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THE EFFECT OF SKIN CONTACT ON THE AROMATIC COMPOSITION OF THE WHITE WINE OF *VITIS VINIFERA* L. CV. MUSCAT OF ALEXANDRIA GROWN IN SOUTHERN ANATOLIA

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Free and bound aroma (precursors) compounds of the Muscat of Alexandria wines and the effect of skin contact (7 h, at 15 °C) on aroma composition have been investigated. The aroma compounds, extracted with the pentan-dichloromethane (2:1) method and Amberlite XAD-2 resin, were analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). A total of 41 free volatile compounds (12 esters, 8 acids, 7 terpenes, 5 alcohols, 4 phenols, 3 C-6 compounds, 2 carbonyl compounds) and 28 bound compounds (10 terpenes, 2 alcohols, 2 C-6 compounds, 5 fatty acids, 6 phenols, 2 C-13 norisoprenoids, 1 carbonyl compound) were identified in Muscat of Alexandria wines. Skin contact treatment increased the total free and bound aroma compound levels, and improved the wine quality by affecting the intensity as well as the quality of aroma.

Keywords: skin contact, aroma, white wine, precursors, Muscat of Alexandria

Hitherto several techniques have been used to obtain maximum intensity of the characteristic varietal aromas in muscat and related aromatic grape varieties. One of them is skin contact. Certain aroma compounds are predominant in grape skins (GUNATA et al., 1985a; WILSON et al., 1986; GOMEZ et al., 1994) and they are present partly as free, volatile forms and partly as glycosidically-bound, non-volatile precursors (GUNATA et al., 1986). Consequently, skin contact between juice and skins prior to pressing generally results in higher concentrations of aroma compounds in the juices and corresponding wines (BAUMES et al., 1989a, b; MOYANO et al., 1994; CABAROGLU et al., 1997). The increase could be caused by the transformation of free aroma compounds (DI STEFANO, 1981) and the liberation of aroma compounds from their non-volatile bound glycosidic form (WILLIAMS et al., 1980; DUGELAY et al., 1993). However, contact of the must with grape skins may also result in increasing the concentration of phenolic compounds which may be detrimental to white wine quality (ARNOLD & NOBLE, 1979; SINGLETON et al., 1980; TEST et al., 1986). In addition, skin-contact technique frequently displays higher colour intensity and sensitivity to oxidation of white wines (CHEYNIER et al., 1989; MACHEIX et al., 1991). The success of this

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technique seems to depend not only on skin contact conditions but also on the grape cultivar (ARNOLD & NOBLE, 1979; CABAROGLU et al., 1997).

Muscat of Alexandria, which is known as Muscat of Iskenderiye in Turkey, is produced widely in Ege and Akdeniz region in Turkey (Southern Anatolia) (ANON, 1990a). Till now, advanced studies have been carried out on the characteristic aroma of muscat grapes and wines (HARDY, 1970; BAYONOVE & CORDONNIER, 1971; RIBEREAU-GAYON et al., 1975; GUNATA et al., 1985a). White cultivar wines, produced in warm wine producing countries, often lack sufficient and characteristic aromas (MARAIS & RAPP, 1988). The aromatic composition of Muscat of Alexandria wines produced in Turkey has not been reported yet. This study was conducted to investigate the effect of skin contact on wine quality as well as the free and bound aroma (precursors) composition of Muscat of Alexandria wines.

1. Materials and methods

1. 1. Wine samples

Healthy grapes (150 kg) from *Vitis vinifera* L. cv. Muscat of Alexandria grown at the University's experimental vineyard in Çukurova, Akdeniz region of Turkey, were manually harvested and transported to the experimental winery at the Department of Food Engineering, Faculty of Agriculture, University of Çukurova. Grapes were divided into two parts. One was treated in the standard way with minimal skin contact: grapes were pressed in a horizontal press and 50 mg l^{-1} of sulphur dioxide was added. The juice was then settled at 15 °C for 24 h and racked. For the skin-contact experiment, the grapes were destemmed and crushed. The pomace was mixed with 50 mg kg^{-1} of sulphur dioxide, kept at 15 °C for 7 h and then pressed in a horizontal press. The juice was settled and racked as mentioned above. Both batches spontaneously fermented at 18 °C. During fermentation, the decrease in juice density was checked twice daily. When most of the lees settled the wines were racked, 50 mg l^{-1} of sulphur dioxide was added, and the wine was stored at 15 °C in tanks.

