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Analysis of Carotenoids in Grapes To Predict Norisoprenoid Varietal Aroma of Wines from Apulia

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To determine a correlation between carotenoid precursors in grapes and norisoprenoid varietal aroma of wine, carotenoids were identified and quantified by HPLC-DAD-MS (ESI^+) from four representative wine grape varieties of the Apulian region (Chardonnay, Merlot, Negroamaro, Primitivo) in two years of study (2006−2007), and C_{13}-norisoprenoid aroma potential, ΔC (μg/kg), was calculated from the difference of total carotenoid concentration between véraison and maturity. C_{13}-norisoprenoids were analyzed by GC-MS in the obtained wines from 2006 and 2007 vintages. Higher ΔC values, found in Chardonnay and Merlot grapes, corresponded to higher norisoprenoid contents in the respective wines, particularly characterized by highly flavorant compounds such as β-damascenone and 3-oxo-α-ionol. A linear regression was determined that was significant at the 0.01% level (F = 36.12, p = 0.00096) with R = 0.9261, between grape ΔC values and total norisoprenoid contents in wine. These findings support the hypothesis that ΔC could be a useful technological tool to predict norisoprenoid aroma of wine and, consequently, to identify grapes with higher aroma potential.

KEYWORDS: Carotenoids; wine grapes; C_{13}-norisoprenoids; aroma potential

INTRODUCTION

Some of the most important key aroma components present in wine include C_{13}-norisoprenoids such as β-ionone or β-damascenone (*1*−*3*). These molecules have an important sensorial impact on wine aroma as they have very low olfactory perception thresholds (*4*−*7*). Norisoprenoids contribute characteristic aromas to many varieties of *Vitis vinifera* (*8*, *9*). In Chardonnay, Williams et al. (*10*) showed that "grassy", "tea", "lime", "honey", and "pineapple" aromas were derived from norisoprenoids and their precursors. Both red and white nonfloral varieties, including Chenin blanc, Semillon, Sauvignon blanc, Cabernet Sauvignon, and Syrah, are known to contain significant levels of norisoprenoids. Even in the floral varieties (e.g., White Riesling and Muscat), which derive most of their aroma impact from terpenes, norisoprenoid concentrations up to 40% higher than those of terpenes have been observed (*11*).

C_{13}-Norisoprenoids are thought to arise from photochemical and enzymatic oxidation of carotenoids (*12*−*14*) and occur in grapes as glycosidically bound precursors; β-carotene and some xanthophylls (neoxanthin, flavoxanthin, and lutein) are abundant before véraison and subsequentely decrease dramatically (*15*−*17*), giving rise to different derivatives, each specific to the initial precursor compounds and highly flavorant (*18*, *19*). The most important norisoprenoid aroma compounds are formed by complex chemical rearrangements of the odorless aglycones present in grapes that happen during winemaking and wine storage (*18*−*22*).

β-Damascenone is particularly interesting, because of its very low sensory threshold of approximately 2 ng/L in water (*5*, *39*). It is believed to arise through the degradation of allenic carotenoids, such as neoxanthin, and the subsequent glycosylation of the derived alcohols. This norisoprenoid ketone is principally generated from hydrolyzable precursors (*5*). β-Damascenone exhibits a complex aroma, which, depending on its concentration, is reminiscent of tropical flowers with fruity and berry undertones (*7*). Its sensory detection threshold is 50 ng/L in hydroalcoholic solution (*3*), which indicates that it may be important to wine flavor.

β- and α-ionone have detection thresholds in wines, respectively, of 90 (*4*) and 400 ng/L (*26*): the first has multiple descriptors, including violets, floral, and fruity (*26*), whereas the latter has been described as dry fruit and raspberry-like (*26*).

Considering that carotenoids are precursors of norisoprenoids (*13*), knowledge of the biogenic pathway of the formation of these compounds has already been suggested as a useful tool to predict the potential for volatile compounds to be formed in wine (*27*). To our knowledge there are few studies (*27*, *28*) on the quantitative relationship between carotenoid level in grapes and total norisoprenoids in respective wines. The purpose of the present work was to develop a means to estimate norisoprenoid aroma of wine based on the difference (ΔC) between the total concentration of carotenoids in grapes between véraison and maturity stages. A parameter such as ΔC would be a useful technological tool to identify grapes with a higher aroma potential before processing.

MATERIALS AND METHODS

Samples. Changes in carotenoid levels between véraison and maturity stages during grape ripening were studied for two seasons (2006 and 2007) in four *Vitis vinifera* varieties, Chardonnay b. clone 76, Merlot n. clone 181, Primitivo n. clone UBA 55A, and Negroamaro, grown in the Apulian

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region and planted in the same nonirrigated trial site on a sandy-clay soil composed of 50% sand, 12% slit, and 38% clay, with a root zone depth of 1 m, of the Agricultural Research Council Research Unit for table grapes and wine growing in Mediterranean environment (CRA-UTV) in Turi, Bari, Italy. To minimize the effect of nonenvironmental factors, special care was taken to achieve maximum uniformity in viticultural conditions. All vines were planted in 1998 on 1103P (*Vitis berlandieri* × *Vitis rupestris*), with a planting density of 4130 vines ha^{-1}, and a vine spacing of 2.2 m between rows and 1.1 m within a row. Vines were fertilized once a year just before bud break with 400 kg ha^{-1} of a complex fertilizer (Nitrophosca blu spezial 12.12.17.2), and the trellising system was a double Guyot. Yield was limited by pruning to 18−20 buds/vine.

Three replicated random samples of 10 bunches, for each variety, were picked between the third and seventh nodes from random plants at weekly intervals from véraison until harvest. From each sample of bunches, approximately 500 g of berries was then chosen at random, immediately frozen, and stored in the dark at −20 °C until analysis.

Wines from the 2006 and 2007 vintages were produced from the grapes harvested from the same vineyard plots, in three replications and under the same conditions. Harvest was done manually (in the third decade of August for Chardonnay and Merlot and in the third decade of September for Primitivo and Negroamaro), and grapes were separated from the stalks, crushed, and maintained in 100 L capacity stainless steel vats with a commercial sulfiting agent (20 g/100 kg of must, corresponding to 10 mg L^{-1} of free SO_2) ($K_2S_2O_5$−N° CE 240-795-3, EVER s.r.l., Pramaggiore, Venice, Italy). The maceration period was 10 days (only for black varieties) with two daily reconstitutions at 20 °C. The must was separated from the solid parts and transferred to 80 L capacity stainless steel vats. With regard to white grapes, the must was clarified by bentonite (0.5 g L^{-1}), gelatin (0.1 g L^{-1}), and commercial enzymes with pectinolytic activity (15 mg L^{-1}).

To start alcohol fermentation, active yeast *Saccharomyces cerevisiae* 206 at a 0.25 g L^{-1} dose was added to the musts. Fermentation temperature was about 17 °C for white wine and about 20 °C for red wines. Once the alcohol fermentation had finished, the wines were chilled to 15−20 °C for 20 days. $K_2S_2O_5$ (35 mg L^{-1} of free SO_2 on average) was added, and then they were bottled. All of the samples were 12 months old at the time of analysis.

Climatic Data. Climatic data used in this study were collected from a meteorological station situated in close proximity to the vineyards. The data consisted of daily recordings of maximum, minimum, and mean temperatures, thermal amplitude, rainfall, and radiation, collected during the fruit ripening periods of the four wine-grape varieties.

