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Volatile composition of red wine from *cv.* Kalecik Karası grown in central Anatolia

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Abstract

Volatile composition of red wines made from *cv*. Kalecik Karası, a native grape variety of *Vitis vinifera* grown in Turkey, was investigated during two vintages. Volatile compounds were extracted with dichloromethane, and then analysed by gas chromatography–flame ionisation detection (GC/FID) and gas chromatography–mass spectrometry (GC/MS). Seventy-eight aroma compounds were identified. Isoamyl alcohol, ethyl hexanoate, ethyl octanoate, ethyl decanoate, isoamyl acetate, 2-phenyl ethanol and octanoic acid were potent aroma compounds on the basis of gas chromatography/olfactometry (GC/O) analysis and odour activity values (OAVS).

Keywords: Wine; cv. Kalecik Karası; Volatile compounds; GC-O analysis

1. Introduction

Aroma is one of the important factors determining wine character and quality. Aroma constituents of different grapes and wines have been extensively studied in the last few years. More than 800 volatile compounds, such as alcohols, esters, organic acids, phenols, thiols, monoterpenes and norisoprenoids have been found in wines, only several tens of which can be impact odorants (Aznar, López, Cacho, & Ferreira, 2001; Ferreira, López, Escudero, & Cacho, 1998; Rapp & Mandery, 1986). Varietal flavor of wines are due to monoterpenes (Gunata, Bayonove, Baumes, & Cordonnier, 1986; Williams, Strauss, & Wilson, 1980), norisoprenoids (Sefton, Francis, & Williams, 1993; Strauss, Wilson, Anderson, & Williams, 1987), methoxypyrazines (Kotseridis, Anocibar-Beloqui, Bertrand, & Doazan, 1998; Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2002) and thiols compounds (Tominaga, Baltenweck-Guyot, Peyrot Des Gachons, & Dubourdieu, 2000; Tominaga, Murat, & Dubourdieu, 1998). Among the volatiles derived from yeast metabolism, esters are mainly known to be important contributors to wine flavour (Nykänen, 1986; Rapp & Mandery, 1986).

Kalecik Karası is a medium, round and thick-skinned grape. It is a native and major red grape variety of Ankara district (Central Anatolia Region), and is used for the production of one of the highest quality red wines of Turkey (Akman & Yazıcıoğlu, 1960; Gökçe, 1990). The aim of this research was to determine volatile composition of wines from this cultivar over two consecutive vintages, with the objective of an evaluation of the potent aroma compounds on the basis of GC–O analysis and odour unit values.

2. Materials and methods

2.1. Wine making

Sound red grapes of *cv*. Kalecik Karası (1000 kg) were obtained from the vineyard of Kavaklıdere Winery (Ankara region) during the 1998 and 1999 vintages and transported to the experimental winery at the Department of Food Engineering, University of Cukurova (Adana province) in 20 kg plastic boxes.

The grapes were destemmed and crushed on a commercial grape destemmer-crusher and then transferred

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into a stainless-steel tank for maceration and treated with sulfur dioxide (35 mg/kg). The maceration time was 3 days at about 25 °C each year. During this time the mixture was stirred twice per day to increase the extraction of polyphenolic and aroma compounds. After maceration time was completed, pomaces were pressed gently in a horizontal press. The musts were fermented (25 °C) with spontaneous yeasts. After the alcoholic fermentation, the young wines were allowed to stand for malolactic fermentation (20 °C). The wines were then racked and added with sulfur dioxide (75 mg/l). They were stored at 15 °C in stainless steel tank prior to analysis.

2.2. Standart chemical analysis

Density, ethanol, extract, total acidity, pH, volatile acidity, acetaldehyde, reducing sugars, total and free SO_2, total phenolic compounds (280 index), tannins and anthocyanins 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. Thirty-four micrograms of 4-nonanol were added as internal standard. Dichloromethane (40 ml) was added and the mixture was stirred at 700 rpm for 15 min (Kotseridis, Razungles, Bertrand, & Baumes, 2000; Moio, Chambellant, Lesschaeve, Issanchau, Schlich, & Etiévant, 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 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 a means of three repetitions.

