Distribution of Free and Glycosidically Bound Monoterpenes in the Skin and Mesocarp of Muscat of Alexandria Grapes during Development

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The major free and glycosidically bound monoterpenes of Muscat grapes accumulated during ripening. Approximately 90% of the monoterpenes were glycosidically bound, while only 10% were in the free odor-producing form in whole berries. The distribution of free and bound monoterpenes in the skins and mesocarp changed constantly during ripening of the berries. At harvest, 4.6% and 5.9% of the three major monoterpenes (linalool, geraniol, and nerol) occurred as free terpenes located in the skin and mesocarp, respectively, whereas 31% and 59% of total terpenes occurred as glycosides in the skin and mesocarp. Fluctuations in bound monoterpene concentrations during grape development seemed to correspond to changes in temperature. However, due to fairly low levels of free monoterpenes, the response of the free monoterpenes was less clearly related to temperature changes than that of the bound forms.

INTRODUCTION

Monoterpenes contribute to the characteristic varietal aroma of Muscat grapes (Webb et al., 1966; Terrier et al., 1972; Ribéreau-Gayon et al., 1975; Strauss et al., 1986). At present, about 50 monoterpene compounds have been identified in *Vitis vinifera* L. grapes (Strauss et al., 1986), of which the most abundant are geraniol, linalool, and nerol. Lower amounts of citronellol, nerol oxide, α-terpineol, diendiol I, and various forms of linalool oxides have also been found.

In addition to the free odor-producing forms of monoterpenes, the presence of glycosidically bound monoterpenes [or monoterpene glycosides (MTG)] was first suggested by Cordonnier and Bayonove (1974). Later, with the development of chromatographic techniques for the isolation of bound monoterpenes, these MTG were shown to be present at higher concentrations in grapes than the free monoterpenes (Wilson et al., 1984, 1986; Gunata et al., 1985). Although MTG have been shown to be tasteless at the levels on which they are found in wine (Noble et al., 1987), they can contribute significantly to aroma upon hydrolysis (Noble et al., 1987; Shoseyov et al., 1990), as suggested previously by Wilson et al. (1984, 1986) and Gunata et al. (1985).

To obtain the maximum intensity of such characteristic floral aromas in Muscat and related aromatic grape varieties, several factors can be manipulated: first, harvesting grapes when total terpene levels are at their highest concentrations (Marais and van Wyk, 1986); second, obtaining the maximum available aroma components by using the extended skin contact or appropriate pressing systems (Kinzer and Schreier, 1980); and third, hydrolyzing bound terpenes enzymatically (Aryan et al., 1987).

Conflicting papers have been published regarding the optimum maturity at which the highest levels of terpenes are found in Muscat of Alexandria grapes. Wilson et al. (1984) found that both free and bound terpenes increased after the time at which grapes were normally harvested in Australia. In contrast, Gunata et al. (1985) reported that the highest free terpene levels were reached before the normal harvest in France, although MTG increased postharvest. In this paper changes in both free and bound monoterpenes were monitored during development of Muscat of Alexandria to determine the optimum harvest time for this variety in California. In addition, the distribution of monoterpenes between the skin and mesocarp was determined.

EXPERIMENTAL PROCEDURES

Grape Samples. Grapes from *V. vinifera* L. cv. Muscat of Alexandria grown at the University's experimental vineyard at Davis, CA, were evaluated in the 1988 season. Two rows, each with 23 vines, were used for the experimental blocks. The grapes were sampled biweekly starting July 12, 1988 (Julian day 194), approximately 2 weeks before véraison (July 25, Julian day 207), until harvest for wine production (September 9, Julian day 253). A final sampling was made 1 week after the harvest.

Daily temperature and humidity minima and maxima were recorded from early July to the middle of September at a weather station near the experimental vineyard in Davis. No precipitation was recorded during the entire sampling period.

Sample Preparation. On each sampling date, one cluster was harvested from alternate vines from each of two rows. Ten berries were removed from the middle of each cluster for standard maturity analysis (berry weight, juice volume, pH, and soluble solids) and the remaining berries were stored at −17 °C for 3–4 months until used for monoterpene analysis. For analysis of monoterpenes, 40–50 g of frozen berries was taken from the middle of each of 10 frozen clusters to make a total of 400–500 g of sample. The skins were carefully peeled with forceps from the grapes, which were frozen in dry ice. The seeds were removed and discarded; the skins and mesocarp were weighed separately, the sum representing the berry pericarp. The partially thawed skin and mesocarp fractions were homogenized separately with NaCl and adjusted to pH 7 with NaCl-saturated citrate–phosphate buffer to avoid acid hydrolysis of bound monoterpenes. The homogenates were then centrifuged at 9500*g* at 5 °C for 25 min and the supernatants stored at −23 °C until extracted.

