AROMA COMPOUNDS OF *VITIS VINIFERA* L. CV. EMIR GROWN IN CENTRAL ANATOLIA

COMPOSÉS AROMATIQUES DU CÉPAGE EMIR CULTIVÉ DANS LA RÉGION DE L'ANATOLIE CENTRALE

T. CABAROGLU[1*], A. CANBAS[1] and Z. GUNATA[2]

1: University of Cukurova, Faculty of Agriculture, Department of Food Engineering, 01330 Adana, Turkey
2 : Université Montpellier II, Laboratoire de Génie Biologique et Science des Aliments, 34095 Montpellier, France

Abstract: Free and glycosidically bound aroma compounds of Emir grape juice obtained with or without skin-contact were investigated. The aroma compounds were isolated by adsorbtion on Amberlite XAD-2 resin and analysed by gas chromatography-mass spectrometry (GC-MS). 32 free and 37 glycosidically bound compounds were identified. - Shikimate - derived compounds were major components. C13-norisoprenoid compounds usually detected in bound forms in grapes were also present in free forms in Emir grape. Monoterpenes were in lower levels that normally found in floral grape cultivars. The amounts of free and bound aroma compounds of the juice increased by skin-contact treatment. The levels of some compounds were significantly different in control and skin-contact jices. Additionally, skin-contact treatment resulted in an increase in pH, colour intensity, total phenolics, potassium, sodium and calcium.

Résumé : Les composés aromatiques libres et glycosidically liés du moût de cépage Emir obtenu avec ou sans macération pelliculaire ont été étudiés. Les composés aromatiques ont été isolés par adsorbtion sur la résine de l'Amberlite XAD-2 et analysés par chromatographie en phase gazeuse-spectrométrie de masse (CPG-SM). 32 composés libres et 37 composés glycosidically liés ont été identifiés. Les composés shikimate dérivés étaient les composants principaux. Les composés de C13-norisoprenoid habituellement détectés sous les formes liées en moût étaient également présents sous les formes libres. Les monoterpènes qui sont normalement trouvés dans les cépages floraux étaient dans des niveaux plus bas. Les teneurs en composés libres et liés ont augmenté par macération pelliculaire. Les teneurs en certains composés sont significativement différentes entre le moût témoin et le moût macéré. De plus, la macération pelliculaire a entrainé une augmentation du pH, de l'intensité colorante, de l'indice de polyphénols totaux et des teneurs en potassium, sodium et calcium.

Mots-clés : cépage Emir, composition aromatique, volatiles libres et liés, macération pelliculaire.

Key words: Emir grape, aroma composition, free and bound volatiles, skin contact.

INTRODUCTION

Vitis vinifera L. cv. Emir is a native grape variety which is one of the best white wine cultivar in Turkey and is grown in the central Anatolia (Cappadoce region). The Emir cultivar is largely predominant in this region. The soil of this region is mainly formed from volcanic ashes and has a tuffaceous character. Emir is a medium, green-yellow, moderately tough-skinned grape that ripens in mid September and continues through October (CANBAS *et al.*, 1992).

It is well known that aroma plays an important role in wine quality. Grape derived flavour compounds are the important contributors to wine flavour (STRAUSS *et al.*, 1986; WILLIAMS *et al.*, 1989; VOIRIN *et al.*, 1990). It is now clear that some important flavor compounds belonging to the class of monoterpenes, norisoprenoids and shikimates occur in grape berries in free and glycoconjugated forms (GUNATA *et al.*, 1985a; STRAUSS *et al.*, 1986; WILLIAMS *et al.*, 1989; VOIRIN *et al.*, 1992). Although the aroma compounds of Emir wines were published previously (CABAROGLU *et al.*, 1997), the aroma compounds of Emir grapes have not been reported yet in international publications. This study was undertaken to investigate the aroma composition of Emir cultivar and the effect of prolonged contact between juice and skins, i.e, skin-contact treatment, on the concentration of aroma compounds. Indeed, free and bound aroma compounds are mainly localized in skin part of berry. Skin-contact technique is applied before fermentation in order to increase the

*Correspondance : tcabar@mail.cu.edu.tr

J. Int. Sci. Vigne Vin, 2002, **36**, n°4, 213-219

Table I - Composition of Emir musts*

Composition des moûts du cépage Émir

	Control	Skin-contact
Reducing sugar (g/l)	195.5	195.0
pH	3.50	3.62
Total acidity (meq/l)	79.2	73.8
Absorbance (280 nm)	0.087	0.096
Absorbance (420 nm)	0.040	0.062
K (mg/l)	901	978
Na (mg/l)	20	25
Ca (mg/l)	151	182
Mg (mg/l)	105	105

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

aroma compounds of wines (SINGLETON *et al.*, 1975; ARNOLD and NOBLE, 1979; GUNATA *et al.*, 1985a; DUBOURDIEU *et al.*, 1986; BAUMES *et al.*, 1986; 1989a; GOMEZ *et al.*, 1994; FALQUE and FERNANDEZ, 1996).