Reagents and Commercial Standards. NaOH (0.1 N), Na_2SO_4 (dry), HPLC grade solvents hexane and acetone, and HPLC grade water were purchased from J. T. Baker. LC-MS grade solvents methanol and *tert*-butyl methyl ether were purchased from Chromasolv. Bromothymol blue solution 0.4% in ethanol, methanol RPE, and dichloromethane RPE were purchased from Carlo Erba. Triethylamine (TEA), 3-*tert*-butyl-4-hydroxyanisole (BHA), and diethyl ether ACS ≥ 99.8% were purchased from Fluka. β-Apo-8′-carotenal and 2-octanol (Fluka) were used as HPLC and GC internal standard, respectively. β-Carotene (Sigma-Aldrich), lutein, and zeaxanthin (Extrasynthese) and (9′*Z*)-neoxanthin, violaxanthin, lutein-5,6-epoxide, (9*Z*)-β-carotene, and (13*Z*)-β-carotene (CaroteNature) were used as HPLC reference standards. β-Damascenone (Sigma-Aldrich) and α-ionone, β-damascone, and β-ionone (Fluka) were used as GC reference standards.

Chemical Analysis on Grapes. Total soluble solids (TSS), titrable acidity (TA), and pH were determined according to protocols established by the OIV (*29*). Berries were crushed to determine TSS (expressed as g L^{-1}) of berry juice using a portable refractometer (ATAGO PR32). Even TA (as g of tartaric acid L^{-1}) was determined, diluting the juice with deionized water and titrating with 0.1 N sodium hydroxide to the bromothymol blue end point according to Regulation CEE 2676/90 (1990). Finally, juice pH was measured, too, by means of pH-meter Crison Basic 20.

Extraction of Carotenoids from Grapes. The carotenoid extraction procedure was adapted from the method of Guedes de Pinho et al. (*30*). Approximately 50 g of fresh berries with 25 μL of BHA (12 mg/mL in EtOH) added, placed in liquid nitrogen, was crushed in a homogenizer for 5 min. The provided sample was spiked with 200 μL of internal standard solution in acetone (100 μg/mL of β-apo-8′-carotenal) and diluted with 40 mL of water (HPLC grade, J. T. Baker). Extraction was done with 40 mL of hexane/diethyl ether (1:1, v/v) by agitating the mixture for 30 min. The resulting upper layer was separated. The extraction procedure was repeated twice for the lower phase using 20 mL of hexane/diethyl ether (1:1, v/v). The pooled extract was evaporated to dryness using a rotovapor Buchi-R-205. The residue was dissolved in 2 mL of acetone/hexane (1:1, v/v) and used for carotenoid analysis by HPLC. Sample handling, homogenization, and extraction were carried out on ice under dim yellow light to minimize light-induced isomerization and oxidation of carotenoids.

HPLC-DAD-MS Analysis. The HPLC-DAD-MS system adopted in this work consisted of a HPLC 1100 equipped with a degasser, quaternary pump solvent delivery, thermostated column compartment, diode array detector, and MSD Trap XCT Plus in a series configuration (Agilent Technologies, Palo Alto, CA).

The reversed stationary phase employed was a YMC pack C30 (YMC Inc., Wilmington, NC), 5 μm (250 × 3 mm i.d.), with a precolumn C30 5 μm (20 × 3 mm i.d.). The following gradient system was used with H_2O (solvent A), methanol (solvent B), and *tert*-butyl methyl ether (solvent C) in the presence of 0.05% of TEA to the three LC mobile phases: 0−2 min, % A−% B−% C, 40−60−0; 5 min, 20−80−0; 10 min, 4−81−15; 60 min, 4−11−85; stop time to 71 min. The flow was maintained at 0.2 mL/min; sample injection was 10 μL.

Diode array detection was between 250 and 650 nm, and absorbance was recorded at 447 nm.

MSD Trap XCT Plus was an ion trap mass spectrometer equipped with an ESI source. Positive electrospray mode was used for ionization of molecules with acquisition of mass spectra between m/z 100 and 1200, capillary voltage was set at −4000 V, and skimmer voltage was set at 30 V.

All data were acquired and processed using ChemStation software (Agilent Technologies, Palo Alto, CA).

Compound identification was achieved by combining different information: positions of absorption maxima (λ_{max}), the degree of vibrational fine structure (% III/II), the ratio of the absorbance of the *cis* peak to the absorbance of the second absorption band in the visible region known as *Q* ratio or D_B/D_{II} (*25*), the capacity factor values k', and mass spectra were compared with those from pure standards and/or interpreted with the help of structural models already hypothesized in the literature.

Quantification of xanthophylls and carotenes was made by using the calibration curves of pure standards, (*all-E*)-lutein and β-carotene, with R^2 = 0.9921 and 0.9953, respectively.

Quantification was performed as described by Zulueta et al. (*32*); briefly, the chromatogram was separated into two parts: all of the carotenoids up to k' = 4.78 and including (*all-E*)-lutein were quantified as such, and the remaining *Z*/*E* carotene isomers were quantified with the β-carotene standard. Finally, single carotenoid quantities were added together.

The variation coefficient, based on three replicates, was ≤10% for the sum of carotenoids.

Extraction of Norisoprenoids from Wines. C_{13}-Norisoprenoids, such as β-damascenone or β-ionone, are recognized as compounds of intermediate analytical accessibility. The analysis of these compounds is possible after a powerful isolation−preconcentration step and further GC-MS (*33*−*36*). The method of Lopez et al. (*33*) was used with slight modifications. Briefly, cartridges with a styrene−divinylbenzene sorbent (Phenomenex, STRATA-X 33 μm, 500 mg/6 mL) were placed in the extraction system and rinsed with 10 mL of dichloromethane, 10 mL of methanol, and, finally, 10 mL of a water−ethanol mixture (12%, v/v). Wine (125 mL), containing 60 μL of BHA in ethanol (12.66 mg/mL), was passed through the SPE cartridge at around 2 mL/min. When the wine volume was completely absorbed, the cartridge was dried by letting air pass through it for 20 min. Analytes were recovered by elution with 5 mL of dichloromethane. Organic fractions were dried over Na_2SO_4, spiked with 200 μL of the internal standard solution containing 2-octanol (5 mg/mL) in dichloromethane, and concentrated first in vacuo at room temperature and then under a nitrogen stream until 0.3 mL. The extracts were then capped and stored at −80 °C until GC-MS analysis.

