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ARTICLE

Evolution of Aroma and Phenolic Compounds during Ripening of 'Superior Seedless' Grapes

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The evolution of aroma and phenolic compounds was studied during ripening of *Vitis vinifera* cv. 'Superior Seedless' grapes in two consecutive years. The major free detected compounds were citral, geraniol, and benzyl alcohol whereas geraniol, citral, nerol, citronellol, dienediol I, linalol oxide I, linalol oxide II, benzyl alcohol, and 2-phenylethanol were identified in the glycosidically bound fraction. Concentrations of the main free terpene alcohols responsible for 'Superior Seedless' aroma decreased during grape development, and bound compounds became predominant at grape maturity. Calculation of odor activity values showed that geraniol was the most active odorant followed to a lesser extent by citral and nerol. With regard to phenolic compound evolution, flavan-3-ols and flavonols were maximal at veraison and decreased throughout the ripening, stilbenes content decreased from the first stage, and total phenolics increased to show a maximum in the ripe grapes. At ripening, quercetin 3-*O*-glucoside and catechin were the main compounds detected in 'Superior Seedless'.

KEYWORDS: Aroma; phenolic compounds; grape; terpenes; glycosides

INTRODUCTION

The region of Murcia (southeastern Spain) has a large agricultural sector (cultivated area ~ 606019 ha) with 1% dedicated to table grapes (*1*). 'Superior Seedless' is an early, seedless table grape cultivated in Spain, with yellow-white flesh, light muscat aroma, and green skin color, which is becoming highly appreciated all over Europe. 'Superior Seedless' was obtained from the Superior Farming Co. (California) and is also known as 'Regular Superior Seedless' or 'Sugraone'.

Varietal aroma is one of the important grape quality factors and is characteristic for every grape variety (*2*, *3*). This parameter has been widely studied, mainly in muscat grape varieties (*4*−*11*), in which numerous terpenes have been identified as responsible for the varietal flavor (*12*). The terpenic alcohols (linalool, nerol, geraniol, α-terpineol, and citronellol) are known to be principally responsible for the aroma of muscat grape (*4*). Other aroma compounds such as hydrocarbons, norisoprenoids, and some alcohols are also of great importance. These aroma compounds are distributed between the berry pulp and skin, with higher concentrations in the latter (*13*).

The aroma components in grape are present in free and bound glycoside forms. Free forms are volatile compounds directly involved in aroma flavor. In contrast, bound glycoside forms are nonvolatile compounds with no direct contribution to the aroma of the grape. However, the glycosides can be transformed into free volatile compounds by hydrolysis, increasing the grape aromatic characteristics (*12*). In general, bound glycoside forms are more abundant than free ones (*13*). These substances are synthesized during berry maturation and are qualitatively and quantitatively influenced by environmental and agricultural factors (*14*).

On the other hand, phenolic compounds are substances with a great impact on the organoleptic characteristics of grapes, and their regular consumption has been associated with beneficial effects for human health. These compounds are antioxidants contributing to a reduction in the risk of cardiovascular diseases and some types of cancer and diabetes (*15*). Phenolic compounds are classified as non-flavonoid compounds (stilbenes, hydroxycinnamic acids, and benzoic acids) and flavonoid compounds (flavanols, flavones, flavonols, and isoflavones). These compounds are found mainly in the solid parts of the grapes, and their composition is influenced by the grape variety and by other factors that affect berry development, such as soil, geographical location, and weather conditions (*16*, *17*).

Whereas studies about aroma composition in several grape cultivars are reported in the literature, to the best of our knowledge no study has been carried out on 'Superior Seedless'. The main objective of this study was to identify and quantify the principal aroma (free and bound glycoside forms) and phenolic compounds present in 'Superior Seedless' and, thereby, to contribute to the characterization of this cultivar during ripening.

MATERIALS AND METHODS

Materials. *Vitis vinifera* L. cv. 'Superior Seedless' was sampled weekly during two seasons (2005 and 2006), in June, July, and August, at an experimental vineyard of the Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario located in Torrepacheco (Murcia, southeastern Spain). Seven plants were sampled weekly: berries were picked off four bunches per plant. For each sampling, berries were

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classified in six stages of maturity: (1) green berries with a diameter of > 15 mm; (2) berries at veraison (from being hard and green to being soft and translucent). From the second maturity stage, berry density was used as a maturity criterion because this parameter increases as the berries advance toward maturity after veraison. Therefore, subsequent stages of maturity were berries with density of (3) 60−80 g/L, (4) 80−100 g/L, (5) 100−110 g/L, and (6) 110−130 g/L. Berries belonging to the same stage of maturity were mixed, constituting a sample that was divided into three subsamples to be analyzed separately. Berries from each sampling were processed similarly during the two years of the project. Afterward, berries from each subsample were squeezed for measurements of juice pH, total acidity (by titration with NaOH), and total soluble solids (TSS) (using a hand-held refractometer). The maturity index was calculated as TSS/total acidity. A second sample of 500 g of sorted berries was frozen immediately in liquid nitrogen and kept at −80 °C for determination of aroma and phenolics compounds. Grape water content was determined by the difference between fresh and dry weight after drying in an oven at 60 °C.

Standards and Solvents. Nerol, geraniol, α-terpineol, linalol oxides I and II (*trans*-furan linalool oxide and *cis*-furan linalool oxide, respectively), and eugenol were purchased from Fluka (Buchs, Switzerland), dienediol and 2-octanol were purchased from Sigma-Aldrich (St. Louis, MO), and linalool, citronellol, benzyl alcohol, 2-phenylethanol, citral, and thymol were purchased from Acros Organics (Geel, Belgium).

