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Aroma potential of Albillo wines and effect of skin-contact treatment

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Abstract

Free and glycosidically-bound aroma compounds were analysed in Albillo grape must, skin and wines, and the effects of different skin-contact treatments on aroma composition and wine sensory characteristics were evaluated. Musts and wines contained large amounts of C_6 and benzene compounds. Must skin contact, at 18 °C for 15 and 23 h, prompted a considerable increase in free and bound varietal compounds. Therefore, this technique, and the use of glycosidases are two methods recommended as a means for enhancing wine aroma. Skin-contact treatment intensified the fresh and fruity properties that were scored positively by wine tasters.

Keywords: Skin contact; Glycosides; Aroma compounds; Gas chromatography–mass spectrometry

1. Introduction

Wine aroma is formed by hundreds of volatile compounds. More than 800 aroma compounds have been reported in wines, including higher alcohols, aldehydes, ketones, esters, acids, monoterpenes and C_{13}-norisoprenoids. The typical flavour of wines is mainly due to volatile compounds coming from grapes. Grape aroma composition and its influence on wine aroma have been widely reviewed (Flanzy, 2000; Ribereau-Gayon, Glories, Maujean, & Dubourdieu, 2000).

The aromatic complexity of wines varies, depending on the grape variety used, the aroma produced during fermentation and those developed during the ageing process (Schneider, 1979; Rapp & Mandery, 1986).

Grape aroma compounds mainly appear either in their free form, directly contributing to wine aroma, or as non-volatile sugar-bound conjugates (monoglucosides or disaccharide glucosides), these being the predominant form in aromatic varieties (Günata, Bayonove, Baumes, & Cordonnier, 1985a). To release the aglycones that enrich wine aroma, bound forms must be subjected to acid or enzyme hydrolysis, normally using commercial preparations with β-glucosidase activity (Marais & Rapp, 1988; Carballeira, Cortés, Gil, & Fernández, 2001).

Free and bound aroma compounds are abundant in grape skins (Günata, Bayonove, Baumes, & Cordonnier, 1985b; Voirin, Sapis, & Bayonove, 1992; Williams, Sefton, & Wilson, 1989; Winterhalter & Skouroumounis, 1997). Consequently, contact between juice and skin, prior to fermentation, generally results in higher concentrations of potential aroma compounds, both in juice and in wines, by ceding free aroma compounds or releasing them from their non-volatile glycosidically-bound form (Di Stefano, 1991; Dugelay, Günata, Sapis, Baumes, & Bayonnove, 1993).

Skin maceration generally prompts increased concentrations of most aroma components in the final wine, though the end-result is influenced by maceration conditions (time and temperature) and by the grape variety used (Cabaroglu et al., 1997; Falqué & Fernández, 1996; García Romero, Pérez Coello, Cabezudo, Sánchez-Muñoz, & Martín-Alvarez, 1999; Marais & Rapp, 1988; Ramey, Bertrand, Ough, Singleton, & Sanders, 1986; Sánchez-Palomo, Pérez-Coello,

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Díaz-Maroto, González Viñas, & Cabezudo, 2006; Selli, Canbas, Cabaroglu, Erten, & Günata, 2006; Selli et al., 2006). Maceration conditions must therefore be selected with care, since 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). On the other hand, undesirable sensory changes can be produced (Dubourdieu, Olivier, & Boidron, 1986; Test, Noble, & Schmidt, 1986).

Surprisingly, even though 90% of world wine production is from non-aromatic grape varieties (Jurado, Pinilla, Ballesteros, Pérez-Coello, & Cabezudo, 2002), research has tended to concentrate primarily on wine produced from aromatic grape varieties.

The Albillo grape is grown in a small area of the La Mancha region (Spain). Although it is traditionally used for eating, the few published studies on must and wine volatile composition suggest that wines made from this neutral variety contain interesting flavour notes (Jurado et al., 2002; Muñoz-Organero & Ortiz, 1997; Sánchez-Palomo et al., 2006).

The aims of this study were to measure free and glycosidically-bound volatile compounds in must, skin and wines made from Albillo grapes grown in the La Mancha region (Spain), and to investigate the effects of different skin-contact times on wine aroma composition and sensory properties.

2. Materials and methods

2.1. Materials

Grapes from *Vitis vinifera* var. Albillo, cultivated in the 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 with minimal skin contact, and the other two batches were used for the skin-contact experiments. The grapes were destemmed and crushed. The pomace was mixed with 100 mg/kg of sulphur dioxide, kept at 18 °C for 15 or 23 h and then pressed.

For making wines, laboratory fermentations were performed in 10 l vessels. All the samples were inoculated with *Saccharomyces cerevisiae* (CECT no. 10835) and fermentations were conducted at 18 °C. Each fermentation was carried out in duplicate.

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

2.2. Standard chemical analyses of wines

Total acidity, ethanol, pH, volatile acidity, total and free SO_2 were analysed (O.I.V, 1990). 3-Flavanols were measured using the method described by Amerine and Ough (1980). The phenolic compounds were analysed using the method proposed by Mazza, Fukumoto, Delaquis, Girard, and Ewert (1999).

2.3. Extraction and gas chromatography analysis of free and bound compounds

2.3.1. Preparation of samples

Prior to analysis, the macerated skin solution and the must control were centrifuged at 2 °C (15.000 rpm, 30 min).

