Effect of Skin Contact Before and During Alcoholic Fermentation on the Chemical and Sensory Profile of South African Chenin Blanc White Wines

J.L. Aleixandre-Tudo[1], C. Weightman[2], V. Panzeri[3], H.H. Nieuwoudt[2], W.J. du Toit[1*]

(1) Department of Viticulture and Oenology, Stellenbosch University, Private Bag X1, 7602 Matieland, Stellenbosch, South Africa
(2) Institute of Wine Biotechnology, Stellenbosch University, Private Bag X1, 7602 Matieland, Stellenbosch, South Africa
(3) The Institute of Grape and Wine Science (IGWS), Stellenbosch University, Private Bag X1, 7602 Matieland, Stellenbosch, South Africa

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The volatile and phenolic composition of Chenin blanc wines made with different skin contact treatments was studied. One batch of grapes was used to make a dry white wine according to two different treatments, namely pre-fermentative skin contact and complete fermentation on the skins. A white wine fermented without any skin contact was used as control. Fermentation on the skins and skin contact before fermentation led to significantly lower levels of terpenes, esters, acids and thiols, and the highest significant levels of alcohols and phenolic compounds. However, this effect was less pronounced in wines with skin contact before fermentation. Sensory analysis of all the experimental wines was also performed. The results showed a significant shift from the sensory attributes of fresh and tropical fruits of the control Chenin blanc wines towards riper fruit notes in the skin contact treatments. This observation was correlated with the length of the skin contact period. Possible reasons to explain the results observed in this study are discussed.

INTRODUCTION

Wine aroma is important in determining wine quality and is influenced mainly by volatile compounds. The aromatic complexity of wines depends on the grape variety used, and on the aromas produced during fermentation and those developed during the ageing process (Schneider, 1979: Rapp & Mandery, 1986). Almost one thousand different aroma compounds, such us alcohols, esters, organic acids, volatile phenols, aldehydes, ketones and monoterpenes, have already been detected in wine (Rapp & Mandery, 1986; Selli *et al*., 2006a). Interactions between different concentrations of these compounds can differentiate one wine from another in terms of their sensory perception (Moreno-Pérez *et al*., 2013; Van Wyngaard *et al*., 2014).

In an extremely competitive international market, wine producers need to invest in technology to improve production processes and product quality to remain competitive. The production of new styles of wines would thus give producers the opportunity to find and fill a new gap in the market. Moreover, consumers are increasingly demanding wines with different flavour characteristics (Swiegers *et al*., 2006a). Improving overall quality, flavour complexity and ageing potential could be the desirable quality factors to achieve.

Chenin blanc grapes are used in South Africa (SA) to produce many styles of wine, including dry wines, sparkling wines and dessert wines (Marais, 2005). Historically, most of the Chenin blanc crop was distilled for producing brandy or other spirits, or used for making lower quality wines (Clarke, 2007). However, this situation has changed significantly. In recent years, South African Chenin blanc has caught the attention of the international market because it offers both quality and good value for money (Hanekom, 2012). Currently, Chenin blanc is the most planted grape variety in South Africa, which makes it the country with the most Chenin blanc vineyards in the world (SAWIS, 2014).

Oenological practices are able to modify the chemical composition of wine and its sensory properties, such as flavour and colour. Skin contact of crushed white grapes can be defined as a pre-fermentative process with the aim to obtain the maximum intensity of varietal aroma. This technique is characterised by a longer period of contact between the juice and the skins of the grapes after they are

**Corresponding author: wdutoit@sun.ac.za*

crushed, but before pressing (Darias-Martin *et al*., 2000). The results when applying this technique are dependent mainly on the grape cultivar, temperature and time of skin contact (Cabaroglu & Canbas, 2002). However, skin contact may also result in herbaceous aroma, increased bitterness, etc. (Selli *et al*., 2006a). Maceration conditions must therefore be chosen carefully.

Several studies have been published in the literature on the use of skin contact, but, according to what was observed, none has been performed on the Chenin blanc variety. Rodriguez-Bencomo *et al*. (2008) reported on the effect of skin contact on the aroma of Listan Blanco wines. The results suggest that wine aroma might only be affected by some terpenes and phenols. However, no differences in free volatiles were observed in relation to the skin contact treatment. Moreover, the aroma potential of Albillo wines subjected to skin contact was assessed by Sanchez-Palomo *et al*. (2007). The authors reported a considerable increase in free varietal compounds, together with an intensified fresh and fruity character, which was perceived positively by a sensory panel. The same authors also reported an increase in varietal compounds in Muscat à Petits Grains wines. Floral, fruity and fresh notes were enhanced, in combination with changes in body and acidity perceptions (Sanchez-Palomo *et al*., 2006). A significant increase in the total free aroma compounds has also been reported by Selli *et al*. (2003; 2006a; 2006b) for the white grape varieties Muscat of Bornova and Narince exposed to skin contact.

In addition, the use of skin contact before and sometimes even during fermentation has recently increased in the production of certain Chenin blanc wines, for instance from the Swartland region in South Africa (Goode, 2013). The aim of this study thus was to determine the influence of pre-fermentative skin contact, as well as skin contact during the entire alcoholic fermentation, on the volatile, phenolic and sensory composition of South African Chenin blanc wines. As far as we know there are no studies in the literature addressing the effect of skin contact and complete fermentation on the skins of SA Chenin blanc wines.

MATERIALS AND METHODS

Materials

Dichloromethane (DCM) (≥ 99.8%), acetonitrile LC-MS CHROMASOLV (≥ 99.0%), methanol (≥ 99.9%), iso-propanol LC-MS CHROMASOLV (≥ 99.0%), sodium chloride (≥ 99.5%), potassium metabisulphite, sodium borohydride, ethanolamine (EA), o-phthaldialdehyde (OPA), anhydrous sodium sulphate (≥ 99.0%), phosphoric acid (85 to 90%), 4-methyl-2-pentanol GC (≥ 97.5%), diethyl ether (≥ 99.9%) and 2,6-dimethyl-6-hepten-2-ol GC (≥ 96.0%) were purchased from Sigma-Aldrich (St Louis, MO, USA). Calcium carbonate, boric acid and acetonitrile (≥ 99.9%) (HPLC phenolics) were purchased from Merck (Merck Millipore, Modderfontein, South Africa). Polyvinylpolypyrrolidone (PVPP) resin was purchased from Dal Cin Gildo Spa (Milan, Italy). Water for ultra-performance liquid chromatography (UPLC) was obtained from a Milli-Q filtration system (EMD Millipore, Bedford, MA, USA). 3-Mercaptohexan-1-ol (3MH) was purchased from Acros Organics (Geel, Belgium) and 3-mercapto-hexylacetate (3MHA) from Oxford Chemical (Hartlepool, England).

