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Aroma components of cv. Muscat of Bornova wines and influence of skin contact treatment

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Abstract

Aroma components of white wine made from cv. Muscat of Bornova, a natives grape variety of *Vitis vinifera* grown in Turkey, and the effect of skin contact treatment (15 °C, 6 and 12 h) on the aromatic profile of this wine were investigated. Aroma components were extracted with dichloromethane and then analysed by gas chromatography(GC)–flame ionisation detection and GC–mass spectrometry. A total of 72 components were identified and quantified. Skin contact treatment increased the amount of total aroma components. Wine produced with 6 h skin contact was the most preferred. From the 72 compounds identified, β-damascenone, ethyl hexanoate, ethyl butanoate, isoamyl acetate, 2-phenyl ethyl acetate, linalool, geraniol, and 2-phenyl ethanol were impact odourants of Muscat of Bornova wine on the basis of odour activity values (OAVs).

Keywords: Wine; cv. Muscat of Bornova; Aroma components; Skin contact

1. Introduction

Muscat of Bornova is a white and aromatic grape variety of *Vitis vinifera* which produces well-balanced and typical wines with an intense fruity and floral aroma. It is largely predominant in the Izmir province of the Agean region, producing one of the best aromatic wines of Turkey (Selli, Cabaroglu, & Canbas, 2001; Selli, Cabaroglu, Canbas, Erten, & Nurgel, 2003). The flavour of aromatic wines, particularly of Muscat varieties, has been extensively studied (Etiévant & Bayonove, 1983; Günata, Bayonove, Baumes, & Cordonnier, 1986; Schneider, Razungles, Augier, & Baumes, 2001). However, the flavour compounds of Muscat of Bornova wine have not yet been studied.

The aroma profile of wines depends on grape variety, ripeness, yeast activities, pre-fermentative and vinification procedures and ageing (Ebeler, 2001; Schreirer, 1979). In the literature, more than 800 aroma compounds have been reported in wines, including higher alcohols, aldehydes, ketones, esters, acids and monoterpenes (Aznar, Lopez, Cacho, & Ferreira, 2001). The aroma compounds responsible for the characteristics of muscat flavour are mainly derived from the grape and they are mainly monoterpenic compounds (Marais, 1983). In white wine-making, prefermentative skin contact (maceration) has been widely used to enrich the wine in aroma compounds. This technique is characterized by a longer period of contact between the juice and the skins of the grapes, after they are crushed but before pressing. It generally provides good results, depending on the grape cultivar, temperature and time (Cabaroglu & Canbas, 2002; Goilloux-Benatier, Le Fur, & Feuillat, 1998; Ho et al., 1999; Schmidt & Noble, 1983; Selli et al., 2003). The compounds responsible for varietal aroma

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are predominant in grape skins (Günata, Bayonove, Baumes, & Cordonnier, 1985). Varietal aroma is the main contributor to the fruity and flowery attributes of white wines (Rapp, 1998). The varietal characteristics of the wine may be enhanced with the skin contact treatment due to the extraction of aroma compounds from the skin. However, skin contact may also result in increasing the astringency, browning potential, and herbaceous character of wines, depending on the maceration conditions and grape ripening (Cabaroglu & Canbas, 2002; Schmidt & Noble, 1983).

The aroma compounds of the Muscat of Bornova must are dominated by monoterpenic compounds (Selli et al., 2003). The aim of this study was to determine potent aroma compounds in wines produced from this cultivar on the basis of odour activity values (OAV) and the effect of the skin contact on wine aroma composition.

2. Materials and methods

2.1. Preparation of wine samples

Healthy grapes of cv. Muscat of Bornova (2000 kg) were manually harvested at optimum maturity in the 1999 season in Izmir, and transported to the Experimental Winery at the Department of Food Engineering, University of Cukurova (Adana province). Muscat of Bornova must had a titratable acidity, as tartaric acid, of 6.4 g/l, pH 3.3, and reducing sugar 218 g/l. After harvest, grapes were divided into three batches. The first batch was treated in the standard way with minimal skin contact and considered as control. In this way, grapes were pressed in a horizontal press and 50 mg/l of sulphur dioxide was added. The juice was then settled at 15 °C for 24 h, and then racked. For the skin contact experiment, the grapes were destemmed and crushed. The second batch was subjected to skin contact for 6 h and the third batch for 12 h with addition of 40 mg/kg of sulphur dioxide, and then pressed in a horizontal press. The juice was settled and racked, as mentioned above. All batches spontaneously fermented at 18 °C. During fermentation, the decrease in density was checked at intervals. When most of the lees had settled, the wines were racked, 50 mg/l of sulphur dioxide were added, and the wine was stored at 15 °C in tanks.

