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Contribution of free and glycosidically-bound volatile compounds to the aroma of muscat “a petit grains” wines and effect of skin contact

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Abstract

Free and glycosidically-bound volatile compounds of must, skin and wine from Muscat “a petit grains” cultivated in Spain were determined, and the effect of different skin contact times on the aroma composition and sensory characteristics of wines was investigated. Must and wine had a large quantity of mono-terpenes, mainly linalool, geraniol, and nerol. The most abundant glycosylated fraction was the mono and poly-oxygenated terpenes, followed by benzene compounds and norisoprenoids. Must skin contact at 18 °C during 15 and 23 h produced an important increase in the free and bound varietal compounds, which suggests the use of glycosidic enzymes, together with skin maceration, to increase wine aroma. From a sensory standpoint, must skin contact was very positive, since it brought about an increase in the fresh and fruity characteristic of the wines, and they also had more body.

Keywords: Skin contact; Glycosides; Aroma compounds; Muscat; GC–MS

1. Introduction

Today, there is an increasing demand for young white wines with a fresh and fruity aroma, one of the most important factors in determining wine character and quality.

The typical flavour of Muscat wines is mainly due to volatile compounds coming from grapes. Grape aroma composition and its influence on the aroma of wines has been reviewed by many authors (Flanzy, 2000; Ribereau-Gayon, Glories, Maujean, & Dubourdieu, 2000).

Grape volatile compounds include monoterpenes, C13 norisoprenoids, benzene derivatives and aliphatic alcohols. Muscat grapes are characterized by a special aroma, the terpenols being the most important aromatic fraction (Bayonove, 1993). Some of the monoterpenes alcohols are the most odoriferous, especially linalool, α-terpineol, nerol, geraniol, citronellol and hotrienol, which have floral aroma. Many polyoxygenated forms of these terpenes have been identified in grape varieties with Muscat character. They have little olfactory impact in wines, due to their high perception thresholds, but they may be precursors of odour-active monoterpenols (Vasserot, Arnaud, & Galzy, 1995).

Grape aroma compounds are present as free forms, which may contribute directly to odour and, in much larger concentrations, as non-volatile sugar-bound conjugates. These forms occur mainly as monoglucosides or disaccharide glycosides: 6-*O*-(α-L-arabinofuranosyl)-β-D-glucopyranosides, 6-*O*-(α-L-rhamnopyranosyl)–β-D-glucopyranosides, 6-*O*-(β-D-apiofuranosyl)-β-D-glucopyranosides, and to a lesser extent β-D-glucopyranosides (Günata, Bayonove, Baumes, & Cordonnier, 1985a;

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Williams, Sefton, & Wilson, 1989; Winterhalter & Skouroumounis, 1997). These flavourless glycosides, and the cysteine derivative ones (Tominaga, Peyrot des Gachons, & Dubourdieu, 1998) accumulate in grape berries during maturation (Günata, Bayonove, Baumes, & Cordonnier, 1985b).

The hydrolysis of glycoconjugates by acids or enzymes can yield odour-active aglyconcs such as monoterpenes, C13 norisoprenoids and benzenic compounds (Günata, Bitteur, Brioullet, Baumes, & Bayonove, 1988; Sefton, Francis, & Williams, 1993; Williams et al., 1989). Since grape and yeast glycosidases show low activity under fermentation conditions (Günata, Dugelay, Sapis, Baumes, & Bayonove, 1993; Lecas, Günata, Sapis, & Bayonove, 1991), exogenous enzymes can be used in order to increase the aroma of wines (Castro Vázquez, Pérez Coello, & Cabezudo, 2002; Günata, Dugelay, Sapis, Baumes, & Cordonnier, 1990). Nevertheless, releasing of undesired compounds, such as volatile phenols could occur by using glycosidase enzymes (Dugelay, Günata, Sapis, Baumes, & Bayonnove, 1993).

Aroma precursors are located mostly in the skin (Bayonove, 1993), so the must skin contact technique has been proposed to increase the aroma of white wines and enhance the varietal character (Baumes, Bayonove, Barrillere, Samson, & Cordonnier, 1989; Test, Noble, & Schmidt, 1986).

The effect of skin maceration on wines depends on the grape variety and the skin contact conditions, such as time and temperature (Falqué & Fernández, 1996; Garcıa Romero, Pérez Coello, Cabezudo, Sánchez-Muñoz, & Martın-Alvarez, 1999; Marais & Rapp, 1988; Ramey, Bertrand, Ough, Singleton, & Sanders, 1986). During maceration, the concentration of the aroma components sometimes increases (Cabaroglu et al., 1997; Falqué & Fernández, 1996; Garcı́a Romero et al., 1999; Jurado, Pinilla, Ballesteros, Pérez-Coello, & Cabezudo, 2001), but sensory changes are not always produced (Dubourdieu, Olivier, & Boidron, 1986; Test et al., 1986). Otherwise there is a risk of negative effects, such herbaceous aroma, bitter flavour or over-strong colour (Cheynier, Rigaud, Souquet, Barillere, & Moutounet, 1989; Ramey et al., 1986). For this reason, maceration conditions must be carefully chosen.

Volatile aroma compounds of wines from Muscat "a petit grains" have been less studied than other more aromatic Muscat varieties, mainly the glycosidically-bound fraction. In the La Mancha region (Spain), this variety is cultivated in small areas with special climatological conditions (warm summers, cold winters and low rain) that could influence its aroma composition (Bureau, Razungles, & Baumes, 2000; Jackson & Lombard, 1993). Knowledge of the free and bound aroma fraction of this variety may help the selection of the best culture practices and the optimum date of harvest, as well as the development of an adequate wine-making technology in order to increase the aroma quality of wines.