2.4. Gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) 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. The flow rate of carrier gas hydrogen was 1.8 ml/min. 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°/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 °C to 245 °C at 3 °C/min, then held 20 min at 245 °C. The F.I.D. 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 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 ionization (PCI) modes. The transfer line temperature was 250 °C with source temperature of 250 °C. Mass spectra were scanned at 70 eV (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.

2.5. Gas chromatography–olfactometry analysis (GC–O)

GC–O analysis was done for the 1999 vintage and carried out using a Hewlett-Packard gas chromatography 5890 series II with the DB-Wax capillary column. The injection (1 μl) of the extract was with splitless/split mode (split ratio 1/10). The carrier gas was hydrogen with a flow rate of 2 ml/min. The oven temperature programme was the same as above (GC-FID). The GC effluents were split to a sniffing port and a flame ionisation detector (3/1).

The sniffing test were performed alternatively by two testers trained for GC/O of wines from the UMR Sciences pour l'Oenologie.

3. Results and discussion

3.1. General wine composition

The general compositions of must and wines are shown in Table 1. The general composition of wines was in accordance with previous studies carried out on Turkish wines (Cabaroglu, Canbas, Lepoutre, & Gunata, 2002; Canbas, Unal, Deryaoglu, Erten, & Cabaroglu, 1995). The titratable acidity of the 1998 vintage was found higher than that of the 1999. As a result of this, pH and reducing sugar were slightly increased in the 1999 vintage. The wine made with the 1998 vintage were lower in ethanol, extract, colour intensity, and anthocyanin content than the 1999 vintage which may be attributed to climatic variations.

3.2. Aroma composition of wines

The volatiles from wines were extracted with dichloromethane. Representative wine aroma extracts for

Table 1
General composition of cv. Kalecik Karası must and wine

	1998	1999
Must composition		
Titratable acidity[a] (g/l)	9.3	8.3
pH	3.5	3.6
Reducing sugars (g/l)	191	195
Wine composition		
Density (20 °C)	0.9955	0.9927
Ethanol (%, v/v)	10.3	11.0
Extract (g/l)	24	20
Titratable acidity[a] (g/l)	5.9	5.0
pH	3.4	3.6
Total phenolics (280 index)	27	24
Anthocyanins (mg/l)	136	180
Tannin (g/l)	1.7	1.2
Volatile acidity[b] (g/l)	0.30	0.18
Acetaldehyde (mg/l)	65	45
Reducing sugar (g/l)	1.76	0.72
Free SO_2 (mg/l)	9	15
Total SO_2 (mg/l)	77	103

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

chemical and olfactory analysis were obtained using this solvent (Moio et al., 1995). Table 2 shows the aroma compounds of Kalecik Karası wines, expressed as means (μg/l) of the GC analyses of triplicate extractions. A total of 65 and 56 free aroma compounds were identified in the 1998 and 1999 Kalecik Karası wines, respectively, including alcohols, esters, monoterpene, carbonyl compounds, lactones, acids, volatile phenols, acetal compounds and C-13 compounds compounds (Table 2). In the 1999 wine, the total concentration of aroma compounds (141 mg/l) was greater than in the 1998 wine (116 mg/l).

Alcohols and esters were the largest groups and made up more than 90% of the free volatiles. Higher alcohols and esters, produced during alcoholic fermentation, play an important role in the flavour of the wines, depending on the types of compounds and their concentrations (Valero, Moyano, Millan, Medina, & Ortega, 2002). The most abundant compounds were the higher alcohols, in accordance with the literature (Baumes, Cordonnier, Nitz, & Drawert, 1986; Nykänen, 1986). At concentrations below 300 mg/l they certainly contribute to the desirable complexity of wine; when their concentrations exceed 400 mg/l, higher alcohols are regarded as a negative quality factor (Mateo, Jiménez, Pastor, & Huerta, 2001; Rapp & Mandery, 1986). The total concentration of higher alcohols in Kalecik Karası wines was below 300 mg/l (Table 2). Among the aliphatic alcohols, isoamyl alcohol showed the highest concentration in both vintages. Another alcohol, present at very high concentration, was 2-phenylethanol. According to the sniffing analysis and OAVs, isoamyl alcohol and 2-phenylethanol were contributors to wine aroma (Table 3). These two alcohols are characterised by fruity and floral attributes, respectively. In the 1998 vintage, the level of 2-phenylethanol was higher than that in the 1999 vintage.