GC-MS Analysis. The analysis was carried out using an Agilent HP 6890N gas chromatograph fitted with an Agilent 5973N mass spectrometer detector equipped with a DB-Wax capillary column (60 m × 0.25 mm i.d., film thickness, 0.25 μm; J&W Scientific Inc., Folsom, CA) and an automatic injector (HP 6890 series injector). A microliter of the extract was injected using an automatic sampler in splitless mode. The split/splitless injector was maintained at 250 °C with a flow of 30 mL/min and a split time of 0.5 min. The pressure of the carrier gas (helium 5.6) was 20 psi with a linear velocity of

1 mL/min; the oven temperature was 40 °C (for 5 min), then increased at 2 °C min^{-1} to 200 °C, and held at this temperature for an additional 15 min. The detector was maintained at 280 °C. Mass spectra were acquired in the electron impact mode (EI 70 eV). The mass range was from m/z 28 to 300, and the electron multiplier was set in the relative mode autotune procedures. The identification of C_{13} norisoprenoids in GC-MS was confirmed by comparing mass spectra in scan mode with those from pure commercially available standards (for α-ionone, β-ionone, β-damascone, and β-damascenone) and Kovats indices and mass spectra present in the NIST05 MS library (for vitispirane, TDN, 3-OH-β-damascone, 3-oxo-α-ionol 3-oxo-7,8-dihydro-α-ionol, 3-OH-7,8-dihydro-β-ionol, 2,3-dehydro-4-oxo-β-ionol, and dehydrovomifoliol) or in the literature (*48*, *49*). The compounds were measured using selected ion monitoring (SIM) mode and quantified as α-ionone, β-ionone, or β-damascenone equivalents. Ions shown in **Table 7** for compounds and ions m/z 115, 97, and 45 for 2-octanol (internal standard) were used for quantification. Calibration graphs were prepared by the GC-MS analysis of dichloromethane solutions containing known amounts of the standards, α-ionone, β-ionone, and β-damascenone, and of the internal standard, 2-octanol. The square of the correlation coefficient of the regression line, obtained from the calibration data for β-damascenone, α-ionone, and β-ionone, gave R^2 values of 0.9988, 0.9978, and 0.9973, respectively.

The detection and quantification limits were calculated by least-squares linear regression increasing 3.3 and 10 times standard deviation of slope over slope ratio (σ_b/b) and for the standard compounds were as follows: 0.20/0.64, 0.41/1.25, and 0.08/0.24, respectively, for β-damascenone, α-ionone, and β-ionone. Because of the lack of commercial standard, the other identified norisoprenoids were quantified, according to their structural characteristics, as β-damascenone, α-ionone. or β-ionone equivalents with the same detection limits.

The variation coefficients for the analyses, based on three consecutive determinations by three extractions applied to the same wine, ranged from 5 to 20% for individual compounds.

Statistical Analysis. The data were statistically analyzed using the Statistica 6.0 software package (StatSoft Inc., Tulsa, OK). Factorial analysis of variance (ANOVA) and the Tukey HSD post hoc test were applied to norisoprenoid data. Correlation analysis was carried out between total norisoprenoid level and climatic parameters of the two seasons (2006 and 2007). To establish a relationship between the difference of total carotenoids between véraison and maturity, ΔC, found in grapes and total norisoprenoids determined in their respective wines, regression analysis and principal component analysis (PCA) were carried out.

Table 1. Climatic Parameters of Grape Ripening Seasons for 2006 and 2007[a]

	temperatures (°C)					
	minimum	maximum	mean	thermal amplitude	rainfall (mm)	radiation (kJ/m^2)
			Chardonnay/Merlot[b]			
2006	13.9	35.4	24.7	21.5	11.9	24636.5
2007	16.8	37.0	26.9	20.2	0	22430.7
			Primitivo/Negroamaro[c]			
2006	13.2	27.2	20.2	14.0	74.4	20251.8
2007	9.4	29.6	19.5	20.2	15.5	17501.1

[a] Values are means for the weather station network near the vineyards. [b] Data were collected between the second and third decades of August. [c] Data were collected between the first and third decades of September.

RESULTS AND DISCUSSION

Climatic Data. The general climate of the Apulian region is Mediterranean, warm, and characterized by a large temperature range. The coolest month is January, with temperatures of 2−10 °C, whereas the hottest one is July (16−30 °C); rarely does the temperature

Table 2. HPLC-DAD-MS (ESI^+) Characteristics of Carotenoids in Grapes

compound	k'	λ_{max} (nm)	% (III/II)[a]	D_B/D_{II}[b]	identification	MS
violaxanthin	3.15	416; 439; 469	86		std; UV;[c] MS[d]	600; 564; 520
(9′*Z*)-neoxanthin	3.21	414; 436; 464	86		std; UV;[c] MS[d]	600; 582; 564; 520
lutein-5,6-epoxide	3.35	416; 439; 468	88		std; UV;[c] MS[d]	584; 566; 548
(*all-E*)-lutein	4.05	(422); 445; 472	42		std; UV;[c] MS[d]	568; 550
zeaxanthin	4.31	(425); 450; 475	22		std; UV;[c] MS[d]	568; 550
(9*Z*) or (9′*Z*)-lutein	4.47	330; (418); 440; 468	52	0.075	UV; MS	568; 550
(9*Z*) or (9′*Z*)-lutein	4.78	330; (418); 440; 468	43	0.067	UV; MS	568; 550
β-carotene	6.67	(428); 452; 478	25		std; UV;[c] MS[d]	536; 444
(9*Z*)- β-carotene	6.94	342; (424); 446; 474	17	0.03	std; UV;[c] MS[d]	536; 444
β-apo-8′-carotenal		460			internal standard	

[a] %III/II is the ratio of the height of the longest wavelength absorption peak, designated III, and that of the middle absorption peak, designated II, taking the minimum between the two peaks as baseline (*47*). [b] *Q* ratio, which is the quotient between the *cis* peak band and band II (normally λ_{max}) (*31*). [c] Identification by comparison with UV−vis spectrum of the standard compound. [d] Identification by comparison with MS spectrum of the standard compound.

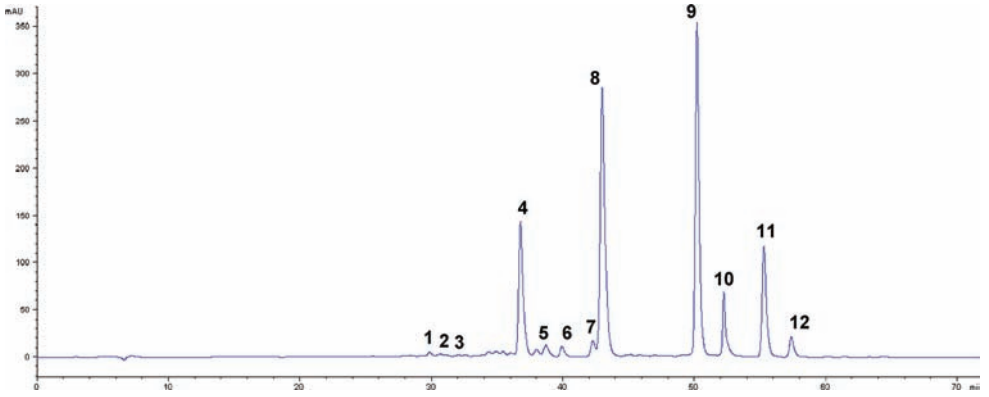

Figure 1. HPLC-DAD chromatogram of Chardonnay at harvest time (August 30, 2007). Peaks: (1) violaxanthin; (2) (9′*Z*)-neoxanthin; (3) lutein-5,6-epoxide; (4) (*all-E*)-lutein; (5) zeaxanthin; (6, 7) (9*Z*)- and (9′*Z*)-lutein; (8) internal standard; (9) pheophytin *b*; (10) pheophytin *a*; (11) β-carotene; (12) (9*Z*)-β-carotene.