MATERIAL AND METHODS

I- JUICE PREPARATION

Grapes (ca. 1 000 kg) at optimum maturity (table I) for winemaking were manually harvested from the Nevsehir-Urgup province in Cappadoce region and transported to the experimental winery at the Department of Food Engineering, Faculty of Agriculture, University of Cukurova (Adana/Turkey). They were divided into two equal parts. First part was treated in the standard way without skin-contact. Grapes were pressed in a horizontal press and 40 mg/l of sulphur dioxide was added. The must was then settled (16 °C , 24 h) and racked. For the skin-contact experiment, second part of the grapes were destemmed and crushed. The pomace was mixed with 40 mg/l of sulphur dioxide, kept at 16 °C for 6 h, and then pressed in a horizontal press. The must was settled and racked as mentioned above.

II- GENERAL JUICE COMPOSITION

Juices were analysed for reducing sugar, pH, total acidity, potassium, sodium, calcium and magnesium by standard methods (ANON, 1990). Total phenolics were estimated by absorbance at 280 nm (RIBÉREAU-GAYON *et al.*, 1976) and colour at 420 nm (SINGLETON and KRAMLING, 1976) .

III- ANALYSIS OF FREE AND BOUND AROMA COMPOUNDS

100 ml of must sample was used for each extraction. For the determination of aroma compounds, triplicate analyses were done for each sample. 30 mg of 4-nonanol (Fluka) was added into the samples as internal standard for free aroma compounds. The samples were passed through an Amberlite XAD-2 resin to isolate free and glycosidically bound aroma compounds (GUNATA *et al.*, 1985a). The free and bound compounds were eluted successively with 50 ml each of pentane-dichloromethane (2/1, v/v) and ethyl acetate/methanol (9/1,v/v) (VERSINI *et al.*, 1994). Pentane-dichloromethane eluate was dried over anhydrous sodium sulphate and concentrated to ca. 500 ml through a Vigreux column prior to GC and GC-MS analysis. The ethyl acetate/methanol eluate was concentrated to dryness in vacuo and dissolved in 0.2 ml of 0.2 M citrate-phosphate buffer (pH 5.0). The glycosidic extract was then subjected to enzymatic hydrolysis with an enzyme preparation (1.2 mg of Pectolase 3PA,Grindsted, Denmark) (GUNATA *et al.*, 1985a; 1985b). The mixture was incubated at 40 °C for 12 h. Released aglycones were extracted with pentane-dichloromethane mixture mentionned above. The organic phase was recovered, added with 30 mg of 4-nonanol as internal standard, concentrated to ca. 500 ml as above and analysed by GC and GC-MS.

GC analysis of free volatiles and released aglycones was performed using a Varian 6000 Chromatograph equipped with a fused capillary column coated with DB-Wax (30 m x 0.32 mm i.d., 0.5 mm film thickness, J&W, Folsom, CA, USA). Operating conditions were as follows. On-column injector : from 25 to 250 °C at a rate of 180 °C/min, oven temperature : 60 °C for 3 min, from 60 to 220 °C at 2 °C/min, from 220 to 245°C at 3 °C/min, then isothermal for 20 min, detector (FID) temperature : 250 °C, carrier gas flow : 2 mL/min. Electron impact mass spectra were obtained by using a Finnigan Mat ITD 700. The GC conditions were the same as described above except for the carrier gas (helium). The identification of compounds was carried out by comparing the linear retention index and electronic mass spectra with published data or with reference compounds (VOIRIN *et al.*, 1990; CABAROGLU *et al.*, 1997). The significance of the effect of skin-contact treatment on aroma compound concentrations was determined by analysis of variance (Anova).