Myricetin, quercetin 3-rhamnosylglucoside (rutin), *trans*-resveratrol, gallic acid, (−)-epicatechin, (+)-catechin, and *p*-dimethylaminocinnamaldehyde (DMACA) were purchased from Sigma-Aldrich. Quercetin 3-*O*-glucoside and kaempferol 3-*O*-glucoside were obtained from Extrasynthèse (Genay, France), and *trans*-piceid was supplied by Polyphenols Laboratories AS (Sandnes, Norway). Folin−Ciocalteu reagent was obtained from Fluka. *cis*-Resveratrol and *cis*-piceid were prepared by UV irradiation (sunlight) of *trans*-resveratrol and *trans*-piceid, respectively, for 4 days (total conversion).

All solvents used in this study were of high purity and were supplied by Scharlau (Barcelona, Spain).

Extraction of Aroma Compounds. Extraction of free and glycosidically linked aroma compounds was carried out according to the method of Di Stefano (*18*) with some modifications. Two hundred grams of berries was deseeded and ground under liquid nitrogen using a Dangoumau ball grinder. Fifty grams of ground berries was suspended, with a Polytron PT2000 homogenizer (Kinematica AG, Lucerne, Switzerland), in 100 mL of pure water containing 0.5 g of D-gluconic acid lactone (Sigma) to inhibit grape β-glucosidase activity. Five microliters of 2-octanol (0.4 g/L) was added as internal standard. After stirring for 15 min at 4 °C, the mixture was centrifuged (9000*g*; 20 min; 4 °C) with an Eppendorf model 5810R centrifuge (Hamburg, Germany). The supernatant was filtered through glass wool. The juice was stirred in the presence of 1 g of polyvinylpolypyrrolidone (Sigma) to eliminate the high levels of phenolic compounds capable of inhibiting the glycosidase activities. The mixture was filtered again through glass wool. The clear juice was passed through the SPE column containing 0.5 g of C_{18} Varian (Palo Alto, CA), already activated with 10 mL of methanol and 20 mL of water, at a flow rate of 1 mL/min. The column was rinsed with 50 mL of pure water to eliminate sugars, acids, and other low molecular weight polar compounds. The free fraction was eluted with 100 mL of dichloromethane. The extract was dried free of water over Na_2SO_4, and the volume of solvent was reduced to 2 mL by distillation through a Vigreux column at 35 °C.

The bound fraction was eluted with 50 mL of methanol, and the extract was concentrated to 1 mL under vacuum with a Büchi model R-205 rotavapor (Flawil, Switzerland) at 35 °C. The extract was then transferred into a small tube and concentrated to dryness at 40 °C under a stream of nitrogen. The dried glycosidic extract was dissolved in 1 mL of citrate−phosphate buffer (0.2 M, pH 5). The mixture was washed five times with 1.5 mL of dichloromethane to eliminate possible traces of free volatiles. Enzymatic hydrolysis was carried out using a commercial preparation, AR-2000, with glycosidase side activities (Gist Brocades, Seclin, France). After stirring, the tube was sealed and placed in a water bath at 40 °C for 16 h. After the addition of 5 μL of 2-octanol (0.4 g/L) as internal standard, the mixture was then extracted five times with 0.4 mL of dichloromethane. The extract was dried over Na_2SO_4 and stored at −20 °C until analysis. All analyses were performed in triplicate.

Extraction of Phenolic Compounds. Homogeneous grape samples were separated into skin and pulp. Phenolic compounds were extracted and determined following a slight modification of the method described by Cantos (*19*). Three gram samples of skin were homogenized for 5 min in 20 mL of extraction solution containing methanol/formic acid (97:3 v/v) using a Polytron (PT-MR 3100, St. Gallen, Switzerland). The extracts were centrifuged at 10000*g* for 10 min in an Eppendorf centrifuge (Biotech International, Witten, Germany), and the remaining pellet was re-extracted using fresh extraction solvent, vortexed for 1 min, and centrifuged. This procedure was repeated two times. Finally, the combined extracts were evaporated to dryness under vacuum at < 35 °C, and the residue was dissolved in 2 mL of extraction solution. All samples were passed through 0.45 μm filters prior to HPLC and spectrophotometric techniques. Triplicate extractions were prepared from each maturity stage. All extractions and analyses were performed in the dark to protect the phenolic compounds from degradation.

Gas Chromatography and Mass Spectrometry Analysis of Aroma Compounds. Analysis of the final extract was performed on an Agilent (Waldbronn, Germany) model HP 6890 gas chromatograph equipped with a flame ionization detector and automatic split/splitless injector model Agilent 7683. The columns used were an HP-5MSI (30 m × 0.25 mm i.d.) with 0.25 μm film thickness and a DB-WAX (30 m × 0.32 mm i.d.) with 1.0 μm film thickness. Both stationary phases were supplied by Agilent Technologies. Helium was used as the carrier gas (constant pressure eluting, thymol, 18.55 min for the HP-5MSI column and 55.66 min for DB-WAX). A 2 μL sample was injected into the GC using the splitless mode. The injector and detector were operated at 250 and 280 °C, respectively. The temperature program run for the HP-5MSI column was 60−240 °C at 3.0 °C/min and for the DB-WAX column, 60−175 °C at 2.5 °C/min; after this, it was increased to 195 °C at a rate of 3.5 °C/min followed by a final ramp to 220 °C at a rate of 2.5 °C/min and held for 15 min. The total analysis time for the HP-5MSI and DB-WAX columns was 60.00 and 76.71 min, respectively. The equilibrium time was 1 min.