2.3.2. Fractionation of free and bound fractions of aroma

The free and bound fractions were separated by adsorption/desorption on preconditioned styrene–divinylbenzene cartridges (Bond Elut, Varian, 1 g of phase) according to the method proposed by Günata et al. (1985a).

The samples, must, wine and macerated skin solutions were passed through the Bond Elut column at a flow rate of 1 ml/min. The column was rinsed with 100 ml of pure water to eliminate sugars and other low-molecular-weight polar compounds.

The free fraction was eluted with 50 ml of pentane–dichloromethane (2:1 v/v). The extract was concentrated to a final volume of 500 μl, using a Vigreux column at 37 °C.

The bound fraction was eluted with 50 ml of ethyl acetate. The ethyl acetate extract was evaporated to dryness under vacuum, and then re-dissolved with 1 ml of methanol.

2.3.3. Enzymatic hydrolysis of bound fraction

An aliquot of 500 μl of methanol extract was evaporated to dryness under a nitrogen stream. The dried glycosidic extract was dissolved in 100 μl of citrate–phosphate buffer (0.2 M, pH 5). Enzymatic treatment with AR2000 (Gist Brocades) was conducted at 40 °C for 18 h, according to optimum conditions described previously (Sánchez-Palomo et al., 2006). The mixture was then extracted five times with 2 ml of pentane–dichloromethane (2:1 v/v). After addition of 10 μl of 4-nonanol (1 g/l) as internal standard, the extract was concentrated to a final volume of 500 μl, using a Vigreux column at 37 °C.

2.3.4. Gas chromatography–mass spectrometry (GC–MS) analysis

An Agilent gas chromatograph, model 6890 N, coupled to a mass selective detector, model 5973 *inert,* equipped with a BP-21 capillary column (60 m × 0.32 mm i.d.; 0.25 μm film thickness) was used. Operating conditions were as follows: oven temperature programme was, 70 °C (5 min) raised at 1 °C/min – 95 °C (10 min) then 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. One microlitre (1 μl) was injected in splitless mode. Carrier gas was helium (1 ml/min).

The identification was based on comparison of the GC retention times and mass spectra with authentic standards from Sigma–Aldrich when standards were available; for these compounds, calibration curves were calculated with the purpose of quantification. When the authentic standards were not available, the identification was based on comparison with the spectral data of the Wiley A library and with the chromatographic dated from the literature; semi-quantitative analyses of these compounds were done, assuming response factors equal to one.

2.4. Analysis of alcoholic fermentation volatiles

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) raised at 4 °C/min to 120 °C. Injector and detector temperature were 250 and 280 °C, respectively. Carrier gas was He (0.7 ml/min).

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

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 using standard compounds.

2.5. Sensory analysis

Wines were evaluated, in duplicate, by a panel of eight experienced wine-testers. Assessment took place in a standard sensory-analysis chamber (ISO 8589, 1998) equipped with separate booths. Wines were sniffed and tasted.

Three wines were presented in each session, in coded standard wine-testing glasses, according to standard 3591 (ISO 3591, 1997) and covered with a watch-glass to minimize the escape of volatile components. Testing temperature was 10 °C.

Physical–chemical standards were used to help define attributes (Noble et al., 1984). The panellists used a 10 cm unstructured scale to rate the intensity of each attribute. The left-hand end of the scale was "attribute not perceptible" and the right-hand end was "attribute strongly perceptible".

2.6. 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 14.0 for Windows statistical package.

3. Results and discussion

3.1. Influence of skin-contact time on general wine composition

The general composition of Albillo control wines and wines obtained using different skin-contact treatments is shown in Table 1. Wine composition was clearly affected by skin-contact treatment; wines made from skin-contact musts generally displayed higher levels of total nitrogen and phenol compounds (total phenols, catechins, tartaric esters and flavonols) than did controls, while values for pH, volatile acidity and total acidity were lower. Similar findings have been reported for a number of grape varieties (Cabaroglu & Canbas, 2002; Cabaroglu et al., 1997; Darias-Martín, Díaz-González, & Díaz-Romero, 2004; Selli, Canbas, Cabaroglu, Erten, & Günata, 2006; Selli et al., 2006).

3.2. Influence of skin-contact time on volatile compounds formed during alcoholic fermentation

Although compounds from the grapes themselves are responsible for the varietal character of wines, the compounds formed during alcohol fermentation via yeast metabolism may have a positive or negative influence on wine sensory properties (Ferreira, Fernandez, Peña, Escudero, & Cacho, 1995). It is therefore of interest to determine possible changes in the concentrations of these compounds due to modifications in processing techniques.

Table 1
General composition of Albillo wines

	Control wine	Skin-contact wine 15 h/18 °C	Skin-contact wine 23 h/18 °C
Fructose (g/l)	0.41[a]	0.34[a,b]	0.28[b]
Glucose (g/l)	0.07[a]	0.09[b]	0.09[b]
Total nitrogen (mg/l)	115[a]	162[b]	182[c]
Total phenols (mg/l eq. gallic acid)	111[a]	194[b]	200[b]
Tartaric esters (mg/l eq. caffeic acid)	54.8[a]	99.6[b]	101[c]
Total flavonols (mg/l eq. quercetin)	26.8[a]	46.9[b]	53.0[c]
Citric acid (g/l)	0.20[a]	0.20[a]	0.18[a]
Succinic acid (g/l)	0.50[a]	1.21[b]	0.90[c]
Lactic acid (g/l)	0.09[a]	0.15[a]	0.14[a]
Catechins (mg/l eq. (+)-catechin)	27.5[a]	57.3[b]	56.0[b]
Volatile acidity[A] (g/l)	0.18[a]	0.14[b]	0.15[b]
Free SO_2 (mg/l)	22.0[a]	41.5[b]	26.5[a]
Total SO_2 (mg/l)	90.5[a]	80.0[a]	85.5[a]
Titrable acidity[B] (g/l)	8.01[a]	6.60[b]	6.60[b]
Ethanol (% v/v)	12.0[a]	11.8[a]	11.8[a]

[a,b,c] Different superscripts in the same row indicate statistical differences at the 0.05 level according to the Student–Newman–Keuls test.
[A] As acetic acid.
[B] As tartaric acid.

