2)

variables	PCA factor 1	PCA factor 2
P[a]-unidentified peak (2.0)	0.106	0.109
P-unidentified peak (2.5)	0.095	−0.044
P-unidentified peak (2.7)	−0.021	0.098
P-unidentified peak (3.0)	−0.029	**0.183**[b]
P-unidentified peak (3.3)	0.088	**0.162**
P-gallic acid (3.9)	**-0.154**	0.056
P-unidentified peak (4.4)	−0.135	0.100
P-unidentified peak (5.1)	**-0.140**	0.093
P-unidentified peak (5.2)	**-0.126**	−0.040
P-unidentified peak (5.5)	**-0.150**	0.048
P-unidentified peak (5.8)	0.007	−0.065
P-unidentified peak (6.4)	**-0.154**	0.056
P-unidentified peak (8.1)	**-0.154**	0.056
P-unidentified peak (8.4)	0.088	**0.162**
P-3,4-dihydroxyphenylacetic acid (8.9)	0.015	−0.068
P-unidentified peak (9.5)	0.000	**0.160**
P-protocatechualdehyde (10.7)	0.074	**0.172**
P-unidentified peak (11.2)	0.044	**-0.146**
P-unidentified peak (11.4)	0.088	**0.162**
P-4-hydroxybenzoic acid (11.8)	**-0.154**	0.056
P-caffeic acid (15.6)	−0.033	−0.052
P-syringic acid (16.9)	0.015	−0.068
P-unidentified peak (18.5)	−0.154	0.056
P-unidentified peak (19)	0.015	−0.068
S-unidentified peak (2.2)	**-0.154**	0.056
S-unidentified peak (2.5)	**-0.154**	0.056
S-unidentified peak (2.9)	**-0.154**	0.056
S-unidentified peak (4.7)	**-0.154**	0.056
S-unidentified peak (10.4)	0.014	−0.042
S-unidentified peak (10.9)	**-0.154**	0.056
S-4-hydroxybenzoic acid (11.8)	**-0.141**	0.016
S-chlorogenic acid (13.2)	0.088	**0.162**
S-caffeic acid (15.3)	0.014	−0.042
S-unidentified peak (15.6)	**-0.142**	0.017
S-syringic acid (16.9)	−0.078	−0.007
S-unidentified peak (19.3)	0.037	**-0.108**
S-unidentified peak (19.9)	**-0.137**	0.011
S-unidentified peak (21.4)	0.088	**0.162**
S-unidentified peak (22.6)	0.088	**0.162**
S-unidentified peak (23.6)	0.096	**0.150**
S-unidentified peak (24.6)	0.088	**0.162**
S-unidentified peak (25.2)	0.088	**0.162**
S-unidentified peak (25.9)	0.088	**0.162**
S-peonidin-3-*O*-glucoside (26.7)	0.088	**0.162**
S-malvidin-3-*O*-glucoside (27.5)	0.088	**0.162**
S-unidentified peak (27.7)	**-0.154**	0.056
S-unidentified peak (27.9)	**-0.154**	0.056
S-quercetin-3-*O*-glucopyranoside (28.5)	0.088	**0.162**
S-unidentified peak (28.7)	−0.032	**-0.161**
S-unidentified peak (29.1)	0.088	**0.162**
S-unidentified peak (38.3)	0.088	**0.162**
S-unidentified peak (43.8)	0.088	**0.162**
S-*trans*-cinnamic acid (44.6)	0.088	**0.162**
S-unidentified peak (46.7)	0.088	**0.162**
Se-gallic acid (3.9)	0.053	**0.172**
Se-unidentified peak (6.1)	**-0.154**	0.056
Se-procyanidin B1 (10.3)	**-0.154**	0.056
Se-4-hydroxybenzoic acid (11.8)	**-0.154**	0.056
Se-catechin (12.9)	0.041	0.011
Se-unidentified peak (13.8)	**-0.154**	0.056
Se-unidentified peak (14.5)	**-0.154**	0.056
Se-procyanidin B2 (16.5)	−0.066	**0.100**
Se-epicatechin (18.6)	0.022	−0.038
Se-unidentified peak (18.9)	**-0.154**	0.056
Se-unidentified peak (19.9)	**-0.154**	0.056
Se-unidentified peak (20.5)	**-0.154**	0.056
Se-unidentified peak (21.9)	0.011	**0.190**
Se-unidentified peak (23.5)	**-0.154**	0.056
Se-unidentified peak (27.7)	**-0.154**	0.056
Se-unidentified peak (28.6)	**0.101**	0.058
Se-unidentified peak (28.8)	**-0.154**	0.056
S-TPC	**-0.125**	0.089
Se-TPC	−0.093	0.083
P-TPC	−0.086	−0.088
S-TA	0.088	**0.162**
S-TF	−0.033	**0.143**
Se-TF	**-0.139**	0.042
S-F	**-0.120**	0.061
Se-F	−0.093	**0.108**
S-P	−0.029	0.041
Se-P	−0.101	0.109
P-HCTA	**-0.131**	−0.067
S-DPPH	0.053	**-0.122**
S-ABTS	0.081	−0.080
Se-DPPH	0.003	**0.100**
Se-ABTS	−0.028	−0.008
P-DPPH	0.020	**0.154**