1. 2. Standard chemical analysis

Wines were analysed for ethanol, extract, pH, total acidity, volatile acidity, ash, ash alkalinity and potassium by the methods in standard use for wines as recommended by OIV (ANON, 1990b). Total phenolics was measured as the absorbance at 280 nm on 1/100 dilution of wine by spectrophotometric method (RIBEREAU-GAYON et al., 1976). Colour was measured as the absorbance at 420 nm as recommended by SINGLETON & KRAMLING (1976).

1. 3. Free and bound aroma compounds analysis

Before extraction, 30 µg of 4-nonanol (Fluca 98%) was added to the wine samples (100 ml) as internal standard for free aroma compounds because of its high recovery

(VOIRIN et al., 1992a). Amberlit XAD-2 resin (20–50 mesh, Merck) was used to isolate free and glycosidically bound aroma compounds (GUNATA et al., 1985b). This polymeric adsorbent was chosen as it had the advantage that free and glycosidically bound aroma compounds can be isolated and separated in one sample preparation step. The samples were eluted through an Amberlite XAD-2 column (120 mm×7 mm i.d.) with 1.5 ml min^{-1} flow rate. The free and bound compounds were eluted successively with 50 ml each of pentane-dichloromethane (2:1) and ethyl acetate-methanol (9:1) (CABAROGLU et al., 1997). Pentane-dichloromethane eluate was dried over anhydrous sodium sulphate, and then concentrated by fractional distillation through a Vigreux column prior to GC and GC-MS analysis. The ethyl acetate/methanol eluate was concentrated to dryness in vacuum and dissolved in 0.2 ml of 0.2 M citrate-phosphate buffer (pH 5.0). For enzymatic release of aglycones, 1.2 mg of Pektolase 3PA (Grinsted, France) was added to this extract and the mixture was incubated at 40 °C for 12 h. Released aglycones were extracted with pentane-dichloromethane and the organic layer, after addition of 30 µg of 4-nonanol as standard, was concentrated and then analyzed by GC and GC-MS. GC analysis of free and released volatile compounds was performed using a Shimadzu GC-14B Chromatograph equipped with a fused capillary column coated with DB-Wax (30 m×0.32 mm i.d., 0.5 µm film thickness, JW, Folsom, CA, USA). On column injector was used and programmed from 20 to 250 °C at 180 °C min^{-1}. Oven temperature was at 60 °C for 3 min, from 60 to 220 °C at 2 °C min^{-1}, from 220 to 245 °C at 3 °C min^{-1}, then held for 20 min. The FID temperature was 250 °C. The carrier gas was helium with a flow rate of 1.1 ml min^{-1}.

Identification was performed using a "Hewlett Packard (HP)-5890 Series II" gas chromatograph coupled to a "HP 5989A" series mass spectrometer with a quadrupole mass filter. The GC conditions and the capillary column were the same as described above. Mass spectra were recorded in the electron-impact (EI) mode. Source temperature was 250 °C. Mass spectra were scanned at 70 eV in the range m/e 29–350 at 1-s intervals. Identifications were carried out by comparing retention times and EI mass spectra with published data or with authentic compounds (VOIRIN et al., 1992b).

Concentrations of aroma compounds were calculated using 4-nonanol as internal standard. The statistical significance of the effect of the skin contact on free and bound aroma compound concentrations obtained in duplicate analyses (three injections for each replication) was determined by analysis of variance (Anova).

