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ARTICLE

Chemical Characterization and Enological Potential of Raboso Varieties by Study of Secondary Grape Metabolites

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DNA studies supported by matrix-assisted laser desorption ionization−time of flight mass spectrometry analysis of seed proteins showed that the *Vitis vinifera* red grape variety Raboso Veronese is the progeny of a spontaneous cross between Raboso Piave (red) and Marzemina Bianca (white) varieties. In the present work, the main secondary grape metabolites of Raboso varieties were studied, and their enological potential was evaluated and compared with that of other red varieties reported in the literature. In general, Raboso grapes had high flavonoid contents and high percentages of polyphenols extractable in winemaking and substantial contents of norisoprenoid aroma precursors. Raboso Veronese stood out for its high content of cyanidin and had higher (+)-catechin and (−)-epicatechin contents than Raboso Piave and abundant quercetin glucoside, indicating substantial plant biosynthesis toward compounds dihydroxylated in the B-ring. Study of secondary grape metabolites is confirmed as an effective tool in differentiating similar varieties, in particular on the basis of polyphenol profiles.

KEYWORDS: Anthocyanins; polyphenols; terpenols; norisoprenoids; grape; Raboso

INTRODUCTION

Raboso Piave, also called "Friularo", is a *Vitis vinifera* red grape variety cultivated in the Veneto region of northern Italy, particularly in the Piave valley and the province of Padova. Ancient documents issued by the Most Serene Republic of Venice report that this variety was introduced into the province of Treviso and the Friuli region in the 17th and 18th centuries (*1*). The grapes are characterized by high acidity, and the resulting wines have high polyphenol contents.

Raboso Veronese is a *V. vinifera* red grape variety, which was widespread in the Po River plane area in the 19th century. Its wines are characterized by pronounced astringency and color (*2*). Two Raboso varieties have very similar ampelographic features, and their monovariety wines are similar; as a consequence, Italian legislation does not distinguish between the two varieties, and the grape generically admitted for wine production is "Raboso".

DNA studies have revealed that the two varieties do have different genetic profiles and that Raboso Veronese is the progeny of a spontaneous cross between Raboso Piave and a white grape variety called Marzemina Bianca (*3*,*4*). A study on grape seed protein profiles by matrix-assisted laser desorption ionization−time of flight mass spectrometry (MALDI-TOF/MS) confirmed these results, showing that Raboso Veronese (the progeny) mainly contains Raboso Piave proteins (the female parent), although it is characterized by the presence of some proteins of the other parent (Marzemina Bianca), which are not found in the Raboso Piave profile (*5*).

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Study of secondary grape metabolites, such as polyphenol compounds (anthocyanins, flavonols, flavan-3-ols), volatile compounds present in grapes in both free and glycoside forms (monoterpenols, norisoprenoids, benzenoids), and compounds resulting from acid hydrolysis of grape precursors, allows us to characterize grape varieties and to choose the best winemaking practices to maximize their enological potential (e.g., maceration conditions, barrel and bottle aging). In variety differentiation, the study of these parameters is linked with data on DNA (microsatellite markers), isoenzymes, and ampelography (*6*).

In the present work, the main secondary metabolites of Raboso grape varieties were studied, and their enological potential was evaluated and compared with that of other red varieties reported in the literature.

MATERIALS AND METHODS

Reagents and Standards. Quercetin, myricetin, kaempferol, quercetin-3-β-D-glucoside, and (+)-catechin were purchased from Fluka; kaempferol-3-*O*-glucoside was from Extrasynthese, 4′,5,7-trihydroxyflavone was from Aldrich, and *trans*-resveratrol was from Sigma (Sigma-Aldrich srl, Milan, Italy). Solid phase extraction was performed with Sep-Pak C_{18} 360 mg, 1 g, and 10 g cartridges purchased from Waters (Milford, MA). Hydrolysis of *trans*-piceid was performed with a β-glucosidase enzyme from almonds (7.55 U/mg) from Fluka. Standards of 1-heptanol, 1-octanol, and 1-decanol used for GC-MS analyses came from Carlo Erba Reagents (Milan, Italy). Hydrolysis of volatile compound glycosides was performed with commercial enzyme AR 2000 (Gist Brocades, France).

Samples. Grape samples from three Raboso Piave and two Raboso Veronese vineyards were collected. The vineyards are situated in various areas of the province of Treviso (Veneto, Italy), and the grapes were harvested at full ripening stage in 2004 and 2005 (see the

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Table 1. Total Skin Flavonoid and Anthocyanin Indices of Raboso Grape[a]

	total flavonoids (mg of (+)-catechin/kg of grape)		total anthocyanins (mg of malvidin/kg of grape)	
sample	2004	2005	2004	2005
Raboso Piave I	4432 ± 33	4847 ± 357	1349 ± 75	1614 ± 169
Raboso Piave II	5503 ± 5	4846 ± 109	1989 ± 239	1660 ± 32
Raboso Piave III	4655 ± 135	4317 ± 178	1674 ± 160	1705 ± 92
Raboso Veronese I	4339 ± 370	3445 ± 133	1356 ± 174	1272 ± 31
Raboso Veronese II	5237 ± 61	5819 ± 260	2367 ± 167	1748 ± 74
mean Raboso Piave	4863	4670	1670	1659
mean Raboso Veronese	4788	4632	1861	1510

[a] Mean data of two samples.