The 1998 and 1999 wines contained 6761 and 7244 μg/l of esters, respectively. High levels were observed for isoamyl acetate, ethyl hexanoate, ethyl lactate, ethyl octanoate, 4-hydroxy-ethyl butanoate, and monoethyl succinate. These compounds are important in young wine aroma and are synthesized during must fermentation. They are among key compounds in the fruity flavours of wines (Herraiz, Reglero, Martin-Alvarez, Herraiz, & Cabuzedo, 1991; Nykänen, 1986; Rapp & Mandery, 1986). OAVs and GC–O analysis have shown that four esters, ethyl hexanoate, ethyl octanoate, ethyl decanoate and isoamyl acetate, were impact odorants of Kalecik Karası wine.

Among monoterpene compounds, only citronellol was detected in both vintages. The 1998 vintage had more citronellol (20 μg/l) than the 1999 vintage (5 μg/l). However, its contribution to Kalecik Karasi wine seems negligible when its odour threshold is considered, (i.e. 100 μg/l in water/ethanol solution, 90/10 (w/w), (Guth, 1997). This first analysis on Kalecik Karası volatiles over two vintages shows that the aroma of this cultivar is not monoterpene-dependent.

High levels of γ-butyrolactone were detected in both vintages. Among the lactones, pantolactone, and 4-carbethoxy-γ-butyrolactone were detected in the wine by sniffing analysis (Table 3). Similar results were found for Cinsaut, Carignan, Grenache, Cabernet sauvignon and Syrah wines by Baumes et al. (1986) and for Öküzgüzü and Boğazkere wines by Cabaroğlu et al. (2002). The odour threshold values of these compounds have not yet been established. They have a pleasant aroma, with spicy and caramel attributes.

In the 1999 vintage, concentrations of fatty acids were higher than in the 1998 vintage. Hexanoic, octanoic, and decanoic acids occurred abundantly. The contents of 6-, 8-, and 10-carbon atom fatty acids, although high, were in agreement with those found by Versini, Orriols, and Dalla-Serra (1994) and Falque, Fernandez, and Dubourdieu (2002). Among fatty acids, the octanoic acid level was highest for both vintages (Table 2). The OAV for octanoic acid was 8. A cheese flavour was detected during GC–O analysis of this compound.

The total volatile phenol concentration was higher in the 1999 wine. The only volatile compounds of this group found at significant levels were tyrosol and 4-vinyl phenol. The latter was not detected in the 1999 vintage. This compound is formed through the decarboxylation of coumaric acid by yeast decarboxylase (Dugelay, Gunata, Sapis, Baumes, & Bayonove, 1993). The other volatile phenols detected were phenol, guaiacol, ethyl phenol, eugenol and vanillin. Among these, vanillin was detected in GC–O analysis. Due to its low OAV, its