An Agilent model HP 6890 gas chromatograph equipped with a model 5973N mass spectrometer operating in electron impact ionization mode with an ionizing energy of 70 eV, scanning from *m*/*z* 40 to 300 at 3.21 scans/s, was used. The ion source temperature was 230 °C and the quadrupole temperature, 150 °C. The electron multiplier voltage (EM voltage) was maintained at 1300 V, and a solvent delay of 6.0 min was employed. Gas chromatography was performed under the same conditions used for GC-FID. Analysis was performed with the selected ion monitoring (SIM) mode using target (T, base ion) and qualifier ions (Q_1, Q_2 Q_3, other characteristic ions of lower intensity as primary ionization). Aroma compounds were identified according to their retention indices, which were obtained by using two GC columns with stationary phases of differing polarities and compared with those of known compounds and by comparison of mass spectra using the NBS75K library (U.S. National Bureau of Standards, 1986) and spectra obtained from the standard. Also, target and qualifier ions and the qualifier-to-target abundance ratios (Q/T %) of pure reference standards were compared to those obtained for the aroma compounds under study. The target and qualifier abundances were determined by injection of individual chemical standards under the same chromatographic conditions using full scan with the mass/charge ratio ranging from *m*/*z* 40 to 300. **Table 1** lists the compounds along with their retention index, the target and qualifier ions, and their qualifier-to-target abundance ratios. Qualifier-to-target ratios had to be within a 10% range for positive confirmation.

Odor Activity Values (OAVs). OAVs were calculated by using the equation OAV = *c*/*t*, where *c* is the total concentration of each compound in the grape samples and *t* is the odor threshold value of the compound in water (*20*). In this work, *t* values were taken from information available in the literature (*21*−*25*). According to other authors, only compounds with OAVs > 1 were considered to be active odorants (*26*). Water content values were used to calculate the concentrations of aroma compounds expressed in micrograms per liter to determine the OAVs of these compounds.

HPLC Analysis of Phenolic Compounds. Grape extracts were analyzed using an HPLC system (Hewlett-Packard, Böblingen, Germany) equipped with a G1311A quaternary pump and G1315A photodiode array UV−vis detector. The separation was performed on a 250 mm × 4 mm i.d., 5 μm, reversed phase Lichrocart C_{18} column (Merck, Darmstadt,

Germany) with water/formic acid (95:5 v/v) (A) and acetonitrile (B) as mobile phase. The flow rate was 1 mL/min. Elution was performed using a gradient starting with 5% B to reach 15% at 40 min, 20% at 50 min, and 30% at 70 min; it then became isocratic for 5 min. Chromatograms were recorded at wavelengths of 360, 320, and 280 nm. Peaks were identified by comparison of their elution times and absorbance spectra with commercially available standards. (−)-Epicatechin and (+)-catechin were determined at 208 nm, *trans*-resveratrol at 320 nm, and myricetin, quercetin, kaempferol, quercetin 3-*O*-glucoside, kaempferol 3-*O*-glucoside, and rutin at 360 nm. Analyses were carried out in triplicate. Total flavonols were quantified as the sum of rutin, quercetin, kaempferol, kaempferol-3-*O*-glucoside, and quercetin-3-glucoside. Stilbenes were calculated as the sum of *cis*- and *trans*-resveratrol and their *cis*- and *trans*-glucosides.

Analysis of Total Phenolics. Total polyphenol content in the grape skin extract was determined with the Folin−Ciocalteu method (*27*, *28*) adapted to a microplaque. In a 1.5 mL plaque, 170 μL of distilled water, 10 μL of sample (diluted appropriately), and 25 μL of Folin−Ciocalteu reagent were added and vortexed. After 10 min, 2% aqueous sodium carbonate (50 μL) was added, and the mixture was vortexed and allowed to stand at 45 °C in the dark for 20 min. The absorbance was read at 750 nm, and the total polyphenol concentration was calculated from a calibration curve, using gallic acid as a standard. The results were expressed as milligrams per liter of gallic acid equivalents (GAE).

Table 1. Retention Index (RI), Target Ion (T), Qualifier Ions (Q_1, Q_2, and Q_3) (*m*/*z*), and Abundance Ratios of Qualifier Ion/Target Ion (Q_1/T and Q_2/T, %)[a] of the Studied Compounds

	RI							
compound	HP-5MSI	DB-WAX	T	Q_1	Q_2	Q_3	Q_1/T	Q_2/T
linalol oxide I	1088	1468	59	94	93	68	63.0	47.4
linalol oxide II	1070	1496	59	94	93	111	61.2	48.2
linalool	1101	1565	71	93	80	121	86.3	35.0
α-terpineol	1202	1718	59	93	121	136	87.9	78.8
citral	1284	1756	69	84	94	137	30.8	19.7
citronellol	1241	1782	69	67	82	95	63.5	53.2
nerol	1241	1822	69	93	68	67	60.3	26.4
geraniol	1268	1870	69	93	68	67	27.3	19.8
benzyl alcohol	1024	1917	79	108	107	77	94.8	66.0
2-phenylethanol	1116	1954	91	92	122	65	57.6	26.8
dienediol I	1201	1976	82	71	67	55	63.4	36.5
dienediol II	1288	2161	67	71	82	55	85.1	52.6
eugenol	1366	2215	164	149	131	103	35.2	27.0
internal standards								
2-octanol	986	1440	45	55	97	84	26.10	12.50
thymol	1305	2226	135	150	91	136	30.12	14.58

[a] Q/T (%) ratios are the results of dividing the abundance values of the qualifier ion (Q_1, Q_2) by the abundance of the target ion (T), × 100.

Analysis of Total Flavan 3-ols. The total flavanol content was estimated using the DMACA method (*29*). Grape extract (25 μL, diluted appropriately) was introduced into a 1.5 mL Eppendorf tube, and 1 mL of DMACA solution (0.1% in HCl/MeOH, 8:98 v/v) was added. The mixture was vortexed and allowed to react at room temperature for 30 min. The absorbance was read at 638 nm, and the concentration of total flavanols was estimated from a calibration curve, constructed using known concentrations of (+)-catechin. The results were expressed as milligrams per liter of catechin equivalents.