[a] The letters P, S, and Se before each variable stand for pulp, skins, and seeds, respectively, and mean that the variable refers to one of the parts of the grape. [b] Values in bold characters represent the highest absolute values between factors 1 and 2.

Despite the high number of standards tested in the present study and also the application of the HPLC−MS analysis (Figure 2), several phenolic compounds of skins, seeds, and pulps remained unidentified and need to be further investigated. Nevertheless, they were used to tentatively discriminate cultivars from each other, together with the identified molecules.

Antioxidant Activity of Skins, Seeds, and Pulp Juices. The results of the antioxidant measurements are reported in Table 3. In terms of DPPH radical scavenging capacity, within the skins of white grapes, the highest antioxidant activity was detected on Baresana and Italia whereas the red Crimson Seedless showed the highest antioxidant property among all the considered

grapes. Concerning seeds, the highest antioxidant activity was exerted by Italia and Michele Palieri within white and red grapes, respectively. Concerning pulp juices, the highest DPPH radical scavenging capacity was exerted by Baresana and Crimson Seedless within white and red grapes, respectively.

Table 3 also contains the results of the antioxidant activity of skins and seeds expressed as inhibition of the ABTS radical cation formation. Among the white grapes, the highest antioxidant activity was detected on Baresana skins and Italia seeds and pulps. Among the red grapes, the best performances were shown by the Crimson Seedless skins and the Red Globe seeds.

Between skins and seeds, the latter gave the highest contribution to such antioxidant activity, as they had higher phenolic content than the former and contained high quantities of proanthocyanidins (flavonoids known for their high antioxidant properties) and galloylated flavanols, compounds that have a higher antioxidant activity in aqueous medium than their non-galloylated homologues.[28] These results confirm the findings of Negro et al.[29] in a study on the red grape Marc. But the very big surprise was the strongest antioxidant activity of the pulp juices whose phenolic content was the lowest. This result can be explained by the presence, among pulp phenolic compounds, of hydroxycinnamyl acids that, according to Sánchez-Moreno et al.,[30] had stronger antioxidant activity than compounds such as tannic acid, α-tocopherol, rutin, quercetin, ferulic acid, 3-*tert*-butyl-4-hydroxyanisole, BHA, and resveratrol (in decreasing order).

Also for antioxidant activity, the coefficients of variation within each cultivar were very low, being generally below 20 and, anyway, below 26%.

Because the phenolic distribution is known to influence the antioxidant activity of grapes, it was reasonable to expect a certain correlation. First of all, simple correlations between phenolic composition (TPC, TA, TF, F, P, HCTA) and antioxidant activity of skins, seeds, and grapes were tested. According to the R^2 adjusted values and the correlation coefficient (at $p < 0.05$) only flavans reactive with vanillin, proanthocyanidins, and total phenolic content were found to be positively and significantly correlated to the antioxidant activity of skins. The most significant equations are the following:

$$\mathrm{DPPH_{SKINS}} = 175.0758 - 0.0059F_{\mathrm{SKINS}},\ R^2_{\mathrm{adjusted}} = 0.682,\ R = 0.826 \quad (1)$$

$$\mathrm{DPPH_{SKINS}} = 197.5646 - 0.0398P_{\mathrm{SKINS}},\ R^2_{\mathrm{adjusted}} = 0.545,\ R = 0.738 \quad (2)$$

$$\mathrm{ABTS_{SKINS}} = 280.5028 - 0.0042\mathrm{TPC_{SKINS}},\ R^2_{\mathrm{adjusted}} = 0.718,\ R = 0.847 \quad (3)$$

$$\mathrm{ABTS_{SKINS}} = 281.5928 - 0.0618P_{\mathrm{SKINS}},\ R^2_{\mathrm{adjusted}} = 0.613,\ R = 0.783 \quad (4)$$