Table 2. Percentages of Individual Anthocyanins Identified in HPLC Profiles of Raboso Piave and Raboso Veronese Grape Skin Extracts

	% area			
	mean Raboso Piave		mean Raboso Veronese	
anthocyanin	2004	2005	2004	2005
delphinidin-monoglucoside	7.6	7.3	12.6	11.9
cyanidin-monoglucoside	14.3	12.0	23.8	24.0
petunidin-monoglucoside	8.7	8.9	11.7	11.5
peonidin-monoglucoside	30.4	30.1	14.2	13.6
malvidin-monoglucoside	28.0	29.4	23.4	22.9
delphinidin-monoglu acetate	0.5	0.5	1.4	1.2
cyanidin-monoglu acetate	0.9	0.9	2.7	2.9
petunidin-monoglu acetate	0.6	0.6	1.4	1.3
peonidin-monoglu acetate	1.4	1.5	1.1	1.0
malvidin-monoglu acetate	1.3	1.8	2.0	2.5
delphinidin-monoglu *p*-coumarate	0.3	0.5	0.4	0.7
malvidin-monoglu caffeate	0.1	0.2	0.1	0.1
cyanidin-monoglu *p*-coumarate	1.1	1.2	2.0	2.5
petunidin-monoglu *p*-coumarate	0.5	0.6	0.5	0.7
peonidin-monoglu *p*-coumarate	2.2	2.6	1.1	1.2
malvidin-monoglu *p*-coumarate	2.1	2.1	1.5	2.0

Supporting Information). Nine bunches were collected of each sample, from plants falling in lines crossing the center of the vineyard, and immediately frozen.

Parameters Studied. The main parameters useful for evaluating the ripening state of grapes at harvest were determined: soluble solids, pH and acidity of juice, and total flavonoids and anthocyanins (calculated as mg of (+)-catechin/kg of grape and mg of malvidin-3-*O*-glucoside/kg of grape, respectively) of skins (see the Supporting Information). Skin anthocyanin and flavonol profiles, seed monomer flavanols, and free and total (free + glucoside) *trans*-resveratrol were determined by HPLC analysis. Profiles of aglycones produced by enzymatic hydrolysis and of volatile compounds formed after acid hydrolysis of pulp and skin extracts were determined by GC-MS analysis.

Sample Preparation for Polyphenol Analyses. Methods previously reported in the literature and modified for our purposes were used (*7*). Samples were prepared from 300 frozen berries: 20 berries were selected (50 for seed extraction), defrosted, dried, and weighed. As soon as the grapes were defrosted, but when the berries were still cold, skins were separated from pulps and seeds, dried, and immersed in 50 mL of a buffer tartrate, pH 3.2, solution containing sodium metabisulfite 2 g/L and ethanol 12% (v/v). Extraction was performed for 3 h at room temperature in the dark. The solution was homogenized and centrifuged at 4000 rpm for 5 min, and the supernatant was frozen until analysis. Seeds were washed in distilled water, dried, immersed in 25 mL of the same solution used for skins, and extracted at 25 °C for 10 days in the dark. A volume of 12.5 mL of extract was evaporated to dryness, and the residue was then dissolved with 10 mL of methanol. The solution was transferred to a tube and kept at −20 °C overnight, to separate the fat layer, and centrifuged at −7 °C and 4500 rpm for 5 min. The supernatant was transferred to a separatory funnel, washed twice with 20 mL of hexane to remove any fat residue, concentrated to 1 mL, and added to 20 mL of water.

Table 3. Mean Contents of Flavonols Identified in Raboso Piave and Raboso Veronese Grape Skin[a]

	mg/kg of grape			
	Raboso Piave		Raboso Veronese	
flavonol	2004	2005	2004	2005
myricetin-glucuronide (**1**)	1.16 ± 0.31	1.36 ± 0.22	1.20 ± 0.72	1.12 ± 0.66
myricetin-glucoside (**2**)	8.11 ± 2.37	8.48 ± 1.56	13.94 ± 4.87	8.93 ± 4.45
quercetin-glucuronide (**3**)	5.88 ± 3.37	6.41 ± 1.83	9.78 ± 2.08	7.34 ± 4.03
quercetin-glucoside (**4**)	10.98 ± 6.42	16.19 ± 7.59	42.03 ± 16.64	33.59 ± 22.63
kaempferol-glucoside	1.23 ± 0.29	1.21 ± 0.61	3.81 ± 0.62	2.73 ± 2.90
$\sum$ Mr/$\sum$ Q	0.55	0.44	0.30	0.24

[a] Data ± standard deviation are reported. $\sum$ Mr/$\sum$ Q = (1 + 2)/(3 + 4).

Monomer flavanols and oligomer proanthocyanidins were separated from higher molecular weight (MW) compounds by fractionation on C_{18} cartridges, the low MW proanthocyanidin fraction being recovered with 5 mL of H_3PO_4, pH 2.0, and 20% (v/v) acetonitrile solution (*8*).

The methods proposed by Mattivi et al. were used to determine indices of skin and seed extractable anthocyanins and polyphenols (*9*). Skins and seeds of 60 berries were extracted with 250 mL of 12% (v/v) hydroalcoholic solution, pH 3.2, containing SO_2 100 mg/L for 5 days at 30 °C. The extracts were then centrifuged and analyzed.

Free and glycoside resveratrol were determined according to the methods reported in the literature and modified for our goals (*10*, *11*). Twenty berries deprived of seeds were pressed and extracted with 30 mL of methanol for 1 h at room temperature in the dark. After homogenization and centrifugation at 4000 rpm for 10 min, methanol was removed under vacuum, the aqueous residue was adjusted to 5 mL by water, and the solution was transferred to a separatory funnel. After the addition of 100 μL of 4′,5,7-trihydroxyflavone 100 mg/L as internal standard and 0.5 g of NaCl, the solution was extracted 3 × 3 mL with ethyl acetate. Organic extracts were combined, the solution was evaporated to dryness, and the residue was dissolved in 2 mL of methanol/formic acid 0.5% 3:7 (v/v) solution. The aglycone of *trans*-resveratrol glucoside (*trans*-piceid) was determined after enzymatic hydrolysis, according to the methods reported in the literature (*12*).

Sample Preparation for Analysis of Aroma Precursors. One hundred berries were weighed, and pulps were separated from skins and seeds, transferred to a flask containing 50 mg of sodium metabisulfite, homogenized, and centrifuged. The volume was adjusted to 200 mL by water, and the solution was clarified by treatment with 40 mg of pectolytic enzyme for 4 h at room temperature.