Table 3. Changes in Carotenoid Content (Expressed as Micrograms per Kilogram of Berries, $m^a \pm \sigma^b$) in Chardonnay Grapes from Véraison to Maturity in the Two Years of Study (2006 and 2007)

2006						
sampling date	July 30	Aug 6[c]	Aug 13	Aug 21	Aug 28	
TSS[d]	78[a] ± 5[b]	112 ± 9	186 ± 17	212 ± 20	218 ± 20	
pH	2.55 ± 0.08	3.02 ± 0.15	3.15 ± 0.16	3.49 ± 0.10	3.55 ± 0.11	
TA[e]	25 ± 3	10.1 ± 0.9	6.1 ± 0.3	5.5 ± 0.3	5.2 ± 0.3	
TSS/TA[f]	3.12 ± 0.18	11.09 ± 0.17	30.49 ± 0.14	38.55 ± 0.14	41.92 ± 0.15	
compound						
violaxanthin[g]	61 ± 3	108 ± 3	102 ± 5	103 ± 7	42 ± 3	
(9′*Z*)-neoxanthin[g]	47.6 ± 1.8	75 ± 3	119 ± 3	91 ± 3	35.7 ± 1.7	
lutein-5,6-epoxide[g]	57 ± 3	78 ± 3	66 ± 3	85 ± 5	42.7 ± 1.7	
(*all-E*)-lutein	265 ± 16	1210 ± 16	1320 ± 20	271 ± 13	253 ± 18	
zeaxanthin[g]	59.8 ± 1.7	261 ± 16	270 ± 14	277 ± 15	182 ± 15	
Z-lutein[h]	46 ± 4	87 ± 3	131 ± 18	131 ± 30	75 ± 3	
β-carotene	540 ± 60	1890 ± 50	980 ± 50	1000 ± 50	690 ± 60	
(9*Z*)-β-carotene	67.7 ± 1.8	280 ± 70	310 ± 60	490 ± 60	170 ± 60	
total[i]	1140 ± 90	3990 ± 170	3300 ± 170	2450 ± 180	1490 ± 160	ΔC^j = 2500 ± 300
2007						
sampling date	July 31	Aug 8	Aug 16[c]	Aug 23	Aug 30	
TSS	68 ± 5	89 ± 6	136 ± 11	158 ± 13	178 ± 16	
pH	2.62 ± 0.08	2.70 ± 0.14	3.27 ± 0.16	3.47 ± 0.14	3.52 ± 0.12	
TA	27 ± 3	15.7 ± 1.4	10.4 ± 0.5	7.0 ± 0.4	5.1 ± 0.3	
TSS/TA	2.52 ± 0.18	5.67 ± 0.16	13.08 ± 0.13	22.57 ± 0.14	34.90 ± 0.15	
compound						
violaxanthin	63 ± 5	100 ± 5	86 ± 5	74 ± 5	89 ± 7	
(9′*Z*)-neoxanthin	57 ± 4	69 ± 3	53 ± 4	76 ± 5	66 ± 5	
lutein-5,6-epoxide	46 ± 3	68 ± 3	88 ± 5	62 ± 5	71 ± 5	
(*all-E*)-lutein	480 ± 20	793 ± 16	580 ± 14	286 ± 14	181 ± 15	
zeaxanthin	98 ± 13	158 ± 15	110 ± 17	73.6 ± 1.7	68.6 ± 1.7	
Z-lutein	130 ± 3	154 ± 3	105 ± 3	81 ± 3	84 ± 4	
β-carotene	1850 ± 60	3400 ± 60	1970 ± 50	1460 ± 50	890 ± 60	
(9*Z*)-β-carotene	710 ± 60	930 ± 60	430 ± 60	270 ± 70	190 ± 60	
total	3430 ± 170	5670 ± 170	3420 ± 160	2380 ± 160	1640 ± 160	ΔC = 4000 ± 300

[a] Means of three replicates. [b] Standard deviation at $p \leq 0.05$. [c] Veraison: phenologic phase showing 10% of berries softening and/or coloring. [d] Total soluble solids are expressed in g/L. [e] Total acidity expressed in g/L as tartaric acid. [f] Maturation index. [g] Expressed as lutein equivalent. [h] (9*Z*) + (9′*Z*)-lutein. [i] Sum of identified carotenoids. [j] Difference of total carotenoids concentration between véraison and maturity.

fall below 0 °C. Annual rainfall in the region is scarce (640 mm per year averaged over 30 years), and summer is usually dry.

According to **Table 1**, higher temperatures occurred in the last two decades of August, during which Chardonnay and Merlot grapes ripen, in 2007 than in 2006, whereas for the ripening period for Primitivo and Negroamaro grapes (September) a slightly lower mean temperature was observed in 2007. Furthermore, in both ripening periods, there was a considerable decrease in rainfall and a slight decrease in radiation impact from 2006 to 2007 vintage.

HPLC Analysis of Carotenoids in Grapes and C_{13}-Norisoprenoid Aroma Potential Determination. Among carotenoids detected (**Table 2**; **Figure 1**) the compounds revealed in higher amounts were (*all-E*)-lutein ($k' = 4.05$) and β-carotene ($k' = 6.67$). The 5,6-epoxyxanthophylls violaxanthin ($k' = 3.15$), (9′*Z*)-neoxanthin ($k' = 3.21$), and 5,6-epoxylutein ($k' = 3.35$) were also identified. Very interesting was the identification of zeaxanthin, a structural isomer of lutein, which is commonly found at low levels in grapes and has chromatographic properties similar to those of the very abundant (*all-E*)-lutein (*37*). In our experimental conditions zeaxanthin and lutein were efficiently resolved by a selective factor of >1 ($\alpha = 1.06$).

Finally, some *cis*-isomers of lutein and β-carotene were identified by λ_{maxs} slightly lower than those of the *all-E*-carotenoids and by the presence of the *cis* peak at about 140 nm below the longest wavelength absorption maximum of the *all-E* form. The location of the *Z*-double bond was indicated by D_B/D_{II}, which is the ratio of the height of the *cis* peak, designated D_B, and that of the middle main absorption peak, designated D_{II} (*38*). This ratio indicates the intensity of the *cis* peak, which is greater as the *Z*-double bond is closer to the center of the molecule.

The compounds with capacity factors of 4.47 and 4.78, respectively, were tentatively identified as (9*Z*)- or (9′*Z*)-lutein on the basis of the low intensity of their *cis* peaks ($D_B/D_{II} = 0.075$ and 0.067, respectively) and the hypsochromic shift of their absorption maxima (4 nm with respect to *all-E* -lutein) (*31*). The identification of the last peak of the chromatogram ($k' = 6.94$) as the isomer (9*Z*)-β-carotene ($D_B/D_{II} = 0.03$) was done by matching its chromatographic and spectrometric properties with those of the pure standard (CaroteNature, Switzerland).