RESULTS AND DISCUSSION

I- GENERAL GRAPE MUST COMPOSITION

The composition of the juices obtained from no skin-contact (i.e. control) and skin-contact are shown in table I. The juice obtained from skin-contact had higher values for pH, total phenolics, colour, potassium, sodium and calcium, and lower value for total

Table II - Effect of skin-contact on free aroma compound levels of Emir musts

Incidence de la macération pelliculaire sur les teneurs des composés aromatiques libres des moûts Emir

			Concentration (μg/l)^x		
Compound	RI^v	ID^y	Control	Skin-contact	Sig^z
Alcohols					
n-butanol	1142	b	3.4	4.2	ns
2-methyl-1-butanol	1209	a	37.8	49.2	ns
3-methyl-1-butanol	1209	a	76.5	83.6	ns
n-pentanol	1250	c	1.8	6.3	**
4-methyl-1-pentanol	1320	b	4.3	5.0	ns
Benzyl alcohol	1870	a	35.0	59.5	*
2-phenyl ethanol	1905	a	64.2	75.0	ns
Total			223.0	282.8	
C-6 alcohols					
1-Hexanol	1356	a	124.6	420.0	***
Z-3-hexen-1-ol	1385	a	37.8	58.9	**
E-2-hexen-1-ol	1407	b	256.6	864.8	***
Total			419.0	1343.7	
Monoterpenes					
α-terpineol	1698	a	5.2	5.7	ns
geraniol	1848	a	8,5	13,3	**
ρ-menthen-7,8-diol	2519	b	2.5	3.6	ns
Total			16.2	22.6	
Fatty acids					
Hexanoic acid	1838	b	12.3	36.8	ns
Octanoic acid	2060	b	7.8	7.5	ns
Nonanoic acid	2158	b	5.4	6.1	ns
Decanoic acid	2350	b	3.6	3.2	ns
Benzoic acid	2415	b	2.6	2.2	ns
Total			31.7	55.8	
Phenols					
Isoeugenol	2135	b	5.6	6.1	ns
4-vinylphenol	2380	b	3.5	4.8	ns
Vanilline	2543	a	7.0	8.2	ns
Acetovanillone	2617	a	trace	2.6	**
3,4-dimethoxyphenol	-	c	2.4	3.6	ns
Zingerone	2783	b	6.1	9.6	ns
2-(4-guaiacyl)-ethanol	-	c	2.6	9.2	ns
Syringaldehyde	2930	b	38.6	63.0	ns
Methyl syringoate	-	c	14.6	20.3	ns
Tyrosol	3012	b	30.7	39.0	ns
Total			111.1	166.4	
Nitrogen compound					
2-Phenyletyl acetamide	-	c	64.6	73.4	ns
C-13 norisoprenoids					
3-Hydroxy-β-damascone	2530	a	13.9	17.2	ns
3-Oxo-α-ionol	2629	a	22.1	31.7	*
3-Hyroxy-7,8-dehydro-β-ionol	2748	b	6.4	9.4	ns
Total			42.4	58.3	
Total of volatiles			**908.0**	**2003.0**	

x : The data are mean values of triplicates (maximum SD : ± 10 %). ; v : RI: Linear retantion index calculated on DB WAX capillary column
Y : Identification: a:GC retention and MS data in agreement with that of pure compound, b: GC retention and MS data in agreement with mass spectra library, c: tentatively identified by MS matching with the library spectra only. z : 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.

Table III - Effect of skin-contact on bound aroma compound levels of Emir musts

Incidence de la macération pelliculaire sur les teneurs des composés aromatiques des moûts Emir