Winemaking

Chenin blanc grapes (25.6°B, pH 3.40, TA 6.38 g/L) were harvested manually during the 2013 vintage and transported to the experimental winery at the Department of Viticulture and Oenology (DVO), Stellenbosch University (Stellenbosch, South Africa). Before processing, the grapes were mixed thoroughly and divided into three treatments, designated control with no skin contact, skin contact before fermentation (ScBF) and fermentation on the skins (FoS). Grapes were destemmed, crushed, mixed and divided into triplicate ferments for each treatment. Fifty-five kilograms of grapes were used in each of the nine vinifications performed (three repeats per treatment). Sulphur dioxide at 30 mg/L and pectolytic enzymes (Lafazym Press, Laffort, Bordeaux, France), according to the manufacturer's instructions, were added just after crushing. The control treatments were then pressed in a basket press up to a pressure of 0.5 bars. Press juice and free-run juice were mixed and left at 4°C for one day for settling of the juice. The clear juice was then racked off the grape lees and inoculated with *Saccharomyces cerevisiae* QA23 (QA23, Lallemand, Blagnac Cedex, France) and fermented at 15°C. The Balling levels were monitored daily. At the end of the fermentation, SO_2 at 50 mg/L was added to the wines. Bentonite was then added at 600 mg/L and the wine was left at room temperature for two hours, and then placed at -4°C for two weeks. After cold stabilisation the wines were racked off and the free SO_2 was adjusted to 35 mg/L. Finally, the wines were filtered and closed under screw cap. The second treatment was submitted to skin contact before fermentation after crushing (ScBF). Grapes were placed at 4°C for 12 hours before pressing. After the skin contact period, wines were fermented following the fermentation procedure for the control wines. Finally, the third batch was fermented on the skins for the duration of the fermentation (FoS). The same yeast and fermentation temperatures were used as in the other treatments, but the skins were mixed manually with the must once a day and then pressed after fermentation. Post-fermentative stabilisation and bottling treatments in all three treatments were the same. For each treatment (Control, ScBF, FoS), three biological repeats per treatment were performed, therefore nine wines were made. Analyses were performed after a year of bottle storage.

Wine analysis

Degrees Balling (°B) were measured using a Balling meter. Total acidity (g/L tartaric acid), volatile acidity (g/L acetic acid), malic acid (g/L malic acid), glucose (mg/L), fructose (mg/L), glycerol (g/L) and ethanol (%vol) concentrations were analysed using a Grapescan™ FT 120 instrument (Foss Electric, Denmark) (Nieuwoudt *et al*., 2004). Methods used for the quantification of phenolic compounds, esters, higher alcohols, fatty acids, carbonyl compounds and monoterpenes have been reported elsewhere (Coetzee *et al*., 2013). Absorbance at 280 nm (total phenolics), 420 nm (yellow colour) and 440 nm (brown colour) were also recorded (Glories, 1984).

The volatile thiols, 3-mercaptohexan-1-ol (3MH)

and 3-mercapto-hexylacetate (3MHA), were quantified following the method developed by Piano *et al*. (2015). A liquid-liquid extraction of the volatile thiols was performed. Briefly, internal standard (3MH, 3MHA, deuterised 3MH and deuterised 3MHA) was added to 180 mL of wine already containing $K_2S_2O_5$ (equivalent of 3 g/L SO_2). Samples were stirred for 10 minutes after the addition of PVPP (5 g/L). After centrifugation, the pH of the supernatant was adjusted to 5 using $CaCO_3$. $NaBH_4$ (3.84 g/L) was later added to the sample under the fume hood. A total of 110 mL of dichloromethane (DCM) was added to the samples, which were then stirred for 20 min. After stirring, the samples were allowed to separate for approximately 10 minutes. The DCM layer was recovered and washed with 100 mL of distilled water. Na_2SO_4 (3 g) was incorporated to eliminate possible water traces in the sample. Finally, the DCM was evaporated under nitrogen and replaced with methanol. Then, 50 µL of extracted sample in methanol was derivatised with 5 µL OPA (5 g/L in methanol) and 5 µL EA (10 g/L in borate buffer, 80 mmol at pH 7.3). The samples then were injected into the ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) system (Waters Acquity UPLC system fitted to a Waters Xevo triple quadrupole mass spectrometer (MS/MS), Waters Corporation). Data were acquired and processed with MassLynx software, version 4.1 (Waters Corporation). The instrument settings, quantification and gradient programme have been reported elsewhere (Piano *et al*., 2015).

Sensory analysis

Descriptive analysis was the chosen sensory method, and 10 panellists (three male and seven female), with an average age of 28, were trained using the consensus method (Lawless & Heymann, 2010). During the first training session the panel was presented with the Chenin blanc wine samples and asked to smell them and generate descriptors to describe the aroma of each sample. The panellists were subsequently asked to taste the wines and comment on differences they observed. In the following training session, the panel was presented with 30 aroma standards for the descriptors they had generated previously. Thereafter the judges were required to smell the wine samples and describe them using the standards as a guide. During the subsequent training sessions, the list of 30 general Chenin blanc aroma standards was discussed by the panel and reduced to the most relevant 13 (Table 4) that best characterised the wines. The aroma attributes and the reference standards used in the descriptive analysis are reported in Table 1.

Testing was conducted in air-conditioned, light-controlled rooms (ISO NORM 8589, 1988). Panellists were supplied with water and crackers for palate cleansing between samples. The samples were coded with randomised three-digit numbers to eliminate bias and serving order effect. For each sample, the panellists rated the intensity of each of the 13 descriptors on an unstructured 10 cm linear scale, anchored "None" at the left end and "Intense" at the right. The panel also rated the wines for the taste and mouthfeel attributes sweetness, sourness, bitterness and astringency. Testing was done in triplicate and all ratings were measured by hand and recorded in Microsoft Excel 2010.