Changes in carotenoid concentration of berries (expressed in μg/kg of berries) during fruit ripening were established (**Tables 3**−**6**; see also the Supporting Information) in 2006 and 2007. Total carotenoids increased until véraison (10% of berries softening and/or coloring) and decreased steadily during grape ripening in all varieties (**Tables 3**−**6**). Carotenoids formed at an early stage progressively degraded without fresh synthesis taking place (*16*). Degradation occurs during berry metabolism either enzymatically or by a chemical pathway in acid medium (*13*, *16*). Chardonnay and Merlot had a higher concentration of

Table 4. Changes in Carotenoid Content (Expressed as Micrograms per Kilogram of Berries, $m^a \pm \sigma^b$) in Merlot Grapes from Véraison to Maturity in the Two Years of Study (2006 and 2007)

2006						
sampling date	July 30	Aug 6[c]	Aug13	Aug 21	Aug 28	
TSS[d]	69[a] ± 5[b]	130 ± 10	152 ± 12	210 ± 19	228 ± 20	
pH	2.65 ± 0.08	3.08 ± 0.15	3.39 ± 0.17	3.40 ± 0.14	3.54 ± 0.11	
TA[e]	23 ± 3	9.4 ± 0.8	6.5 ± 0.3	4.6 ± 0.2	4.6 ± 0.2	
TSS/TA[f]	3.0 ± 0.2	13.82 ± 0.17	23.38 ± 0.13	45.65 ± 0.13	49.56 ± 0.13	
compound						
violaxanthin[g]	104 ± 5	100 ± 5	95 ± 5	135 ± 7	86 ± 5	
(9′*Z*)-neoxanthin[g]	131 ± 3	141 ± 3	91 ± 3	86 ± 5	114 ± 5	
lutein-5,6-epoxide[g]	74 ± 3	63 ± 4	47 ± 3	98 ± 5	143 ± 5	
(*all-E*)-lutein	1069 ± 19	1240 ± 20	937 ± 18	764 ± 14	682 ± 16	
zeaxanthin[g]	80.7 ± 1.7	200 ± 16	137 ± 16	112 ± 16	110 ± 16	
Z-lutein[h]	91 ± 3	110 ± 3	93 ± 3	110 ± 3	79 ± 4	
β-carotene	590 ± 50	1260 ± 50	1550 ± 50	1090 ± 50	800 ± 50	
(9*Z*)-β-carotene	210 ± 70	220 ± 70	510 ± 60	360 ± 60	200 ± 60	
total[i]	2350 ± 160	3330 ± 170	3460 ± 160	2760 ± 160	2210 ± 160	ΔC[j] = 1100 ± 300
2007						
sampling date	July 31	Aug 8	Aug 16[c]	Aug 23	Aug 30	
TSS	52 ± 4	56 ± 5	93 ± 7	138 ± 12	173 ± 16	
pH	2.58 ± 0.09	2.65 ± 0.13	2.96 ± 0.15	3.15 ± 0.13	3.52 ± 0.14	
TA	26 ± 3	25 ± 2	12.8 ± 0.6	7.0 ± 0.4	4.1 ± 0.2	
TSS/TA	2.00 ± 0.19	2.24 ± 0.17	7.75 ± 0.13	19.71 ± 0.15	42.20 ± 0.14	
compound						
violaxanthin	99 ± 5	149 ± 6	119 ± 5	119 ± 7	97 ± 7	
(9′*Z*)-neoxanthin	106 ± 3	147 ± 4	110 ± 4	95 ± 5	76 ± 5	
lutein-5,6-epoxide	44 ± 3	62 ± 4	68 ± 3	55 ± 3	76 ± 5	
(*all-E*)-lutein	871 ± 19	923 ± 17	1007 ± 19	542 ± 14	459 ± 15	
zeaxanthin	89.4 ± 1.8	155 ± 17	115 ± 16	65.8 ± 1.7	57.5 ± 1.6	
Z-lutein	144 ± 4	180 ± 4	171 ± 3	132 ± 3	94 ± 3	
β-carotene	1980 ± 40	2710 ± 50	2840 ± 50	2010 ± 50	1610 ± 50	
(9*Z*)-β-carotene	420 ± 70	640 ± 70	540 ± 60	370 ± 70	200 ± 70	
total	3750 ± 150	4970 ± 170	4970 ± 160	3390 ± 150	3670 ± 160	ΔC = 2300 ± 300

[a] Means of three replicates. [b] Standard deviation at $p \leq 0.05$. [c] Veraison: phenologic phase showing 10% of berries softening and/or coloring. [d] Total soluble solid are expressed in g/L. [e] Total acidity expressed in g/L as tartaric acid. [f] Maturation index. [g] Expressed as lutein equivalent. [h] (9*Z*) + (9′*Z*)-lutein. [i] Sum of identified carotenoids. [j] Difference of total carotenoids concentration between véraison and maturity.

carotenoids at véraison than Negroamaro and Primitivo. Moreover, carotenoids underwent a higher reduction during ripening in Chardonnay (both years) and in Merlot (in 2007) than in the other cultivars (**Tables 3**−**6**). Therefore, Chardonnay and Merlot had the highest ΔC over the two years (3300 and 1700 μg/kg, respectively, combined over years), approximately 3−9-fold higher than those of Primitivo and Negroamaro (400 and 600 μg/kg, respectively).

Furthermore, all analyzed samples showed higher (*all-E*)-lutein and β-carotene contents compared to other compounds. At grape maturity, β-carotene was 2−4-fold higher than (*all-E*)-lutein in all varieties.

Zeaxanthin was the second xanthophyll in concentration in grapes with a pattern during ripening similar to that of (*all-E*)-lutein in the four varieties. Concerning the identified 5,6-epoxyxanthophylls (violaxanthin, neoxanthin, and lutein-5,6-epoxide), they followed a less consistent pattern than the other carotenoids. In particular 5,6-epoxylutein increased during fruit ripening, especially in the Merlot variety, reaching at maturity a content ∼2−3-fold higher than that at veraison (**Table 4**).

GC-MS Analysis of Norisoprenoids in Wines. The results of GC-MS analysis of Chardonnay, Merlot, Primitivo, and Negroamaro wines (2006−2007 vintages) are reported in **Table 7**. The mean values of total C_{13}-norisoprenoid content (expressed in μg/L of wine) over the two years were higher in Chardonnay and Merlot (1000 and 330 μg/L, respectively) than in Primitivo and Negroamaro (216 and 108 μg/L, respectively).

Among identified norisoprenoids, the major product, in all analyzed samples, was the flavorless 3-hydroxy-β-damascone: a 7-oxygenated megastigmane derived, like β-damascenone, by neoxanthin degradation. Typically, the ratio of 3-hydroxy-β-damascone to β-damascenone generated in wines is 10:1 (*5*); in the analyzed extracts (**Table 7**), this ratio was confirmed in red wines, but in Chardonnay this value was reduced to ∼3:1 because of a higher concentration of damascenone. This finding could be due to a higher presence, in Chardonnay grapes, of megastigma-4,6,7-triene-3,9-diol (**I** in **Figure 2**), precursor of both 3-hydroxy-β-damascone and β-damascenone, glycosylated at the C9 position and favoring ionization at C3, would produce β-damascenone (**II** in **Figure 2**) (*11*, *18*). Conversely, in red grapes, the diol (**I** in **Figure 2**) could be predominantly glycosylated at the C3 hydroxyl group, thus favoring ionization at the C9 position and formation of the corresponding glycoside of 3-hydroxy-β-damascone (**III** in **Figure 2**).