Compound	RI[v]	ID[y]	Concentration (μg/l)[x] Control	Skin-contact	Sig[z]
Alcohols					
2-methyl-1-butanol	1209	b	11.3	14.0	ns
3-methyl-3-butenol + pentanol	1251	b	7.0	8.8	ns
2-methyl-2-buten-1-ol	1322	b	2.7	2.7	ns
3-octenol	1445	b	0.7	0.9	ns
Benzyl alcohol	1870	a	284.8	317.4	*
2-phenyl ethanol	1905	a	61.6	70.0	ns
Total			368.1	413.8	
C-6 alcohols					
1-hexanol	1356	a	11.0	14.1	ns
Z-3-hexen-1-ol	1385	a	3.9	4.6	ns
E-2-hexen-1-ol	1407	b	2.5	3.8	ns
Total			17.4	22.5	
Monoterpenes					
α-terpineol	1700	a	0.4	0.5	ns
Nerol	1799	a	1.1	1.2	ns
Geraniol	1850	a	8.0	10.0	*
Geranic acid	2330	a	5.7	4.1	ns
ρ-menthen-7,8-diol	2519	b	4.9	6.2	**
Total			20.1	22.0	
Fatty acids					
Hexanoic acid	1838	b	5.9	5.1	ns
Octanoic acid	2060	b	1.1	1.4	ns
Nonanoic acid	2158	b	0.3	0.6	ns
Decanoic acid	2355	b	2.3	1.7	ns
Benzoic acid	2415	b	4.3	2.8	ns
Total			13.9	11.6	
Phenols					
Methyl salicylate	1747	b	3.6	4.4	ns
Eugenol	2140	b	2.2	2.1	ns
4-Vinylphenol	2382	b	0.6	1.4	**
Vanilline	2545	a	0.8	1.2	ns
Acetovanillone	2620	a	7.5	6.9	ns
3,4-dimethoxyphenol	-	c	2.9	2.7	ns
Zingerone	2786	b	12.1	15.3	ns
2-(4-guaiacyl)-ethanol	-	c	2.3	2.2	ns
Syringaldehyde	2932	b	11.4	13.1	ns
4-hydroxy-methyl benzoate	-	c	3.9	4.5	ns
Methyl syringoate	-	c	3.0	5.1	*
Tyrosol	3014	b	8.6	9.4	ns
Total			58.9	68.3	
Nitrogen compound					
2-Phenyletyl acetamide	-	c	4.2	3.7	ns
C-13 norisoprenoids					
3-Hydroxy-β-damascone	2532	a	10.0	14.0	ns
3-Oxo-α-ionol	2630	a	7.0	8.2	ns
3-Oxo-α-retroinol	-	c	0.8	2.0	ns
3-Hyroxy-7,8-dehydro-β-ionol	2749	b	2.3	3.0	ns
Total			20.1	27.2	
Total of precursors			**502.7**	**569.1**	

v, x, y, z : See table 2 for footnotes

titratable acidity than the juice obtained from no skin-contact as reported previously (SINGLETON, 1974; DUBOURDIEU *et al.*, 1986; RAMEY *et al.*, 1986; TEST *et al.*, 1986; BAUMES *et al.*, 1989a). Indeed the skin-contact enriches the juice in phenolics and cations aforementioned.

II- FREE AROMA COMPOUNDS

32 volatile aroma compounds identified in Emir grape musts are listed in table II. These included 10 phenols, 7 higher alcohols, 5 volatile acids, 3 monoterpenes, 3 six-carbon alcohols, 3 C13- norisoprenoid compounds and 1 nitrogen compound. Aroma compounds were found to be in higher concentration in the skin-contact juice than the control juice as observed previously (DUBOURDIEU *et al.*, 1986; RAMEY *et al.*, 1986; BAUMES *et al.*, 1989a; MOYANO *et al.*, 1994). The total concentration of volatiles in the control and skin-contact juices were 908 μg/l and 2 003 μg/l respectively.

Free volatiles were dominated by aliphatic alcohols and phenols. 3-methyl-1-butanol, 2-phenyl ethanol, 2-methyl-1-butanol and benzyl alcohol were the most abundant higher alcohols in Emir juices. Skin-contact increased the total concentration of alcohols, in agreement with previous findings (RAMEY *et al.*, 1986; BAUMES *et al.*, 1989a). From these compounds benzyl alcohol level, which has a characteristic pleasant, fruity odour, increased significantly with skin-contact. 2-Phenylethanol which has a floral, rose-like aroma is also important in the aroma of grapes (NYKANEN and SUOMALAINEN, 1989). Both compounds are preferentially located in berry skins (NYKANEN and SUOMALAINEN, 1989; GUNATA *et al.*, 1985b).

Skin-contact treatment considerably increased the amount of six-carbon alcohols as reported in previous studies (BAUMES *et al.*, 1989a). This can be explained by the abundance of fatty acid precursors in the skins. The levels of E-2-hexen-1-ol were much higher than those of 1-hexanol and Z-3-hexen-1-ol. Six-carbon alcohols are characterized by herbaceous and leafy odours. Their concentrations detected in Emir juice were below their detection thresholds (ETIEVANT, 1991).