2.2. Standard chemical analysis

Density, ethanol, extract, total acidity, pH, volatile acidity, acetaldehyde, reducing sugar, total nitrogen, ash, ash alkalinity, total and free SO_2 and total phenolic compounds (absorbance at 280 nm), and colour (absorbance at 420 nm) were analysed (O.I.V., 1990; Ough & Amerine, 1988).

2.3. Extraction of volatiles

A 100 ml portion of wine was transferred into a 500 ml Erlenmeyer flask and cooled to 0 °C in an ice bath under nitrogen; 34 μg of 4-nonanol was added as internal standard because of its high recovery (Voirin et al., 1992). Dichloromethane (40 ml) was added and the mixture was stirred at 700 rpm for 15 min (Kotseridis, Razungles, Bertrand, & Baumes, 2000; Moio et al., 1995). Then the mixture was centrifuged at 4 °C (9000*g*, 15 min). The organic phase was recovered. The aqueous phase was then re-extracted twice, as mentioned above. The organic extracts were combined, dried over sodium sulfate and concentrated to a volume of 1 ml with a Vigreux distillation column prior to gas chromatography/mass spectrometry (GC/MS) analysis (Schneider, Baumes, Bayonove, & Razungles, 1998). Each sample was extracted in triplicate and the concentration of volatiles, as 4-nonanol equivalents, was obtained as the mean of three repetitions.

2.4. Gas chromatography and gas chromatography–mass spectrometry analysis of volatiles

GC analysis of volatiles was performed using a Varian 3300 chromatograph equipped with a fused capillary column coated with DB-Wax (30 m × 0.32 mm dia., 0.5 μm film thickness, JW, Folsom, CA,USA) and a flame ionisation detector (FID). The flow rate of carrier gas hydrogen was 1.8 ml/min. The injection mode system was on column and the injection volume was 1 μl. The injector temperature was programmed from 20 to 250 °C at 180 °C/min, then held at 250 °C for 80 min. The oven temperature was at 60 °C for 3 min, from 60 to 220 °C at 2 °C/min, from 220 to 245 °C at 3 °C/min, then held 20 min at 245 °C. The FID temperature was 250 °C.

Identification of the components was performed by a Hewlett–Packard 5890 Series II Chromatograph coupled with a Hewlett–Packard 5989 mass spectrometer with a quadrupole mass filter (Les Ulis, France). The chromatograph was equipped with the same DB-Wax capillary column as mentioned above. The flow rate of helium (carrier gas) was 1.5 ml/min. The injection on column volume was 1 μl. The oven and injector temperature programmes were as above. Mass spectra (MS) were recorded in the electronic impact (EI) and positive chemical ionisation (PCI) modes. The transfer line temperature was 250 °C with source temperature of 250 °C. Mass spectra were scanned at 70 (EIMS) and 230 eV (PCIMS) in the range *m/e* 29–350 amu at 1 s intervals (Bureau, Razungles, & Baumes, 2000; Schneider et al., 1998). Identification of the components was done on the basis of retention index and the comparison of EI mass spectra with published data or with reference compounds.

3. Results and discussion

3.1. The influence of skin contact time on general wine composition

General composition of wines obtained with different skin contact times from cv. Muscat of Bornova is given in Table 1. The wine composition was affected by the skin contact treatment. The wines made with skin contact, with 6-h and 12-h treatments had higher values for extract, ash, ash alkalinity, total nitrogen, total phenol compounds (absorbance at 280 nm) and browning index (absorbance at 420 nm) than did to the control wine. On the other hand, the wines with skin contact treatments had lower values for ethanol and titratable acidity values. Similar results were reported in the literature (Cabaroglu & Canbas, 2002; Cabaroglu et al., 1997; Darias-Martin, Diaz-Gonzalez, & Diaz-Romero, 2004; Ho et al., 1999).