Statistical treatment

The data corresponding to the volatile and phenolic composition of the control, skin contact before fermentation and fermentation on skins were analysed by analysis of variance (ANOVA) to determine differences between the treatments. Moreover, sensory data analysis was performed using principal component analysis (PCA). STATISTICA 10 software (www.statsoft.com) was used for the statistical treatment of the data.

RESULTS AND DISCUSSION

Influence of skin contact treatments on general wine composition

The conventional composition of the Chenin blanc control wines and wines obtained using different skin contact treatments is shown in Table 2. Wine composition was affected

TABLE 1
Aroma attributes and reference standards used for descriptive analysis.

Attribute	Definition	Composition
Pineapple	Odour associated with fresh pineapple	¼ slice of pineapple
Banana	Odour associated with banana sweets/candy	1 µL isoamyl acetate + 30 mL water
Citrus	Odour associated with lemon and grapefruit	¼ slice lemon + ¼ slice grapefruit
Yellow apple	Odour associated with yellow apple	1 cm wedge of Golden Delicious apple
Passion fruit	Odour associated with fresh passion fruit	1 Tsp fresh passion fruit pulp
Dry grass	Odour associated with dry grass/hay	Handful, finely chopped
Marmalade	Odour associated with orange marmalade	1 tsp marmalade (All Gold)
Stone fruit	Odour associated with peach and apricot	15 mL peach juice + 15 mL apricot juice (LiquiFruit)
Honey	Odour associated with acacia honey	1 tsp in 10 mL water (Woolworths)
Raisin	Odour associated with raisins	5 raisins chopped (Safari)
Mint	Odour associated with fresh mint	1 sprig chopped
Dried fruit	Odour associated with mixed dried fruit	1 piece each of apple, apricot, peach, prune, pear chopped (Safari)
Cooked vegetable	Odour associated with canned green bean and artichoke	20 mL artichoke brine + 20 mL green bean brine (Goldcrest)

by skin contact treatments. FoS wines had significantly lower pH and higher total acidity than the ScBF and Control wines. In contrast to our results, other researchers have found that skin contact may result in an increased TA and decreased pH due to the liberation of potassium ions from the grape skins. However, in a study by Cai *et al*. (2014), they reported higher TA and lower pH at the end of the alcoholic fermentation in Cabernet Sauvignon wines made with skin contact, results that are in accordance with our study. The solubilisation of organic acids from the skins counteracting the liberation of potassium ions could be the reason for these results. Moreover, the FoS wines showed significantly lower levels of fructose together with an observed increase in alcohol content, although not statistically significant. During skin contact, some nitrogen is extracted and dissolved into the must (Sanchez-Palomo *et al*., 2007). The availability of the extra nitrogen might influence yeast activity, which could have caused the decrease in certain residual sugars (fructose) observed in the FoS wines. However, this significant effect was not observed in ScBF, probably because the skin contact period was not long enough to cause a significant increase in the available nitrogen (Table 2). In the ScBF wines, the fructose concentration was significantly lower compared to the control, but this was not the case for glucose levels. Finally, the FoS wines showed a significant increase in glycerol levels. Glycerol forms during fermentation and can have a considerable impact on the organoleptic properties of wines (Lubbers *et al*., 2001).

Influence of skin contact treatments on colour and phenolic compounds of Chenin blanc wines

In white wine vinification, skin contact treatment is a process often applied to increase the wine's varietal character (Peinado *et al*., 2004, Selli *et al*., 2003). However, skin contact increases the phenolic compounds of wines, and this may also increase the astringency and bitterness (Selli *et al*., 2006a). Table 3 shows the colour parameters (absorbances at 280, 420 and 440 nm) and concentrations of phenolic compounds in the wines elaborated with different skin contact treatments. A total of eight different phenolic compounds were quantified in the white wines, together with the total polymeric phenols. The levels of gallic acid, catechin, dimer B1, total polymeric phenols, coutaric acid, quercetin-3-glycoside and quercetin were significantly higher in the FoS wines compared to the Control and ScBF wines. Only the grape reaction product (GRP) was significantly higher in the ScBF wines when compared to the Control wines, where GRP, in turn, also was significantly higher than in the FoS wines.

On the other hand, caftaric acid concentrations showed a different trend, since the Control and FoS wines had significantly higher values when compared with the ScBF wines. GRP is formed through enzymatic oxidation during crushing and must preparation. The compound is produced by the interaction of four components: glutathione, caftaric (or coutaric) acid, active polyphenoloxidase and oxygen (Singleton *et al*., 1985). The formation kinetics of this compound could partially explain the highest and lowest values observed in the ScBF wines for GRP and caftaric acid respectively, due to the more oxidative conditions during the pre-fermentative cold maceration step when applying this technique.

In addition, a similar trend as observed for the phenolic compounds was found for the 280, 420 and 440 nm measurements, where FoS wines had significantly higher absorbance values. As expected, the FoS wines showed higher yellow and brown intensity, together with higher phenolic content – results in accordance with the study reported by Darias-Martin *et al*. (2000).

Influence of skin contact treatments on volatile compounds of Chenin blanc wines

The quantitative data of volatile compounds found in Chenin blanc white wines are shown in Table 4. Forty-three volatiles were quantified in the wines, consisting of higher alcohols, esters, fatty acids, terpenes, carbonyl compounds and thiols. Volatile compounds that were present at the highest levels were terpenes, followed by higher alcohols, acids and esters. Concerning the total concentration of volatile compounds, the FoS wines in general had lower levels of volatiles.

TABLE 2
General composition of Control, pre-fermentative (ScBF) and fermentative macerated (FoS) Chenin blanc wines.