The objective of this study was to determine the free and glycosidically-bound volatile compounds of skins, must and wines from Muscat "a petit grains" cultivated in La Mancha region (Spain), and to investigate the effects of different skin contact times on the aroma composition and sensory profile of wines.

2. Materials and methods

2.1. Materials

Grapes from *Vitis vinifera* var. Muscat "a petit grains" cultivated in La Mancha region (Spain), were harvested at the optimal maturity stage.

Grapes were divided into three batches. One batch was treated in the standard way without skin contact, and the other two batches were used for the skin-contact experiments. The grapes were destemmed, crushed, mixed with 100 mg/kg of sulphur dioxide and kept with skins at 18 °C for 15 or 23 h and then pressed.

Laboratory fermentations were performed in 10 l vessels at 18 °C in duplicate. The samples were inoculated with *Saccharomyces cerevisiae* (CECT No. 10835).

For the analysis of free volatile compounds of skins, the skins left from pressing were collected and then macerated in water with 5 g/l tartaric acid (pH 3.5) for 72 h at 8 °C (400 g of skin/l).

2.2. Isolation of free and glycosidically-bound volatile compounds

Prior to analysis, macerated skin solution, must and wines were centrifuged at 2 °C (15,000 rpm, 30 min). Isolation procedure followed the method developed by Günata et al. (1985a). Two hundred millilitres of macerated skin solution, must or wines were fractionated on preconditioned styrene–divinylbenzene cartridges (Bond Elut, Varian, 1 g of phase) adding 4-nonanol as internal standard. This type of cartridge has been used by other authors to analyse volatile compounds in wine with good quantitative results (Ferreira, Sharman, Cacho, & Dennis, 1996).

Free and bound fractions were eluted successively with 50 ml of pentane–dichloromethane (2:1) and 50 ml of ethyl acetate, respectively. Free fraction was concentrated at 36 °C on a Vigreux column and analysed by GC–MS.

Ethyl acetate extracts were evaporated to dryness under vacuum, then re-dissolved with 1 ml of methanol. An aliquot of 500 μl was evaporated to dryness under nitrogen and taken up in citric/phosphate buffer pH = 5 (100 μl). Enzymatic treatment with AR2000 (Gist Brocades) was conducted at 40 °C for 18 h.

Released aglycones were recovered by liquid–liquid extraction with 5 × 2 ml of pentane–dichloromethane (2:1) (Schneider, 2001).

Organic extracts were concentrated at 36 °C on a Vigreux column, adding 4-nonanol as internal standard, and analysed by GC–MS.

The other aliquot of 500 μl of the ethyl acetate extract was used to analyse the glycosides. The eluates were evaporated to dryness under nitrogen, and then taken up in anhydrous pyridine (20 μl) and 40 μl of *N*-methyl-bis-(trifluoroacetamide) as derivatization agent. Phenyl-β-D-glucopyranoside was used as internal standard. The derivatization reaction was performed at 60 °C for 30 min and the extracts were analysed by GC–MS.

2.3. Chromatographic conditions for the analysis of free and enzymatically-released volatile compounds

An Agilent gas chromatograph, model 6890 N, coupled to a mass selective detector, model 5973 *inert*, was used. An amount of 1 μl of extract was injected in splitless mode on a BP-21 capillary column (50 m × 0.32 mm i.d.; 0.25 μm film thickness). Oven temperature programme was: 70 °C (5 min) – 1 °C/min – 95 °C (10 min) – 2 °C/min – 190 °C (40 min). Injector and transfer line temperatures were 250 °C and 280 °C, respectively. Mass detector conditions were: electronic impact (EI) mode at 70 eV; source temperature: 178 °C; scanning rate: 1 scan/s; mass acquisition: 40–450 amu.

The identification was based on comparison of the GC retention times and mass spectra with authentic standards from Sigma–Aldrich. For quantification purposes, calibration curves were calculated when standards were available; otherwise semi-quantitative analyses were carried out assuming a response factor equal to one.

2.4. Chromatographic conditions for the analysis of glycosides

An Agilent gas chromatograph, model 6890 N, coupled to a mass selective detector, model 5973 *inert*, was used under the same conditions as indicated above. The column used was a SPB-1 capillary column (50 m × 0.25 mm i.d.; 0.25 μm film thickness). Oven temperature programme was: 125 °C (1 min) – 3 °C/min – 220 °C (1 min) – 1 °C/min – 270 °C (2 min).

The tentative identification of the glycosides was based on comparison of their mass spectra with those from the literature (Aldave, 1999; Sefton, Francis, & Williams, 1994; Voirin, Baumes, & Bayonove, 1990; Voirin, Sapis, & Bayonove, 1992; Williams, Strauss, Wilson, & Massy-Westropp, 1983) and from the Wiley G 1035 A spectral library. Semi-quantitative analysis of positively identified compounds was performed by the internal standard method assuming a response factor equal to one.

2.5. Analysis of volatile compounds from the alcoholic fermentation

The major volatile compounds in wines were analysed by direct injection on a HP-5890 GC with a FID detector, using a CP-Wax-57 capillary column (50 m × 0.25 mm i.d.; 0.25 μm film thickness). Oven temperature programme was: 40 °C (5 min) – 4 °C/min – 120 °C. Carrier gas was He (0.7 ml/min). Injector temperature: 250 °C. Detector temperature: 280 °C.

Minor volatiles compounds from alcoholic fermentation were extracted by continuous liquid–liquid extraction with *n*-pentane–dichloromethane (60:40), concentrated on a Vigreux column and then analysed by GC–FID, using the same conditions as for the analysis of the free and released volatile compounds.