3.2. The influence of skin contact time on aroma compounds

Table 2 shows the aroma compounds of Muscat of Bornova wines, expressed by the means (μg/l) of the three analytical replicates. GC–MS analysis of cv. Muscat of Bornova wines identified 72 volatile compounds. These were sixteen higher alcohols, twelve terpenes, sixteen esters, four C-6 alcohols, ten volatile acids, six volatile phenols, three lactones, two C-13 norisoprenoids, two carbonyl compounds and one acetal. Alcohols and esters were the main volatiles of the wines. These compounds are mainly produced by yeast metabolism during fermentation (Rapp & Mandery, 1986). Odour threshold, odour activity value (OAV), and sensory description of some volatiles detected in wine, in view of the literature data, are shown in Table 3. Skin contact treatment increased the total concentration of volatiles in wines compared to the control wine. Similar results were found by Cabaroglu and Canbas (2002); Cabaroglu et al. (1997), and Falqué and Fernandez (1996). The control, skin contact for 6 and 12 h wines contained 158, 168, and 172 mg/l of volatiles, respectively.

As indicated in Table 2, higher alcohols were the major constituents of the Muscat of Bornova wines. The concentration of higher alcohols was generally independent of with skin contact times. However, the levels of 1-propanol, 2-phenyl ethanol, and 1,2-propanediol increased with skin contact, but amounts of 3-ethoxy-1-propanol and 2,3-butanediol decreased. Their concentrations were much lower than their threshold values reported by Simpson (1979) and Etiévant (1991). Higher alcohols positively affect the quality of wines in quantities not above 400 mg/l (Rapp & Mandery, 1986). The total concentration of these components in Muscat of Bornova wines was below 400 mg/l (Table 2). Among the higher alcohols, the OAV for 2-phenyl ethanol was 1.3 (Table 3). This alcohol has a pleasant aroma, resembling that of a rose.

Twelve terpenes, including terpene alcohols and some of their oxides, were identified in Muscat of Bornova wines. Among these, linalool, *t*-furan linalool oxide, citronellol, α-terpineol, 3,7-dimethyl-octa-1, 7-dien-3,6-diol, geraniol, and geranic acid significantly increased with skin contact treatments. The results are in agreement with other studies, namely by Bueno, Peinado, Moreno, Moyano, and Zea (2003); Cabaroglu and Canbas (2002) and Cabaroglu et al. (1997). In the present study, linalool, geraniol, α-terpineol, and *t*-pyran linalool oxide were the most abundant terpenes in wines. Among these, linalool and geraniol were at levels significantly above the odour threshold values mentioned by Guth (1997) and Lopez, Ferreira, and Cacho (1999) (Table 3). Terpenes are responsible for the characteristic aroma of muscat and aroma related wines (Marais, 1983). About 50 terpene compounds are reported in the literature (Rapp & Mandery, 1986). The most prominent terpenes are linalool, geraniol, nerol, citronellol, 3,6-dimethyl-octa-1,5-octa-1,7-diol and α-terpineol, located in the skin and the solid parts of the cells in the berries, particularly muscat and related grapes (Günata et al., 1985; Marais, 1983).

cv. Muscat of Bornova wines had low amounts of C-6 compounds. Although, skin contact treatment did not significantly increase the levels of these compounds, the highest level was obtained with the 12 h treatment. 1-Hexanol was the most abundant among these compounds (Table 2). C-6 compounds are unfavourable to wine quality, giving herbaceous and leafy notes. Their amounts detected in wines were much lower than their threshold levels reported by Etiévant (1991).

Table 1
General composition of cv. Muscat of Bornova wines

	Skin contact time		
	Control	6 h	12 h
Density (20 °C)	0.9920	0.9926	0.9922
Ethanol (%, v/v)	12.3	11.7	11.9
Extract (g/l)	19.4	19.8	20.0
Titratable acidity[a] (g/l)	5.1	4.8	4.6
pH	3.4	3.4	3.4
Absorbance at 280 nm	14	15	18
Absorbance at 420 nm	0.126	0.132	0.182
Volatile acidity[b] (g/l)	0.18	0.36	0.18
Reducing sugar (g/l)	4.0	4.3	1.9
Total nitrogen (mg/l)	87	98	123
Ash (g/l)	1.3	1.6	2.1
Ash alkalinity (meq/l)	24	25	26
Free SO_2 (mg/l)	17	17	18
Total SO_2 (mg/l)	78	91	87

[a] As tartaric acid.
[b] As acetic acid.