	Control	ScBF	FoS	p-value	Significance
pH	3.39 ± 0.02^{b}	3.41 ± 0.02^{b}	3.34 ± 0.02^{a}	0.004	**
VA	0.52 ± 0.04^{b}	0.50 ± 0.04^{ab}	0.42 ± 0.05^{a}	0.064	NS
TA	5.52 ± 0.04^{a}	5.63 ± 0.03^{a}	6.23 ± 0.09^{b}	0.000	**
MA	2.67 ± 0.05^{a}	2.67 ± 0.07^{a}	2.80 ± 0.10^{a}	0.143	NS
Glucose	2.20 ± 0.77^{a}	2.73 ± 1.23^{a}	1.47 ± 0.26^{a}	0.269	NS
Fructose	7.92 ± 0.73^{c}	5.21 ± 1.60^{b}	1.77 ± 1.02^{a}	0.002	**
Ethanol	15.74 ± 0.11^{a}	15.76 ± 0.04^{a}	16.06 ± 0.30^{a}	0.325	NS
Glycerol	8.79 ± 0.33^{a}	9.04 ± 0.19^{a}	9.88 ± 0.06^{b}	0.003	**

Mean values and standard deviation of three repeats. Different letters mean significant differences ($p < 0.05$) between the average values for the LSD Fisher test. Total acidity (mg/L tartaric acid); VA: volatile acidity (mg/L acetic acid); MA: malic acid (mg/L malic acid); glucose, fructose and glycerol (mg/L); ethanol (%vol); Control: non-pre-fermentative macerated wines; ScBF: skin contact before fermentation; FoS: fermentation on skins.
* Significance at $p < 0.05$
** Significance at $p < 0.01$

TABLE 3
Mean values and standard deviations of colour parameters and phenolic compounds identified by HPLC analysis of Control, pre-fermentative (ScBF) and fermentative macerated (FoS) Chenin blanc wines.

	Control	ScBF	FoS	p-value	Significance
280 nm	0.63 ± 0.01[a]	0.64 ± 0.01[a]	1.35 ± 0.18[b]	0.000	**
420 nm	0.10 ± 0.00[a]	0.09 ± 0.00[a]	0.15 ± 0.01[b]	0.000	**
440 nm	0.07 ± 0.00[a]	0.07 ± 0.00[a]	0.10 ± 0.01[b]	0.000	**
Gallic acid (mg/L)	3.68 ± 0.16[a]	3.87 ± 0.23[a]	14.02 ± 2.53[b]	0.000	**
(+)-Catechin (mg/L)	7.87 ± 0.09[a]	8.45 ± 0.15[a]	18.32 ± 3.47[b]	0.001	**
B1 (mg/L)	1.39 ± 0.04[a]	1.89 ± 0.21[a]	6.91 ± 2.28[b]	0.004	**
Polymeric phenols (mg/L)	8.58 ± 0.94[a]	8.86 ± 1.85[a]	39.24 ± 12.35[b]	0.003	**
GRP (mg/L)	2.56 ± 0.60[b]	4.55 ± 0.75[c]	1.34 ± 0.27[a]	0.001	**
Caftaric acid (mg/L)	12.15 ± 2.88[b]	5.40 ± 0.72[a]	10.97 ± 1.77[b]	0.013	*
Coutaric acid (mg/L)	1.45 ± 0.01[a]	1.39 ± 0.04[a]	3.47 ± 0.73[b]	0.001	**
Quer-3-glyc (mg/L)	0.28 ± 0.07[a]	0.41 ± 0.09[a]	4.66 ± 1.08[b]	0.000	**
Quercetin (mg/L)	0.79 ± 0.00[a]	0.81 ± 0.01[a]	0.90 ± 0.06[b]	0.020	*

Different letters mean significant differences ($p < 0.05$) between the average values for the LSD Fisher test. B1: proanthocyanin; GRP: grape reaction product.
* Significance at $p < 0.05$
** Significance at $p < 0.01$

TABLE 4
Effect of skin contact treatments on the aroma compound concentrations (µg/L) of Control, pre-fermentative (ScBF) and fermentative macerated (FoS) Chenin blanc wines. Mean values and standard deviation are shown.

Compounds	Control	ScBF	FoS	p-value	Significance
Alcohols					
Methanol	78.86 ± 7.01[a]	102.80 ± 1.23[b]	224.30 ± 16.74[c]	0.000	**
Propanol	48.07 ± 1.8[a]	59.80 ± 2.07[b]	74.82 ± 2.80[c]	0.000	**
Isobutanol	27.87 ± 0.47[a]	31.51 ± 0.59[a]	48.08 ± 3.86[b]	0.000	**
Butanol	1.56 ± 0.01[a]	1.47 ± 0.07[a]	2.51 ± 0.09[b]	0.000	**
Isoamyl alcohol	237.60 ± 12.20[a]	269.80 ± 6.56[b]	369.40 ± 6.33[c]	0.000	**
Pentanol	0.41 ± 0.00[a]	0.42 ± 0.00[a]	0.52 ± 0.03[b]	0.000	**
3-Methyl-1-pentanol	0.25 ± 0.02[a]	0.24 ± 0.01[a]	0.30 ± 0.03[b]	0.029	*
Hexanol	1.96 ± 0.10[a]	2.34 ± 0.10[a]	3.80 ± 0.44[b]	0.000	**
3-ethoxy-1-propanol	8.26 ± 0.36[a]	10.35 ± .59[c]	9.16 ± 0.31[b]	0.003	**
2-Phenylethanol	28.81 ± 2.18[a]	33.00 ± 0.46[b]	57.37 ± 1.16[c]	0.000	**
Total	433.66[a]	511.72[b]	790.25[c]	0.000	**
Terpenes					
Linalooloxide 1	14.07 ± 1.25[a]	18.92 ± 1.17[a]	48.45 ± 7.47[b]	0.000	**
Linalool	13.22 ± 3.51[a]	15.03 ± 1.00[a]	20.80 ± 7.78[a]	0.228	NS
Linalyl acetate	8.02 ± 0.34[a]	8.76 ± 0.38[a]	21.42 ± 2.62[b]	0.000	**
Citronellol	8.71 ± 0.40[a]	10.44 ± 0.73[a]	16.02 ± 1.38[b]	0.000	**
Nerol	116.21 ± 1.79[b]	122.12 ± 3.43[c]	6.31 ± 1.61[a]	0.000	**
Geraniol	1150.53 ± 102.16[c]	946.60 ± 96.06[b]	353.60 ± 51.47[a]	0.000	**
α-Ionone	8.96 ± 0.89[a]	19.62 ± 1.99[a]	41.81 ± 13.57[b]	0.006	**
B-Farnesol 2	16.60 ± 4.24[a]	14.57 ± 2.39[a]	28.04 ± 10.43[a]	0.156	NS
B-Farnesol 3	35.49 ± 3.57[a]	34.09 ± 3.62[a]	26.41 ± 16.90[a]	0.537	NS
Total	1329.52[c]	1122.11[b]	548.59[a]	0.000	**