For quantification purposes calibration curves were calculated using authentic standards from Sigma–Aldrich.

2.6. Sensory descriptive analysis

Wines were evaluated in duplicate by a panel consisting of nine assessors, staff members of the Department of Food Science and Technology, of ages between 25 and 40 and experienced in wine testing. The assessment took place in a standard sensory analysis chamber (ISO 8589–1998) equipped with separate booths.

The wines were presented (one after another), in coded standard wine-testing glasses ISO standard 3591 (1997) and covered with a watch-glass to minimize volatile components released, at 10 °C. Three wines were evaluated in each session and mineral water was provided for rinsing.

Five training sessions were held before applying the rating test. Standards were used to assist in the attributes definition (Noble et al., 1984). Seven aroma terms (fresh, citrus, green apple, floral, fruity, tropical fruit and muscat), and eight gustative attributes (fruity, green apple, tropical fruit, acidity, body, and aftertaste intensity and quality) were selected to describe Muscat wines.

The formal evaluation consisted of two sessions that were held on different days. The panellists used a 10 cm unstructured scale from 0 to 10 to rate the intensity of each attribute previously selected. The left-hand end of the scale was "attribute not perceptible" and the right-hand end of the scale was "attribute strongly perceptible".

2.7. Statistical analysis

The Student–Newman–Keuls test was applied to discriminate among the means of chemical data. The mean ratings and Fischer's least significant differences (LSD) for each sensory descriptor were calculated by analysis of variance (ANOVA). Statistical processing was carried out by using the SPSS 11.0 for Windows statistical package.

3. Results and discussion

3.1. Free aroma compounds of skins, must and wines

Free volatile compounds were analysed in musts and wines of Muscat "a petit grains" with and without skin contact, and in the skins from the must pressing control. Mean values of concentrations and standard deviations are shown in Table 1.

The must contained a significant quantity of C_6 alcohols, mainly (*E*)-2-hexen-1-ol and 1-hexanol. Monoterpenes (linalool which was the most abundant), were found in lower concentrations than in other Muscat varieties, such as "Muscat of Alexandria" which is rich in linalool and geraniol (Bayonove, 1993).

Among poly-oxygenated terpenes, the 2,6-dimethyl-3,7-octadiene-2,6-diol is predominant in musts and wines. Although, the sensory relevance of these compounds is small, they can be transformed into monoterpenol odorants by hydrolysis at acid pH during wine-making or storage (Strauss, Wilson, & Gooley, 1986).

All analysed compounds were found in important quantities in the skins. That was especially relevant for 1-hexanol, nerol, geraniol and benzene derivatives, which indicates that skin maceration is a good technique for increasing their concentrations in musts.

Total C_6 compounds in control wines were similar to that in the must, but 1-hexanol was at greater concentration and (*E*)-2-hexen-1-ol and 2-hexenal were not detected in wines, probably due to transformations by yeast metabolism.

Table 1
Mean concentrations of free varietal compounds (μg/l) and relative standard deviations ($n = 2$)

Compounds	Skins[a]	Must	Control wine[b]	Skin-contact wine 15 h/18 °C	Skin-contact wine 23 h/18 °C
2-Hexenal	116 (6.7)	75.7 (5.4)	n.d.	n.d.	n.d.
1-Hexanol	486 (0.1)	165 (11.6)	442^{c} (10.0)	548^{b} (7.2)	660^{a} (9.0)
(*E*)-3-Hexen-1-ol	11.6 (15.0)	n.d.	51.9^{a} (22.8)	38.6^{c} (5.3)	39.8^{b} (2.0)
(*Z*)-3-Hexen-1-ol	20.2 (5.7)	34.6 (9.1)	38.0^{b} (10.6)	36.1^{b} (0.9)	42.3^{a} (5.7)
(*E*)-2-Hexen-1-ol	461 (2.8)	206 (8.5)	n.d.	n.d.	n.d.
Total C_6 alcohols	1094	481	533	622	743
Linalool	350 (0.6)	91.8 (2.1)	126^{b} (1.5)	148^{b} (7.0)	172^{a} (3.2)
Hotrienol	Tr.	5.7 (6.2)	73.9^{ba} (9.4)	93.9^{b} (4.2)	192^{a} (1.4)
α-Terpineol	5.8 (0.9)	4.1 (9.7)	52.3^{a} (10.9)	39.2^{a} (9.5)	35.3^{a} (12.0)
Citronellol	11.6 (22.5)	2.1 (7.1)	82.2^{a} (1.7)	101^{a} (1.4)	87.0^{a} (16.8)
Nerol	65.9 (0.1)	17.7 (15.5)	Tr.	Tr.	Tr.
Geraniol	220 (0.7)	31.8 (16.5)	55.9^{a} (10.8)	61.6^{a} (3.1)	57.8^{a} (27.5)
Geranic acid	383 (5.5)	41.6 (8.4)	7.4^{b} (9.9)	78.2^{a} (15.6)	119^{a} (0.3)
Geraniol hydrate	160 (2.4)	15.6 (9.2)	n.d.	42.4^{a} (15.0)	71.2^{b} (10.5)
Total monoterpenes	1196	210	398	564	735
cis-Furan-linalool oxide	10.6 (7.6)	4.7 (3. 7)	Tr.	Tr.	Tr.
trans-Furan-linalool oxide	5.3 (15.6)	15.2 (15.2)	25.9^{a} (3.6)	31.4^{a} (21.6)	31.3^{a} (1.3)
cis-Pyran-linalool oxide	47.0 (1.9)	58.0 (5.2)	67.7^{c} (1.7)	105^{a} (2.1)	94.6^{b} (0.2)
trans-Pyran-linalool oxide	15.5 (1.8)	28.6 (4.7)	27.7^{a} (4.0)	30.7^{a} (0.7)	27.3^{a} (6.3)
2,6-Dimethyl-3,7-octadiene-2,6-diol	364 (1.3)	191 (5.9)	389^{a} (4.2)	397^{a} (10.3)	490^{a} (1.3)
2,6-Dimethyl-1,7-octadiene-3,6-diol	41.0 (1.1)	18.8 (10.7)	32.7^{a} (2.2)	34.7^{a} (2.3)	39.9^{a}(15.6)
3,7-Dimethyl-1,7-octanediol	n.d.	7.3 (3.7)	16.6^{a} (11.5)	82.0^{b} (9.2)	135^{b} (5.3)
Total polyoxygenated terpenes	484	324	560	681	818
Benzaldehyde	4.5 (11.7)	6.9 (3.7)	4.2^{a} (2.7)	6.6^{a} (3.6)	8.1^{a} (1.2)
Benzyl alcohol	28.9 (33.7)	46.2 (5.6)	266^{b} (1.6)	327^{b} (0.1)	455^{a} (6.7)
2-Phenylethanol	86.6 (1.2)	26.6 (10.9)	$22{,}886^{a}$ (0.9)	$15{,}767^{b}$ (7.2)	$13{,}004^{c}$ (4.2)
4-Vinylguaiacol	15.3 (4.6)	n.d.	283^{a} (6.9)	269^{b} (0.46)	236^{b} (23.9)
Total benzenic compounds	135	79.7	23,439	16,369	13,703