Analyses of Monoterpenes. An internal standard (8.03 μg of 2-octanol) was added to each homogenate before extraction. Free monoterpenes were extracted from the homogenates (corresponding to 250 g of mesocarp or 60 g of skins) with Freon 11 at 30 °C for 72 h with solvent changes every 24 h, using a continuous liquid/liquid extraction system similar to that of Williams et al. (1982). The solvent was concentrated to 200 μL at 27 °C by using a modified Kuderna-Danish concentrator and stored at −23 °C until analyzed.

To recover the bound monoterpenes, filtered homogenate (corresponding to 30–50 g of mesocarp or skin) was pumped at 1.5 mL/min through a column packed with 9 g of Davisil C_{18} reverse-phase adsorbent (90–130 μm; Alltech Associates, Deerfield, IL) as described by Williams et al. (1982). Sugars and acids retained on the C_{18} adsorbent were washed off with 100 mL of distilled/deionized water, and then MTG were eluted with 50

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mL of double-distilled methanol. The methanolic eluate was evaporated to dryness at 37 °C under reduced pressure, resuspended in 25 mL of a citrate–phosphate buffer, pH 5, washed with Freon 11 to remove residual free monoterpenes, and then evaporated to remove residual Freon 11. The glycosidic solution was incubated at 40 °C for 24 h with 20 mL of the pH 5 buffer containing 20 mg of crude pectinase with β-glucosidase activity (Rohapect 7104; Röhm, Darmstadt, W. Germany). The hydrolyzed monoterpenes were extracted and concentrated as described above.

Gas Chromatography. A Hewlett-Packard gas chromatograph Model 5890A equipped with an on-column injection system and FID was used. The column was Supelcowax 10 bonded phase capillary column (60 m × 0.32 mm i.d., film thickness 0.5 μm; Supelco, Bellefonte, PA) protected by a 1.0 m × 0.32 mm i.d. deactivated fused silica precolumn (J&W Scientific, Folsom, CA). The operating conditions were the following: carrier gas flow (He), 1.3 mL/min; H_2, 37 mL/min; air, 364 mL/min; make-up gas (N_2), 30.5 mL/min; injector, 40 °C; detector, 250 °C. The oven temperature was held at 50 °C for 10 min and then raised to 185 °C at 2 °C/min and held for 25 min. One microliter of sample from the final concentrate was injected directly into the capillary column. Maxima 820 (Dynamic Solutions, Ventura, CA) was used for acquiring and processing the signal from the FID.

Gas Chromatography–Mass Spectrometry. A VG-Trio 2 quadrupole mass spectrometer was interfaced to a Hewlett-Packard 5890A GC equipped with an on-column injector (J&W Scientific). A DB-Wax capillary column, 60 m × 0.32 mm i.d., film thickness 0.25 μm, was used (J&W Scientific). The GC conditions were the same as described previously except for the linear velocity of carrier gas which was 40 cm/s. The mass spectra were taken over the m/z range 30–300, utilizing an ionizing voltage of 70 eV. Monoterpenes were identified by comparing the mass spectra and retention times of authentic monoterpenes. Identification was confirmed by spiking the samples with the known compounds and comparing retention times and mass spectra.

Quantitative Analysis. For recovery studies, authentic monoterpenes were serially diluted with ethanol to make standard solutions ranging from 2 to 21.78 μg/mL of ethanol. After addition of the internal standard, the calibration samples were extracted with Freon 11 as described previously. Quantification of the components in extracts was also carried out with a Maxima 820 data processing system, by comparing the response to that for a known amount of internal standard. The recovery efficiency was shown to be greater than 95% for each of the monoterpenes examined. The recovery efficiency of glycosides from the C_{18} adsorbent was determined by using p-nitrophenyl β-D-glucopyranoside (PNG, Sigma). PNG was dissolved in citrate–phosphate buffer, pH 5, and 10 mL (6363 μg of PNG) was pumped through the C_{18} column. The PNG that eluted from the C_{18} column and a control solution of PNG were incubated with the enzyme Rohapect 7104 as described previously. The recovery was determined by measuring absorbance at 405 nm and found to be 100%.