Monoterpenes which often possess floral odors were detected at low levels in Emir juice compared to many floral grape varieties such as Muscat, Riesling and Gewurztraminer (GUNATA *et al.*, 1985a; STRAUSS *et al.*, 1986). The compounds identified were geraniol, α-terpineol and ρ-menthen-7-8-diol. Skin-contact treatment caused an increase in the level of monoterpenes in Emir juice as previously reported for some cultivars (BAYANOVE *et al.*, 1976; MARAIS and RAPP, 1988; BAUMES *et al.*, 1989a). This increase was significant for geraniol.

Volatile phenols were dominant in Emir juice both in number and concentration. Importantly, this cultivar was distinguished from many cultivars by the abundance of volatile phenols (BAUMES *et al.*, 1989a; FALQUE and FERNANDEZ, 1996). Among the volatile phenols detected, isoeugenol, which has a clove-like aroma, and vanilline, which has a vanilla-like aroma note, are potential contributors to wine aroma (DUBOIS, 1994; GUNATA, 1994). Syringaldehyde was the dominant volatile phenol in Emir juice.

C13-norisoprenoid compounds were detected in free forms in Emir juice while they have been mainly found in bound forms in many cultivars (BAUMES *et al.*, 1989a; WILLIAMS *et al.*, 1989; SEFTON *et al.*, 1993). This is the case for 3-hydroxy-β-ionone and 3-oxo-α-ionol which are often major bound norisoprenoids from many cultivars. They were the dominant free norisoprenoids in Emir juice. Skin-contact treatment increased the concentrations of free norisoprenoids and there was a significant increase for 3-oxo-α-ionol. The concentration of 3-hydroxy-7,8-dehydro-β-ionol was 6,4 μg/l in control juice and 9,4 μg/l in skin-contact juice. This compound is precursor of β-damascenone (SEFTON *et al.*, 1993), a potent flavorant which has a flowery and quince-like odor and very low odor threshold (50 ng/L in 10 % alcohol) (GUTH, 1997).

Small amounts of fatty acid were detected in Emir juice. Among them, hexanoic acid was the most abundant. Only hexanoic acid and nonanoic acid levels increased in the skin-contact Emir juice. 2-phenyl ethyl acetamide was detected in Emir juice as nitrogen-containing compound and its concentration was higher in the skin-contact juice than the control juice.

III- BOUND AROMA COMPOUNDS

37 bound aroma compounds were identified in Emir grape juices after enzymatic hydrolysis of glycosidic extract (table III). These included 12 phenols, 7 higher alcohols, 5 volatile acids, 5 monoterpenes, 3 six-carbon alcohols, 4 C13-norisoprenoid compounds, and 1 nitrogen compound. Their total concentrations in control and skin-contact juices were respectively 502.7 μg/l and 569.1 μg/l, indicating that glycosidic compounds were extracted from the berry skin where they are located (GUNATA *et al.*, 1985b; GOMEZ *et al.*, 1994). Similar findings were reported by Baumes *et al.* (1989b).

The aglycone composition of Emir cultivar is quite similar to that observed for other cultivars since they are made up of aliphatic alcohols, shikimate-derived compounds, monoterpenes and C13-norisoprenoid compounds (GUNATA *et al.*, 1985a; WILLIAMS *et al.*, 1989; VOIRIN *et al.*, 1990). As for the case of free volatiles, Emir cultivar was particularly rich in shikimate-derived compounds. The most abundant compounds were benzyl alcohol and 2-phenylethanol. Bound monoterpenes were at quite low levels, as observed for free ones.

Bound C13-norisoprenoid compounds were the same detected in free form, except for 3-oxo-α-retroionol. The total concentration of bound norisoprenoids was lower than that of free ones. This is indicative of the particularity of Emir grape since the opposite situation was reported for many cultivars (BAUMES *et al.*, 1989a; WILLIAMS *et al.*, 1989; SEFTON *et al.*, 1993).

The concentration of geraniol, ρ-menthene-7,8-diol, 4-vinylphenol, methyl syringoate levels increased significantly with skin-contact treatment.

CONCLUSION

This study shows that Emir grape belongs to the class of non floral cultivars with respect to its composition in free and bound aroma compounds. The varietal aroma of this cultivar was dominated by shikimate-derived compounds, the influence of which on wine flavor needs to be clarified. The skin-contact treatment was found to be interesting in increasing the amounts of free and bound aroma compounds in the juice.

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Manuscrit reçu le 8 juillet 2002 ; accepté pour publication le 18 octobre 2002