Table 2 shows concentrations of volatile compounds formed during the alcoholic fermentation of Albillo wines expressed as mg/l of two replicates.

Wines made from skin macerated musts had higher concentrations of methanol than had control wines, since methanol is derived from the demethylation of skin pectins.

The concentration of higher alcohols generally declined with skin-contact time, probably as a result of blockage of the Ehrlich mechanism – the main pathway for the formation of these compounds – due to increased levels of nitrogenous substances in musts (Rapp & Versini, 1995).

Skin-contact treatment resulted in significant increases in the concentrations of esters, including isoamyl acetate, hexyl acetate, ethyl hexanoate, ethyl octanoate; similar results have previously been reported (Baumes, Bayonove, Barrillere, Samson, & Cordonnier, 1989; Cabaroglu & Canbas, 2002; Falqué & Fernández, 1996; Sánchez-Palomo et al., 2006; Selli, Canbas, Cabaroglu, Erten, & Günata, 2006; Selli et al., 2006). Esters are one of the major components of wine aroma, providing delicate odours (Etièvant, 1991). By contrast, ethyl acetate and ethyl lactate levels declined significantly with skin-contact treatment.

Butyric acid, hexanoic acid, octanoic acid and decanoic acid were the most abundant acids in Albillo wines. Fatty acid production is governed by the initial composition of the must and by fermentation conditions (Schneider, 1979). In general, fatty acid concentrations increased slightly in skin-contact wines (Baumes et al., 1989; Falqué

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
Aldehydes			
Acetaldehyde	23.4^{a} (0.2)	19.3^{a} (0.2)	13.9^{b} (2.4)
Alcohols			
Methanol	9.25^{a} (0.2)	25.9^{b} (1.2)	28.5^{c} (0.5)
1-Propanol	16.9^{a} (2.0)	16.5^{a} (0.5)	18.1^{a} (0.4)
Isobutanol	23.2^{a} (0.1)	20.1^{b} (0.1)	12.8^{c} (0.3)
3-Methyl-1-butanol	143^{a} (2.1)	105^{b} (4.1)	102^{b} (1.7)
2-Methyl-1-butanol	54.6^{a} (0.1)	45.4^{b} (2.7)	33.7^{c} (0.6)
3-(methylthio)-1-propanol	1.44^{a} (2.6)	1.39^{b} (3.1)	1.38^{b} (9.0)
4-vinylguaiacol	0.29^{a} (7.8)	0.29^{a} (10.6)	0.30^{a} (2.7)
2-Phenylethanol	4.67^{a} (7.0)	5.41^{b} (6.3)	4.76^{a} (4.2)
Esters			
Ethyl acetate	27.7^{a} (0.7)	21.0^{b} (0.4)	14.4^{c} (0.1)
Isoamyl acetate	2.63^{a} (6.2)	3.66^{b} (0.3)	3.49^{b} (7.5)
Hexyl acetate	0.02^{a} (0.5)	0.03^{b} (7.1)	0.04^{c} (2.4)
Phenylethyl acetate	0.02^{a} (0.5)	0.03^{b} (7.1)	0.04^{b} (2.4)
Ethyl lactate	1.23^{a} (0.3)	1.09^{a} (4.9)	1.19^{a} (5.1)
Ethyl hexanoate	0.35^{a} (0.5)	0.66^{b} (8.8)	0.70^{b} (1.7)
Ethyl octanoate	0.35^{a} (9.3)	0.79^{b} (6.1)	0.80^{b} (9.1)
Ethyl decanoate	0.18^{a} (4.9)	0.26^{b} (4.2)	0.22^{c} (1.1)
Diethyl succinate	0.12^{a} (5.0)	0.14^{b} (6.2)	0.13^{a,b} (4.2)
Diethyl monosuccinate	0.21^{a} (6.8)	0.23^{a} (9.1)	0.21^{a} (11.1)
3-(Methylthio)-propanoic acid, ethyl ester	0.02^{a} (7.8)	0.02^{a} (4.5)	0.01^{b} (2.9)
2-Furancarboxylic acid, ethyl ester	0.01^{a} (2.3)	0.01^{a} (7.1)	0.01^{a} (6.0)
Ethyl 3-hydroxybutyrate	0.01^{a} (5.4)	0.02^{a} (2.2)	0.05^{b} (8.8)
Ethyl 4-hydroxybutyrate	6.77^{a} (1.6)	6.80^{a} (1.0)	6.49^{b} (7.9)
Ethyl malonate	Tr	Tr	Tr
Ethyl malate	0.02^{a} (4.6)	0.02^{a} (9.0)	0.03^{b} (1.2)
Acids			
Isobutyric acid	1.91^{a} (2.8)	1.69^{b} (11.0)	1.89^{a} (1.2)
Butyric acid	0.50^{a} (1.5)	0.56^{b} (8.7)	0.76^{c} (10.0)
Isovaleric acid	0.42^{a} (8.6)	0.46^{b} (7.6)	0.42^{a} (2.0)
Hexanoic acid	1.46^{a} (9.6)	2.13^{b} (2.0)	1.85^{c} (7.5)
Heptanoic acid	0.03^{a} (1.0)	0.03^{a} (1.5)	0.02^{b} (2.6)
2-Hexenoic acid	0.03^{a} (0.1)	0.04^{a} (1.9)	0.03^{a} (5.5)
Octanoic acid	2.48^{a} (0.6)	3.40^{b} (2.5)	3.35^{b} (4.0)
Decanoic acid	0.55^{a} (4.5)	1.01^{b} (0.6)	0.79^{c} (2.4)
Dodecanoic acid	0.03^{a} (10.9)	0.07^{b} (3.5)	0.07^{b} (8.7)
Hexadecanoic acid	0.07^{a} (1.0)	0.08^{a} (5.1)	0.09^{a} (3.4)
γ-butyrolactone	3.08^{a} (6.9)	2.87^{b} (3.1)	3.44^{c} (8.8)