Statistical Analysis. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS 15.0) program (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Changes in Classic Parameters during Berry Development. TSS, pH, and maturity index increased and total acidity decreased in 'Superior Seedless' over the berry maturation period (**Table 2**). In agreement with previous results reported for different grape varieties, the largest increase of TSS was observed at the beginning of the maturation period (*30*). This increase coincided with a large decrease in total acidity and a consequent increase in maturity index.

Changes in Free and Bound Volatile Compounds. To assess the aromatic potential of 'Superior Seedless' grapes, the concentrations of free and bound aroma compounds were determined during 2005 and 2006. In both years, a total of 13 compounds in both forms were identified and quantified during ripening, 10 of which were monoterpenes, whereas the other three were alcohols (benzyl alcohol, 2-phenylethanol, and eugenol) (**Tables 3** and **4**). At the first maturity stage (diameter > 15 mm), 3,7-dimethyl-1,5-octadiene-3,7-diol (dienediol I) was the most-abundant free compound detected, followed by geraniol and *trans*-furan linalool oxide (linalol oxide I) (**Table 3**). The concentrations of the free dienediol I and linalol oxide isomers showed a large decrease during maturation. Dienediol I is not considered to contribute directly to grape aroma, but it is a precursor of hotrienol and nerol oxide (*31*, *32*). However, we did not find these compounds in 'Superior Seedless' grapes. Free 3,7-dimethyl-1,7-octadiene-3,6-diol (dienediol II) was detected only at the first maturity stages, at concentrations much lower than those of dienediol I. Glycosidically bound dienediol and linalol oxide were also identified in 'Superior Seedless' grapes (**Table 4**). These compounds decreased during maturation. In 2006, at the first maturity stage, linalol oxide I showed the highest concentration after benzyl alcohol. However, in 2005, it was the most abundant of the volatile compounds. This difference may be attributed to differences in environmental conditions (light, temperature, etc.) between years.

Table 2. Total Soluble Solids (TSS, °Brix), pH, Total Acidity (Grams of Tartaric per Liter), and Maturity Index in Developing 'Superior Seedless' Grapes

	maturity stage[a]					
	>15 mm	véraison	60−80 g/L	80−100 g/L	100−110 g/L	110−130 g/L
			2005			
TSS	5.8 ± 0.3	10.4 ± 0.1	11.4 ± 0.2	14.6 ± 0.1	16.7 ± 0.2	17.9 ± 0.2
pH	2.6 ± 0.0	2.8 ± 0.0	2.9 ± 0.0	3.2 ± 0.0	3.6 ± 0.0	3.6 ± 0.0
acidity	34.18 ± 0.52	18.40 ± 0.33	12.09 ± 0.75	6.31 ± 0.33	4.63 ± 0.13	3.44 ± 0.16
maturity index	0.17 ± 0.01	0.57 ± 0.01	0.95 ± 0.07	2.32 ± 0.11	3.62 ± 0.13	5.25 ± 0.28
			2006			
TSS	5.6 ± 0.2	10.9 ± 0.2	11.4 ± 0.1	14.0 ± 0.1	17.2 ± 0.1	18.4 ± 0.2
pH	2.6 ± 0.0	2.9 ± 0.0	3.0 ± 0.0	3.2 ± 0.0	3.5 ± 0.0	3.5 ± 0.0
acidity	33.66 ± 0.39	19.52 ± 0.47	16.33 ± 0.28	9.04 ± 0.10	4.85 ± 0.10	4.61 ± 0.11
maturity index	0.17 ± 0.01	0.56 ± 0.01	0.70 ± 0.02	1.54 ± 0.02	3.55 ± 0.08	3.99 ± 0.06

[a] Values are mean ± standard error (*n* = 3).

Table 3. Evolution of Free Volatile Compounds (Micrograms per Kilogram) in Developing 'Superior Seedless' Grapes