Table 2
Volatile compounds of Kalecik Karası wines over two vintages

Compounds (μg/l)	LRI[a]	1998	1999	ID[b]
Alcohols				
1-Butanol	1119	173±21.6	185±10.1	A
Isoamyl alcohol	1210	85 499±393.2	108 846±758.2	A
3-Methyl-3-buten-1-ol	1240	74±15.0	nd	A
1-Pentanol	1249	nd	22±2.2	A
2-Methyl-2-buten-1-ol	1315	nd	9±2.3	A
1-Hexanol	1356	891±14.7	917±22.0	A
(E)-3-Hexen-1-ol	1384	30±3.7	24±0.7	A
(Z)-3-Hexen-1-ol	1387	11±2.0	34±1.3	A
(E)-2-Hexen-1-ol	1409	9±1.9	8±0.3	A
(Z)-2-Hexen-1-ol	1416	24±4.5	nd	A
4-Methyl-1-pentanol	1301	15±0.6	29±2.8	A
3-Methyl-1-pentanol	1313	50±2.3	51±1.3	A
3-Ethoxy-1-propanol	1364	61±6.6	82±6.7	A
Heptanol	1457	14±1.4	23±2.9	A
1,2-Propanediol	1603	27±2.9	nd	B
Benzyl alcohol	1869	247±2.7	153±3.3	A
2-Phenylethanol	1905	16 195±104	12 854±69.3	A
Furaneol	2031	66±5.2	nd	B
Homofuraneol	2105	8±0.7	nd	B
Total		*103 394*	*123 237*	
Esters				
Isoamyl acetate	1132	1972±39.9	641±24.0	A
Ethyl hexanoate	1230	1722±19.0	647±14.1	A
Ethyl pyruvate	1242	16±7.0	20±1.4	A
Hexyl acetate	1251	3±0.9	6±1.3	A
Ethyl lactate	1353	215±15.1	1063±19.7	A
Ethyl octanoate	1430	336±23.7	543±12.3	A
Ethyl-3-hydroxy-butanoate	1524	74±5.6	188±9.5	A
Isoamyl lactate	–	2±0.4	nd	B
Ethyl decanoate	1635	171±8.2	313±15.5	A
Ethyl-9-decenoate	1694	4±0.1	nd	A
Diethyl succinate	1690	nd	758±78.3	A
2-Phenylethyl acetate	1786	15±1.2	71±5.5	A
Ethyl-4-hydroxy-butanoate	1819	1259±29.9	1830±28.5	A
Ethyl dodecanoate	1851	29±3.4	4±0.6	A
Diethyl malate	2041	68±3.1	75±3.2	A
Ethyl hexadecanoate	2259	26±3.0	76±6.2	A
Ethyl-hydroxy-glutarate	–	nd	37±1.4	C
Ethylphenyl lactate	–	4±1.2	49±2.7	B
Monoethyl succinate	2440	845±25.9	906±13.3	A
Ethyl octadecanoate	–	nd	17±1.8	B
Total		*6761*	*7244*	
Terpenol				
Citronellol	1767	41±7.1	5±0.3	A
Total		*41*	*5*	
Carbonyl compounds				
Acetoin	1291	1365±15.5	2508±18.3	A
2-Hydroxy- propanone	–	3±0.4	nd	B
Isobenzofuranone	–	4±0.7	nd	B
Benzaldehyde	1538	tr	nd	B
Total		*1372*	*2508*	
Lactones				
γ-Butyrolactone	1635	296±21.2	505±33.8	B
γ-Nonalactone	–	13±1.6	nd	C
4-Carbethoxy-γ-butyrolactone	–	62±5.1	144±4.2	B
Pantolactone	–	nd	36±1.5	C
Total		*371*	*685*	

(*continued on next page*)

Table 2 (*continued*)