β-Damascenone has a very low sensory threshold of approximately 2 ng/L in water (*5*, *39*); therefore, it can have an important direct or indirect (*40*) impact on the wine aroma. β-Damascenone contents found in the red wines (Merlot, Primitivo, Negroamaro) in our research were slightly higher than those found by Kotseridis et al. (*4*) in Merlot wines (0.2−1.3 μg/L); however, our results were

Table 5. Changes in Carotenoid Content (Expressed as Micrograms per Kilogram of Berries, $m^a \pm \sigma^b$) in Primitivo Grapes from Véraison to Maturity in the Two Years of Study (2006 and 2007)

2006						
sampling date	Aug 31	Sept 6[c]	Sept 12	Sept 18	Sept 25	
TSS[d]	94[a] ± 7[b]	140 ± 11	158 ± 13	168 ± 15	186 ± 17	
pH	2.75 ± 0.07	3.20 ± 0.16	3.37 ± 0.17	3.37 ± 0.13	3.51 ± 0.11	
TA[e]	23 ± 2	7.5 ± 0.4	5.9 ± 0.3	5.6 ± 0.3	4.9 ± 0.3	
TSS/TA[f]	4.09 ± 0.16	18.67 ± 0.13	26.77 ± 0.13	30.00 ± 0.14	37.96 ± 0.15	
compound						
violaxanthin[g]	85 ± 2	104 ± 5	129 ± 5	126 ± 5	147 ± 5	
(9′*Z*)-neoxanthin[g]	107 ± 3	65 ± 5	92 ± 4	80 ± 5	93 ± 5	
lutein-5,6-epoxide[g]	68 ± 2	106 ± 3	138 ± 3	158 ± 3	153 ± 5	
(*all-E*)-lutein	440 ± 20	590 ± 20	750 ± 10	290 ± 8	130 ± 11	
zeaxanthin[g]	110 ± 10	80.3 ± 1.1	97 ± 10	74.6 ± 1.1	84.4 ± 1.1	
Z-lutein[h]	62 ± 3	45 ± 3	53 ± 2	53 ± 2	53 ± 2	
β-carotene	660 ± 40	840 ± 50	550 ± 20	650 ± 30	590 ± 30	
(9*Z*)-β-carotene	180 ± 40	260 ± 40	190 ± 40	310 ± 40	300 ± 40	
total[i]	1710 ± 120	2090 ± 130	2000 ± 100	1740 ± 110	1540 ± 160	ΔC^j = 600 ± 300
2007						
sampling date	Sept 2	Sept 9[c]	Sept 16	Sept 23	Sept 30	
TSS	67 ± 5	116 ± 9	156 ± 12	178 ± 16	213 ± 19	
pH	2.75 ± 0.10	2.94 ± 0.15	3.14 ± 0.16	3.27 ± 0.12	3.41 ± 0.12	
TA	24 ± 3	12.8 ± 0.5	7.7 ± 0.3	6.7 ± 0.4	5.4 ± 0.4	
TSS/TA	2.79 ± 0.19	9.06 ± 0.13	20.25 ± 0.12	26.52 ± 0.15	39.44 ± 0.16	
compound						
violaxanthin	50 ± 3	65 ± 4	80 ± 7	78 ± 7	59 ± 5	
(9′*Z*)-neoxanthin	41 ± 3	63 ± 4	82 ± 5	80 ± 5	80 ± 5	
lutein-5,6-epoxide	38 ± 3	52 ± 4	65 ± 5	67 ± 5	70 ± 5	
(*all-E*)-lutein	178 ± 15	292 ± 14	157 ± 15	166 ± 16	190 ± 15	
zeaxanthin	45.6 ± 1.8	65.4 ± 1.7	47.0 ± 1.7	53.9 ± 1.7	50.5 ± 1.7	
Z-lutein	48 ± 3	80 ± 3	57 ± 3	65 ± 4	65 ± 3	
β-carotene	680 ± 70	870 ± 60	750 ± 60	810 ± 60	780 ± 60	
(9*Z*)-β-carotene	130 ± 70	170 ± 70	160 ± 60	180 ± 70	180 ± 60	
total	1210 ± 170	1660 ± 160	1400 ± 160	1500 ± 170	1480 ± 160	ΔC = 200 ± 300

[a] Means of three replicates. [b] Standard deviation at $p \leq 0.05$. [c] Veraison: phenologic phase showing 10% of berries softening and/or coloring. [d] Total soluble solid are expressed in g/L [e] Total acidity expressed in g/L as tartaric acid. [f] Maturation index. [g] Expressed as lutein equivalent. [h] (9*Z*) + (9′*Z*)-lutein. [i] Sum of identified carotenoids. [j] Difference of total carotenoids concentration between véraison and maturity.

consistent with those reported by Simpson and Miller for Chardonnay (*2*), who also found high β-damascenone concentrations, ranging from 66 to 170 μg/L.

The major contribution of β-damascenone to wine aroma is often attributed to its odor activity value (OAV), defined as the ratio of a wine's β-damascenone content over its perception threshold in water or hydroalcoholic solution (*41*). In our research, the flavor activity of β-damascenone was greatly evident in Chardonnay wine, in both vintages (OAVs were 68500 and 95000 for 2006 and 2007, respectively), which was also confirmed in the sensory evaluation of the wines by the intensity of honey and exotic fruit aromas, typical of this compound (data not shown) (*42*, *43*).

In red wines, β-damascenone also had a high OAV (ranging from 1000 and 1300), indicative of its major contribution to wine aroma; indeed, even if its concentration as free aglycone in Merlot, Primitivo, and Negroamaro was less than its odor threshold in wine (4 μg/L), it is known that β-damascenone has a more indirect than direct impact on red wine aroma (*40*).

Higher concentrations of vitispirane and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), two important norisoprenoid compounds considered (as for β-damascenone) to derive from neoxanthin, were determined in Chardonnay and Merlot than in Primitivo and Negroamaro, in both vintages. Norisoprenoids of the ionone series (such as lutein and zeaxanthin derivatives 3-oxo-α-ionol and 3-hydroxy-7,8-dihydro-β-ionol) were also identified in the free fractions analyzed, especially for Chardonnay and Merlot wines (*44*). α-Ionone was not found in any sample, but this could be due to the absence in grapes of its precursor, α-carotene, whereas β-ionone was found at levels close to its olfactory detection limit, 90 ng/L (*4*), in Chardonnay and Merlot. Further research is necessary to explain the absence of β-ionone in Primitivo and Negroamaro wines even if its carotenoid precursor, β-carotene, was detected in corresponding grapes.

An important finding of this study was the higher norisoprenoid levels in wines in 2007 as compared to 2006 (**Table 4**). As viticultural conditions were uniform throughout the experiment, this trend could be due to the differences in climatic conditions between the two ripening periods.

For Chardonnay and Merlot, full ripeness was reached in the third decade of August, (TSS/acidity ratio, between 35 and 50). Conversely, for Primitivo and Negroamaro, suitable maturation indices (ranging from 35 to 40) were reached in the third decade of September (**Tables 3**−**6**).