Skins were extracted with 30 mL of methanol for 4 h. After homogenization and centrifugation, the methanol content of the solution was reduced under vacuum, and the volume was adjusted to 200 mL by water; 4 g of PVPP was added to the solution to reduce the polyphenol content, and then the solution was centrifuged.

A volume of 100 mL of juice solution was reunified with an equal volume of skin extract, and 200 μL of 1-heptanol 180 mg/L as internal

Table 4. Mean Contents of Flavan-3-ols in Raboso Grape Seeds[a]

	(+)-catechin (mg/100 g of seeds)		(−)-epicatechin (mg/100 g of seeds)		(+)-catechin/(−)-epicatechin	
seed	2004	2005	2004	2005	2004	2005
Raboso Piave	67.71 ± 11.37	63.73 ± 22.12	57.93 ± 11.98	34.23 ± 12.24	1.17	1.87
Raboso Veronese	213.92 ± 20.15	95.29 ± 4.64	120.54 ± 15.27	61.94 ± 8.26	1.78	1.55

[a] Data ± standard deviation of three Raboso Piave and two Raboso Veronese samples collected in duplicate for each harvest.

Table 5. Mean Content of Free and Total (Free + Glucoside) *trans*-Resveratrol in Grape[a]

	trans-resveratrol (mg/kg of grape)			
	2004		2005	
sample	free	total	free	total
Raboso Piave	0.62 ± 0.24	1.04 ± 0.61	0.36 ± 0.05	0.63 ± 0.17
Raboso Veronese	0.62 ± 0.04	1.11 ± 0.01	0.42 ± 0.10	0.86 ± 0.44

[a] Data ± standard deviation of three Raboso Piave and two Raboso Veronese samples collected in duplicate for each harvest.

standard was added to the resulting solution, which was used for sample preparation for GC-MS analysis.

Three fractions containing volatile compounds, glycoside compounds, and compounds formed by acid hydrolysis were isolated by solid phase extraction (SPE) with C_{18} 10 g, C_{18} 1 g, and C_{18} 360 mg Sep-Pak cartridges, respectively, according to literature methods (*13*).

Spectrophotometric Analyses. Total anthocyanins, total flavonoids, extractable anthocyanins, and extractable polyphenols indices were determined with the analytical methods proposed by Di Stefano et al. for wines (*14*). A Uvikon 930 UV−vis spectrophotometer (Kontron Instruments, Milan, Italy) was used.

HPLC Analyses. A ThermoFinnigan-Spectra System (Thermo, San Jose, CA) was used, composed of a P4000 pump, an AS3000 autosampler, and a UV6000LP diode array detector and equipped with an SCM100 degasser.

For analysis of anthocyanins, 1 mL of skin extract was diluted with 1 mL of H_2SO_4 0.1 N, and the solution was passed through a C_{18} 360 mg cartridge, yielding anthocyanins with 2 mL of methanol. The organic solution was filtered through an Acrodisc GHP 0.22 μm syringe filter (Waters) and used for analysis. Anthocyanin profiles were determined with the analytical conditions reported in the literature (*7*, *15*−*17*). Chromatograms were recorded both at a fixed wavelength (520 nm) and over a range of 250−650 nm. As most standards were not available, each compound was quantified as an area percentage of the total area of anthocyanins identified in the chromatogram.

For the analysis of seed flavan-3-ols, the extract was filtered and 20 μL directly injected into the column. Analysis involved a binary solvent composed of (A) acetonitrile and (B) H_3PO_4 aqueous solution, pH 2.6. The gradient elution program was 5% A isocratic for 10 min, from 5 to 12% A in 20 min, isocratic for 20 min, from 12 to 32% A in 30 min, from 32 to 50% A in 10 min, from 50 to 5% A in 5 min, and isocratic for 10 min. The flow rate was 0.5 mL/min. Chromatograms were recorded at 280 nm, as were the 200−700 nm absorption spectra (*8*, *18*).

For the analysis of skin flavonols, 4.5 mL of extract was acidified by the addition of 0.5 mL of H_3PO_4 1.0 M solution; for the analysis of flavonols in pulp, 1.0 mL of extract was diluted with 4.0 mL of H_3PO_4 0.125 M. The column used was a 200 mm × 2.1 mm i.d., 5 μm ODS Hypersil RP-18 (Thermo Hypersil-Keystone, Bellefonte, PA), and a binary solvent composed of (A) H_3PO_4 10^{-3} M and (B) methanol was used. The gradient elution program was from 5 to 10% B in 5 min, from 10 to 30% B in 15 min, from 30 to 60% B in 10 min, from 60 to 100% B in 10 min, isocratic for 10 min, from 100 to 5% B in 4 min, and isocratic for 30 min. The flow rate was 0.25 mL/min, and sample injected was 10 μL. Chromatograms were recorded at 360 nm, and 200−700 nm absorption spectra were also recorded. Myricetin glucoside, quercetin glucuronide, and myricetin glucuronide were identified according to the elution sequence column and comparisons of the absorption spectra with data reported in the literature (*19*). Analytes were quantified on quercetin, myricetin, and kaempferol calibration curves.

Analysis of *trans*-resveratrol was performed with the same column used for flavonols and a binary solvent composed of (A) methanol and (B) aqueous formic acid 0.5% (v/v) at a flow rate of 0.25 mL/min. The gradient elution program was from 25 to 40% A in 40 min, from 40 to 85% A in 10 min, and isocratic for 5 min. Chromatograms were recorded at 307 nm.

GC-MS Analysis. Analyses were performed by an HP 5890 gas-chromatograph equipped with an HP Innowax 30 m × 0.25 mm, 0.25 μm, column (Agilent Technologies, Santa Clara, CA) and coupled with an HP 5971A mass spectrometer and a 6890 series injector autosampler. The oven temperature program was as follows: 36 °C isothermal for 3 min, increased at 2 °C/min until 160 °C, increased at 3 °C/min until 230 °C, and 230 °C isothermal for 5 min. Other experimental conditions were injector temperature, 230 °C; carrier gas, He; column head pressure, 12 psi; sample volume injected, 0.5 μL; splitless mode injection; and transfer line temperature, 280 °C. Compounds were identified according to retention times of available standards and comparisons of fragmentation spectra with NIST98 and CRA-VIT databases.