Tr: trace.
[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 ($p < 0.05$).

& Fernández, 1996; Cabaroglu & Canbas, 2002; Selli, Canbas, Cabaroglu, Erten, & Günata, 2006; Selli et al., 2006; Sánchez-Palomo et al., 2006).

3.3. Influence of skin-contact time on free and bound volatile compounds

Table 3 shows free aroma compounds for Albillo skin extracts, musts and wines, expressed as the mean (μg/l) of duplicate samples. GC–MS analysis of skin extracts, musts and wines identified 32, 32 and 30 varietal aroma compounds, respectively, including C_6 alcohols, higher alcohols, volatile phenols, terpene compounds and C_{13}-norisoprenoids.

Skin extracts showed higher concentrations of most varietal compounds – and especially of terpene compounds and norisoprenoids – than did musts or wines, confirming pre-fermentation maceration as a useful technique for enhancing the varietal character of wines (Sánchez-Palomo et al., 2006; Selli, Canbas, Cabaroglu, Erten, & Günata, 2006; Selli et al., 2006).

Predominant compounds in Albillo musts included the C_6 aldehydes and alcohols, the highest values being recorded for 1-hexanol and (*E*)-2-hexen-1-ol. Wine C_6 compound concentrations were generally similar to those of musts, although the highest values were found for 1-hexanol, while (*E*)-2-hexenal was not detected, probably due to its transformation via yeast metabolism (Sánchez-Palomo et al., 2006). Skin contact with musts significantly increased C_6 compound concentrations in wines (Sánchez-Palomo et al., 2006; Selli, Canbas, Cabaroglu, Erten, & Günata, 2006; Selli et al., 2006). These compounds could be associated with the fresh and green notes in wine aroma.

Terpene compounds, characteristic of aromatic varieties such as Muscat, have a low olfactory threshold and are generally associated with floral and citric aromas (Etièvant, 1991; Güth, 1997). Levels of both terpene compounds and C_{13}-norisoprenoids tend to be lower in the musts and wines of neutral grape varieties such as Albillo. Even so, maceration of must with skin doubled the amount of these compounds in Albillo wines, exceeding the olfactory thresholds in the case of linalool (15 μg/l) and β-damascenone (0.05 μg/l) (Güth, 1997).

Benzene compounds were the most abundant in musts and wines; concentrations were higher in skin extracts, although some compounds were not found, including guaiacol, isoeugenol and eugenol. Concentrations of a number of compounds, including vanillin and its derivatives – with their characteristic vanilla aroma – and benzyl alcohol in final wines, increased significantly with skin-contact time. By contrast, there was no significant increase in other compounds, including volatile phenols and benzaldehyde. Volatile phenols are considered characteristic components of wine aroma, although their influence on the final product may be positive or negative depending on their concentrations. In our case, volatile phenols did not attain levels sufficient to produce off-flavours.

The volatile compounds released from the bound fraction by enzyme hydrolysis in musts and wines are shown in Table 4. A total of 48 and 46 enzyme-released compounds were identified in musts and wines, respectively, including C_6 compounds, terpene compounds, benzene compounds, C_{13}-norisoprenoids and aliphatic compounds.

The ability of the enzyme preparation used to release glycosidically-bound compounds from grapes, AR-2000, has been confirmed in numerous studies (Aldave, 1999; Baek & Cadwallader, 1999; Sánchez-Palomo, Díaz-Maroto Hidalgo, González Viñas, & Pérez-Coello, 2005; Sánchez-Palomo et al., 2006).

Skin contact, prior to alcohol fermentation, significantly increased the concentration of glycosidically-bound aroma compounds in wines, as was also reported by other authors for a number of grape varieties (Baumes et al., 1989; Cabaroglu & Canbas, 2002; Cabaroglu et al., 1997; Sánchez-Palomo et al., 2006; Selli, Canbas, Cabaroglu, Erten, & Günata, 2006; Selli et al., 2006).

Benzene and terpene compounds were the most abundant bound compounds in musts and wines, followed by aliphatic compounds and C_{13}-norisoprenoids.