No significant correlations were found between phenolic distribution and antioxidant activity of seeds and pulps. For prediction of antioxidant activity, multiple regression models were applied but no significant equations were found. The ability as radical scavengers of phenolic extracts of skins, seeds, and pulps depended not only on the phenolic concentration of the various classes of phenolic compounds but also on the specific chemical structure of each phenolic (degree of hydroxylation and extent of conjugation), and some research in literature reports examples of hierarchies for antioxidant activity and reduction potential of phenols.[31] Furthermore, particular compounds may act additively or synergistically with other compounds and the total antioxidant activity may depend on the relative proportions of each compound in the system.[19] The absence of correlation between TPC and DPPH values was due to the different reactions of antioxidants to the DPPH free radicals with respect to the Folin−Ciocalteau reagent in the total-phenol assay. The Folin−Ciocalteau reagent is sensitive to a broad range of substrates, which are easily oxidized, whereas the DPPH free radicals exhibit different sensitivity to various antioxidants.[32] Other authors found strong correlation between antioxidant activity and TPC or TA.[33]

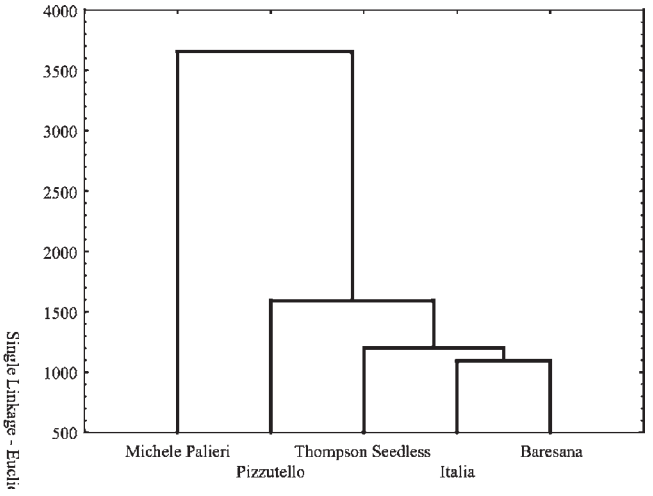

Figure 4. Cluster analysis for the samples of the seven table grape cultivars.

In order to tentatively discriminate samples, principal component analysis was applied to all the experimental variables. The resulting graph is reported in Figure 3 and illustrates the relationships among cultivars. The first two principal components (factors 1 and 2) accounted for up to 73.62% of the explained variance (43.37 and 30.25%, respectively). Italia and Michele Palieri samples were clearly separated whereas Baresana, Pizzutello and Thompson Seedless were very close. Crimson Seedless and Red Globe did not compare in the factor plane.

According to the eigenvectors (Table 4), several original variables were associated with factors 1 and 2. The variables negatively associated with factor 1 were represented by several unidentified compounds of skins, pulps, and seeds, concentration of gallic and 4-hydroxybenzoic acids in pulps, concentration of TPC and F in skins, and concentration of procyanidin B1, 4-hydroxybenzoic acid, and TF in seeds. Several unidentified peaks of skins, pulps, and seeds, concentration of protocatecualdehyde in pulps, concentration of chlorogenic acids, peonidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, quercetin-3-*O*-glucopyranoside, and *trans*-cinnamic acid in skins, and concentration of gallic acid and procyanidin B2 in seeds were positively associated with factor 2 whereas several unidentified compounds of pulps and skins were negatively associated with factor 2. The sample

Table 5. Descriptive Statistics of Hierarchical Cluster Analysis: Mean Values and Standard Deviation of the Variables for Each of the Two Clusters Found by HCA