	maturity stage[a]						
compound	>15 mm	véraison	60−80 g/L	80−100 g/L	100−110 g/L	110−130 g/L	P^b
			2005				
linalol oxide I	13.20 ± 0.69 d	6.02 ± 0.91 c	2.54 ± 0.24 b	2.00 ± 0.23 ab	0.61 ± 0.03 a	0.67 ± 0.09 a	0.000
linalol oxide II	4.92 ± 0.47 b	2.76 ± 0.50 a	2.09 ± 0.23 a	1.21 ± 0.11 a	nd	nd	0.001
linalool	5.34 ± 0.36 c	5.18 ± 0.56 c	3.46 ± 0.32 b	0.95 ± 0.04 a	1.16 ± 0.15 a	0.96 ± 0.05 a	0.000
α-terpineol	1.39 ± 0.07	1.12 ± 0.04	1.08 ± 0.00	1.33 ± 0.13	1.30 ± 0.07	1.17 ± 0.05	ns
citral	6.40 ± 0.26 b	5.60 ± 0.17 ab	5.03 ± 0.58 ab	5.40 ± 0.38 ab	5.08 ± 0.33 ab	4.04 ± 0.18 a	0.010
citronellol	9.73 ± 0.52 d	5.47 ± 0.49 c	2.85 ± 0.18 b	1.09 ± 0.05 a	0.43 ± 0.03 a	0.46 ± 0.04 a	0.000
nerol	2.23 ± 0.12 bc	1.78 ± 0.12 ab	2.44 ± 0.14 c	1.87 ± 0.13 abc	2.01 ± 0.01 bc	1.37 ± 0.17 a	0.001
geraniol	49.86 ± 1.31 d	24.77 ± 1.45 c	19.27 ± 0.27 ab	22.17 ± 0.67 bc	15.00 ± 1.10 a	15.07 ± 0.58 a	0.000
benzyl alcohol	3.79 ± 0.17 abc	3.91 ± 0.56 bc	2.53 ± 0.24 ab	2.23 ± 0.24 a	3.13 ± 0.35 ab	4.88 ± 0.34 c	0.001
2-phenylethanol	3.42 ± 0.20 c	3.22 ± 0.21 c	1.17 ± 0.08 b	1.09 ± 0.11 a	0.94 ± 0.03 a	0.89 ± 0.07 a	0.000
dienediol I	102.96 ± 16.55 c	27.07 ± 5.25 b	11.17 ± 2.04 ab	4.29 ± 0.47 a	1.79 ± 0.16 a	0.83 ± 0.09 a	0.000
dienediol II	0.40 ± 0.03	0.21 ± 0.02	nd	nd	nd	nd	ns
eugenol	0.13 ± 0.03	nd	nd	0.12 ± 0.02	nd	nd	ns
			2006				
linalol oxide I	17.49 ± 3.99 c	5.89 ± 0.67 b	2.97 ± 0.40 ab	3.17 ± 0.49 ab	0.45 ± 0.07 a	1.55 ± 0.05 a	0.000
linalol oxide II	5.62 ± 1.16 b	3.15 ± 0.31 ab	1.78 ± 0.25 a	3.48 ± 0.35 ab	nd	nd	0.000
linalool	5.19 ± 1.09 c	3.82 ± 0.49 bc	2.29 ± 0.24 ab	1.53 ± 0.22 ab	1.26 ± 0.20 a	1.02 ± 0.22 a	0.001
α-terpineol	1.12 ± 0.12	1.35 ± 0.19	0.99 ± 0.05	1.42 ± 0.25	0.96 ± 0.21	1.19 ± 0.02	ns
citral	4.68 ± 0.18	4.82 ± 0.54	4.51 ± 0.35	5.02 ± 0.87	4.73 ± 0.36	3.72 ± 0.35	ns
citronellol	8.10 ± 0.71 b	2.92 ± 0.26 a	3.37 ± 0.22 a	2.08 ± 0.22 a	nd	nd	0.000
nerol	1.22 ± 0.13 a	1.87 ± 0.05 ab	2.34 ± 0.16 b	2.52 ± 0.15 b	2.28 ± 0.36 b	1.79 ± 0.04 ab	0.003
geraniol	40.53 ± 1.24 c	20.33 ± 0.07 ab	19.09 ± 1.03 ab	26.41 ± 1.31 b	20.74 ± 2.47 ab	18.91 ± 0.48 a	0.000
benzyl alcohol	2.88 ± 0.39	1.69 ± 0.34	1.51 ± 0.22	1.64 ± 0.02	2.30 ± 0.47	2.98 ± 0.67	ns
2-phenylethanol	0.89 ± 0.09	0.89 ± 0.09	0.70 ± 0.11	0.66 ± 0.01	0.63 ± 0.02	0.73 ± 0.07	ns
dienediol I	80.46 ± 3.02 d	19.05 ± 0.59 c	10.83 ± 0.25 b	7.44 ± 0.50 b	1.02 ± 0.33 a	0.48 ± 0.08 a	0.000
dienediol II	0.48 ± 0.02	nd	nd	nd	nd	nd	ns
eugenol	nd	nd	nd	nd	nd	nd	ns

[a] Values are mean ± standard error ($n = 3$). Different letters within rows indicate statistical differences according to Tukey's test ($P < 0.05$). nd, not detected; ns, nonsignificant differences according to ANOVA. [b] P values for statistical significance according to ANOVA.

The terpene alcohols (linalool, nerol, geraniol, α-terpineol, and citronellol) are synthesized from glucose by acetyl-coenzyme A (*7*) and are compounds that impart a floral character to berries (*4*). Terpenols are discriminatory among cultivars (*8*), and their synthesis depends on environmental and agricultural factors (*9*, *10*). In 2005 and 2006, free geraniol, citronellol, and linalool levels decreased during the first stages of berry development, and they remained constant until the end of ripening, except for citronellol in 2006, which was not detected in the two last maturity stages. The high concentration of free geraniol observed in the green berry suggests a significant biosynthetic role of this compound during ripening (*33*). On the other hand, free nerol and α-terpineol were found at low concentrations, and their levels stayed almost constant during berry development. Glycosidically bound forms of monoterpene compounds reached higher values than free forms during all of the maturation period. These results are in agreement with those found in other varieties (*6*, *30*). In general, for both seasons, the concentrations of geranyl and neryl derivatives increased during ripening, whereas citronellol glycoside decreased during berry development. Concentrations of linalool and α-terpineol glycosides remained almost constant during berry development. Finally, free citral levels stayed constant, whereas the citral glycoside concentration increased during development.

Benzyl alcohol, eugenol, and 2-phenylethanol, which are aromatic alcohols, were detected (except eugenol in 2006) at low concentrations in their free forms, and their levels fluctuated during ripening. For both seasons, benzyl alcohol, eugenol, and 2-phenylethanol were also identified in the glycosidically bound fraction. During maturation, the concentrations of these volatile alcohol components were higher than those of free alcohols at all stages of maturation. At all maturation stages, bound benzyl alcohol was more abundant than bound 2-phenylethanol, and their concentrations increased during ripening. At the mature stage, benzyl alcohol, nerol, and geraniol glycosides were the most abundant precursor compounds in the grapes.

Voirin et al. (*5*) indicated that the presence of aromatic alcohols is associated with neutral cultivars. Thus, the levels of benzyl alcohol and 2-phenylethanol are quite high in nonmuscat grape varieties in which terpenols are less abundant (*5*, *10*, *14*). 'Superior Seedless' is considered to have a light muscat aroma and, as a consequence, concentrations of terpenols, benzyl alcohol, and 2-phenylethanol in this variety were shown to be intermediate between those of typical muscat and neutral varieties (*13*, *30*).