The small amounts of C_6 compounds in the bound fraction of Albillo musts and wines have also been observed for other varieties (Cabaroglu, Selli, Canbas, Lepoutre, & Günata, 2003; Sánchez-Palomo et al., 2006); the increase prompted by skin contact was slight but statistically significant.

Some terpene compounds – including linalool oxide, furan isomers and α-terpineol – were not present in the free fraction of wines but were detected in the bound fraction. In the bound fraction of others, β-citronellol, nerol and hydroxylinalool, were more abundant than in the free fraction.

Skin contact caused a discrete increase in bound terpene compounds, that was not affected by maceration time.

The bound fraction contents of some benzene compounds were considerable, particularly in skin extracts. Compounds, which increased significantly as a function of skin contact, included 2-phenylethanol, eugenol and isoeugenol, benzoic acid and vanillin.

Norisoprenoids detected in negligible amounts or not found in the wine and must free fraction, such as 3-oxo-α-ionol and 3-hydroxy-7,8-dehydro-β-ionol, were relatively abundant in the bound fraction. By contrast, β-damascenone – detected in the free fraction in wines – was not detected in bound form.

Skin-contact treatment for 15 h increased the total concentration of bound compounds in wines, but the maceration of 23 h did not significantly increase the levels of some bound compounds in wines. The same effect has been observed in Muscat a petit grains wines macerated under the same conditions (Sánchez-Palomo et al., 2006). This bound fraction can be considered to be a potential aroma source if a skin-contact technique is used in conjunction with a glycosidic-enzyme wine treatment (Cabaroglu et al., 2003; Sánchez-Palomo et al., 2005, 2006).

Table 3
Mean concentrations of free volatile compounds (μg/l) and relative standard deviations ($n = 2$)

Compounds	Control skin extract	Control must	Control wine[A]	Skin-contact wine 15 h/18 °C	Skin-contact wine 23 h/18 °C
2-Hexenal	5.4 (0.3)	7.6 (4.2)	n.d.	n.d.	n.d.
1-Hexanol	88.8 (0.5)	87.4 (9.0)	230[a] (2.4)	318[b] (4.7)	381[c] (2.5)
(*E*)-3-Hexen-1-ol	44.8 (4.0)	14.3 (3.6)	32.7[a] (1.2)	24.0[b] (0.1)	20.0[b] (9.1)
(*Z*)-3-Hexen-1-ol	n.d.	32.4 (9.5)	6.5[a] (11.2)	1.8[b] (1.7)	11.2[c] (4.9)
(*E*)-2-Hexen-1-ol	43.6 (0.2)	148 (2.2)	n.d.	n.d.	n.d.
Total C_6 compounds	183	290	269	343	412
Linalool	18.1 (5.9)	nd	11.8[a] (8.2)	13.8[a] (5.4)	12.2[a] (8.5)
cis-Pyran-linalool oxide	9.2 (0.2)	4.6 (5.2)	n.d.	24.1[a] (5.7)	17.5[b] (9.2)
β-Citronellol	7.7 (3.1)	n.d.	n.d.	n.d.	n.d.