TABLE 4 (CONTINUED)

Compounds	Control	ScBF	FoS	p-value	Significance
Esters					
Ethyl acetate	72.71 ± 3.74^{b}	65.99 ± 6.74^{ab}	56.94 ± 1.86^{a}	0.016	*
Ethyl butyrate	0.59 ± 0.02^{b}	0.52 ± 0.06^{b}	0.26 ± 0.02^{a}	0.000	**
Isoamyl acetate	4.73 ± 0.59^{b}	3.25 ± 1.12^{a}	1.81 ± 0.22^{a}	0.008	**
Ethyl hexanoate	1.36 ± 0.06^{c}	1.14 ± 0.13^{b}	0.56 ± 0.03^{a}	0.000	**
Hexyl acetate	0.14 ± 0.07	n.d.	n.d.		
Ethyl lactate	17.58 ± 0.23^{a}	18.36 ± 0.17^{a}	21.94 ± 1.46^{b}	0.017	**
Ethyl caprylate	1.60 ± 0.11^{b}	1.39 ± 0.21^{b}	0.29 ± 0.01^{a}	0.000	**
Ethyl-3-hydroxybutanoate	1.33 ± 0.02^{a}	1.37 ± 0.03^{a}	1.39 ± 0.07^{a}	0.323	NS
Ethyl caprate	0.76 ± 0.04^{b}	0.68 ± 0.10^{b}	0.18 ± 0.01^{a}	0.000	**
Diethyl succinate	1.09 ± 0.04^{a}	1.20 ± 0.05^{a}	1.44 ± 0.13^{b}	0.006	**
Ethyl phenylacetate	0.63 ± 0.01^{a}	0.67 ± 0.02^{a}	n.d.	0.038	*
2-Phenylethyl acetate	0.85 ± 0.04^{c}	0.72 ± 0.08^{b}	0.59 ± 0.03^{a}	0.002	**
Total	103.38^{c}	95.28^{b}	85.38^{a}	0.000	**
Acids					
Acetic acid	556.30 ± 20.28^{b}	511.80 ± 25.25^{b}	403.10 ± 56.53^{a}	0.006	**
Propionic acid	5.26 ± 2.44^{a}	3.59 ± 2.42^{a}	10.13 ± 9.64^{a}	0.424	NS
Isobutyric acid	1.91 ± 0.11^{a}	2.34 ± 0.07^{a}	2.64 ± 0.69^{a}	0.162	NS
Butyric acid	2.26 ± 0.05^{b}	2.13 ± 0.19^{b}	1.79 ± 0.26^{a}	0.056	NS
Isovaleric acid	2.05 ± 0.10^{a}	2.20 ± 0.02^{a}	3.37 ± 0.51^{b}	0.003	**
Valeric acid	0.39 ± 0.01^{a}	0.39 ± 0.00^{a}	0.52 ± 0.18^{a}	0.289	NS
Hexanoic acid	5.17 ± 0.20^{c}	4.49 ± 0.35^{b}	2.83 ± 0.29^{a}	0.000	**
Octanoic acid	6.67 ± 0.36^{c}	5.74 ± 0.49^{b}	2.05 ± 0.21^{a}	0.000	**
Decanoic acid	3.96 ± 0.17^{b}	3.59 ± 0.12^{b}	2.38 ± 0.60^{a}	0.004	**
Total	584.01^{b}	536.27^{b}	428.82^{a}	0.000	**
Carbonyl compounds					
Acetoin	3.12 ± 0.211^{a}	2.83 ± 0.43^{a}	5.50 ± 1.5^{b}	0.022	*
Thiols					
3MHA (ng/L)	34.76 ± 8.49^{a}	19.26 ± 14.5^{a}	n.d.	0.185	NS
3MH (ng/L)	395.71 ± 49.44^{a}	554.2 ± 32.9^{b}	365.1 ± 67.3^{a}	0.009	**
Total (ng/L)	430.47^{b}	573.50^{c}	365.11^{a}	0.000	**
TOTAL (mg/L)	2884.11^{b}	2841.57^{b}	2223.61^{a}	0.000	**

Different letters mean significant differences ($p < 0.05$) between the average values for the LSD Fisher test. Control: non-pre-fermentative macerated wines; ScBF: skin contact before fermentation; FoS: fermentation on skins.
NS: non-significant
n.d.: not detected
* Significance at $p < 0.05$
** Significance at $p < 0.01$

Almost the same concentration of volatiles was observed in the ScBF and Control wines (Table 4). These findings are not in agreement with other authors, who found a higher volatile concentration in skin contact-macerated wines (Rodriguez-Bencomo *et al*., 2008: Sanchez-Palomo *et al*., 2006; Selli *et al*., 2003; 2006a; 2006b).

Three distinct styles are often used by the South African Chenin Blanc Association when classifying Chenin blanc wines (Chenin Blanc Association, 2013). These are fresh and fruity, rich and ripe unwooded, and rich and ripe wooded. Based on the grapes' characteristics at harvest and also on the measured enological parameters (Table 2), the wines used in this study could probably be classified in the rich and ripe unwooded group.

Terpenes can contribute to floral, fruity and perfume odours in wine. Monoterpenes are regarded as typical grape varietal character impact odorants, mostly in floral varieties such as Moscatel, Riesling and Gewürztraminer (Fisher,

2007). In a study performed by Antwerpen (2012), the terpenes linalool oxidise 1, linalyl acetate, citronellol and β-farnesol 2 and 3 were identified in concentrations ranging from 9.79 to 70.29 µg/L in rich and ripe unwooded Chenin blanc wines, which is accordance with our study. Surprisingly, geraniol and nerol, compounds that showed the highest concentrations in our study, were not detected in the work reported by Antwerpen (2012). On the other hand, Lawrence (2012) analysed 11 rich and ripe unwooded SA Chenin blanc wines and geraniol and nerol were found at concentrations of between 30 and 60 µg/L, far below the levels detected in this study. Only the FoS wines showed nerol concentrations lower than the range reported by Lawrence (2012).