Reference Compounds. Linalool, geraniol, citronellol, nerol oxide, α-terpineol were obtained from Aldrich Chemicals. Hotrienol, diendiol I, and trans cis forms of pyran and furan linalool oxides were donated by P. J. Williams of the Australian Wine Research Institute (Glen Osmond, South Australia).

RESULTS

Terpene Composition at Harvest. Muscat of Alexandria grapes were harvested for winemaking on Julian day 253 at 23.2 °Brix. Fifteen monoterpenes were identified in the fruit including monoterpene alcohols, oxides, and aldehydes. The amounts of the free and bound monoterpenes in the skins and mesocarp (expressed per kilogram pericarp) are provided in Table I. Clearly, the bound forms are found in far higher concentrations than the corresponding aglycons. In addition to those components previously reported in Muscat grapes, myrcen-2-ol, geranial, and neral were also identified. Although myrcen-2-ol has not been reported in fresh grape juice, it was identified as a significant constituent of heated juice (Rapp et al., 1984; Williams et al., 1980). In this study, a relatively large amount of free myrcen-2-ol was found in the fresh Muscat mesocarp at harvest, although no bound myrcen-2-ol was found. Traces of neral and geranial, which have previously been shown to occur as the intermediate products of the conversion from geraniol to its cis isomer nerol (Charlwood and Banthorpe, 1978; Paisarnrat and Ambid, 1985), were also found in the mesocarp. Given the thresholds for sensory detection of monoterpenes in a sugar–water model solution similar to grape juice, the only free monoterpene that was found at levels near threshold at harvest was geraniol, whereas the bound linalool and geraniol were found at levels which on hydrolysis would yield concentrations 4–5 times the threshold.

Table I. Quantification of Free and Bound Monoterpenes in the Mesocarp and Skin of Muscat of Alexandria Grapes at Harvest (Julian Day 253) and Odor Threshold Values for the Free Terpenes

components	concn, μg/kg of pericarp: mesocarp free	mesocarp bound[a]	skin free	skin bound[a]	threshold value, μg/L
t-furan linalool oxide	tr[d]	–[d]	tr	–	>6000[b]
c-furan linalool oxide	–	1.5	–	2.5	>6000[b]
nerol oxide	4.0	5.5	–	tr	~100[b]
linalool	28.0	280.5	4.5	136.0	100[b]
hotrienol	1.5	1.5	–	1.5	~110[b]
neral	1.9	–	*–	–	–[c]
geranial	2.4	–	–	–	–[c]
α-terpineol	–	14.5	–	14.0	400–500[b]
t-pyran linalool oxide	24.0	23.5	3.5	5.5	3000–5000[b]
c-pyran linalool oxide	tr	–	–	–	3000–5000[b]
citronellol	10.0	–	1.5	2.2	–[c]
nerol	10.0	114.5	9.0	55.5	400–500[b]
geraniol	37.0	349.5	45.0	208.5	130[b]
diendiol I	20.0	71.0	3.0	10.0	–[c]
myrcen-2-ol	40.0	–	3.0	–	–[c]

[a] Expressed as concentration of free terpene. [b] In sugar–water (Ribéreau-Gayon et al., 1975). [c] Data not available. [d] tr, trace, (<1 μg/kg fresh weight). –, not detected.

Changes during Development. Figure 1 shows the changes in juice volume, berry weight, pH, and soluble solids (degrees Brix) during berry development. From these data and field observations, véraison, the onset of ripening, occurred on or about Julian day 207. The maximum and minimum temperatures during the seven sampling dates are provided in Figure 2.

In Figure 3, the concentrations of the major free and bound monoterpenes (linalool, geraniol, and nerol) in the skin and in the mesocarp are shown throughout development. Concentrations of these and most other monoterpenes were lowest at véraison when the berries began to soften and lose their green color. After véraison, free monoterpenes increased slowly, while MTG increased more rapidly and reached higher concentrations. All three of the major monoterpenes continued to increase even a week after the grapes had reached normal commercial maturity. In fact, the rates of increase observed in the week after harvest were more rapid than those observed at any other developmental stage. The increase in concentration of the three major bound monoterpenes (geraniol, linalool, and nerol), despite a plateau in sugar accumulation as the fruit reached commercial maturity, is consistent with previous reports that the accumulation of monoterpenes is independent of sugar accumulation (Hardy, 1970; Wilson et al., 1984; Williams et al., 1985).