Compounds (μg/l)	LRI[a]	1998	1999	ID[b]
Acids				
Isobutanoic acid	1584	308±24.6	340±30.0	C
Butanoic acid	1622	81±7.6	122±2.6	B
Isovaleric acid + 2 methyl butyric acid	1686	35±12.2	nd	C
Valeric acid	–	nd	21±1.2	B
Hexanoic acid	1838	788±59.4	129±2.8	B
2-Hexenoic acid	–	53±3.8	nd	C
Heptanoic acid	–	nd	8±1.5	B
Octanoic acid	2060	1362±44.3	4008±70.5	B
Nonanoic acid	2158	nd	57±2.2	B
Decanoic acid	2357	614±43.6	801±20.6	B
Dodecanoic acid	2449	88±14.6	49±2.3	B
Tetradecanoic acid + propiovanillone	–	53±14.2	nd	C
Tetradecanoic acid	2692	nd	77±5.0	B
Hexadecanoic acid	2886	154±13.2	235±10.6	B
Total		*3536*	*5847*	
Volatile phenols				
4-Ethylphenol	2195	nd	19±3.6	A
4 Vinylphenol	2379	151±7.1	nd	A
Vanilline	2545	nd	8±0.7	A
Methyl vanillate	2600	20±1.0	nd	A
Zingerone	2786	5±1.3	nd	A
Guaiacly propanol	–	3±0.2	nd	B
Guaiacly ethanol	–	5±0.5	16±2.1	B
Vanilloylmethyl ketone	2800	12±2.4	3±0.7	A
Acetovanillone	2995	24±2.9	nd	A
Tyrosol	3012	221±18.5	818±33.5	A
Total		*441*	*864*	
Acetal compounds				
2-Methyl-4-hydroxy-1,3-dioxane	–	147±4.93	nd	B
2-Methyl-5-hydroxy-1,3-dioxane	–	11±3.1	50±3.2	B
Total		*158*	*50*	
C_{13} norisoprenoids				
3-Oxo-α-ionol	2651	4±1.4	nd	A
3-hydroxy-7,8-dihydro-β- ionol	2726	10±1.4	nd	B
3-hydroxy-7,8-dehydro-β-ionol	2775	Nd	14±4.2	A
Vomifoliol	3167	27±2.5	4±0.5	A
Total		*41*	*18*	
TOTAL		*116 115*	*140 458*	

[a] LRI: Linear retention index calculated on DB-WAX capillary column.

[b] 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 dedected, tr, trace. Results are the means of three repetitions.

contribution to Kalecik Karası wine seems insignificant (Table 3).

The two acetal compounds identified in wines were 2-methyl-4-hydroxy-1,3-dioxane and 2-methyl-5-hydroxy-1,3-dioxane (Table 2). These compounds have been reported in sherry wines (Rapp & Mandery, 1986), Australian port wine (Simpson, 1980), sweet fortified wines (Schneider et al., 1998) and other table wines (Baumes et al., 1986; Herraiz et al., 1991). During sniffing analysis, at the retention time of this compound, the sniffing panellists observed a herbaceous and hot pepper odour (Table 3).

Among the carbonyl compounds, acetoin was identified in both vintages, whereas 2-hydroxy-propanone and isobenzofuranone were identified only in the 1998 vintage. Acetoin was detected by sniffing panellists, and was described as an unpleasant fatty and toasted bread attribute.

The norisoprenoid compounds were present at low concentrations in both vintages. Among these, 3-hydroxy-7,8-dehydro-β-ionol is an important compound, since it is transformed to β-damascenone, a potent wine flavorant generated during wine storage (Cabaroglu et al., 2002; Simpson, 1980).

Table 3
Odours detected by GC–O analysis of a Kalecik Karası wine extract and odour threshold and odour activity values of aroma compounds

Compound	Odour threshold values (μg/l)	Odour activity values[a]	Aroma description
Isoamyl alcohol	40 000[b]	2.7	Red fruit, framboise
Ethyl hexanoate	5[c]	129.4	Floral, fruity
Acetoin	150 000[b]	< 1	Fatty, toasted bread
Ethyl octanoate	2[c]	271.5	Floral, fruity
Ethyl decanoate	200[b]	1.6	Fruity
Isoamyl acetate	30[b,c]	21.4	Fruity, banana
2-Methyl-5-hydroxy-1,3-dioxane	–	–	Herbaceous, hot pepper
Citronellol	100[c]	< 1	Citrusy, citron
2-Phenyl ethanol	10 000[c]	1.3	Floral, rose, lavender
Pantolactone	–	–	Spicy, caramel
Octanoic acid	500[b]	8.0	Cheese
4-Carbetoxy-γ-butyrolactone	–	–	Spicy, curry
Ethyl phenyl acetate	250[c]	< 1	Honey
Vanillin	200[c]	< 1	Vanilla, chocolate

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

Furaneol and homofuraneol were detected in the 1998 vintage. The concentration of the former (66 μg/l) was higher than that of its odour threshold (37 μg/l) determined in a model wine solution (Kotseridis et al., 2000). The furaneol imparts a caramel odour to wines.

This first study on volatiles of Kalecik Karası wine shows that the main aroma contributors are the compounds originating from yeast metabolism.

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