Statistical Analysis. ANOVA, principal component, and cluster analyses were performed with the Statistica Program for Windows, version 4.5 (StatSoft Inc., Tulsa, OK).

RESULTS AND DISCUSSION

Polyphenol Contents and Profiles. Grapes collected in 2005 had higher sugar content and lower acidity than those of 2004. In general, Raboso Veronese was characterized by lower acidity and higher sugar content than Raboso Piave, matching the results of Crespan et al. (*4*). Particularly high sugar content was found in Raboso Veronese II samples collected in 2005.

Table 1 lists the total anthocyanin and flavonoid indices in skin extracts. No significant differences between the two Raboso varieties were observed. The higher values of Raboso Veronese II (expressed as mg of (+)-catechin/kg of grape) were probably due to the fact that the berries of this sample were smaller and consequently had a greater surface/weight ratio. Comparison with polyphenol indices of other red grape varieties reported in the literature and determined by the same methods showed that Raboso has the highest total flavonoids and anthocyanins comparable with the richer varieties such as Negroamaro and Carmenère.

The enological potential of Raboso was evaluated by determining indices of polyphenols extractable in winemaking using a model wine solution and comparing reported data for 25 other red grape varieties determined with the same method (*9*). Compared with the other varieties, Raboso Veronese and Raboso Piave had some of the highest values of extractable anthocyanins (1465 and 1185 mg of malvidin/kg of grape, respectively) and skin and seed extractable polyphenols (3366 and 3093 mg of (+)-catechin/kg of grape, respectively).

Table 2 lists percentages of individual anthocyanins identified by HPLC profiles of Raboso Piave and Raboso Veronese and **Table 3** the five glycoside flavonols identified in grape skins. Identification of compounds was based on their elution order from chromatographic columns, UV−vis spectra, and literature data (*15*, *17*).

In general, the two Raboso grape varieties are characterized by high monoglucoside anthocyanins, whereas acylated compounds are poorly represented and malvidin-3-glucoside caffeate

Table 6. Benzene Compounds, Terpenes, and Norisoprenoid Aglycones Identified in Grape Extract after Enzymatic Hydrolysis[a]

		Raboso Piave (μg/kg of grape)		Raboso Veronese (μg/kg of grape)	
glycoside precursor	RI[b]	2004 mean	2005 mean	2004 mean	2005 mean
benzenoids					
benzaldehyde		11 ± 1	12 ± 4	7 ± 1	12 ± 1
benzyl alcohol		888 ± 126	741 ± 188	647 ± 106	1102 ± 37
2-phenylethanol		307 ± 64	356 ± 116	340 ± 29	415 ± 17
eugenol		20 ± 3	48 ± 24	16 ± 5	30 ± 8
4-vinylguaiacol		216 ± 81	154 ± 50	311 ± 109	328 ± 10
4-vinylphenol		56 ± 1	40 ± 18	52 ± 1	105 ± 21
vanillin		95 ± 34	88 ± 24	258 ± 10	99 ± 5
methyl vanillate (t)[c]	2580	119 ± 30	119 ± 63	104 ± 13	112 ± 14
4-hydroxy-3-methoxybenzyl alcohol (t)	2785	87 ± 50	70 ± 18	142 ± 14	185 ± 8
homovanillic alcohol (t)	2825	454 ± 77	585 ± 91	557 ± 8	900 ± 56
4-hydroxybenzaldehyde		37 ± 8	43 ± 16	30 ± 9	87 ± 21
terpenoids					
trans-furanlinalool oxide (t)	1419	11 ± 5	13 ± 4	22 ± 3	21 ± 2
cis-furanlinalool oxide (t)	1448	13 ± 4	nd	18 ± 5	28 ± 13
α-terpineol		17 ± 3	21 ± 6	2 ± 1	20 ± 1
trans-pyranlinalool oxide (t)	1722	13 ± 7	15 ± 8	17 ± 1	25 ± 7
cis-pyranlinalool oxide (t)	1751	12 ± 5	12 ± 1	19 ± 2	24 ± 3
eso-2-hydroxycineole (t)	1834	48 ± 11	61 ± 11	33 ± 19	74 ± 10
geraniol		28 ± 8	38 ± 12	29 ± 8	39 ± 3
trans-8-hydroxylinalool (t)	2265	66 ± 19	52 ± 10	86 ± 8	76 ± 19
hydroxygeraniol (t)	2299	114 ± 35	68 ± 10	99 ± 5	132 ± 13
cis-8-hydroxylinalool (t)	2302	18 ± 5	14 ± 6	170 ± 44	114 ± 10
geranic acid		34 ± 17	29 ± 14	12 ± 7	31 ± 1
p-menth-1-ene-7,8-diol (t)	2495	342 ± 154	323 ± 113	51 ± 8	294 ± 34
norisoprenoids					
3-hydroxy-β-damascone (t)	2506	60 ± 12	62 ± 20	68 ± 1	77 ± 2
3-oxo-α-ionol (t)	2605	404 ± 129	462 ± 165	344 ± 33	572 ± 25
4-oxo-β-ionol (t)	2614	39 ± 9	55 ± 16	44 ± 11	74 ± 2
dihydroxymegastigma-5-ene (t)	2644	89 ± 27	57 ± 14	105 ± 2	106 ± 14
3-hydroxy-7,8-dihydro-β-ionol (t)	2741	44 ± 10	49 ± 21	50 ± 6	64 ± 7
vomifoliol (t)	3121	1145 ± 275	1000 ± 144	1360 ± 72	1463 ± 118

[a] Mean ± standard deviation of three Raboso Piave and two Raboso Veronese grape samples each collected in duplicate for two harvests. Data are expressed as μg of 1-octanol/kg of grape. [b] RI, linear retention index based on a series of *n*-hydrocarbons. [c] t, tentative identification according to GC retention index and fragmentation spectrum.

is practically absent, as already reported for Raboso Piave (*20*). Comparisons with other varieties reported in the literature (*17*, *20*) showed high levels of cyanidin-3-glucoside in Raboso and, particularly in Raboso Veronese, this anthocyanin is similar to malvidin-3-glucoside. The main flavonols in Raboso Piave were myricetin and quercetin glucoside, matching a previous paper (*20*). Despite the high variability (standard deviation) observed, probably due to grapes grown in different environments and collected in different vintages, and samples I and II, which had differently sized berries and different ripeness at harvest, particularly high contents of quercetin glucoside were found in Raboso Veronese. **Table 4** lists the mean flavan-3-ol contents in seeds. Higher monomer flavanols were found in Raboso Veronese samples in both harvests; in all samples (+)-catechin was higher than (−)-epicatechin.