Correlation analyses with data of the two vintages showed that wine C_{13}-norisoprenoid content was strongly positively correlated with averaged maximum and minimum temperatures and negatively correlated with rainfall (**Table 8**). These results seem to explain the higher norisoprenoid content of wines in 2007 compared to 2006, especially for Chardonnay and Merlot; indeed, an increase of temperature, coinciding with low rainfall,

Table 6. Changes in Carotenoids Content (Expressed as Micrograms per Kilogram of Berries, $m^a \pm \sigma^b$) in Negraoamaro Grapes from Véraison to Maturity in the Two Years of Study (2006 and 2007)

2006						
sampling date	Aug 31	Sept 6[c]	Sept 12	Sept 18	Sept 9	
TSS[d]	84[a] ± 6[b]	138 ± 11	156 ± 12	176 ± 16	192 ± 17	
pH	2.69 ± 0.08	3.28 ± 0.16	3.40 ± 0.17	3.37 ± 0.13	3.47 ± 0.10	
TA[e]	23 ± 3	8.6 ± 0.8	8.0 ± 0.4	7.4 ± 0.4	5.3 ± 0.3	
TSS/TA[f]	3.7 ± 0.2	16.04 ± 0.17	19.50 ± 0.13	23.78 ± 0.14	36.22 ± 0.15	
compound						
violaxanthin[g]	142 ± 6	125 ± 6	195 ± 6	179 ± 6	182 ± 6	
(9′*Z*)-neoxanthin[g]	76 ± 4	84 ± 4	138 ± 4	105 ± 4	109 ± 4	
lutein-5,6-epoxide[g]	127 ± 4	122 ± 4	128 ± 4	121 ± 4	161 ± 4	
(*all-E*)-lutein	730 ± 16	980 ± 16	550 ± 9	420 ± 13	350 ± 11	
zeaxanthin[g]	165 ± 13	200 ± 13	105 ± 16	62.8 ± 1.3	51.7 ± 1.4	
Z-lutein[h]	55 ± 3	53 ± 3	73 ± 3	55 ± 3	59 ± 3	
β-carotene	820 ± 60	1120 ± 60	1280 ± 30	1090 ± 50	870 ± 40	
(9*Z*)-β-carotene	470 ± 60	190 ± 60	210 ± 50	150 ± 50	210 ± 50	
total[i]	2590 ± 170	2880 ± 170	2680 ± 130	2180 ± 130	1990 ± 130	ΔC^j = 900 ± 300
2007						
sampling date	Sept 2	Sept 9[j]	Sept 16	Sept 23	Sept 30	
TSS	74 ± 5	127 ± 10	153 ± 12	176 ± 16	197 ± 18	
pH	2.68 ± 0.09	2.75 ± 0.14	2.91 ± 0.15	3.03 ± 0.12	3.23 ± 0.11	
TA	25 ± 4	16.4 ± 1.5	11.2 ± 0.6	9.5 ± 0.5	5.7 ± 0.3	
TSS/TA	3.0 ± 0.2	7.74 ± 0.17	13.66 ± 0.13	18.52 ± 0.14	34.56 ± 0.14	
compound						
violaxanthin	61 ± 5	76 ± 5	108 ± 8	99 ± 7	79 ± 5	
(9′*Z*)-neoxanthin	46 ± 3	54 ± 4	47 ± 4	84 ± 5	92 ± 5	
lutein-5,6-epoxide	34 ± 3	43 ± 4	42 ± 4	78 ± 5	72 ± 5	
(*all-E*)-lutein	213 ± 15	305 ± 14	201 ± 17	187 ± 16	230 ± 15	
zeaxanthin	39.5 ± 1.7	55.9 ± 1.7	43.4 ± 1.9	43.8 ± 1.8	53.2 ± 1.7	
Z-lutein	57 ± 3	72 ± 3	70 ± 4	68 ± 3	73 ± 4	
β-carotene	680 ± 70	1130 ± 60	770 ± 70	830 ± 60	890 ± 60	
(9*Z*)-β-carotene	130 ± 70	210 ± 70	160 ± 70	200 ± 70	190 ± 70	
total	1260 ± 170	1950 ± 160	1440 ± 180	1590 ± 170	1680 ± 160	ΔC = 300 ± 300

[a] Means of three replicates. [b] Standard deviation at $p \leq 0.05$. [c] Veraison: phenologic phase showing 10% of berries softening and/or coloring. [d] Total soluble solid are expressed in g/L. [e] Total acidity expressed in g/L as tartaric acid. [f] Maturation index. [g] Expressed as lutein equivalent. [h] (9*Z*) + (9′*Z*)-lutein. [i] Sum of identified carotenoids. [j] Difference of total carotenoids concentration between véraison and maturity.

Table 7. Norisoprenoid Concentrations (Expressed in μg/L, $m^a \pm \sigma^b$) in Chardonnay, Merlot, Primitivo, and Negroamaro Wines from 2006 and 2007 Vintages

			2006 vintage				2007 vintage			
compound	KI[c]	ions (*m*/*z*)[d]	Chardonnay	Merlot	Primitivo	Negroamaro	Chardonnay	Merlot	Primitivo	Negroamaro
vitispirane	1567	136, 121	102 ± 10b[e]	100 ± 13b	116 ± 9ab	38 ± 4c	141 ± 11a	132 ± 17a	118 ± 9ab	42 ± 5c
TDN[f]	1766	172, 157	144 ± 14b	102 ± 13c	27 ± 3d	36 ± 4d	194 ± 19a	140 ± 20b	27 ± 2d	38 ± 4d
β-damascone	1841	192, 177	nd[g]	nd	nd	nd	nd	nd	nd	nd
β-damascenone	1845	190, 121	137 ± 16b	2.0 ± 0.4c	2.0 ± 0.4c	2.2 ± 0.4c	190 ± 20a	2.5 ± 0.4c	2.4 ± 0.5c	2.6 ± 0.5c
α-ionone	1874	192, 121	nd	nd	nd	nd	nd	nd	nd	nd
β-ionone	1963	192, 177	1.11 ± 0.10c	1.47 ± 0.14b	nd	nd	1.43 ± 0.08bc	1.82 ± 0.18a	nd	nd
3-OH-β-damascone	2554	208, 121	470 ± 60b	14.5 ± 1.2c	30 ± 3c	17.9 ± 1.8c	640 ± 90a	19.0 ± 1.5c	30 ± 3c	20 ± 2c
3-oxo-α-ionol	2648	208, 108	18 ± 3 cd	58 ± 7b	30 ± 3c	8.6 ± 1.2d	20 ± 4c	77 ± 9a	30 ± 3c	9.1 ± 1.3d
3-oxo-7,8-dihydro-α-ionol	2721	210, 108	nd	2.8 ± 0.4b	6.9 ± 0.9a	nd	nd	3.8 ± 0.6b	7.0 ± 1.0a	nd
3-OH-7,8-dihydro-β-ionol	2753	208, 193	2.23 ± 0.14b	1.76 ± 0.08c	1.83 ± 0.09c	0.97 ± 0.09d	2.9 ± 0.2a	2.26 ± 0.11b	1.95 ± 0.08bc	1.15 ± 0.06d
2,3-dehydro-4-oxo-β-ionol	2785	206, 163	0.84 ± 0.06e	0.96 ± 0.09d	0.86 ± 0.07e	0.80 ± 0.13f	1.01 ± 0.08b	1.19 ± 0.16a	0.99 ± 0.11bc	0.97 ± 0.15 cd
dehydrovomifoliol	2828	222, 124	nd	nd	nd	nd	nd	nd	nd	nd
total[h]			870 ± 110	280 ± 30	210 ± 30	104 ± 11	1190 ± 140	380 ± 50	218 ± 19	114 ± 12

[a] Means of three replicates. [b] Standard deviation at $p \leq 0.05$. [c] Kovats indices calculated in a DB-Wax column according to the literature (*50*). [d] Selected ions for quantification. [e] Different letters in the same line are significantly different at 5% level (Tukey HSD test). [f] 1,1,6-Trimethyl-1,2-dihydronaphthalene. [g] Not detected. [h] Sum of identified norisoprenoids.

corresponds to an increase of norisoprenoids in wines. Reported results agree with studies realized by Marais et al. (*45*, *46*) on the levels of two norisoprenoids, TDN and vitispirane, that were lower in White Riesling wines from relatively cool regions such as northern Italy and Germany compared to wines from warmer South African regions.