n.d.: not detected.
Tr.: traces.

[a] μg/l released in 1 l of aqueous solution from the skins corresponding to 1 l of must.

[b] In each case, according to the result of the Student–Newman–Keuls test, values that do not share a common superscript are significantly different ($p < 0.05$).

Control wines contained higher quantities of some mono and poly-oxygenated terpenes, than the corresponding must. This increase could be due to a release from their bound forms during the fermentation process by a residual activity of grape or yeast glycosidases (Delcroix, Günata, Sapis, Salmon, & Bayonove, 1994; Delfini et al., 2001).

On the other hand, citronellol could be formed by the metabolism of yeasts from nerol and geraniol (Dugelay, Günata, Sapis, Baumes, & Bayonove, 1992). Acid-catalysed cyclisation of nerol, geraniol and linalool can yield α-terpineol, and acid-catalysed elimination of water from 2,6-dimethyl-3,7-octadiene-2,6-diol can yield hotrienol.

The skin contact increased the concentrations of sensorially valuable compounds in wines, e.g., 1-hexanol, linalool, and hotrienol and benzyl alcohol, and these differences were statistically significant, especially in wines macerated for 23 h. Similar findings have been reported by other authors in different grape varieties (Baumes et al., 1989; Cabaroglu & Canbas, 2002; Cabaroglu et al., 1997; Cabaroglu, Selli, Canbas, Lepoutre, & Günata, 2003; Jurado et al., 2001).

2-Phenylethanol and vinylguaiacol are formed by yeast during the alcoholic fermentation, so we expect high quantities of these compounds in wines. The decrease of these compounds in wines elaborated with skin contact could be related to yeast metabolism.

3.2. Volatile compounds from the alcoholic fermentation

Although, grape volatile compounds have a greater sensorial impact, volatile compounds formed during alcoholic fermentation from yeast metabolism can also influence wine characteristics, increasing their fruitiness (Etievant, 1991; Ferreira, Fernandez, Peña, Escudero, & Cacho, 1985) or producing undesirable aromas (ethyl acetate, isoamyl alcohols). Consequently, all wine-making technique innovations should take them into consideration.

Table 2 shows the concentrations of fermentation compounds in the wines. Maceration with skins introduces several changes in the initial composition of the must, that probably affect the fermentation compounds.

The greater quantity of methanol in the skin-macerated musts and wines is logical, since it is derived from the demethylation of the pectins present in skins. The fermentation alcohols show a significantly lower concentration in wines elaborated in contact with skins. This could be due to the greater nitrogen content in these samples, that causes a repression of the Ehrlich reaction forming these compounds (Rapp & Versini, 1995).

Table 2 shows a lower concentration of some acetates, including isoamyl acetate, and medium chain fatty acid esters, and an increase in the corresponding fatty acids. This has also been observed by other authors in

Table 2
Mean concentrations (mg/l) and relative standard deviations ($n = 2$) of volatile compounds formed during alcoholic fermentation