Unlike the patterns seen for the three major monoterpenes, bound diendiol I (3,7-dimethyl-1,5-octadien-3,7-diol) was found in highest concentrations around véraison

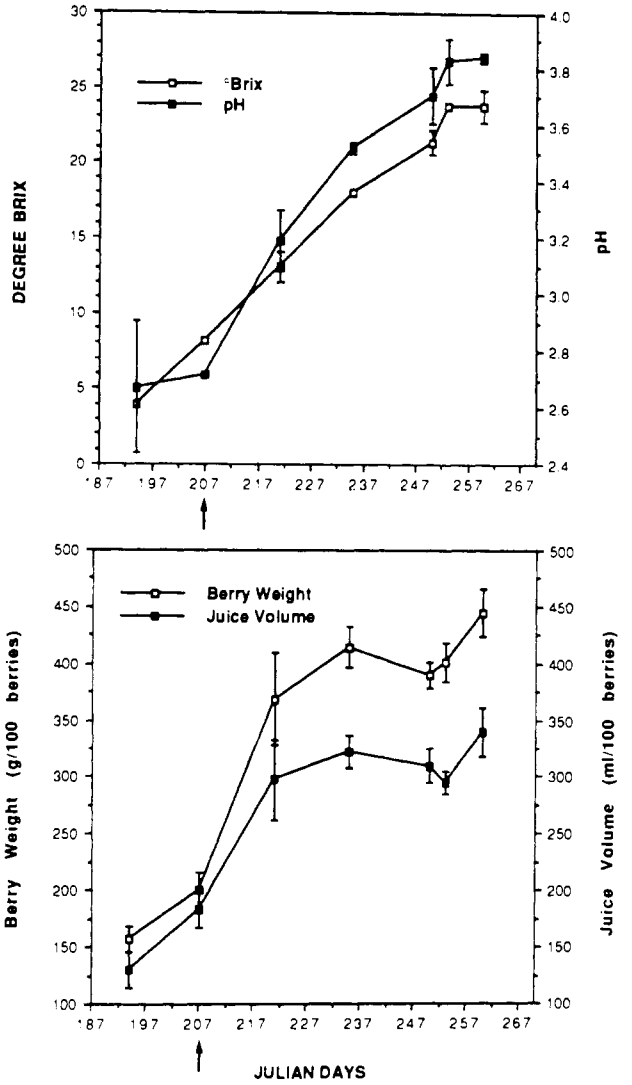

Figure 1. Changes in degrees Brix and pH (top) and in berry weight and juice volume (bottom) during ripening. Arrow at abscissa indicates véraison; vertical bars denote standard deviation (n = 2).

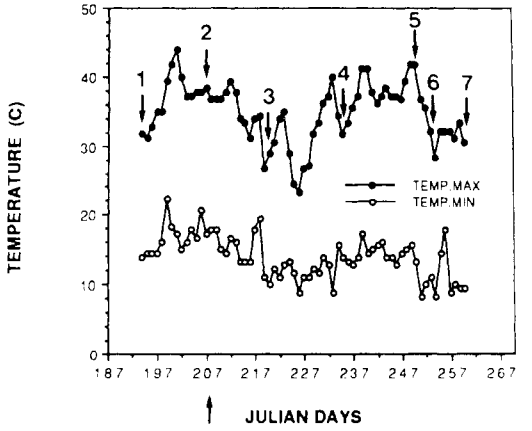

Figure 2. Variation of temperature during sampling period. Numbered arrows indicate sampling dates.

and decreased during ripening (Table II). Upon acidic hydrolysis, diendiol I is a potential source of both hotrienol and nerol oxide, which have low sensory thresholds (Table I), as previously demonstrated by Williams et al. (1980) and Strauss et al. (1988).