Relationship between ΔC in Grapes and Total Norisoprenoid Content in Wines. C_{13}-Norisoprenoid content in wine grapes is greatly affected by carotenoid degradation during ripening (*24*, *25*); study of the total levels of carotenoids at harvesting and their kinetics of decrease could be a useful tool to determine the aroma potential of grapes in wine preparation. Furthermore,

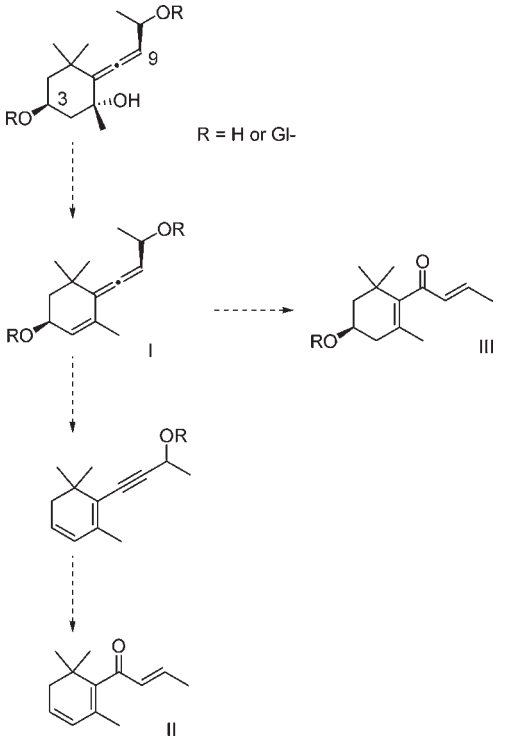

Figure 2. Conversion of the allenic triol glucosides (**I**) to β-damascenone (**II**) and 3-hydoxy-β-damascone (**III**) (*11*, *18*).

Table 8. Correlation Coefficients between Climatic Parameters of the Grape Ripening Seasons for 2006 and 2007 and Total Norisoprenoid Content in Wines (Data Set $n = 8$)

	C_{13}-norisoprenoids	significance
rainfall	−0.5019	$p = 0.012$
maximum temp	0.6985	$p = 0.0003$
minimum temp	0.6043	$p = 0.002$
mean temp	0.7171	$p = 0.0001$
thermal amplitude	0.4176	$p = 0.042$
radiation	0.5495	$p = 0.005$

study of the carotenoid profile in grape is related to the kind of norisoprenoid compounds that can be found in wine; indeed, β-ionone can derive from β-carotene oxidation (*23*), whereas 3-hydroxy-α-ionone and 3-oxo-α-ionol can derive from lutein cleavage.

Figure 3 illustrates the axis factor 1 × factor 2 of the PCA regarding the chemical analyses results of the 2006 and 2007 grapes and wines, which explain 84.64% of the total variance among the data. The factor 1 axis represents 56.06%, and the factor 2 axis represents 28.58% of the total dispersion. Projection of the cases onto the first two axes shows that grape varieties characterized by higher ΔC (Chardonnay and Merlot) were also characterized by highly flavorant compounds in their wines, such as neoxanthin derivatives, especially β-damascenone, in Chardonnay, and lutein derivatives, such as 3-oxo-α-ionol, in Merlot. Conversely, Primitivo and Negroamaro were correlated neither with ΔC in grapes nor with norisoprenoid content in wines (**Figure 3**). Moreover, the linear regression of norisoprenoid concentration (μg/L) in the wines, as a function of ΔC (μg/kg), was significant at the 0.01% level ($F = 36.12$, $p = 0.00096$) with $R = 0.9261$ (**Figure 4**). This correlation demonstrates that an increase of ΔC in grapes corresponds to a proportional increase of C_{13}-norisoprenoids in related wines.

In conclusion, evidence gathered in this study indicates that grapes with higher ΔC have the potential to give more aromatic wines with higher C_{13}-norisoprenoid contents. These results are

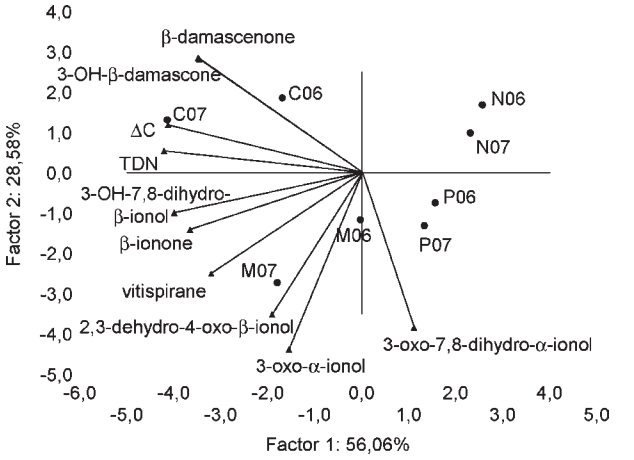

Figure 3. Principal component diagram of carotenoid derivatives in the four grape varieties (C, Chardonnay; M, Merlot; P, Primitivo; N, Negroamaro) in the two years of study. 06 and 07 correspond to the vintages 2006 and 2007, respectively. Factor score plot 1−2: axes 1 and 2 account for 84.64% of the total variance explained. Variables correspond to ΔC values in grape and norisoprenoid compounds quantified in wines.

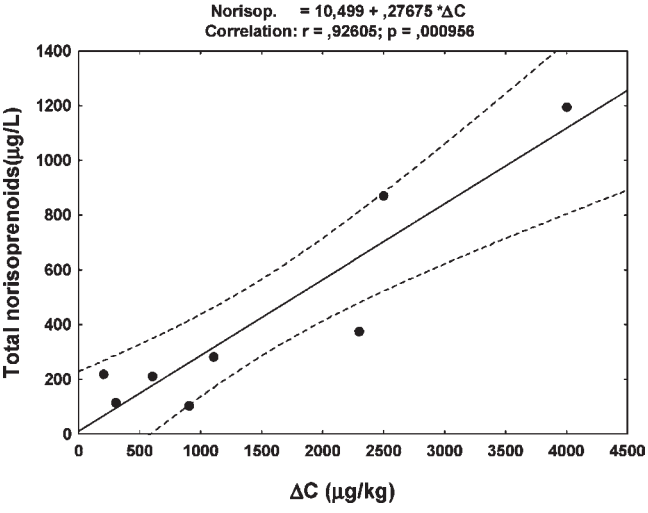

Figure 4. Linear regression between the difference of total carotenoids between véraison and maturity, ΔC (μg/kg), in grape vintages, and total norisoprenoids (μg/L) determined in respective wines in 2006 and 2007.

major arguments in favor of the hypothesis that ΔC could be a useful technological tool to predict norisoprenoid aroma of wine and, consequently, to distinguish grapes with higher aroma potential for winemaking. Complementary studies are in progress to extend this correlation to other floral and nonfloral varieties to confirm the results of this research.

ABBREVIATIONS USED

CRA-UTV, Agricultural Research Council, Research Unit for grape and winegrowing in Mediterranean environment; ESI, electrospray ionization; std, pure standard.

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

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