Odor Activity Values. The OAVs of aroma compounds were calculated to estimate the sensory contribution of these odorants to the general grape flavor. Among all of the compounds identified in the grape samples, only geraniol, citral, and nerol showed OAV levels above their odor thresholds (OAVs > 1) at ripeness (**Table 5**). In general, geraniol had the highest OAVs, with values above the odor threshold (OAVs > 1) throughout the maturation period (except for the second and third maturation stages in 2006). In contrast with previous results for muscat grape aroma in which linalool showed the highest contribution to aroma (*11*, *30*), geraniol was the compound contributing most to aroma in 'Superior Seedless'. Citral and nerol showed OAVs > 1 at the last maturation stages. Recent studies have reported the relevance to the overall aroma of substances present at concentrations representing at least 20% of their threshold value

Table 4. Evolution of Glycosidically Bound Compounds (Micrograms per Kilogram) in Developing 'Superior Seedless' Grapes

	maturity stage[a]						
compound	>15 mm	véraison	60−80 g/L	80−100 g/L	100−110 g/L	110−130 g/L	P^b
			2005				
linalol oxide I	106.76 ± 5.46 d	82.26 ± 6.36 c	160.67 ± 5.81 e	67.21 ± 3.96 bc	48.07 ± 2.44 ab	34.78 ± 2.65 a	0.000
linalol oxide II	35.87 ± 1.39 c	30.38 ± 2.81 c	50.53 ± 1.50 d	27.20 ± 2.66 bc	16.67 ± 1.79 a	18.93 ± 2.19 ab	0.000
linalool	1.78 ± 0.15	1.77 ± 0.12	1.63 ± 0.19	1.72 ± 0.14	1.75 ± 0.06	2.11 ± 0.11	ns
α-terpineol	2.17 ± 0.08 cd	1.41 ± 0.07 ab	2.79 ± 0.09 d	1.89 ± 0.25 bc	1.15 ± 0.13 a	1.41 ± 0.07 ab	0.000
citral	17.75 ± 1.84 bc	5.47 ± 0.55 a	12.47 ± 0.64 ab	23.53 ± 0.87 c	39.48 ± 1.98 d	72.67 ± 4.06 e	0.000
citronellol	33.00 ± 0.87 d	31.16 ± 2.33 cd	23.22 ± 0.90 bc	20.36 ± 0.95 ab	17.16 ± 0.48 a	16.65 ± 1.68 a	0.000
nerol	22.97 ± 0.57 a	28.44 ± 2.22 a	37.72 ± 0.25 a	80.48 ± 6.24 b	280.40 ± 5.95 c	281.23 ± 11.41 c	0.000
geraniol	25.07 ± 1.48 a	14.00 ± 1.80 a	46.95 ± 3.25 ab	63.82 ± 5.59 b	159.24 ± 14.58 c	227.79 ± 11.37 c	0.000
benzyl alcohol	95.08 ± 5.64 a	141.73 ± 10.33 ab	192.47 ± 26.14 bc	164.03 ± 5.09 b	300.24 ± 12.80 d	253.47 ± 10.48 cd	0.000
2-phenylethanol	23.87 ± 1.19 a	30.93 ± 2.92 a	71.70 ± 4.86 b	64.22 ± 6.84 b	78.28 ± 3.39 b	84.67 ± 6.92 b	0.000
dienediol I	23.06 ± 1.10 b	28.18 ± 1.18 c	17.34 ± 1.18 ab	15.95 ± 1.11 a	15.01 ± 1.37 a	14.32 ± 0.61 a	0.000
dienediol II	0.94 ± 0.20 b	1.06 ± 0.15 c	0.64 ± 0.12 ab	0.60 ± 0.07 a	0.51 ± 0.06 a	0.50 ± 0.02 a	0.000
eugenol	0.98 ± 0.04 ab	0.81 ± 0.02 b	0.89 ± 0.06 b	0.90 ± 0.07 b	0.99 ± 0.08 ab	1.20 ± 0.07 b	0.011
			2006				
linalol oxide I	79.47 ± 5.61 a	67.38 ± 4.22 a	150.01 ± 25.63 b	74.09 ± 12.67 a	50.44 ± 1.77 a	36.71 ± 0.50 a	0.000
linalol oxide II	41.90 ± 3.89 a	32.25 ± 0.67 a	76.00 ± 7.32 b	39.82 ± 7.34 a	21.79 ± 1.65 a	20.93 ± 0.30 a	0.000
linalool	2.39 ± 0.42	2.03 ± 0.13	3.09 ± 0.32	2.64 ± 0.39	1.71 ± 0.14	2.18 ± 0.14	ns
α-terpineol	2.24 ± 0.27 a	1.52 ± 0.20 a	3.36 ± 0.05 b	2.36 ± 0.26 a	1.71 ± 0.13 a	1.95 ± 0.25 a	0.001
citral	22.81 ± 5.45 ab	8.50 ± 0.36 a	12.30 ± 0.62 ab	29.45 ± 4.53 b	59.97 ± 4.44 c	91.67 ± 6.65 c	0.000
citronellol	33.25 ± 2.47 b	20.84 ± 0.33 a	38.39 ± 2.65 b	19.53 ± 1.60 a	17.84 ± 1.43 a	17.78 ± 0.32 a	0.000
nerol	4.62 ± 0.76 a	8.88 ± 0.74 a	3.18 ± 0.21 a	36.44 ± 10.40 b	200.41 ± 18.43 c	200.85 ± 3.03 b	0.000
geraniol	7.70 ± 0.42 ab	10.93 ± 0.75 ab	2.84 ± 0.34 a	25.19 ± 6.87 b	160.63 ± 13.65 c	205.02 ± 4.33 d	0.000
benzyl alcohol	122.52 ± 4.35 a	168.43 ± 3.48 ab	394.78 ± 13.69 c	232.65 ± 35.35 b	349.38 ± 32.21 c	349.40 ± 30.29 c	0.000
2-phenylethanol	18.04 ± 1.10 a	31.79 ± 0.79 ab	84.63 ± 6.10 d	54.59 ± 8.59 bc	61.31 ± 3.85 c	66.84 ± 3.68 c	0.000
dienediol I	29.23 ± 3.52 c	13.49 ± 0.45 ab	19.41 ± 1.52 b	12.23 ± 1.58 ab	7.97 ± 0.58 a	8.75 ± 0.05 a	0.000
dienediol II	1.35 ± 0.17 bc	0.71 ± 0.04 a	1.66 ± 0.19 c	0.98 ± 0.16 ab	1.01 ± 0.10 ab	0.60 ± 0.05 a	0.001
eugenol	1.22 ± 0.31 a	1.14 ± 0.03 a	4.20 ± 0.06 c	2.26 ± 0.34 b	1.14 ± 0.09 a	1.34 ± 0.19 ab	0.000