Table 2
Effect of skin contact on the aroma compound levels of Muscat of Bornova wines (μg/l)

Compounds	Skin contact time				Significance[b]	ID[c]
	LRI[a]	Control	6 h	12 h		
C-6 alcohols						
1-Hexanol	1356	1030	1049	1441	ns	A
(E)-3-Hexen-1-ol	1384	27	23	29	ns	A
(Z)-3-Hexen-1-ol	1387	43a	44a	69b	*	A
(E)-2-Hexen-1-ol	1407	6a	4b	5b	*	A
Total		1106	1120	1544		
Higher alcohols						
1-Propanol	1037	326a	517b	538b	**	A
Isobutanol	1085	17,538	18,930	18,908	ns	A
1-Butanol	1119	419	309	351	ns	A
Isoamyl alcohol	1210	91,917	95,512	97,431	ns	A
3-Methyl-2-buten-1-ol	1127	18	19	21	ns	A
3-Methyl-3-buten-1-ol	1240	26	27	30	ns	A
4-Methyl-1-pentanol	1301	16a	5b	14a	**	A
3-Methyl-1-pentanol	1313	42	45	36	ns	A
3-Ethoxy-1-propanol	1364	99a	65b	45b	*	A
Heptanol	1457	26a	30b	22a	*	A
2,3-Butanediol	1583	1756a	1746a	1261b	**	A
1,2-Propanediol	1603	20a	27ab	35b	*	B
2- Methyl thio ethanol	–	67	66	65	ns	B
Methionol	1723	182	221	198	ns	A
Benzylalcohol	1869	111	124	151	ns	A
2-Phenylethanol	1905	13,175a	15,303b	14,833b	*	A
Total		125,738	132,946	133,939		
Terpenes						
Ho-trienol	1449	45	90	58	ns	A
t-Furan linalool oxide	1439	17a	28b	58b	**	A
Linalool	1537	179a	215b	171a	*	A
α-Terpineol	1688	140a	166b	144a	*	A
t-Pyran linalool oxide	1731	243	265	211	ns	A
Citronellol	1737	58a	75b	49ab	*	A
Geraniol	1847	171a	256b	161a	*	A
2-Hydroxy cineol	–	1a	4ab	3b	*	B
Linalool hydrate	1926	94	74	52	ns	A
3,7-Dimethyl octa-1,7-dien-3,7-diol	1969	25	36	36	ns	A
3,7-Dimethyl octa-1,7-dien-3,6-diol	2128	3a	76b	62b	**	A
Geranic acid	2353	54a	124b	84c	*	A
Total		1030	1409	1089		
Esters						
Ethyl butanoate	1044	2327	2350	2395	ns	A
Isoamyl acetate	1132	1255a	1814b	2139c	**	A
Ethyl hexanoate	1230	827a	905ab	969b	*	A
Ethyl pyruvate	1242	21	15	22	ns	A
Hexyl acetate	1251	68a	29b	31b	**	A
Ethyl lactate	1353	4354a	4562a	5944b	*	A
Ethyl octanoate	1430	669a	714ab	774b	*	A
Ethyl-3-hydroxy-butanoate	1524	230a	272a	133b	*	A
Ethyl decanoate	1635	333	321	306	ns	A
Diethyl succinate	1690	366a	396a	784b	***	A
2-Phenylethyl acetate	1786	1931a	1826a	1561b	*	A
Ethyl-4-hydroxy-butanoate	1819	185a	302b	253b	**	A
Diethyl malate	2041	72	64	50	ns	A
Ethyl hexadecanoate	2259	113	130	126	ns	A
Ethylphenyl lactate	–	95a	95a	79b	*	B
Monoethyl succinate	2440	2374	2546	2530	ns	A
Total		15,220	16,341	18,096		

Table 2 (*continued*)