Free and total *trans*-resveratrol determined before and after enzymatic hydrolysis of grape extracts are listed in **Table 5**. No significant differences between the two Raboso varieties were observed; about half the total resveratrol in grape was present in the *trans*-piceid form. However, because phytoalexin synthesis of the plant may be induced, besides the variety, by external factors such as stress conditions (e.g., water deficiency) or attack by pathogens, these parameters cannot be viewed as direct genetic expressions of the plant and were not considered for variety characterization (*10*, *21*).

Volatile Compounds. Because low levels of free volatile compounds were found in Raboso extracts, this study focused on the aglycones released from their glycoside precursors and on the volatile compounds formed after acid hydrolysis of methanolic extracts recovered from SPE cartridges after isolation of the fraction of glycosides added to the solution containing the aglycones released by enzymatic hydrolysis. Identifications were made with the standard compounds, or according to GC retention indices and fragmentation spectra available in the literature and reported in databases (in these cases "tentative identification" is reported) (*22*, *23*).

Glycoside compounds are precursors of volatile aromatic compounds released into must during winemaking. Because compounds were determined in samples prepared by combining equal volumes of skin and pulp extracts, these data represent average berry contents and were calculated as micrograms per kilogram of grape.

No significant qualitative or quantitative differences among samples of the same variety collected from different vineyards were found. Among identified aliphatic compounds, those characterized by significant sensory proprieties were 2-hexenal, hexanol, and 2- and 3-hexenol (herbaceous notes, sensory perception thresholds in water of 17, 500−2500, and 70 μg/L, respectively) (data not shown).

Table 6 lists average contents of benzene compound, terpenols, and C_{13}-norisoprenoid aglycones identified after enzymatic hydrolysis. The most abundant benzene compounds were benzyl alcohol (content between 540 and 1140 μg/kg of grape, pungent note, perception threshold = 10 mg/L) and homovanillic alcohol (380−960 μg/kg of grape, sweet balsamic note). The substantial presence of compounds released from wood was observed, such

as 4-vinylguaiacol (100−340 μg/kg of grape, spicy note), 4-vinylphenol (25−125 μg/kg of grape, "mousy" note), eugenol (10−70 μg/kg of grape, spicy note, perception threshold in wine = 0.3 mg/L), vanillin (60−260 μg/kg grape, vanilla note, perception threshold in wine = 0.3−0.4 mg/L), methyl vanillate, and some methoxyphenols.

Raboso showed a few glycoside terpenols, the main ones being *cis*- and *trans*-8-hydroxylinalool (between 10 and 210 μg/kg of grape and between 40 and 95 μg/kg of grape, respectively), *cis*- and *trans*-furanlinalool, and pyranlinalool oxides; linalool was practically absent. Geraniol (floral note, olfactory perception threshold = 130 μg/L) was present in interesting amounts, as well as hydroxygeraniol (between 60 and 150 μg/kg of grape) and geranic acid. This matches the results of a previous study of Raboso wine, in which glycoside hydroxygeraniol and geraniol prevailed over linalool and hydroxylinalool. The most abundant terpenol was *p*-menth-1-ene-7,8-diol, at 40−500 μg/kg of grape. In any case, due to the total contents of terpenols, between 400 and 1000 μg/kg of grape, a partially aromatic character can be attributed to Raboso according to its chemical profile.

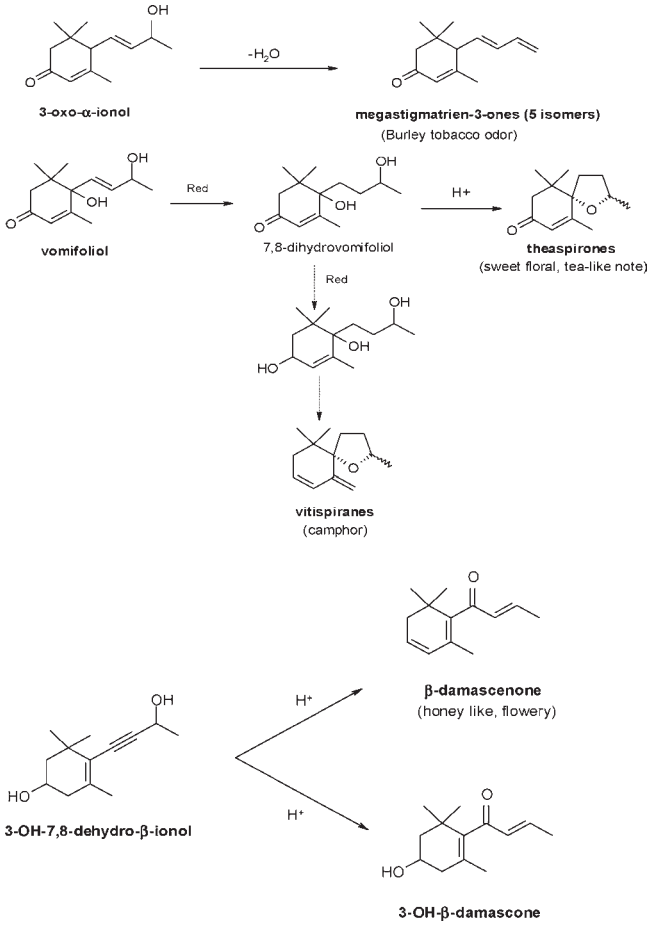

Figure 1. Grape aroma compounds deriving from C_{13}-norisoprenoid precursors.