	cluster 1		cluster 2	
	mean	std dev	mean	std dev
P[a]-unidentified peak (2.0)	**32.80**[b]	0.00	4.02	8.05
P-unidentified peak (2.1)	0.00	0.00	0.00	0.00
P-unidentified peak (2.5)	**35.30**	0.00	26.37	30.46
P-unidentified peak (2.7)	**4.20**	0.00	1.97	2.28
P-unidentified peak (3.0)	**4.80**	0.00	1.27	1.79
P-unidentified peak (3.3)	**1.20**	0.00	0.00	0.00
P-gallic acid (3.9)	0.00	0.00	0.80	1.60
P-unidentified peak (4.4)	2.60	0.00	2.60	4.26
P-unidentified peak (5.1)	1.20	0.00	1.45	2.90
P-unidentified peak (5.2)	0.00	0.00	3.20	2.79
P-unidentified peak (5.5)	1.30	0.00	**12.72**	17.37
P-unidentified peak (5.8)	0.00	0.00	**8.30**	15.49
P-unidentified peak (6.4)	0.00	0.00	0.425	0.85
P-unidentified peak (8.1)	0.00	0.00	0.45	0.90
P-unidentified peak (8.4)	**2.70**	0.00	0.00	0.00
P-3,4-dihydroxyphenylacetic acid (8.9)	0.00	0.00	0.95	1.90
P-unidentified peak (9.5)	**1.40**	0.00	0.50	0.58
P-unidentified peak (10.3)	0.00	0.00	0.00	0.00
P-protocatechualdehyde (10.7)	**20.90**	0.00	0.92	1.16
P-unidentified peak (11.2)	0.00	0.00	1.30	1.56
P-unidentified peak (11.4)	**8.70**	0.00	0.00	0.00
P-4-hydroxybenzoic acid (11.8)	0.00	0.00	4.75	9.50
P-unidentified peak (13.6)	0.00	0.00	0.00	0.00
P-caffeic acid (15.6)	0.00	0.00	4.70	6.79
P-syringic acid (16.9)	0.00	0.00	0.77	1.55
P-unidentified peak (17.9)	0.00	0.00	0.00	0.00
P-unidentified peak (18.5)	0.00	0.00	4.57	9.15
P-unidentified peak (19	0.00	0.00	4.50	9.00
P-unidentified peak (21.8)	0.00	0.00	0.00	0.00
S-unidentified peak (2.2)	0.00	0.00	**150.42**	300.85
S-unidentified peak (2.5)	0.00	0.00	**33.70**	67.40
S-unidentified peak (2.9)	0.00	0.00	**17.07**	34.15
S-unidentified peak (4.7)	0.00	0.00	**19.87**	39.75
S-unidentified peak (10.4)	0.00	0.00	**14.60**	29.20
S-unidentified peak (10.7)	0.00	0.00	0.00	0.00
S-unidentified peak (10.9)	0.00	0.00	**14.42**	28.85
S-unidentified peak (11.1)	0.00	0.00	0.00	0.00
S-4-hydroxybenzoic acid (11.8)	0.00	0.00	**97.72**	96.83
S-unidentified peak (12.4)	0.00	0.00	0.00	0.00
S-chlorogenic acid (13.2	**48.60**	0.00	0.00	0.00
S-unidentified peak (14.3)	0.00	0.00	0.00	0.00
S-2,5-dihydroxybenzoic acid (15.0)	0.00	0.00	0.00	0.00
S-caffeic acid (15.3)	0.00	0.00	**25.70**	51.40
S-unidentified peak (15.6)	0.00	0.00	**22.12**	27.26
S-syringic acid (16.9)	0.00	0.00	**25.25**	30.71
S-unidentified peak (19.3)	0.00	0.00	**65.37**	130.75
S-unidentified peak (19.9)	0.00	0.00	**33.10**	39.80
S-unidentified peak (21.4)	**53.30**	0.00	0.00	0.00
S-unidentified peak (22.3)	0.00	0.00	0.00	0.00
S-unidentified peak (22.6)	**68.50**	0.00	0.00	0.00
S-unidentified peak(23.1)	0.00	0.00	0.00	0.00
S-unidentified peak (23.6)	**212.80**	0.00	19.85	39.70
S-unidentified peak (24.6)	**269.50**	0.00	0.00	0.00
S-unidentified peak (25.2)	**438.80**	0.00	0.00	0.00
S-unidentified peak (25.9)	**635.70**	0.00	0.00	0.00
S-peonidin-3-*O*-glucoside (26.7)	**731.00**	0.00	0.00	0.00
S-cyanidin-3-*O*-glucoside (27.2)	0.00	0.00	0.00	0.00
S-malvidin-3-*O*-glucoside (27.5)	**1510.60**	0.00	0.00	0.00
S-unidentified peak(27.7)	0.00	0.00	**55.60**	111.20
S-unidentified peak(27.9)	0.00	0.00	**20.72**	41.45
S-quercetin-3-*O*-glucopyranoside (28.5)	**2528.30**	0.00	0.00	0.00
S-unidentified peak (28.7)	0.00	0.00	**49.30**	21.33
S-unidentified peak (29.1)	**69.80**	0.00	0.00	0.00
S-unidentified peak(38.3)	**224.90**	0.00	0.00	0.00
S-resveratrol (39.5)	0.00	0.00	0.00	0.00
S-unidentified peak (43.8)	**148.20**	0.00	0.00	0.00
S-*trans*-cinnamic acid (44.6)	**68.20**	0.00	0.00	0.00
S-unidentified peak (46.7)	**912.40**	0.00	0.00	0.00
Se-gallic acid (3.9)	**2359.10**	0.00	688.57	461.94
Se-unidentified peak (6.1)	0.00	0.00	**35.50**	71.00
Se-procyanidin B1 (10.3)	0.00	0.00	**76.72**	153.45
Se-4-hydroxybenzoic acid (11.8)	0.00	0.00	**69.22**	138.45
Se-catechin (12.9)	829.90	0.00	600.82	762.55
Se-unidentified peak (13.8)	0.00	0.00	**31.65**	63.30
Se-unidentified peak (14.5)	0.00	0.00	**35.87**	71.75
Se-procyanidin B2 (16.5)	**207.70**	0.00	136.95	158.22
Se-epicatechin (18.6)	**352.70**	0.00	522.45	590.71
Se-unidentified peak (18.9)	0.00	0.00	**43.47**	86.95
Se-unidentified peak (19.9)	0.00	0.00	**49.05**	98.10
Se-unidentified peak (20.5)	0.00	0.00	**31.47**	62.95
Se-unidentified peak (21.9)	**248.90**	0.00	31.02	62.05
Se-unidentified peak (23.5)	0.00	0.00	**82.72**	165.45
Se-unidentified peak (27.7)	0.00	0.00	**28.72**	57.45
Se-unidentified peak (28.6)	**228.80**	0.00	99.30	114.80
Se-unidentified peak (28.8)	0.00	0.00	**29.12**	58.25
S-TPC	33.10	0.00	35.25	11.66
Se-TPC	111.00	0.00	113.25	80.33
P-TPC	0.35	0.00	0.6165	0.20
S-TA	**10.10**	0.00	0.00	0.00
S-TF	25.70	0.00	21.47	4.77
Se-TF	26.30	0.00	**57.00**	45.05
S-F	9.59	0.00	11.89	5.54
Se-F	69.20	0.00	61.77	44.18
S-P	2.30	0.00	2.39	0.90
Se-P	7.86	0.00	7.19	5.31
P-HCTA	0.01	0.00	0.02	0.00
S-DPPH	66.90	0.00	**89.60**	44.42
S-ABTS	132.70	0.00	130.65	83.01
Se-DPPH	**20.60**	0.00	13.97	9.64