[a] Values are mean ± standard error ($n = 3$). Different letters within rows indicate statistical differences according to Tukey's test ($P < 0.05$). ns, nonsignificant differences according to ANOVA. [b] P values for statistical significance according to ANOVA.

Table 5. Odor Activity Values of Compounds with Greater Influence on the Aroma of 'Superior Seedless' Grapes

		maturity stage											
		2005						2006					
compound	published odor threshold in water (μg/L)	>15 mm	véraison	60−80 g/L	80−100 g/L	100−110 g/L	110−130 g/L	>15 mm	véraison	60−80 g/L	80−100 g/L	100−110 g/L	110−130 g/L
linalol oxide I	>3000[a]	0.04	0.03	0.06	0.03	0.02	0.01	0.03	0.03	0.06	0.03	0.02	0.02
linalol oxide II	>3000[a]	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.01	0.03	0.02	0.01	0.01
linalool	6[c]	1.28	1.32	0.96	0.52	0.60	0.64	1.36	1.11	1.01	0.81	0.61	0.67
α-terpineol	330[d]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
citral	32[c]	0.81	0.39	0.62	1.06	1.72	3.00	0.93	0.47	0.59	1.26	2.50	3.73
citronellol	40[b]	1.15	1.04	0.74	0.63	0.54	0.53	1.12	0.67	1.18	0.63	0.55	0.56
nerol	300[b]	0.09	0.11	0.15	0.32	1.16	1.18	0.02	0.04	0.02	0.15	0.84	0.84
geraniol	40[d]	2.02	1.10	1.87	2.51	5.39	7.59	1.30	0.89	0.62	1.51	5.61	7.00
other compounds													
benzyl alcohol	10000[e]	0.01	0.02	0.02	0.02	0.04	0.03	0.01	0.02	0.04	0.03	0.04	0.04
2-phenylethanol	1100[e]	0.03	0.04	0.07	0.07	0.09	0.10	0.02	0.03	0.09	0.06	0.07	0.08
eugenol	6[c]	0.20	0.15	0.17	0.20	0.20	0.25	0.22	0.22	0.79	0.44	0.24	0.28

[a] Karagiannis et al. (*21*). [b] Ohloff (*22*). [c] Buttery et al. (*23*). [d] Takeoka et al. (*24*). [e] Buttery et al. (*25*).

(OAV > 0.2) (*34*). According to this, linalool and citronellol could contribute to the final aroma, with OAVs of 0.5−0.7 at the end of maturation. When results from 2005 were compared with those of 2006, the OAVs at the end of maturation were similar for each compound. Therefore, the results obtained during two consecutive years provide representative information about which compounds are potentially the principal contributors to aroma in this variety.

Changes in Phenolic Compounds during Berry Development. The flavan-3-ols and flavonols concentrations had a typical evolution during ripening in white grapes (**Figure 1**). For total flavan-3-ols, initial concentrations were about 3 mg/kg of berry, and then a sharp increase was observed with the maximum concentration (about 22 mg/kg of berry) occurring during the veraison stage (**Figure 1a**). This peak was followed by a rapid decrease, leading to the final concentration of 8.3 mg/kg. The content of flavonols was calculated as the sum of rutin, quercetin, kaempferol, kaempferol-3-*O*-glucoside, and quercetin-3-glucoside, which represent the majority of flavonols. At the end of ripening, the main flavonol by far was quercetin 3-glucoside (80%), followed by

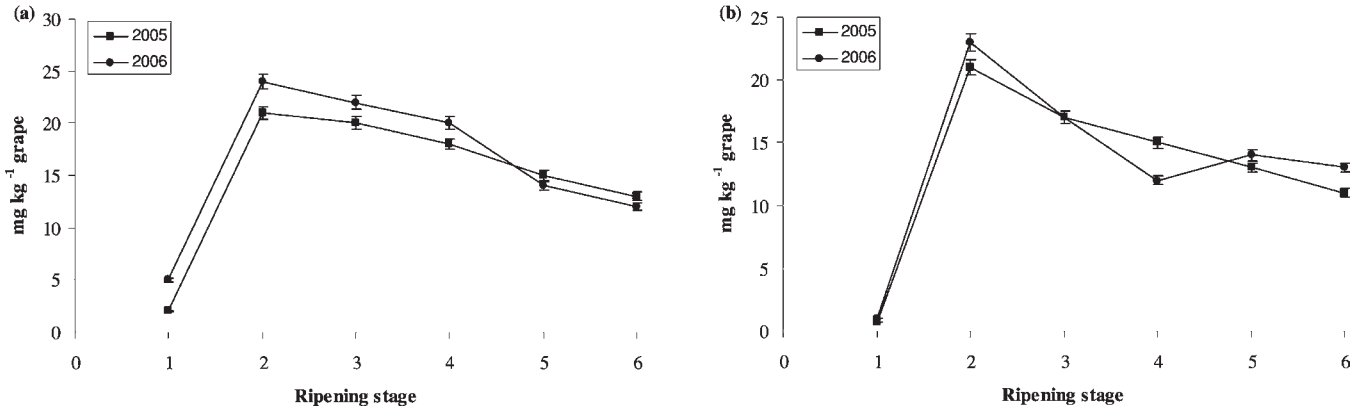

Figure 1. Evolution of flavan-3-ols, expressed as catechin (**a**), and flavonols, calculated as the sum of rutin, quercetin, kaempferol, kaempferol-3-*O*-glucoside, and quercetin-3-glucoside (**b**), in 'Superior Seedless' during ripening in the 2005 and 2006 seasons. Ripening stages: 1, green berries with a diameter of >15 mm; 2, berries at véraison; and 3, berries with density of 60−80 g/L; 4, 80−100 g/L; 5, 100−110 g/L; and 6, 110−130 g/L.