Conversely, many glycoside norisoprenoids were found, particularly 3-hydroxy-β-damascone (40−80 μg/kg of grape), 3-oxo-α-ionol (275−600 μg/kg of grape), 4-oxo-β-ionol (30−75 μg/kg of grape), 3,9-dihydroxymegastigma-5-ene (3-hydroxy-7,8-dihydro-β-ionol, 40−120 μg/kg of grape), 3-hydroxy-7,8-dehydro-β-ionol (30−70 μg/kg of grape), and vomifoliol (up to 1.6 mg/kg of grape). 3-Hydroxy-β-damascone and 3-oxo-α-ionol are associated with tobacco aroma. The finding of 3-hydroxy-7,8-dehydro-β-ionol was interesting, as it is a precursor of β-damascenone, a compound characterized by a complex fragrance (flowers, exotic fruit, honey; olfactory threshold in wine = 5 μg/L) and of 3-hydroxy-β-damascone. The main relationships among these grape noisoprenoids and their precursors are shown in **Figure 1** (*24*, *25*).

In general, total glycoside norisoprenoid contents were particularly high (1.2−2.9 mg/kg of grape). Comparisons among samples collected in different years showed considerable harvest effects, with higher levels of glycoside compounds such as benzyl alcohol, 2-phenylethanol, homovanillic alcohol, *cis*-pyranlinalool oxide, 2-hydroxycineole, geraniol, 3-oxo-α-ionol, and 4-oxo-β-ionol, particularly for Raboso Veronese in the 2005 samples and higher vanillin in the 2004 ones (**Figure 2**).

Volatiles formed by acid hydrolysis are involved in the evolution of wine aroma during aging (*26*). The average contents of benzene compounds, terpenols, and norisoprenoids are listed in **Table 7**. After hydrolysis, increases in 4-vinylphenol and benzoic acid were observed in all samples and in 4-vinylguaiacol in two Raboso Veronese samples. The formation of new terpenols such as *cis*- and *trans*-ocimenol and endiol and increases in *cis*- and *trans*-furanlinalool oxide were observed, with the concomitant disappearance of geraniol, pyranlinalool oxides, *cis*- and *trans*-8-hydroxylinalool, and hydroxygeraniol, matching reports of the transformation of terpenols in acid conditions (*27*). The formation of new norisoprenoids, such as vitispiranes, β-damascenone, and actinidols, and an increase in 3-hydroxy-β-damascone is concomitant with a decrease in their precursors vomifoliol and

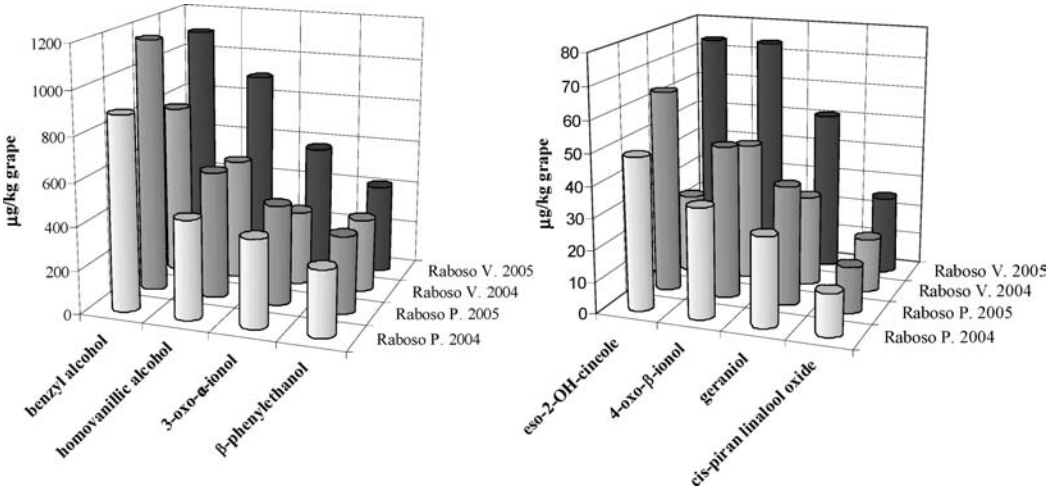

Figure 2. Main glycoside compounds, the contents of which in grape are influenced by harvest year.

Table 7. Benzene Compounds, Monoterpenes, and Norisoprenoids Identified after Acid Hydrolysis[a]