Table 5. Continued

	cluster 1		cluster 2	
	mean	std dev	mean	std dev
Se-ABTS	11.70	0.00	**18.82**	14.05
P-DPPH	**8.71**	0.00	5.18	1.37

[a] The letters P, S, and Se before each variable stand for pulp, skins, and seeds, respectively, and mean that the variable refers to one of the parts of the grape. [b] Values in bold characters represent the highest mean values between clusters 1 and 2.

discrimination was made including phenolic compounds that, although clearly separated, were not identified on the basis of the comparison of their spectra, mass, and retention times with those of the pure standards. Many unidentified compounds had very close retention times, but the risk of overlapping was carefully excluded. The unknown compounds are actually submitted to further investigation aimed to their identification, but they were included as variables in the discriminant analysis because of the low number and concentration of phenolic compounds found in the cultivars that were the object of the research.

The application of hierarchical cluster analysis was used to separate the cultivars, gave the dendrogram of Figure 4, and suggested the classification of the cultivars into two clusters that coincided with classification based on skin color. Cluster 1 contained table grape samples of Michele Palieri whereas cluster 2 included samples of Baresana, Italia, Pizzutello, and Thompson Seedless cv., only. Crimson Seedless and Red Globe were not classified. Within the second cluster, the distances of the samples from the respective cluster center varied from 43.49 (Baresana) to 144.79 (Michele Palieri) whereas Michele Palieri was just at the center of the second cluster. The Euclidean distance between the two clusters was 370.44.

Cluster 1 was characterized (Table 5) by the highest mean concentrations of protocatechualdehyde in pulps, chlorogenic acid, peonidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, quercetin-3-*O*-glucopyranoside, and *trans*-cinnamic acid in skins, gallic acid, catechin, and epicatechin in seeds. Cluster 2 was a heterogeneous group (as demonstrated by the high values of standard deviations, mainly due to the differences between Italia and the other cultivars), and showed the highest concentrations of several unidentified compounds and of 4-hydroxybenzoic, caffeic, and syringic acids in skins and of procyanidin B1, 4-hydroxybenzoic, and TF in skins.

Funding Sources

The authors would like to express their gratitude to REGIONE PUGLIA-ACCORDO DI PROGRAMMA QUADRO IN MATERIA DI "RICERCA SCIENTIFICA" (Project PS_026) for its financial support.

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