Compounds	Control wine[A]	Skin-contact wine 15 h/18 °C	Skin-contact wine 23 h/18 °C
Acetaldehyde	46.5^{a} (4.43)	44.5^{a} (8.58)	20.6^{b} (9.43)
Methanol	10.5^{a} (16.60)	21.5^{b} (4.32)	27.4^{c} (0.24)
1-Propanol	13.5^{a} (12.72)	24.0^{b} (4.14)	24.2^{b} (0.23)
Isobutanol	31.4^{a} (6.81)	28.9^{b} (4.90)	22.2^{c} (2.27)
3-Methyl-1-butanol	222.4^{a} (1.18)	167.2^{b} (2.04)	111^{c} (0.14)
2-Methyl-1-butanol	64.9^{a} (3.44)	42.5^{b} (4.29)	25.3^{c} (1.29)
3-(Methylthio)-1-propanol.	1.93^{a} (10.22)	0.90^{b} (3.48)	0.40^{c} (9.90)
Ethyl acetate	30.2^{a} (0.78)	24.9^{b} (9.43)	44.9^{c} (2.45)
Isoamyl acetate	3.21^{a} (6.85)	1.75^{b} (0.19)	1.22^{c} (3.51)
Hexyl acetate	0.02^{a} (13.52)	0.01^{b} (9.95)	0.02^{b} (9.04)
Phenylethyl acetate	0.01^{a} (8.64)	0.01^{a} (17.33)	0.02^{b} (23.66)
Ethyl lactate	0.94^{a} (9.84)	0.62^{b} (2.14)	0.65^{b} (5.07)
Ethyl hexanoate	0.35^{a} (2.35)	0.45^{b} (4.62)	0.68^{c} (4.42)
Ethyl octanoate	0.44^{a} (4.92)	0.30^{b} (3.13)	0.83^{c} (1.22)
Ethyl decanoate + isovaleric acid	2.42^{a} (8.12)	2.20^{a} (22.59)	0.81^{b} (14.45)
Diethyl succinate	0.05^{a} (15.44)	0.06^{a} (5.35)	0.07^{a} (5.97)
Ethyl 4-hydroxybutyrate	8.42^{a} (9.12)	7.65^{a} (24.35)	4.20^{a} (16.18)
Isobutyric acid	1.87^{a} (16.18)	1.54^{a,b} (5.05)	1.05^{b} (5.95)
Butyric acid	0.62^{a} (4.88)	0.78^{a} (17.82)	0.93^{b} (1.52)
Hexanoic acid	1.78^{a} (1.87)	1.96^{a} (5.16)	3.20^{b} (9.10)
Octanoic acid	2.79^{a} (6.74)	3.04^{a} (6.08)	4.84^{b} (11.13)
Decanoic acid	0.65^{a} (6.27)	0.69^{a} (0.62)	1.28^{b} (8.04)
γ-Butyrolactone	1.94^{a} (4.35)	1.99^{a} (0.53)	2.28^{a} (13.54)

[A] In each case, according to the result of the Student–Newman–Keuls test, values that do not share a common superscript are significantly different ($p < 0.05$).

wines made after maceration with skins (Cabaroglu et al., 1997; Falqué & Fernández, 1996). These changes could be relatively insignificant in the overall aroma of wines from aromatic varieties such as "Muscat", but they must be considered in wines made from more neutral varieties, where there are fewer varietal compounds.

3.3. Bound aroma compounds enzymatically released from must and wines

Table 3 shows the concentrations of the compounds enzymatically released from the glycosylated fraction in optimal enzyme activity conditions in must and wines. The enzymatic preparation used (AR2000) contains the enzyme activities required to hydrolyse grape glycosides, and it has been tested by other authors (Aldave, 1999; Baek & Cadwallader, 1999).

In the must, concentrations of bound compounds are particularly important in the case of monoterpenes, such as nerol and geraniol, furanyl oxides, and benzene derivatives (benzyl alcohol and 2-phenylethanol), that in many cases exceeded the quantities found in the free fraction (Table 1). The same has been observed by other authors in must and wines elaborated with different Muscat varieties (Aldave, 1999; Baek & Cadwallader, 1999; Mateo & Jiménez, 2000).

Table 3
Mean concentrations (μg/l) and relative standard deviations ($n = 2$) of bound volatile compounds released by enzymatic hydrolysis