Nerol	5.3 (1.5)	2.8 (8.1)	n.d.	n.d.	n.d.
Geraniol	26.5 (2.4)	11.1 (7.9)	13.2[a] (8.2)	12.4[b] (12.9)	12.6[b] (4.7)
2,6-Dimethyl-3,7-octadiene-2,6-diol	8.0 (3.4)	7.5 (6.8)	23.5[a] (3.5)	27.1[b] (0.7)	28.9[b] (6.4)
3,7-Dimethyl-1,7-octadienol	63.9 (3.1)	14.7 (6.8)	n.d.	33.5[a] (1.0)	35.2[b] (5.3)
8-Hydroxylinalool	46.2 (7.3)	n.d.	n.d.	n.d.	n.d.
Geranic acid	32.3 (1.8)	16.3 (7.0)	n.d.	n.d.	n.d.
Total terpenic compounds	217	60.2	48.5	111	106
β-Damascenone	n.d.	n.d.	6.6[a] (1.8)	11.5[b] (0.9)	13.3[b] (5.6)
3-Hydroxy-β-damascone	21.3 (2.1)	tr	13.8[a] (3.2)	30.1[b] (0.5)	24.6[c] (4.7)
3-Oxo-α-ionol	Tr	n.d.	n.d.	n.d.	n.d.
3-Hydroxy-7,8-dihydro-β-ionol	Tr	n.d.	n.d.	n.d.	n.d.
Total C_{13}-norisoprenoids	23.3	tr	20.4	41.6	37.9
Vanillin	n.d.	14.7	15.4[a] (2.2)	63.8[b] (1.8)	15.6[b] (1.8)
Acetovanillone	n.d.	36.5	41.7[a] (1.3)	98.8[b] (6.8)	98.2[b] (0.5)
Propiovanillone	n.d.	n.d.	11.3[a] (6.8)	23.6[b] (5.8)	29.3[b] (5.6)
Zingerone	n.d.	n.d.	16.8[a] (4.4)	52.6[b] (7.0)	147[c] (10.5)
Methyl vanillyl ether	Tr	7.0	5.2[a] (3.4)	23.9[b] (5.9)	39.2[c] (6.5)
Benzaldehyde	2.3 (10.2)	14.4 (2.8)	16.4[a] (2.4)	13.6[b] (5.7)	7.0[c] (1.9)
3 (2 H)Tiophenone, dihydro, 2-methyl	n.d.	n.d.	46.4[a] (2.7)	52.1[b] (2.7)	39.2[c] (6.5)
Benzene, 1-(methyl-2-cyclopropen-1-yl)	9.5 (1.1)	3.2 (6.1)	3.6[a] (1.4)	3.2[a] (1.1)	tr
Benzeneacetaldehyde	12.6 (4.3)	8.3 (7.4)	5.6[a] (5.6)	4.4[a] (2.7)	4.9[a] (1.9)
Ethylbenzaldehyde	0.9 (0.6)	9.2 (0.6)	16.0[a] (0.3)	16.4[a] (5.4)	15.5[a] (1.1)
Benzyl alcohol	194 (0.2)	53.8 (4.2)	17.9[a] (11.7)	35.6[b] (9.1)	35.4[b] (1.0)
2-Phenylethanol[B]	106 (0.0)	24.9 (8.1)			
4-Vinylguaiacol[B]	Tr	tr			
Guaiacol	n.d.	5.3 (8.9)	13.2[a] (5.3)	6.2[b] (9.9)	6.3[b] (10.5)
Benzothiazole	11.4 (5.1)	5.1 (2.4)	4.3[a] (12.0)	13.7[b] (6.9)	17.8[c] (5.5)
2,3-Dihydrobenzofuran	n.d.	n.d.	9.5[a] (5.8)	26.5[b] (7.6)	24.7[b] (9.5)
Eugenol	n.d.	3.2 (7.2)	tr	tr	tr
Isoeugenol	n.d.	29.1 (1.4)	10.2[a] (12.4)	14.8[b] (2.2)	10.2[a] (8.3)
Cinnamic acid	24.2 (17.9)	1.9 (8.4)	n.d.	n.d.	n.d.
Acetophenone	10.4 (1.6)	7.7 (6.4)	7.5[a] (7.4)	10.4[a] (8.0)	10.2[a] (6.0)
Total benzenic compounds	360	216	234	449	490
4-Methyl-1-pentanol	n.d.	n.d.	44.9[a] (2.2)	49.8[a] (7.7)	39.1[b] (4.4)
3-Methyl-1-pentanol	n.d.	n.d.	146[a] (3.2)	170[b] (9.4)	114[c] (7.3)
Hexanoic acid[B]	79.6(0.3)	19.8 (0.8)			
2-Hexenoic acid[B]	13.9 (4.1)	2.5 (14.4)			
Octanoic acid[B]	20.8 (7.1)	8.3 (9.0)			
Nonanoic acid[B]	20.9 (8.1)	3.2 (7.2)			
Decanoic acid[B]	37.9 (8.1)	4.1 (11.6)			
Hexadecanoic acid[B]	128 (2.9)	45.7 (10.2)			
Total aliphatic compounds	311	91.3	199	230	164