2. Results and discussion

2. 1. General wine composition

The wine composition obtained from skin contact differed from the control wine, as is shown in Table 1. The wine made with skin contact had higher values for pH, extract, ash, ash alkalinity, potassium-, phenolic content, and lower values for ethanol, total acidity and volatile acidity. This increase was the consequence of skin compounds being extracted into the juice as reported previously (SINGLETON et al., 1980; DUBOURDIEU et al., 1986; RAMEY et al., 1986). As expected, absorbance at 280 nm and absorbance at 420 nm increased with skin contact, probably because of the same causes. Total phenolics and colour of wines are known to be affected by skin contact treatment (OUGH, 1969; SINGLETON et al., 1980; TEST et al., 1986). It has been reported that skin contact caused an increase in flavonoid content of the juice, and this increase raised the absorbance at 420 nm and browning capacity (SINGLETON & KRAMLING, 1976; SINGLETON, 1974).

Table 1. Composition of Muscat of Alexandria wines[a]

	Control	Skin-contact
Ethanol (% v/v)	10.26	10.03
Titratable acidity (meq l^{-1})	80	75
PH	3.20	3.35
Volatile acidity (meq l^{-1})	6.0	4.1
Absorbance at 280 nm	0.025	0.047
Absorbance at 420 nm	0.058	0.067
Ash (g l^{-1})	1.46	1.54
K (mg l^{-1})	901	978

[a] The results are given as mean values of triplicates (maximum SD: ±5%)

2. 2. Free fraction of aroma compounds of the wines

A total of 41 free volatile compounds were identified in Muscat of Alexandria wines by GC-MS, including 12 esters, 8 acids, 7 terpenes, 5 alcohols, 4 phenols, 3 C-6 compounds, 2 carbonyl compounds (Table 2). Nevertheless, there were no qualitative differences between control and skin contact wines, and the concentration of volatile aroma compounds was higher in the latter, as had been previously reported (BAUMES et al., 1989a, b; FALQUE & FERNANDEZ, 1996; CABAROGLU et al., 1997). The control and skin contact wines contained 102.0 and 118.9 mg l^{-1} of volatiles, respectively, and the amounts of several compounds were significantly different. Skin contact caused significant increases in the total concentration of alcohols, esters and terpenes (Table 2). Alcohols were the most abundant compounds of Muscat of Alexandria wines, followed by acids, esters, C-6 compounds, terpenes, phenols and carbonyl compounds.

Among the alcohols, 3-methyl-1-butanol and 2-phenylethanol were the major alcohols in the wines. Skin contact treatment increased the total level of the alcohol compounds significantly. Except for 3-methyl thio-1-propanol, all compounds were increased significantly by skin contact (Table 2). Similar findings have been reported by BAUMES and co-workers (1989a) and FALQUE & FERNANDEZ (1996). From the alcohols identified, 2-phenylethanol and benzylalcohol are favourable aroma compounds of wines (NYKANEN & SUOMALAINEN, 1989; ETIEVANT, 1991).

Table 2. Effect of skin-contact on free aroma compound levels of Muscat of Alexandria wines[a]