Compounds	Must	Control wine[A]	Skin-contact wine 15 h/18 °C	Skin-contact wine 23 h/18 °C
1-Hexanol	25.0 (9.7)	6.6^{b} (3.7)	20.8^{a} (4.8)	21.2^{a} (4.1)
(*Z*)-3-Hexen-1-ol	8.2 (3.0)	n.d.	n.d.	9.5 (7.1)
(*E*)-2-Hexen-1-ol	11.4 (7.0)	3.7^{b} (3.9)	3.4^{a} (1.8)	8.6^{a} (2.7)
Total C_6 alcohols	44.6	10.3	24.2	39.3
Linalool	21.4 (5.5)	15.4^{b} (7.6)	54.7^{a} (6.2)	20.3^{b} (1.3)
Hotrienol	10.7 (15.4)	2.1^{a} (4.3)	1.6^{b} (0.6)	1.0^{c} (2.3)
α-Terpineol	11.7 (25.4)	23.7^{b} (8.7)	65.6^{a} (6.3)	51.4^{a} (12.4)
Citronellol	4.8 (20.1)	7.3^{c} (1.8)	13.2^{a} (2.1)	10.5^{b} (6.0)
Nerol	83.5 (1.8)	115^{b} (2.7)	208^{a} (2.7)	210^{a} (1.2)
Geraniol	38.7 (1.7)	52.3^{b} (1.1)	110^{a} (8.4)	28.6^{c} (14.4)
Geranic acid	73.6 (6.2)	87.8^{b} (0.5)	192^{a} (10.6)	168^{a} (10.4)
Geraniol hydrate + (*Z*)-8-hydroxylinalool	68.9 (2.6)	30.2^{c} (1.3)	44.3^{b} (3.4)	50.9^{a} (1.4)
Total monoterpenes	313	333	690	540
cis-Furan-linalool oxide	17.7 (3.2)	19.9^{c} (1.8)	65.5^{a} (3.8)	55.0^{b} (0.2)
trans-Furan-linalool oxide	23.1 (0.9)	37.5^{c} (1.0)	114^{a} (8.3)	93.8^{b} (3.3)
cis-Pyran-linalool oxide	35.5 (5.1)	37.2^{b} (4.9)	98.8^{a} (9.6)	88.5^{a} (3.5)
trans-Pyran-linalool oxide	8.5 (4.8)	7.7^{c} (0.9)	28.3^{a} (1.5)	23.1^{b} (3.0)
2,6-Dimethyl-3,7-octadiene-2,6-diol	26.7 (1.6)	2.9^{c} (2.6)	9.2^{a} (5.4)	6.9^{b} (4.8)
2,6-Dimethyl 1,7-octadiene-3,6-diol	7.5 (3.3)	12.8^{a} (7.3)	8.5^{b} (9.1)	n.d.
3,7-Dimethyl-1,7-octanediol	19.3 (2.4)	13.3^{b} (2.9)	37.6^{a} (7.4)	33.1^{a} (0.5)
(*E*)-8-Hydroxylinalool	61.2 (4.0)	25.9^{b} (0.1)	51.3^{a} (2.1)	45.3^{a} (7.2)
Total polyoxygenated terpenes	200	157	413	346
Benzaldehyde	4.7 (7.5)	2.1^{b} (6.9)	3.0^{a} (4.7)	3.2^{a} (2.1)
Benzyl alcohol	244 (5.2)	13.8^{c} (6.9)	38.8^{b} (1.1)	152^{a} (4.2)
2-Phenylethanol	132 (1.1)	170^{a} (11.9)	11.6^{b} (10.7)	22.1^{b} (0.6)
4-Vinylguaiacol	6.3 (9.1)	0.6^{a} (2.1)	2.8^{b} (1.1)	1.6^{c} (9.7)
Total benzenic compounds	387	187	56.2	184
β-Damascenone	1.2 (10.0)	1.1^{b} (1.8)	2.4^{a} (8.7)	2.1^{a} (2.8)
3-Hydroxy-β-damascone	41.6 (2.8)	33.5^{b} (14.7)	73.5^{a} (14.1)	$54.0^{a,b}$ (2.8)
3-Hydroxy-7,8-dihydroβ-ionol	10.2 (14.8)	n.d.	n.d.	8.0 (10.3)
Total norisoprenoids	53.0	34.6	75.9	64.1
3-Methyl-3-buten-1-ol	9.5 (1.6)	n.d.	n.d.	n.d.
3-Methyl-2-buten-1-ol	8.3 (4.3)	n.d.	n.d.	n.d.
Hexanoic acid	4.4 (0.8)	6.0^{c} (2.7)	13.7^{a} (6.9)	10.0^{b} (1.4)
Octanoic acid	10.8 (16.7)	11.8^{a} (11.4)	10.9^{a} (7.7)	8.9^{a} (2.8)
Total aliphatic compounds	33.0	17.8	24.6	18.9

n.d.: not detected.
Tr.: traces.
[A] In each case, according to the result of the Student–Newman–Keuls test, values that do not share a common superscript are significantly different ($p < 0.05$).

The small quantities of enzymatically-released C_6 alcohols and of 4-vinylguaiacol show the limited significance of the bound fraction of these compounds, as occurs in other grape varieties (Cabaroglu et al., 2003).

Some norisoprenoids such as 3-hydroxy-damascone, 3-hydroxy-7,8-dihydro-β-ionol and β-damascenone were identified in the bound fraction, but they were not detected in the free fraction.

β-Damascenone is a potent odorant that has been found in musts and wines from many grape varieties and it is generated by acid hydrolysis from multiple precursors, including 3-hydroxy-damascone and 3-hydroxy-7,8-dihydro-β-ionol (Winterhalter, Baderschneider, & Bonnländer, 1998).

E and *Z*-8-hydroxylinalool, geranic acid, geraniol hydrate and some aliphatic alcohols have been found in the bound fraction from Muscat and Melon B. musts and wines (Bureau et al., 2000; Schneider, Razungles, Augier, & Baumes, 2001).

Skin contact treatment increased the total concentration of bound compounds in wines, due to the presence of glycosylated compounds in the berry skin (Bayonove, 1993). The same effect has been observed by Cabaroglu et al. (1997) and Cabaroglu and Canbas (2002), in Emir and Muscat wines macerated for 7 h at 15 °C. In our case, maceration for 23 h did not significantly increase the levels of some bound compounds in wines.

The bound aroma fraction knowledge of a grape variety can be useful for proposing a wine-making technique that uses both skin contact and glycosidases in order to increase the potential aroma of wines (Cabaroglu et al., 2003).

3.4. Total glycosides of musts and wines

Fig. 1 shows the concentrations of the glycosylated compounds identified in musts and wines. In most Muscat varieties, the glycosylated fraction is largely formed by diglycosides and, to a lesser extent, by β-D-glucosides (Bayonove, 1993). However, in our samples glucosides and apiosylglucosides were the most abundant in the must (37% and 35%, respectively).

A more extensive analysis shows that the glycoside percentage and linked aglycones are different, depending on the grape variety (Aldave, 1999; Bayonove, 1993) and probably on the climatological conditions and culture practices.

The knowledge of glycosides distribution can be of interest when exogenous glycosidic enzymes are used. Arabinosylglucosides were more hydrolysed using Novoferm 12G and AR2000, but the last one was 30% more effective for hydrolysing rutinosides. Both enzymes showed a specific selection of aglycone. In the case of AR2000 enzyme treatment, neryl and geranyl glycosides were hydrolysed to a greater extent (Aldave, 1999).

Glycosidically-linked C_6 alcohols were found in small quantities, and they were found as β-D-glucosides, excepting the 1-hexanol that was found as arabinosyl glucoside.

Glucosides of linalyl oxides were the most abundant, as occurs in other Muscat varieties (Bayonove, 1993), but also important were the apiosylglucosides. Linalyl, geranyl, neryl, benzyl and 2-phenylethyl aglycones were found mainly as rutinosides and, to a lesser extent, as arabinosylglucosides.