		Raboso Piave (μg/kg of grape)		Raboso Veronese (μg/kg of grape)	
volatile compound by acid hydrolysis	RI[b]	2004 mean	2005 mean	2004 mean	2005 mean
benzenoids					
benzaldehyde		8 ± 2	5 ± 1	9 ± 2	7 ± 4
benzyl alcohol		439 ± 102	237 ± 75	207 ± 66	237 ± 8
2-phenylethanol		211 ± 33	90 ± 20	209 ± 86	182 ± 36
eugenol		14 ± 6	19 ± 9	10 ± 3	11 ± 2
4-vinylguaiacol		141 ± 24	40 ± 16	260 ± 194	172 ± 60
4-vinylphenol		137 ± 29	82 ± 14	137 ± 90	146 ± 66
vanillin		84 ± 15	33 ± 8	154 ± 11	57 ± 5
methyl vanillate (t)[c]	2580	97 ± 22	35 ± 6	77 ± 35	48 ± 9
homovanillic alcohol (t)	2825	343 ± 59	172 ± 37	379 ± 206	316 ± 123
terpenoids					
trans-furanlinalool oxide (t)	1419	48 ± 11	42 ± 11	44 ± 19	39 ± 5
cis-furanlinalool oxide (t)	1448	26 ± 6	24 ± 3	25 ± 13	34 ± 8
cis-ocimenol (t)	1645	12 ± 4	5 ± 2	6 ± 2	4 ± 2
trans-ocimenol (t)	1667	15 ± 6	6 ± 4	7 ± 3	5 ± 2
α-terpineol		27 ± 12	13 ± 5	13 ± 4	7 ± 3
eso-2-hydroxycineole (t)	1834	61 ± 11	32 ± 11	29 ± 7	28 ± 11
enediol (t)	1937	11 ± 4	10 ± 4	8 ± 4	7 ± 2
p-menth-1-ene-7,8-diol (t)	2495	230 ± 69	102 ± 20	46 ± 28	34 ± 16
norisoprenoids					
vitispiranes (t)	1510	12 ± 4	19 ± 7	22 ± 10	9 ± 3
β-damascenone		8 ± 2	10 ± 4	9 ± 4	11 ± 3
actinidol 1 (t)	1894	27 ± 2	15 ± 5	37 ± 16	17 ± 6
actinidol 2 (t)	1906	43 ± 5	23 ± 7	61 ± 27	36 ± 7
3-hydroxy-β-damascone (t)	2506	103 ± 12	66 ± 9	99 ± 34	52 ± 18
3-oxo-α-ionol (t)	2605	302 ± 79	135 ± 19	339± 136	152 ± 11
4-oxo-β-ionol (t)	2614	18 ± 2	15 ± 4	27 ± 16	19 ± 5
dihydroxymegastigma-5-ene (t)	2644	60 ± 9	20 ± 3	75 ± 42	30 ± 7
3-hydroxy-7,8-dehydro-β-ionol (t)	2741	18 ± 4	19 ± 4	24 ± 9	29 ± 13
vomifoliol (t)	3121	679 ± 78	273 ± 41	900 ± 478	498 ± 1

[a] Mean ± standard deviation of three Raboso Piave and two Raboso Veronese grape samples each collected in duplicate for two harvests. Data are expressed as μg of 1-decanol/kg of grape. [b] RI, linear retention index based on a series of n-hydrocarbons. [c] t, tentative of identification according to GC retention index and to fragmentation spectrum.

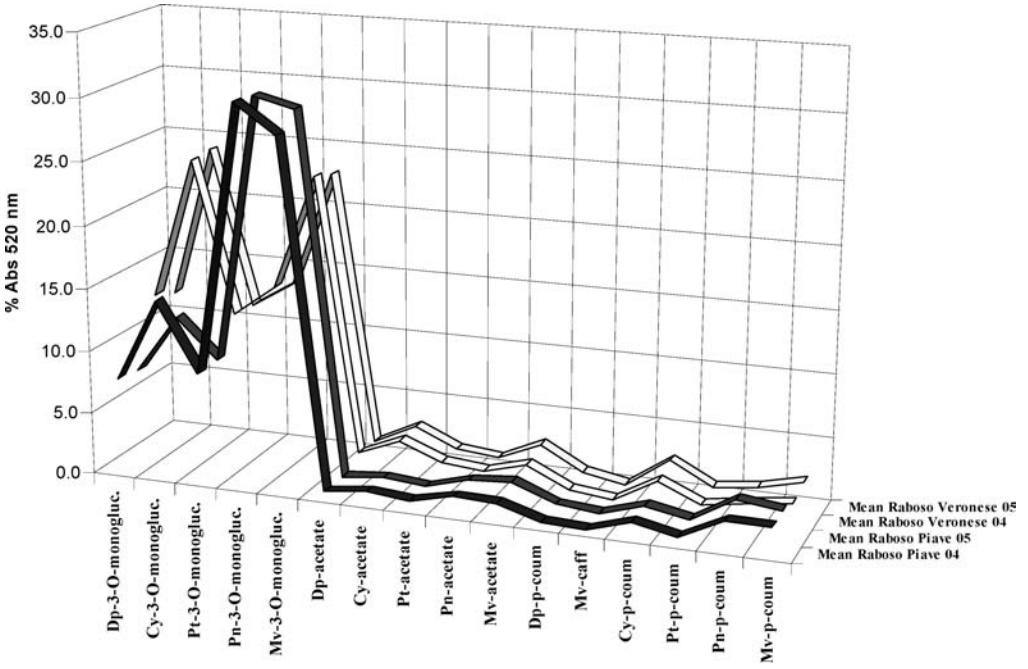

Figure 3. Anthocyanin profiles of Raboso Piave and Raboso Veronese grape samples collected in two harvests.

3-hydroxy-7,8-dehydro-β-ionol (**Figure 1**). The modest level of β-damascenone and increase in 3-hydroxy-β-damascone found matches the slow kinetics of hydrolysis of 3-hydroxy-7,8-dehydro-β-ionol aglycone in acidic conditions (*25*, *26*).

Vitispiranes (formed in wine during bottle aging) and actinidols are associated with a camphor note. Vitispiranes and β-damascenone have been found in Riesling wine up to 200 μg/L and, together with 3-hydroxy-β-damascone, 3-oxo-α-ionol,

3-hydroxy-7,8-dehydro-β-ionol, vomifoliol, and actinidols, are characteristic of other red grape varieties such as Sangiovese, Canaiolo, Grenache, Nebbiolo, and Barbera. These compounds have also been identified in several white grape varieties such as Chardonnay, Prosecco, and Torbato.

Differentiation between Raboso Piave and Raboso Veronese. **Figure 3** shows the Raboso Piave and Raboso Veronese anthocyanin profiles. The former variety had higher levels of peonidin-3-glucoside and malvidin-3-glucoside, matching the results of Mattivi et al. (*28*), and the latter had higher cyanidin-3-glucoside, delphindin-3-glucoside, and acetate anthocyanins. **Figure 3** also shows that harvest year had no effect on grape anthocyanin profiles. With regard to volatile compounds, Raboso Veronese characteristically had higher levels of vanillin and homovanillic alcohol, some glycoside terpenols such as furan and pyranlinalool oxides, *cis*- and *trans*-8-hydroxylinalool, hydroxygeraniol, and norisoprenoids. Instead, *p*-menth-1-ene-7,8-diol was more abundant in Raboso Piave.