Compound (µg l^{-1})	Control	Skin-contact	Sig[b]
Alcohols			
2-methyl-1-propanol	902 ± 31.11	1628 ± 73.53	**
3-Methyl-1-butanol	66922 ± 898.9	77510 ± 1029.5	**
3-methylthio-1-propanol	24 ± 7.48	50 ± 10.34	ns
Benzyl alcohol	47 ± 2.82	72 ± 5.65	*
2-Phenyl ethanol	16372 ± 540.2	20959 ± 1299.6	*
Total of alcohols	***84267 ± 1480.5***	***100219 ± 2418.5***	**
C-6 alcohols			
1-Hexanol	820 ± 25.45	983 ± 16.97	*
E-3-Hexen-1-ol	106 ± 16.97	189 ± 21.21	*
Z-3-Hexen-1-ol	104 ± 31.11	86 ± 8.48	ns
Total of C-6 alcohols	***1030 ± 73.53***	***1258 ± 46.66***	***ns***
Terpenes			
Linalol	60 ± 5.99	68 ± 4.24	ns
α-Terpineol	295 ± 14.14	400 ± 24.04	*
Geraniol	84 ± 0.99	98 ± 3.10	**
3,7-Dimethyl-1,5-octadiene-3,7-diol	65 ± 2.41	241 ± 30.11	*
Linalol hydrate	38 ± 2.82	94 ± 15.55	*
3,7-Dimethyl-1,5-octadiene-3,8-diol	113 ± 7.28	120 ± 12.40	ns
Geranic acid	156 ± 6.75	167 ± 2.96	ns
Total of terpenes	***811 ± 40.38***	***1188 ± 92.40***	*
Esters			
Ethyl butanoate	303 ± 24.25	448 ± 36.56	*
3-Methylbutyl acetate	1124 ± 84.85	1622 ± 92.83	*
Ethyl hexanoate	288 ± 26.87	443 ± 28.28	*
Ethyl lactate	820 ± 56.60	781 ± 8.48	ns
Ethyl octanoate	388 ± 20.81	381 ± 24.04	ns
Ethyl benzoate	21 ± 4.24	26 ± 4.98	ns
Ethyl succinate	116 ± 29.69	151 ± 18.38	ns
Ethyl 4-hydroxy-butanoate	87 ± 9.79	147 ± 26.87	*
2-Phenylethyl acetate	189 ± 29.69	300 ± 10.79	*
Diethyl malate	189 ± 18.38	443 ± 25.45	**
Ethyl hydroxy-glutarate	749 ± 53.74	800 ± 10.07	ns
Monoethyl succinate	862 ± 40.08	1071 ± 94.75	ns
Total of esters	***5136 ± 398.99***	***6613 ± 381.48***	*
Fatty acids			
Isobutanoic acid	12 ± 2.82	16 ± 5.65	ns
Butanoic acid	48 ± 9.89	58 ± 11.31	ns
Isovaleric acid	67 ± 4.24	46 ± 8.48	ns
Hexanoic acid	3300 ± 648.52	3928 ± 138.59	ns
Octanoic acid	5230 ± 377.24	3780 ± 191.73	*
Decanoic acid	970 ± 155.56	541 ± 38.18	ns
Tetradecanoic acid	178 ± 32.52	172 ± 36.46	ns
Hexadecanoic acid	439 ± 18.38	482 ± 84.45	ns
Total of acids	***10244 ± 1249.16***	***9023 ± 514.85***	**ns**

Table 2. continued

Compound (µg l^{-1})	Control	Skin-contact	Sig[b]
Phenols			
Vanilline	23 ± 0.8	29 ± 1.41	*
Syringaldehyde+4-hydroxy benzaldehyde	125 ± 14.70	139 ± 21.21	ns
Acetosyringone	65 ± 5.65	69 ± 4.47	ns
Tyrosol	250 ± 17.34	242 ± 20.68	ns
Total of phenols	***463 ± 38.49***	***479 ± 47.77***	**ns**
Carbonyl compound			
4-Carbethoxy-γ-butyrolactone	161 ± 15.55	179 ± 17.18	ns
Total of volatiles	**102092**	**118959**	

[a] The results are given as mean of two replications (six injections on GC) with standard deviations
[b] Sig: Significance at which means differ as shown by analysis of variance; *, ** denote significances at P<0.05 and P<0.01, respectively; ns: not significant

With regard to C-6 compounds, hexanol was the most abundant compound in the wines. The total concentration of C-6 alcohols was increased by skin contact, as has been reported previously (BAUMES et al., 1989a; CABAROGLU et al., 1997). However, the increase was small and not significant. As it has been known, C-6 compounds have herbaceaus and leafy odour and detract from wine aroma. Their concentrations in Muscat of Alexandria wines were below their taste thresholds (ETIEVANT, 1991).

Among the terpenes identified, α-terpineol was the compound with the highest amount in the control and skin contact wine. Terpenes are responsible for the characteristic aroma of muscat cultivars and aroma related wines (MARAIS, 1983). The total concentration of terpenes increased significantly due to the skin contact. This treatment resulted in significant increase in the concentrations of α-terpineol, geraniol, 3,7-dimethyl-1,5-octadien-3,7-diol and linalool hydrate. Previous studies showed that terpene compounds were mainly located in the skin and the solid parts of the cells, and skin contact improved the quality of aroma increasing the concentration of terpenes (GUNATA et al., 1985a; MARAIS & RAPP, 1988; GOMEZ et al., 1994; BAUMES et al., 1989b; MARAIS, 1983).