The only norisoprenoid glycoside identified was 3-OH-β-damascone glucoside. This glycoside and the benzyl glycoside are the most abundant in some non-Muscat varieties, such as Verdejo and Albarillo (Aldave, 1999).

Total glycoside concentrations in control wines were similar to or lower than those in must, depending on the releasing of aglycones during alcoholic fermentation. Must skin contact caused a significant increase of diglycosides in wines, justifying their higher concentration in skins, but not in the case of glucosides.

3.5. Sensory analysis of wines

All wines were assessed by skilled tasters, using attributes previously agreed upon as the best for describing sensorial characteristics of wines. Fig. 2 shows "spider webs", diagrams for the average scores of the intensities of aroma and taste attributes of wines.

The control wine had a moderately intense floral aroma, with some fruity and fresh notes. The more intense attribute detected by tasters was described as "Muscat aroma", that is a complex aroma generated by a synergic effect of various monoterpenes present in Muscat varieties. The most odoriferous monoterpenes are linalool and geraniol with odour perception thresholds of 15 and 30 μg/l, respectively (Guth, 1997), which were found at concentrations above their odour perception threshold in the control wine.

Skin contact slightly increased some aroma attributes, such as floral and Muscat aroma, correlated with the increase of monoterpenes in skin contact wines, such as linalool, hotrienol and citronellol, that reached their odour perception threshold (100 μg/l). Other authors also found more intense aroma and terpene-like character in Muscat wines elaborated with skin contact (Cabaroglu & Canbas, 2002).

Attributes such as fresh, citrus and fruity had a significant increase in wines elaborated with skin contact, especially after 23 h. Skin contact augments the quantities of many compounds that could exceed their odour perception threshold in these samples. For instance, 1-hexanol and *cis*-3-hexen-1-ol have been correlated with the fresh aroma, citronellol with citrus aroma, and esters (ethyl hexanoate, ethyl octanoate) with fruity

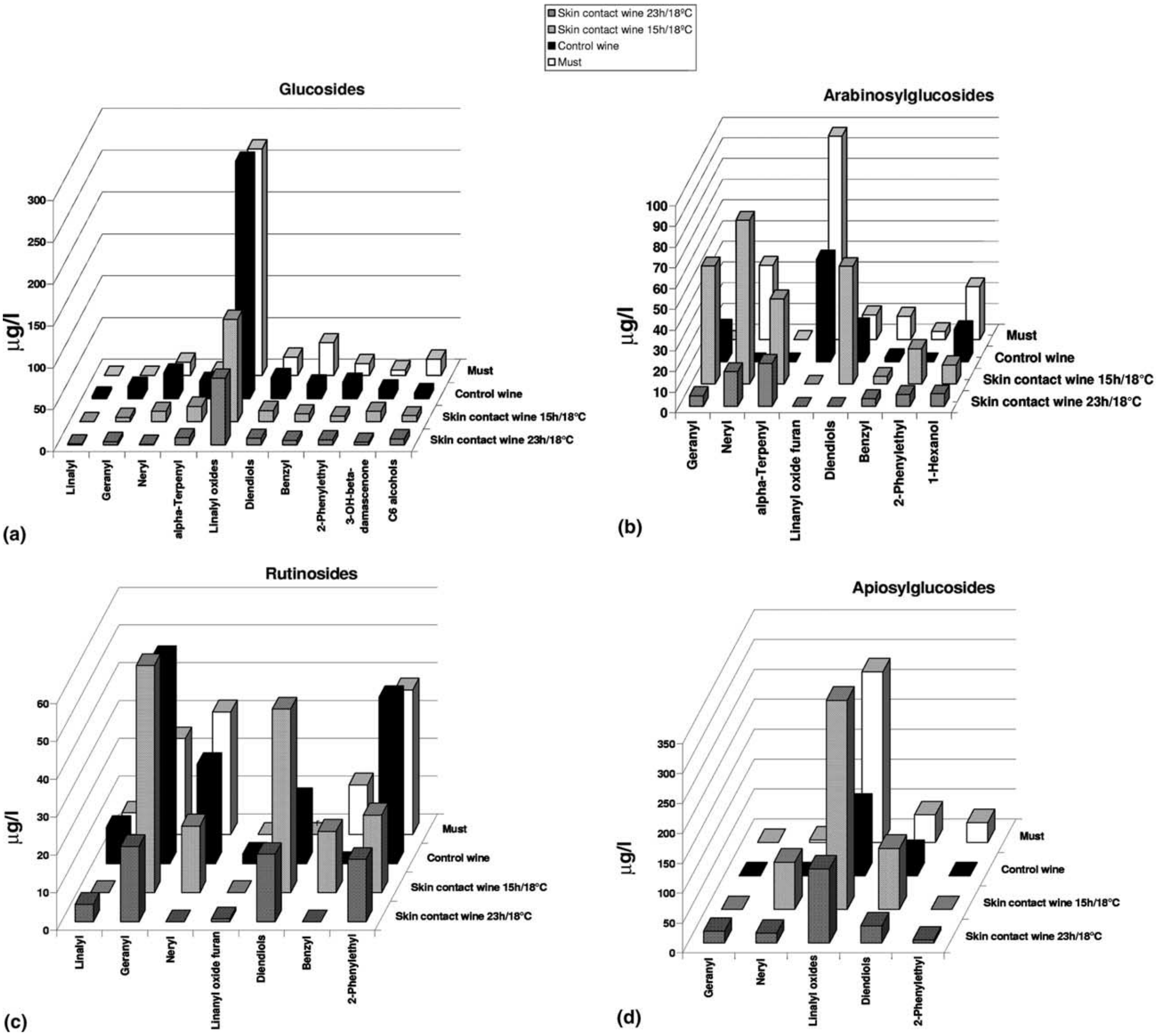

Fig. 1. Mean concentrations (μg/l) of glucosides, arabinosylglucosides, rutinosides and apiosylglucosides in must and wines: (a) glucosides; (b) arabinosylglucosides; (c) rutinosides; (d) apiosylglucosides.

attributes (green apple, apricot and pineapple) (Cabaroglu, Canbas, Lepoutre, & Günata, 2002).