As noted above, the individual free monoterpenes were present in grape berries at concentrations below published

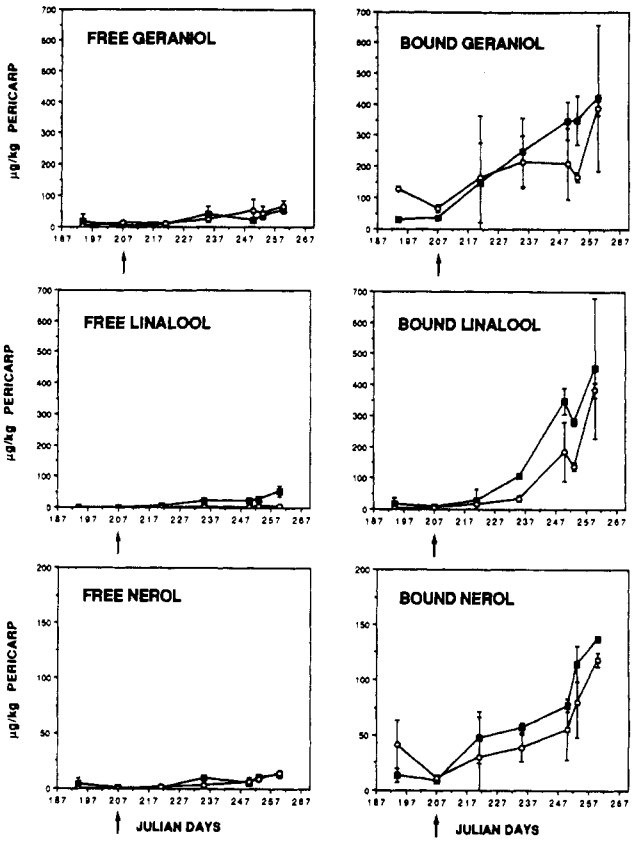

Figure 3. Changes in concentration of free and bound forms of geraniol, linalool, and nerol in mesocarp (■) and skins (○) of Muscat of Alexandria grapes. Arrow at abscissa indicates véraison; vertical bars denote standard deviation (n = 2).

Table II. Free and Bound Diendiol I in Mesocarp and Skin in Muscat Grape (Micrograms per Kilogram of Pericarp)

	free		bound	
Julian day	mesocarp	skin	mesocarp	skin
194	1.5		41.0	41.5
207	4.0		281.5	44.0
221	7.0	3.5	283.0	20.0
235	22.0		135.0	23.5
250	15.5	5.0	19.0	11.0
253	20.0	3.0	71.0	10.0
260	32.5		78.5	38.5

aroma threshold levels (Ribéreau-Gayon et al., 1975). Their relatively low concentrations, and resulting low intensity of varietal aroma in the wine, may be due to the hot and dry weather conditions that occurred during development. Marais (1987) similarly suggested that lack of characteristic aroma in many wines of white cultivars in South Africa is the result of high temperatures during ripening. As shown in Figure 3, both forms of linalool and geraniol exhibit a marked decrease in concentration at harvest (September 9, Julian day 253) with a subsequent rise by the last sampling date. On the other hand, free and bound nerol increased continuously. The decrease in concentration of two of the major bound monoterpenes at harvest corresponds to a decrease in berry volume and weight (Figure 1). All of these changes could be a response by the vine to high temperatures that occurred between Julian days 247 and 252 when the vines experienced several days of high temperatures over 38 °C (100 °F) before harvest (Figure 2). Despite berry dehydration, indicated by the decrease in berry weight and juice volume (Figure 1), the total content per berry of free and bound monoterpenes has the same decreasing pattern as that shown on a

Table III. Total Concentration in Mesocarp and Skin of Free and Bound Forms of the Three Major Monoterpenes in Grapes at Harvest and Last Sampling Date (Micrograms per Kilogram of Pericarp)

	free		bound	
Julian day	mesocarp	skin	mesocarp	skin
253	75.0 (5.9)[a]	58.5 (4.6)	744.5 (59.0)	384.5 (30.5)
260	122.0 (5.8)	87.5 (4.1)	1011.5 (47.9)	889.0 (42.2)

[a] Percentage of total terpene content is indicated in parentheses (n = 2).