In the case of esters 3-methylbutyl acetate, monoethyl succinate, ethyl lactate and ethyl hydroxy glutarate were the major esters of Muscat of Alexandria wines. Esters are one of the most important compounds in the aroma of wines due to the fact that they provide delicate odours to wines (ETIEVANT, 1991). Skin contact caused a significant increase in the total concentration of esters as reported previously (BAUMES et al., 1989a; FALQUE & FERNANDEZ, 1996). From the esters identified, ethyl butanoate, 3-methylbutyl acetate, ethyl hexanoate, ethyl 4-hyroxy-butanoate, 2-phenylethyl acetate and diethyl malate levels increased significantly with the skin contact treatment.

Hexanoic acid and octanoic acid were the most abundant fatty acids in Muscat of Alexandria wines. While certain fatty acid levels (i.e. isobutanoic, butanoic, hexanoic, hexadecanoic) increased with the skin contact treatment, others (i.e. isovaleric, octanoic, decanoic, tetradecanoic) decreased. However, the differences between the concentrations of the acids were significant only for octanoic acid. Thus, it can be said that skin contact treatment does not seem to have a significant effect on the fatty acids levels.

With regard to volatile phenols, the skin contact treatment resulted in increases in total phenol concentration and individually, in vanilline and acetosyringone concentrations. However, only vanilline level was significantly different. Syringaldehyde concentration was not determined, as it coeluted in GC analysis with 4-hydroxy benzaldehyde.

Only 4-carbethoxy-γ-butyrolactone and 4-hydroxy benzaldehyde were detected in Muscat of Alexandria wines as carbonyl compounds. 4-Carbethoxy-γ-butyrolactone was at higher concentration in the skin contact wine than the control, however, the difference between the concentrations was not significant.

2. 3. Bound fraction of aroma compounds of the wines

A total of 28 bound compounds were identified in Muscat of Alexandria wines by GC-MS, including 10 terpenes, 2 alcohols, 2 C-6 compounds, 5 fatty acids, 6 phenols, 2 C-13 norisoprenoids, 1 carbonyl compound (Table 3). The total concentrations of bound aroma released by enzyme hydrolysis in control and skin contact treated wines were 1583 and 2348 µg l^{-1}, respectively. Skin contact treatment increased considerably the total concentration of bound compounds as previously reported (BAUMES et al., 1989b; CABAROGLU et al., 1997). Terpenes were the most abundant bound compounds in Muscat of Alexandria wines followed by phenols, alcohols and acids.

Skin contact resulted in significant increase in the total concentration of terpenes, alcohols and norisoprenoids. Largest increases were found in terpenes. Among them, α-terpineol, trans-pyran linalool oxide, citronellol, nerol, geraniol, 3,7-dimethyl-1,5-octadien-3,7-diol, linalool hydrate and 3,7-dimethyl-1,7-octadien-3,6-diol levels increased significantly with skin contact treatment. Muscat wines made with skin contact were richer in terpenes due to high levels of terpenes in berry skin (GUNATA et al., 1985a; BAUMES et al., 1989b). On the other hand, bound benzylalcohol, 2-phenylethanol, benzoic acid, 4-vinylguaiacol, 4-vinylphenol, 3-hydroxy-β-damascone and 3-oxo-α-ionol levels increased significantly with skin contact treatment. A number of studies have shown that these compounds occur in grapes in glycosidic form (WILLIAMS et al., 1989; VOIRIN et al., 1990; SEFTON et al., 1993). The increase in bound aroma concentrations as a result of skin contact were reported previously (CABAROGLU et al., 1997).