n.d.: No 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 ($p < 0.05$).

[B] Compounds formed principally during the alcoholic fermentation (see Table 2).

Table 4
Mean concentrations and relative standard deviations ($n = 2$) of bound volatile compounds released by enzymatic hydrolysis

Compounds	Control must	Control wine[A]	Skin-contact wine 15 h/18 °C	Skin-contact wine 23 h/18 °C
(*E*)-2-Hexenal	104 (14.9)	n.d.	n.d.	n.d.
1-Hexanol	111 (2.8)	33.9[a] (5.9)	45.4[b] (4.1)	38.5[c] (1.0)
(*E*)-3-Hexen-1-ol	Tr	n.d.	n.d.	n.d.
(*Z*)-3-Hexen-1-ol	13.7 (4.6)	3.9[a] (7.2)	5.6[b] (10.0)	n.d.
(*E*)-2-Hexen-1-ol	24.9 (6.6)	n.d.	n.d.	n.d.
Total C_6 compounds	254	37.8	51.0	38.5
cis-Linalool oxide furan	16.1 (11.2)	7.6[a] (11.8)	11.6[b] (1.0)	n.d.
trans-Linalool oxide furan	5.2 (6.3)	5.1[a] (5.3)	5.6[b] (4.7)	n.d.
Linalool	5.6 (3.8)	6.1[a] (2.8)	8.7[b] (3.0)	7.2[c] (2.5)
α-Terpineol	15.7 (13.1)	3.9[a] (5.6)	7.4[b] (2.5)	6.2[c] (2.5)
cis-Linalool oxide pyran	28.2 (7.8)	3.6[a] (14.0)	3.8[a] (12.2)	3.8[a] (2.5)
trans-Linalool oxide pyran	4.9 (8.0)	n.d.	n.d.	n.d.
β-Citronellol	3.1 (7.5)	5.1[a] (3.9)	5.3[a] (3.8)	4.8[a] (2.5)
Nerol	14.9 (11.9)	8.7[a] (3.2)	11.6[b] (2.6)	6.4[c] (0.5)
Geraniol	14.4 (5.3)	22.9[a] (1.6)	28.6[b] (1.0)	24.2[c] (0.9)
3,7-Octadiene-2,6-diol, 2,6-dimethyl	2.7 (7.2)	2.6[a] (2.2)	3.2[b] (1.0)	n.d.
1,7-Octanediol, 3,7-dimethyl	26.6 (9.0)	21.2[a] (8.9)	27.0[b] (7.2)	14.2[c] (6.6)
(*E*)-8-Hydroxylinalool	120 (16.1)	17.2[a] (5.5)	28.7[b] (2.9)	17.1[a] (2.5)
(*Z*)-8-Hydroxylinalool	n.d.	45.0[a] (5.1)	59.5[b] (3.3)	28.4[c] (3.2)
Geranic acid	36.6 (10.7)	10.2[a] (4.5)	11.4[b] (3.3)	10.5[a,b] (3.0)
Total terpenic compounds	304	159	212	123
4-Oxo-α-isophorone	Tr	n.d.	n.d.	n.d.
3-Hydroxy-β-damascone	29.9 (8.2)	96.2[a] (9.2)	128[b] (3.0)	98.9[a] (8.0)
3-Oxo-α-ionol	11.5 (7.6)	18.3[a] (5.9)	32.5[b] (5.9)	67.0[c] (10.8)
3-Hydroxy-7,8-dihydro-β-ionol	13.1 (9.1)	n.d.	n.d.	n.d.
Vomifoliol	Tr	n.d.	n.d.	n.d.
Total C_{13}-norisoprenoids	54.5	115	160	166
Benzaldehyde	23.1 (8.2)	4.0[a] (8.1)	6.6[b] (0.9)	5.9[c] (3.3)
Guaiacol	5.3 (8.9)	n.d.	n.d.	n.d.
Benzyl alcohol	1098 (5.9)	34.8[a] (2.9)	37.7[a] (2.7)	33.2[a] (6.5)
2-Phenylethanol	259 (13.7)	39.2[a] (4.4)	68.1[b] (2.1)	49.2[c] (0.3)
Eugenol	55.5 (2.5)	26.3[a] (7,4)	43.6[b] (13.9)	31.9[c] (0.9)
Isoeugenol	29.1 (1.4)	10.2[a] (12.4)	14.8[b] (2.2)	10.2[a] (8.3)
4-Vinylguaiacol	45.5 (15.8)	7.1[a] (1.9)	14.3[b] (14.7)	12.8[b] (6.7)
Benzenepropanol	8.6 (2.1)	7.9[a] (0.5)	10.8[b] (1.0)	5.7[a] (8.0)
Benzoic acid	38.2 (4.1)	47.3[a] (6.7)	76.2[b] (3.8)	68.6[c] (7.0)
1,2-Benzothiazole	n.d.	5.0[a] (8.3)	1.1[a] (1.0)	n.d.
2,3-Dihydrobenzofuran	23.1 (7.1)	6.7[a] (7.1)	12.8[b] (3.6)	7.0[a] (3.6)
Phenol	9.4 (6.8)	3.7[a] (6.5)	5.0[b] (1.9)	Tr
Allylsiryngol	n.d.	32.7[a] (15.3)	11.8[b] (1.0)	29.0[a] (11.0)
Cinnamaldehyde	5.4 (9.5)	1.6[a] (6.8)	2.7[b] (3.6)	2.6[a] (8.2)
Cinnamyl alcohol	19.4 (6.1)	12.8[a] (11.2)	14.6[a] (9.8)	7.5[a] (0.7)
3,4,5-Trimethoxy phenol	140 (2.5)	8.7[a] (5.7)	11.8[b] (1.0)	n.d.
Vanillin	1.7 (1.3)	0.7[a] (1.1)	2.4[b] (1.1)	0.9[a] (2.5)
Acetovanillone	18.8 (14.1)	n.d.	n.d.	n.d.
Methyl vanillyl ether	184 (12.5)	n.d.	n.d.	n.d.
Acetophenone	n.d.	3.7[a] (12.2)	5.2[a] (2.4)	6.0[c] (2.5)
Methyl salicylate	14.5 (13.1)	6.8[a] (7.2)	5.1[b] (2.5)	6.8[b] (2.5)
Total benzenic compounds	1973	259	345	277
Butanoic acid	5.3 (9.1)	4.1[a] (3.7)	8.1[b] (1.0)	6.0[c] (0.0)
Butanoic acid, 3-methyl + 2-furanmethanol	8.5 (4.4)	5.1[a] (0.8)	5.2[a] (2.4)	6.8[b] (7.2)
Hexanoic acid	8.6 (2.2)	11.8[a] (0.9)	17.0[b] (0.3)	14.1[c] (13.2)
Heptanoic acid	1.5 (7.3)	n.d.	1.1 (1.1)	n.d.
Octanoic acid	14.4 (7.7)	13.1[a] (14.1)	21.8[b] (9.8)	18.5[c] (1.5)
Decanoic acid	n.d.	7.6[a] (3.0)	9.6[b] (5.4)	10.0[b] (8.4)
Dodecanoic acid	n.d.	38.9[a] (8.2)	42.4[b] (5.5)	53.5[c] (5.4)
Tetradecanoic acid	n.d.	7.0[a] (4.0)	12.2[b] (4.0)	2.0[c] (2.5)

(*continued on next page*)

Table 4 (*continued*)

Compounds	Control must	Control wine[A]	Skin-contact wine 15 h/18 °C	Skin-contact wine 23 h/18 °C
Hexadecanoic acid	188 (9.0)	40.8[a] (10.4)	87.2[b] (14.1)	83.8[b] (1.0)
Total aliphatic compounds	227	128	205	195

n.d.: No 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 ($p < 0.05$).

3.4. Sensory descriptive analysis

Wines were analysed by expert tasters in terms of attributes previously selected by consensus as those best describing the sensory characteristics of Albillo wines. Fig. 1 shows "spider-web" diagrams for average wine aroma-intensity and taste-attribute scores.