An ANOVA was performed to identify the main parameters useful for differentiating the two varieties. Compounds with significance of $p < 0.05$ turned out to be anthocyanins, glucoside flavanols, and some glycoside compounds such as 4-vinylguaiacol, *cis*- and *trans*-furanlinalool oxide, *cis*- and *trans*-pyranlinalool oxide, and *cis*- and *trans*-8-hydroxylinalool.

Factor analysis, calculated with 11 polyphenol variables such as monoglucoside anthocyanins and sum of acylated anthocyanins and flavonols, provided 84% of the cumulative percentage of total variance explained by the first two factors. As **Figure 4A** shows, the cluster of Raboso Piave samples plot to the left and that of Raboso Veronese to the right. Samples were grouped according to cluster analysis with a relatively large cutoff value at the linkage distance of 15, and evident separation between the two varieties was achieved. Factor 1 (F1) separated the two varieties and was mostly charged by five monoglucoside anthocyanins, three glucoside flavonols, and quercetin glucuronide. The separation between two Raboso Veronese samples emphasized their differences, and Raboso Veronese I was also separated from Raboso Piave on the basis of myricetin glucuronide, the main charging variable of F2 (**Figure 4A**).

Instead, factor analysis coupled with cluster analysis with glycoside compounds (15 variables: eugenol, vanillin, homovanillic alcohol, *cis*- and *trans*-furanlinalool oxide, α-terpineol, *cis*- and *trans*-pyranlinalool oxide, geraniol, *trans*-8-hydroxylinalool, hydroxygeraniol, *cis*-8-hydroxylinalool, *p*-menth-1-ene-7,8-diol, 3-hydroxy-β-damascone, 3-oxo-α-ionol, dihydroxymegastigma-5-ene, 3-hydroxy-7,8-dehydro-β-ionol) provided partial separation between Raboso Piave and Raboso Veronese, although the former samples mainly fell in the upper part of the scatterplot and Raboso Veronese in the lower one (**Figure 4B**). In this case, 56% of the cumulative percentage of total variance was explained by the first two factors, and F1 was mostly charged by *cis*- and *trans*-pyranlinalool oxide, geraniol, *trans*-8-hydroxylinalool, hydroxygeraniol, 3-hydroxy-β-damascone, dihydroxymegastigma-5-ene, and 3-hydroxy-7,8-dehydro-β-ionol.

In conclusion, Raboso had high flavonoid contents, high percentages of extractable anthocyanins and polyphenols in winemaking, and substantial contents of norisoprenoid precursors (vitispiranes, β-damascenone, actinidols), which potentially improve wine aroma with aging. From this, we infer that these varieties have high enological potential, which can easily be transferred to the wine by means of suitable winemaking practices.

Raboso Veronese had a high abundance of quercetin glucoside, a compound with demonstrated antioxidant proprieties and beneficial effects on human health (*29*), and stood out for its high content of cyanidin-3-glucoside and higher (+)-catechin and (−)-epicatechin contents compared with Raboso Piave (data in **Tables 2**−**4**), indicating substantial plant biosynthesis toward B-ring disubstituted compounds.

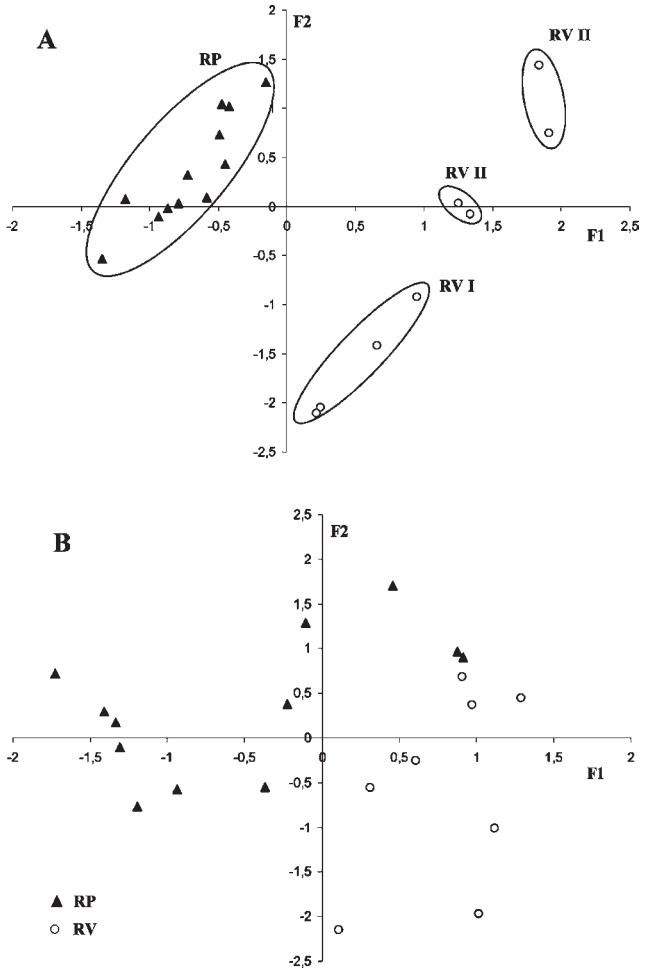

Figure 4. (**A**) Principal component analysis (PCA) calculated with 11 polyphenol variables. Scatterplot of 20 Raboso samples in factor plane (1 × 2); 63% of variance explained by F1 and 23% by F2. Samples are grouped according to cluster analysis. (**B**) PCA calculated with 17 glycoside compound variables. Scatterplot of 20 Raboso samples in factor plane (1 × 2); 39% of variance explained by F1 and 17% by F2. Samples are grouped according to cluster analysis. RP, Raboso Piave; RV, Raboso Veronese.

Study of secondary grape metabolites has been confirmed as an effective tool in differentiating similar varieties, particularly on the basis of polyphenol profiles in cases of crossing between red and white varieties.

Supporting Information Available: Additional figures and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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