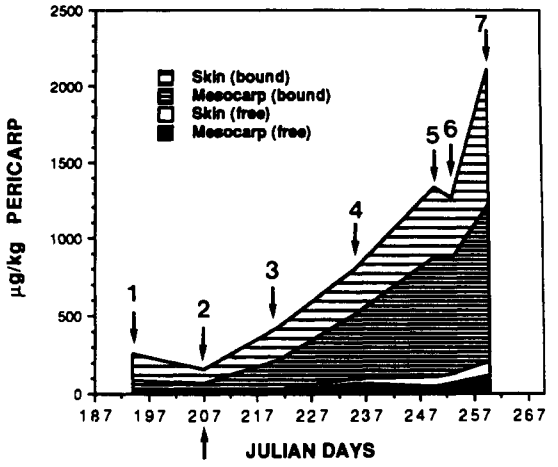

Figure 4. Distribution of the three major free and bound monoterpenes (geraniol, linalool, and nerol) between mesocarp and skins during development. Numbered arrows indicate sampling dates. Arrow at abscissa indicates véraison.

concentration basis in Figure 3 (data not provided). It is interesting to note that no corresponding increase in free linalool and geraniol was observed when bound linalool and geraniol decreased. This may indicate that the monoterpenes are subject to rapid turnover or catabolism in grape berries (Paisarnrat and Ambid, 1985) as in other plants (Loomis and Croteau, 1973; Croteau and Martinkus, 1979; Croteau, 1986). Although no correlations were made previously between accumulation of individual monoterpenes in grapes and temperature or other environmental conditions in grapes, large fluctuations between sampling dates are found in several previous papers (Terrier et al., 1972; Wilson et al., 1984; Williams et al., 1985; Gunata et al., 1985; Bravdo et al., 1989). It may be that transient changes in weather were also involved in the fluctuations in monoterpene concentrations noted in those studies.

Distribution of Monoterpenes. At the final sampling date, 90% of the total monoterpenes occurred in the odorless bound form, with 42.2% in the skins and 47.9% in mesocarp (Table III). A shift in the distribution of free and bound monoterpenes within the grape berry was seen during ripening, as illustrated in Figure 4 for the sum of the three major terpenes (linalool, nerol, and geraniol). Before véraison, on the first sampling date, about 74% of the major bound monoterpenes were found in the skin, whereas only 16% of the free monoterpenes were located there. The proportion of bound monoterpenes in the mesocarp increased steadily from 26% before véraison to 53.2% on the last sampling date. More fluctuations were observed in the distribution of free monoterpenes. Despite the low proportion of skin to mesocarp (<10% skins by berry weight), over 35% and 42% of the major free and bound monoterpenes were found in the skin at harvest and the last sampling date, respectively. Wilson et al. (1986) reported a similar distribution of free and bound monoterpenes between skin and juice in three Muscat varieties. In contrast, Gunata et al. (1985) found the highest levels of the free and bound monoterpenes in the skin. These contradictory results are possibly attributable to differences in grape maturity at harvest or differences in environmental conditions. Moreover, the accumulation and distribution of individual monoterpenes during ripening are variable (Figure 3). Free linalool was primarily located in the mesocarp rather than the skin over the developmental period, whereas free nerol and geraniol were more equally distributed between mesocarp and skin (Figure 3). As the berries matured, bound linalool, nerol, and geraniol also became more equally distributed, indicating dynamic changes in distribution and concentration. This is consistent with the hypothesis that glycosylation effectively allows redistribution of the monoterpenes throughout the berry fractions (Wilson et al., 1986).

CONCLUSIONS

Except for diendiol I, free and bound monoterpenes increased continuously even after sugar accumulation slowed and after the grapes had reached conventional maturity levels for winemaking. This suggests that monoterpene accumulation is not tied to sugar accumulation, which is a maturity indicator used widely in the winegrape industry. Ninety percent of the total terpenes occurred as glycosides, indicating their potentially significant contribution to wine aroma; upon hydrolysis, concentrations 4–5 times the aroma thresholds of both geraniol and linalool would be produced. Over 46% of the total monoterpenes were found in the grape skins, suggesting that extended skin contact to extract terpenes in skins before the grapes are pressed may be an effective way to enhance total wine aroma. However, this beneficial effect of extended skin contact may be overridden by the deleterious effects on wine flavor and color resulting from the simultaneous extraction of bitter and astringent phenolic compounds, which are located primarily in grape skins (Singleton and Noble, 1976).

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Registry No. *t*-Furan linalool oxide, 34995-77-2; nerol oxide, 1786-08-9; linalool, 78-70-6; hotrienol, 20053-88-7; neral, 106-26-3; geranial, 141-27-5; *t*-pyran linalool oxide, 39028-58-5; *c*-pyran linalool oxide, 14009-71-3; citronellol, 106-22-9; nerol, 106-25-2; geraniol, 106-24-1; diendiol I, 13741-21-4; myrcen-2-ol, 543-39-5.