Compounds	Skin contact time				Significance[b]	ID[c]
	LRI[a]	Control	6 h	12 h		
Fatty acids						
Isobutanoic acid	1584	247	237	297	ns	C
Butanoic acid	1622	112	98	89	ns	B
Hexanoic acid	1838	2943a	2966a	3576b	**	B
2-Hexenoic acid	–	5	5	4	ns	C
Octanoic acid	2060	5424a	5568b	6475c	**	B
Nonanoic acid	2158	264	226	168	ns	B
Decanoic acid	2357	1279a	1326b	1517c	**	B
9-Decenoic acid	–	484a	592b	1307c	**	B
Tetradecanoic acid	2692	102	120	154	ns	B
Hexadecanoic acid	2886	803	854	939	ns	B
Total	–	11,663	11,992	14,526	–	
Volatile phenols						
4-Vinyl guaiacol	2181	3a	5a	24b	**	A
4-Vinylphenol	2379	120a	187b	223c	***	A
Guaiacly ethanol	–	65	70	74	ns	B
Acetovanillone	2460	37	52	88	ns	A
Tyrosol	3012	119a	189b	193b	**	A
Ethyl-4-hydroxy-benzoate	–	44	52	46	ns	B
Total	–	388	555	648		
C-13 norisoprenoids						
β-Damascenone	1841	11a	13b	10a	*	A
3-Oxo-α-ionol	2651	44a	72b	148c	**	A
Total		55	85	158		
Carbonyl compounds						
Acetoin	1291	275a	313a	413b	*	A
Isobenzofuranone	–	39	35	39	ns	B
Total		314	348	452		
Lactones						
γ-Butyrolactone	1635	2513a	2579a	1646b	**	B
4-Carbethoxy-γ-butyrolactone	–	279a	267a	183b	*	B
Pantolactone	–	9	2	3	ns	C
Total		2801	2848	1832		
Acetal compound						
2-Methyl-5-hydroxy-1,3-dioxane	–	51a	60b	30c	*	B
Total		51	60	30		
Total		158,366	167,704	172,314		

ns, not significant.

[a] LRI, linear retention index calculated on DB-WAX capillary column.

[b] Significance at which means differ as shown by analysis of variance.

[c] Identification: A = GC retention and MS data in agreement with that of pure compound available in the lab; B = GC retention and MS data in agreement with spectra found in the library; C = tentatively identified by MS matching with library spectra only; nd: not detected, tr: trace. Results are the means of three repetitions.

* Significance at $p < 0.05$.

** Significance at $p < 0.01$.

*** Significance at $p < 0.001$.

Skin contact treatment resulted in significant increase in the concentration of the esters, including isoamyl acetate, ethyl hexanoate, ethyl octanoate, ethyl lactate, diethyl succinate, and ethyl-4-hydroxy-butanoate, similar to the findings of Falqué and Fernandez (1996) and Cabaroglu and Canbas (2002). The amounts of hexyl acetate, 2-phenyl ethyl acetate and ethyl phenyl lactate, however, decreased with the skin contact process. Esters are very important compounds of wine flavour, giving a fruity odour (Etiévant, 1991). Ethyl lactate was the most

Table 3
Odour threshold values and odour activity values of some compounds of Muscat of Bornova wine

	Odour threshold values (μg/l)	Odour activity values (OAVs)[a]	Aroma description
Ethyl butanoate	20[c]	116	Ananas
Isoamyl acetate	30[b,c]	41.8	Fruity, banana
Ethyl hexanoate	5[c]	165	Ripe banana
2-Phenyl ethyl acetate	250[c]	7.7	Powerful fruity, fruit jam
Linalool	25[c]	7.2	Floral, fruity
Geraniol	30[b,c]	5.7	Floral, rose
2-Phenyl ethanol	10,000[c]	1.3	Rose
β-Damascenone	0.05[c]	220	Floral, lilac

[a] Odour activity values calculated by dividing concentration by odour threshold value of the compound.
[b] In water/ethanol (90 + 10, w/w) according to Guth (1997).
[c] In wine according to Lopez et al. (1999).

abundant ester in the Muscat of Bornova wines. It has been reported that its production was mainly due to malolactic fermentation (Henick-Kling, 1993). This fermentation is not performed in Turkey for white wines. Moreover, samples for GC–MS analysis were checked for the absence of malolactic fermentation. Therefore, it could be concluded that it was formed during the alcohol fermentation by yeasts, in agreement with the findings of Antonelli, Castellari, Zambonelli, and Carnacini (1999). Due to high OAVs, ethyl hexanoate (ripe banana), ethyl butanoate (ananas), isoamyl acetate (banana) and 2-phenyl ethyl acetate (fruit jam) should be considered as interesting contributors to the typical aroma of Muscat of Bornova wine (Table 3).