Table 3. Effect of skin-contact on bound aroma compound levels of Muscat of Alexandria wines[a]

Compound (μg l^{-1})	Control	Skin-contact	Sig[b]
Alcohols			
Benzyl alcohol	34 ± 5.65	64 ± 8.54	*
2-Phenyl ethanol	141 ± 9.24	203 ± 18.38	*
Total of alcohols	***175 ± 14.89***	***267 ± 26.92***	*
C-6 compounds			
1-Hexanol	35 ± 7.07	40 ± 4.24	ns
E-3-Hexen-1-ol	9 ± 1.41	9 ± 1.26	ns
Total of C-6 compounds	***44 ± 8.48***	***49 ± 4.24***	**ns**
Terpenes			
Linalol	7 ± 0.8	8 ± 1.45	ns
α-Terpineol	21 ± 0.0	27 ± 1.41	*
Trans pyran linalol oxyde	4 ± 0.62	7 ± 0.85	***
Trans furan linalol oxyde	43 ± 4.24	44 ± 3.10	ns
Citronellol	7 ± 0.60	19 ± 1.28	**
Nerol	140 ± 10.14	195 ± 14.40	*
Geraniol	194 ± 9.79	496 ± 22.62	**
3,7-Dimethyl-1,5-octadiene-3,7-diol	60 ± 1.61	102 ± 9.62	**
Linalol hydrate	46 ± 3.82	150 ± 22.60	*
3,7-Dimethyl-1,7-octadiene-3,6-diol	7 ± 0.42	19 ± 1.96	**
Total of terpenes	***529 ± 48.98***	***1067 ± 79.27***	**
Fatty acids			
Hexanoic acid	58 ± 4.25	60 ± 8.48	ns
Nonanoic acid	17 ± 1.78	17 ± 2.36	ns
Decanoic acid	171 ± 29.69	178 ± 24.04	ns
Benzoic acid	31 ± 4.24	79 ± 11.31	*
Dodecanoic acid	17 ± 4.39	30 ± 5.65	ns
Total of acids	***294 ± 44.35***	***364 ± 51.84***	**ns**
Phenols			
4-Vinylguaiacol	6 ± 1.28	16 ± 2.82	*
4-Vinylphenol	6 ± 1.41	14 ± 3.65	*
Vanilline	7 ± 1.78	8 ± 2.98	ns
2-(4-guaiacyl)-ethanol	62 ± 6.42	49 ± 5.65	ns
Syringaldehyde + 4-hydroxy benzaldehyde	143 ± 19.79	150 ± 20.87	ns
Tyrosol	256 ± 38.29	280 ± 53.84	ns
Total of phenols	***480 ± 68.98***	***517 ± 71.71***	**ns**
C-13 norisoprenoids			
3-Hydroxy-β-damascone	31 ± 0.98	45 ± 1.71	**
3-Oxo-α-ionol	30 ± 2.69	39 ± 2.82	*
Total of C-13 norisoprenoids	***61 ± 3.68***	***84 ± 4.54***	*
Total of precursors	**1583**	**2348**	

[a] The results are given as mean of two replications (six injections on GC) with standard deviations
[b]Sig: Significance at which means differ as shown by analysis of variance; *, **, *** denote significances at $P<0.05$, $P<0.01$ and $P<0.001$, respectively; ns: not significant

2. 4. Sensory evaluation

Sensory analysis of Muscat of Alexandria wines were carried out by 7 trained judges using triangle test. The significance was determined from detailed statistical tables (ROESSLER et al., 1978). Six out of 7 judges indicated that there was a significant difference between control and skin contact treated wines ($P<0.01$). Five judges from those 6 preferred skin contact wine. From aromatic point of view, skin contact treated wine bore more intense and more terpene-like character than control wine.

3. Conclusions

The skin contact treatment at 15 °C, for 7 h affected general and aromatic composition of Muscat of Alexandria wine. The total concentrations of free and bound aroma compounds of the wine increased considerably with the skin contact. The results of the gas chromatographic data and sensory evaluation showed that skin contact treatment under the condition of the investigation, improved the aroma intensity (particularly terpene-like character) and wine quality of Muscat of Alexandria wine. However, this study should be expanded to different skin contact times and temperatures at the same region for the production of high quality wines.

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