Interestingly, although this cultivar has been considered as containing relatively few primary grape-derived compounds (Marais, 2005), terpenes are quantitatively the largest group of volatile compounds in our Chenin blanc wines. A general classification based on terpene concentration has been proposed by Mateo and Jimenez (2000). Varieties can be classified as (1) intensely flavoured muscats (total free terpene concentration as high as 6 mg/L): (2) non-muscat but aromatic varieties (total terpene concentration of 1 to 4 mg/L) and (3) more neutral varieties not dependent on terpenes for their flavour. In this study, the total terpene concentration in the Control and ScBF wines was 1 371.81 and 1 189.34 µg/L respectively (Table 4), with these wines therefore possibly being included in the second group, defined as aromatic varieties. In contrast, the total terpene concentration in the FoS wines was below 1 mg/L (562.85 µg/L). The presence of the skins during the fermentation process had an important effect on the terpene concentration, decreasing the levels of these compounds in the finished wines. Some studies have shown that an extended skin contact period could cause a decrease in the terpene levels. Rodriguez-Bencomo *et al*. (2008) and Selli *et al*. (2006a) found that a short period of skin contact led to increases in terpene concentrations, but longer periods of up to 12 h led to decreases.

The Control wines showed significantly higher values of geraniol compared to ScBF, which were also significantly higher than in the FoS wines (Table 4). These values were also much higher than the sensory threshold value of 300 µg/L (Guth, 1997). Nerol levels were significantly higher in the ScBF wines compared to the Control wines. Moreover, nerol levels in the FoS wines were significantly the lowest. Geraniol has a sweet, rose blossom and geranium smell, while nerol has been defined as rose, floral, fruity and sweet (Swiegers *et al*., 2005). Moreover, the terpenes linalooloxide 1, linalyl acetate, citronellol and α-ionone were present at significantly higher levels in the FoS wines. These compounds have been described by terms such as lychee, green, citrus, lavender, citronella, rose, sweet, floral, violet, tropical fruit, anise, peach, honey and raspberry (Ferreira *et al*., 2000; Swiegers *et al*., 2005). The rest of the detected compounds did not show significant differences between samples.

The total concentrations of alcohols were 790.25, 511.72 and 433.66 µg/L in the FoS, ScBF and Control wines respectively (Table 4). In accordance with the results obtained by other authors, the total concentration of alcohols increased with longer maceration times (Rodriguez-Bencomo *et al*., 2008; Selli *et al*., 2006a). In contrast, Sánchez-Palomo *et al*. (2006) reported a significant decrease in the levels of fermentation alcohols due to the greater nitrogen content in skin contact samples. The regression of the Ehrlich reaction (formation pathway of higher alcohols) has been proposed as the mechanism responsible for this decrease.

Higher alcohols are associated with strong, chemical, pungent aromas as well as herbaceous notes (Gomez-Miguez *et al*., 2007; Vilanova *et al*., 2010). Isobutanol, butanol, pentanol, 3-methyl-1-pentanol and hexanol showed significantly higher levels in the FoS wines compared to the other treatments. The odour of these compounds has been defined as balsamic, solvent, alcohol, fresh and green, but also with descriptors such us fruity and sweet, depending on the final concentration in the wine (Swiegers *et al*., 2005). Higher hexanol levels are due to skin contact, when C6-aldehydes and C6-alcohols are formed from the enzymatic and chemical oxidation of fatty acid precursors extracted from the grape skins. Hexenal is reduced to hexanol during alcoholic fermentation (Callejón *et al*., 2012; Joslin & Ough, 1978). Moreover, the FoS wines showed significantly higher levels of methanol, propanol (sweet, fresh alcohol), isoamyl alcohol (nail polish) and 2-phenyl ethanol (floral, honey, lilac, rose) compared to the ScBF wines, which also were significantly higher than in the Control wines. The increased amount of methanol in the skin contact treatments could be explained by the pathway of methanol formation, since this compound is derived from the dimethylation of skin pectins, with an increase in these compounds in skin-macerated wines (Sanchez-Palomo *et al*., 2007). On the other hand, the compound 3-ethoxy-1-propanol was present at higher levels in the ScBF wines compared to the FoS wines, which also had significantly higher levels than the Control wines. The odour of this compound has been defined as blackcurrant, therefore contributing to the fruity attributes in the wine (Peinado, 2004).

In terms of esters, the skin contact treatments generally showed a decrease in concentrations compared to the Control wines. The Control wines had the highest concentration of these compounds (103.38 µg/L), followed by the ScBF wines (95.28 µg/L) and the FoS wines (85.38 µg/L). Esters are essential in imparting a fruity character to wine (Francis & Newton, 2005). These compounds are formed mainly during fermentation (Riberéau-Gayon *et al*., 2000) and are present in concentrations ranging from ng/L to mg/L (Antalick *et al*., 2010). Only the esters ethyl lactate (cream, coconut, lactic, raspberry) and diethyl succinate (fruity) had significantly higher levels in the FoS wines compared to the other treatments (Table 3). In contrast, the compound isoamyl acetate had significantly higher values in the Control wines than those observed in the ScBF and FoS wines. Moreover, the compounds ethyl butyrate (apple, strawberry, acid fruit), ethyl caprylate (pineapple, pear, floral, sweet, ripe banana) and ethyl caprate (floral, grape, fruity) were significantly lower in the FoS wines in comparison with the other treatments. Finally, the compounds ethyl hexanoate (green apple, banana, violets and strawberry) and 2-phenyl-ethyl acetate (rose, honey and tobacco) were significantly higher in the Control wines than in the ScBF wines, which also were significantly higher than in the FoS wines. As in

our study, esters are often found in wine at concentrations below their individual odour perception thresholds, but recent studies have reported how small changes in the concentration of some esters cause differences in the aroma of model wine solutions (Pineau, 2008; Pineau *et al*., 2009), which indicates the synergistic and/or suppressive effects of some combinations of wine aroma components.