Octanoic acid, hexanoic acid, decanoic acid, 9-decenoic acid, and tetradecanoic acid were the major fatty acids in Muscat of Bornova wines and their levels increased with skin contact treatment. The total fatty acid levels were significantly higher with a 12 h skin contact treatment compared to both control and 6 h treatment (Table 2). However, the increase in fatty acid levels may not have a direct impact on wine aroma since the concentration of fatty acids is far below their threshold values (Etiévant, 1991). Falqué and Fernandez (1996) reported similar results for octanoic acid and hexanoic acid. The production of fatty acids depends on the composition of the must and fermentation conditions (Schreirer, 1979).

Among volatile phenols, significant increases occurred in 4-vinyl phenol, tyrosol, and 4-vinyl guaiacol levels with skin contact times. Volatile phenols are considered among the usual components of the aroma of a wine. Depending on their concentration, they contribute positively or negatively to wine aroma, but the levels of the volatile phenols detected in Muscat of Bornova wines were below those imparting off-flavour to wine aroma (Dominguez, Guillén, & Barroso, 2002).

The two C-13 norisoprenoid compounds identified in wines were β-damascenone and 3-oxo-α-ionol. The total concentration of C-13 norisoprenoid compounds was higher in the skin contact wines. Increasing the skin contact times uniformly increased the 3-oxo-α-ionol level but not the levels β-damascenone (Table 2). C-13 norisoprenoid are substances that come from degradation of carotenoid molecules and also from the hydrolysis of glycosides (Baumes, Wirth, Bureau, Günata, & Razungles, 2002; Schneider et al., 2001). They are mainly present in glycoconjugated forms in young wines, but 3-oxo-α-ionol was found in the free form in Muscat of Bornova wines, as reported in previous studies (Cabaroglu et al., 1997; Cabaroglu, Selli, Canbas, Lepoutre, & Günata, 2003). According to OAVs, β-damascenone was one of the most important odourants of Muscat of Bornova wine (Table 3). It has a pleasant floral aroma, with lilac attribute.

Among carbonyl compounds, acetoin and isobenzofuranone were found in wines. The total amount of these compounds increased with skin contact treatments.

With regard to lactones, γ-butyrolactone, 4-carbethoxy-γ-butyrolactone, and pantolactone were found in wines. γ-Butyrolactone was the quantitatively predominant lactone. Skin contact treatment decreased the amounts of these compounds (Table 2).

3.3. Sensory evaluation

Wines were evaluated using triangle and preference tests (Amerine & Roessler, 1976; Roessler, Pangborn, Sidel, & Stone, 1978). The wine made without a skin contact process was used as the control. The wines were easily distinguished by the judges (ten judges from UMR Sciences) ($p < 0.05$). In the preference test, the most preferred wine was the one produced with a 6 h skin contact treatment, followed by the control wine.

4. Conclusions

In the present work, the aromatic profile of Muscat of Bornova wine was first characterized. According to

OAVs, β-damascenone, ethyl hexanoate, ethyl butanoate, isoamyl acetate, 2-phenyl ethyl acetate, linalool, geraniol, and 2-phenyl ethanol were the most characteristic aroma-active compounds of this wine. For the volatiles identified, the increase in the skin contact time did not uniformly increase the levels of the aromatic constituents. In sensory evaluation, wine produced with a 6 h skin contact was preferred and presented a higher fruity aroma. The 12 h skin contact had no significant effect on the sensory results. Moreover this treatment lowered the wine quality by increasing the total phenolic compounds, browning index, and C 6 alcohol levels (herbaceaous odour) as compared to the immediate pressing and 6 h skin contact.

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