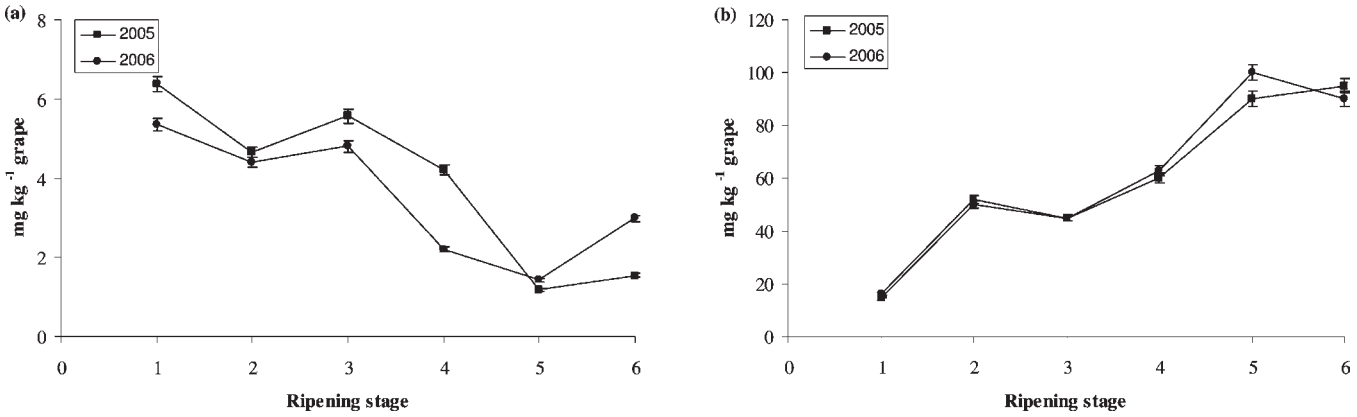

Figure 2. Evolution of stilbenes, calculated as the sum of *cis*- and *trans*-resveratrol and their *cis*- and *trans*-glucosides (**a**), and total phenolics, expressed as gallic acid (**b**), in 'Superior Seedless' during ripening in the 2005 and 2006 seasons. Ripening stages: 1, green berries with a diameter of >15 mm; 2, berries at véraison; and 3, berries with density of 60−80 g/L; 4, 80−100 g/L; 5, 100−110 g/L; and 6, 110−130 g/L.

kaempferol 3-glucoside (16%) and quercetin (3%). The evolution during ripening was similar for individual flavonols, and therefore only the total flavonol profile is represented (**Figure 1b**). Initial concentrations were about 1 mg/kg of berry, and then a sharp increase was observed until 18 mg/kg. The increases in catechin and flavonol occurred from late July to early August, the hottest period; a similar result was reported in previous studies concerning quercetin and catechin (*35*, *36*). The delphinidin-like flavonols (myricetin, laricitrin, and syringetin) were absent in all of the stages investigated, in agreement with the very low mRNA levels of F3′5′H reported in the berry skin of white varieties (*37*, *38*). With respect to flavanols determined in the skin extract, catechin (40%), epicatechin (20%), and two unidentified compounds were the major neutral phenolic constituents. Some authors found that the concentrations of these compounds and other proanthocyanidins were highest at veraison, decreased until just before complete ripeness, and then remained relatively constant (*39*, *40*). No significant year-to-year variations in the amounts of phenolic compounds during ripening were noted.

Stilbenes were calculated as the sum of *cis*- and *trans*-resveratrol and their *cis*- and *trans*-glucosides. The most abundant stilbene was *trans*-piceid (glycosylated form). Because similar evolution during ripening was observed for individual stilbenes, only the total stilbene profile is represented (**Figure 2a**). The stilbene content of whole berries declined steadily between the green stage and complete maturity, approaching zero in ripe fruit in 2005, whereas a small increase was seen at the end of ripening in 2006.

The total polyphenol content in the skin extract was determined with the Folin−Ciocalteu method, as gallic acid content. As expected, it increased throughout ripening, reaching its maximum in the last period before harvest due to increases in the phenolic components such as gallic and cinnamic acids (**Figure 2b**). When the results for 2005 were compared with those of 2006, no statistically significant differences were found at the end of ripening for polyphenolic content. The total value obtained is higher than the sum of the individual phenolic compounds, suggesting that some other phenolic compounds may be present in the skin but not identified in this study.

In this study, the evolution of free and glycosidically bound aroma components and phenolic compounds of 'Superior Seedless' grapes has been studied. With regard to the aroma components, accumulation of the main compounds detected was observed at the end of ripening. The major compounds detected were geraniol, benzyl alcohol, citral, nerol, and α-terpineol in the free fraction and nerol, benzyl alcohol, geraniol, 2-phenylethanol, and citral in the bound fraction. According to other studies on muscat varieties, most compounds showed higher concentrations in the bound fraction than in the free one. Averaged over the two years of this study, the monoterpenes mainly contributing to the final aroma (OAVs > 1) included citral, geraniol, and nerol. With regard to the phenolic composition, flavanols and flavonols showed maxima during veraison, whereas the stilbene content

started to decline from the green berry state onward. At ripening, the main compounds were quercetin 3-glucoside, catechin, and epicatechin.

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