Fatty acids are formed during alcoholic and malolactic fermentation by yeast and lactic acid bacteria metabolism and are associated with lactic and soapy notes (Vilanova *et al*., 2009). However, it seems that these acids play a positive role in the quality of wine aroma, as long as they are present at small concentrations (Etievant, 1991; Ortega-Heras *et al*., 2008) and below odour threshold levels (Ferreira *et al*., 2000). The total concentration of these compounds was again slightly higher in the control wines that in the skin contact-treated wines. Within the skin contact treatments, ScBF resulted in higher fatty acid levels compared to the FoS wines. Decanoic acid and acetic acid showed significantly higher levels in the Control and ScBF wines compared to the FoS wines. Moreover, hexanoic and octanoic acids were significantly higher in the Control wines than in the ScBF wines, in which they also were significantly higher than in the FoS wines. Finally, only the compound isovaleric acid was significantly higher in the FoS wines. Fatty acids have been described in the literature as rancid, butter, cheese, sweat, oily or soapy (Francis & Newton, 2005), although the low concentrations observed in the analysed wines indicates that these compounds could be making a positive contribution to the overall wine aroma.

With regard to carbonyl compounds, acetoin was detected in the Chenin blanc wines elaborated with different skin contact treatments. The presence of this compound in wines is considered unpleasant (Herraiz *et al*., 1991). Its production is mainly due to the early development of apiculate yeasts during must fermentation. The concentration of acetoin in all the treatments was far below its odour threshold (150 mg/L) given by Lopez *et al*. (1999), and therefore it cannot be considered as a negative odorant in our study.

The tropical characters in many white wines come primarily from volatile thiols, with contributions from fermentation-derived esters (Swiegers *et al*., 2009). Two of the most important volatile thiols are 3-mercaptohexan-1-ol (3MH) and 3-mercapto-hexylacetate (3MHA). The odour imparted to wine by these compounds has been defined as passion fruit, grapefruit, gooseberry and guava-type aromas (Coetzee & Du Toit, 2012; Van Wyngaardt *et al*., 2014). These volatile thiols are extremely potent, having perception thresholds of 60 ng/L for 3MH and 4 ng/L for 3MHA (Tominaga *et al*., 1996; 1998). At excessive concentrations, these volatile thiols can impart strong, sweaty aromas reminiscent of cat's urine (Swiegers *et al*., 2009).

The free form of volatile thiols is almost non-existent in grape juice and appears only during fermentation. 3MH exists in the grapes in the form of aroma-inactive, non-volatile, cysteine-bound conjugates, viz. S-3-(hexan-1-ol)-L-cysteine (Cys-3MH) (Darriet *et al*., 1995), as well as glutathionylated S-3-(hexan-1-ol)-glutathione (Coetzee & Du Toit, 2012). Another source of volatile thiols is from the conjugation of *(E)*-2-hexenal and certain sulphur compounds (Harsch *et al*., 2013). Research by Tominaga *et al*. (1998) showed that the amplification of varietal aromas during fermentation occurs by the action of yeast carbon-sulphur lyases through an enzymatic mechanism that releases the volatile thiols. In another study, carried out by Swiegers *et al*. (2006b), it was observed that 3MHA is formed from 3MH by the action of yeast acetyltransferases. It has also been showed that, as the concentration of Cys-3MH decreases during fermentation, the concentration of 3MHA increases (Dubourdieu *et al*., 2006).

The concentration of 3MH in the ScBF wines was significantly higher than in the Control and FoS wines, with no significant difference in 3MH levels between the latter two treatments. Increases in 3MH concentrations in the wines that underwent skin contact before fermentation can be due to an increase in the extraction of the cys-3MH precursor (Maggu *et al*., 2007), or because of hexenol decreases, which, as already mentioned, may serve as additional 3MH precursors (Harsch *et al*., 2013; Roland *et al*., 2011).

No significant differences were observed in 3MHA concentrations between the Control and ScBF wines, although the formation of 3MHA from 3MH was higher in the Control wines. On the other hand, 3MHA was not detected in the FoS wines. Based on the results observed we can conclude that skin contact treatment during the entire fermentation does not enhance the presence of volatile thiols (3MH and 3MHA) in the final wines. This could be due to the higher incidence of hydroxycinnamic acids and catechin oxidation to o-quinones, compounds found at higher levels in FoS wines (Table 3). In the presence of oxygen, these quinones can react with volatile thiols such as 3MH, either via Michael addition reactions or via the generation of peroxides (Coetzee *et al*., 2013; Maggu *et al*., 2007).

The composition of the wine-must medium during fermentation is proposed to be one of the possible reasons that could partially explain the decrease in the volatile levels of the FoS wines observed in the current study. Moreover, the most important flavour and aroma compounds formed from amino acids are higher alcohols and their associated esters and volatile acids. The presence of skins during fermentation could have favoured the availability of different aroma-precursor amino acids, therefore influencing the wine's final volatile composition (Styger *et al*., 2011). In addition, the presence in skin contact wines of higher available assimilable nitrogen levels could influence yeast CO_2 production, which also might cause an important volatilisation with CO_2 evolution during fermentation (Dennis *et al*., 2012). However, the loss of volatile compounds through volatilisation could not account for the total loss of volatile compounds, such as terpenes or esters, observed in this study.

Another possible reason for the observed results could be the adsorption of volatile compounds by certain macromolecules and skin components. Ferreira *et al*. (1995) reported a decrease in C6 alcohols and fatty acids in Chardonnay wines submitted to skin contact treatments due to adsorption by macromolecules and skin components. In addition, Bindon *et al*. (2010) reported interactions between grape-derived proanthocyanidins and cell wall material. The authors reported a high affinity of cell wall material for proanthocyanidin. Robinson *et al*. (2009) also detected

an interaction between wine volatile compounds and grape and wine components such as catechin, results that were in agreement with Dufour & Bayonove (1999). These findings could indicate a possible interaction between skin cell walls and different grape components, which substantiates the observation of decreased volatile concentrations in FoS wines.

In addition, it has been reported that an increase in the ethanol concentration decreases the volatilisation of volatile compounds, supressing the fruit aroma attributes in wine (Robinson *et al*., 2009). The FoS wines showed a slightly higher alcohol content than the ScBF and Control wines (Table 1). Again, this phenomenon could not solely account for the differences observed, but it could have influenced wine aroma.