The aroma profile of control Albillo wines was characterized by moderately-intense fresh and fruity aromas, with occasional sweet notes. Skin contact, prior to fermentation, significantly increased the attributes detected in control wines, and prompted the appearance of new attributes – mainly fruity notes – not detected in control wines, such as peach, apricot, citric, ripe fruit and green apple.

The increase in fresh notes may be linked to greater abundance of C_6 compounds in treated wines. Fruity notes are mainly attributed to esters and C_{13}-norisoprenoids, particularly β-damascenone, with a low perception threshold (0.05 μg/l) (Güth, 1997).

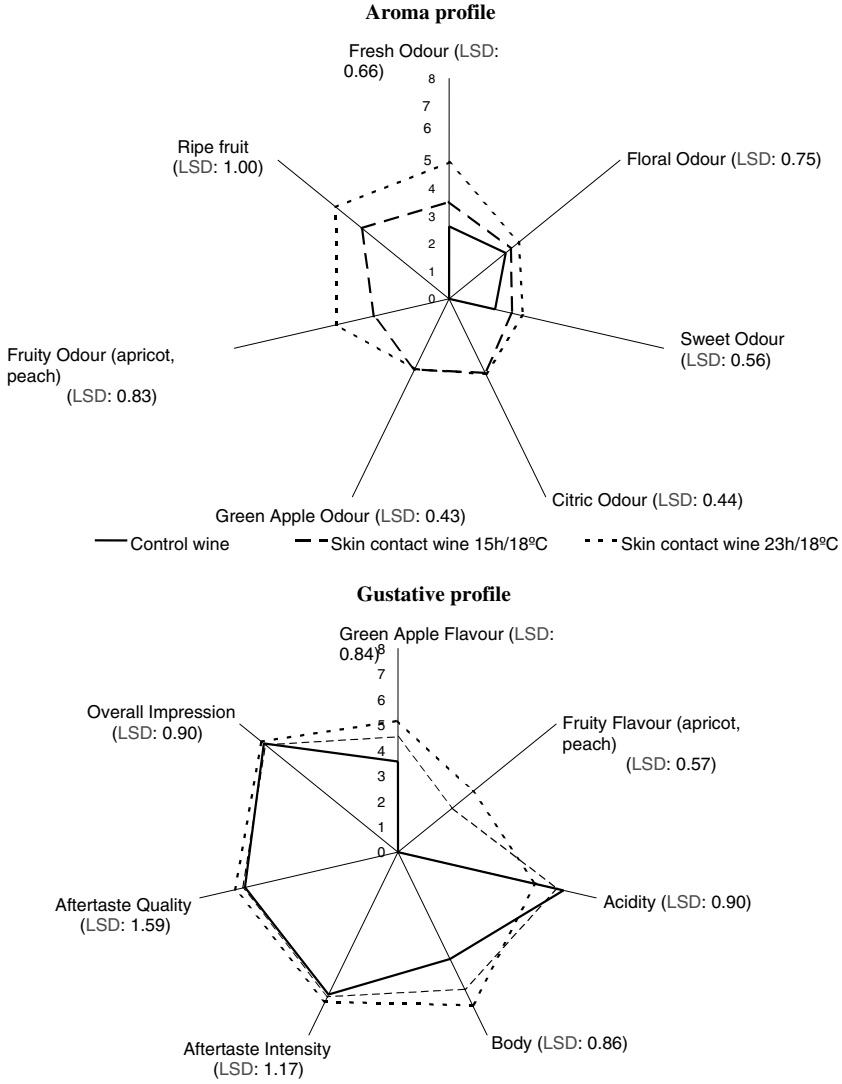

Fig. 1. Sensory descriptive analyses of wines mean scores of eight judges (two replicates).

Skin-contact treatment led to a significant increase in the concentration of vanillin and its derivatives, and thus to the sweet aroma to which these compounds contribute.

Skin contact, over 15 and 23 h, slightly modified the taste profile; there was a significantly higher score for green apple, and some evidence of peach and apricot tastes not found in control wines.

Skin-contact wines were less acid, and had more body, than had control wines, probably due to neutralization of the main wine acids during pre-fermentation maceration of the must (García Romero et al., 1999; Sánchez-Palomo et al., 2006). These skin-treated wines also scored higher than controls for aftertaste intensity and quality, as well as for overall impression.

In conclusion, control Albillo wines displayed high concentrations of C_6 compounds and benzene compounds, including vanillin and its derivatives, with lower values for terpene compounds.

Higher concentrations of all compounds in skin extracts suggest that the skin-contact technique may be an effective means for enhancing wine aroma. Skin contact for 15 and 23 h at 18 °C prompted a considerable increase in the levels of terpenes, norisoprenoids and benzene compounds in Albillo wines. This increase was more significant for free than for bound forms, even though bound forms – especially of terpenes and norisoprenoids – were considerably more abundant than were free forms in the Albillo wines.

Skin-contact treatment enhanced the sensory attributes already noted in control wines, and also gave rise to new aromas – sweet, peach, apricot, and green apple notes – as well as greater intensity, body and fruitiness in treated wines. The differences recorded in chemical analysis and tasting as a function of skin-contact time were slight, and would not warrant risking over-prolonged maceration; 15 h is considered a reasonable time for pre-fermentation maceration.

It may be concluded that pre-fermentation maceration of musts with grape skins, together with glycosidic-enzyme treatment of wines, provides a viable alternative to traditional methods for enhancing the varietal character of Albillo wines.

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