Taste evaluation detected fruity flavours such as green apple or apricot that were more intense in wines elaborated with skin contact.

Furthermore, skin contact wines were less acid and had more body and aftertaste intensity than control wine, probably because of the neutralization of the must acids during the maceration process (García Romero et al., 1999).

With regard to the overall impression and quality, the skin contact wines gave better punctuation than the control wine.

In conclusion, wines made with Muscat "a petit grains" had sufficient quantities of sensorially valuable terpenes to obtain the typical "Muscat character". The major monoterpene in the must was linalool, whereas the skins had an important quantity of geraniol and nerol.

Must skin contact, at a controlled temperature of 18 °C over 15 or 23 h, produced an important increase in volatile compounds that enhanced the floral character and introduced fruity and fresh notes to wines, involving other characteristics such as body and acidity appreciates in white wines.

On the other hand, the bound aroma fraction of musts and wines is important in this grape variety, and must skin contact also increased this aroma fraction in wines. That finding suggests the use of glycosidic enzymes, together with skin maceration, as an alternative for increasing the aromatic potential of wines.

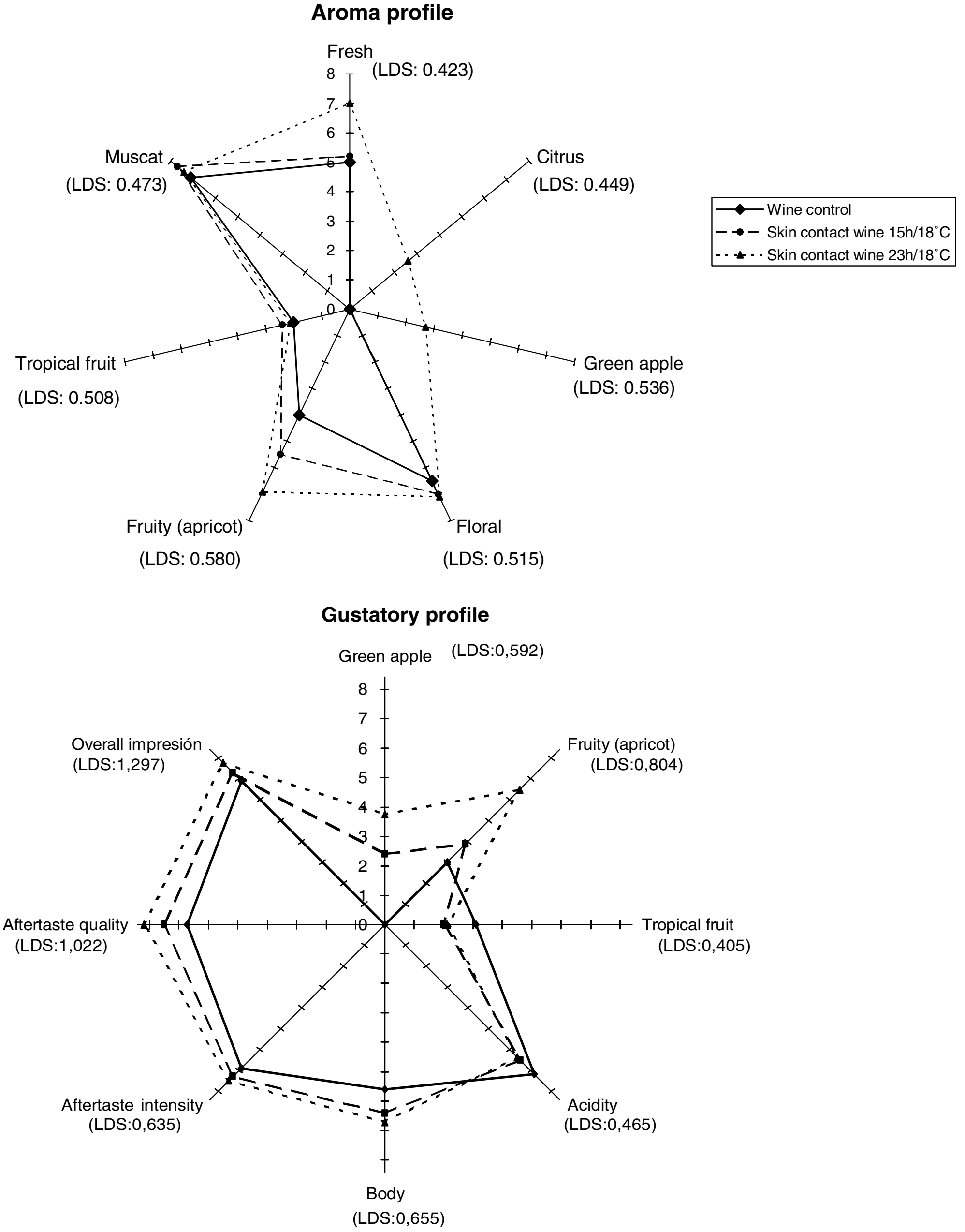

Fig. 2. Sensory descriptive analysis of wines. Mean scores of nine judges (two replicates) and least significant differences (LDS, $p < 0.05$) are shown.

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