Influence of skin contact treatments on sensory profile of Chenin blanc wines

To corroborate the results obtained in the previous sections, and with the aim to identify which are the descriptors that better characterise each skin contact treatment, ANOVA and PCA were performed on the sensory data of the nine analysed wines (Fig. 1 and Table 5 respectively). As can be seen in Fig. 1, a clear separation was observed between the ScBF, FoS and Control wines. The differentiation between wines is reflected in principal component 1 (PC1), which accounts for 73.3% of the variance. The FoS wines appear to be located on the left side of the plot and were associated with dry grass, raisins, honey and marmalade attributes. On the other hand, the Control and ScBF wines are located on the opposite side of the plot. The Control wines were described by attributes such as citrus, banana, pineapple, passion fruit and mint. Moreover, the ScBF wines were associated with yellow apple, stone fruit, dry fruit and cooked vegetable aromas. Principal component 2 (PC2), which accounts for 23.7% of the variance, allows for the separation between the Control and ScBF wines.

It seems that the presence of the skins throughout the fermentation decreased the tropical and fruity notes, with these wines showing significant sweet-associated aromas such as marmalade, raisins, honey and dry grass (Table 5). These findings could be explained by the increase in the concentration of higher alcohols (herbaceous odour), together with the decrease in the levels of terpenes, esters and 3MHA. The highest concentration of α-ionone (sweet, woody, tropical fruit notes), together with the higher concentration of the majority of higher alcohols, could have dominated the aroma of those wines. The presence of phenolics can also influence the aromatic perception of volatile compounds (Lund *et al*., 2009).

The FoS wines were also described by the tasters as more astringent, bitter and sour (Table 5). However, it is important to mention here that, in the preliminary screening session, the wines were not perceived negatively by the panellists regarding their general taste attributes. The presence of acids in astringent media has been shown to affect the intensity of perceived astringency (Kallithraka *et al*., 1997). An increase in the perceived astringency in the presence of tartaric acid along with polyphenolic compounds has been reported (Amerine, 1980; Guinard *et al*., 1986). The FoS wines showed lower pH and higher total acidity than the other treatments. This fact, together with the increased phenolic content of these wines, could have favoured an increased astringency, sourness and bitterness.

Differences were also observed between the Control and ScBF wines (Fig. 1). Differences in higher alcohol and volatile thiol concentrations, together with the slightly lower concentration of esters, terpenes and acids, could explain

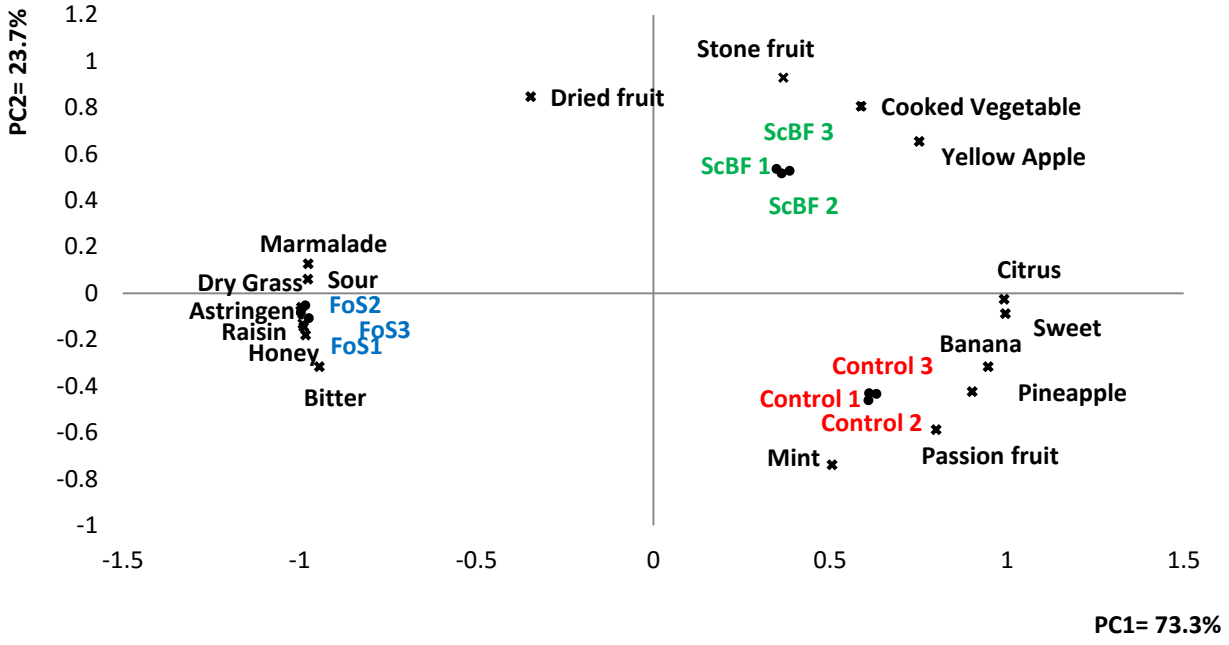

FIGURE 1

Principal component analysis bi-plot of the skin contact treatments on the sensory analysis of Chenin blanc wines
Control: wines without pre-fermentative maceration; ScBF: skin contact before fermentation; FoS: fermentation on skins

TABLE 5
Mean values and significance level of the sensory attributes identified by descriptive analysis of Control, pre-fermentative (ScBF) and fermentative macerated (FoS) Chenin blanc wines.

Attribute	Control	ScBF	FoS	p-values	Significance
Pineapple	22.03^{c}	14.80^{b}	0.00^{a}	0.000	**
Banana	10.96^{b}	7.50^{b}	0.86^{a}	0.001	**
Citrus	24.36^{b}	19.46^{b}	1.03^{a}	0.000	**
Yellow apple	2.83^{ab}	4.66^{b}	0.00^{a}	0.013	*
Passion fruit	9.50^{b}	4.13^{a}	0.00^{a}	0.003	**
Dry Grass	0.00^{a}	0.43^{a}	55.90^{b}	0.000	**
Marmalade	1.60^{a}	3.40^{a}	13.00^{b}	0.000	**
Stone fruit	1.66^{ab}	6.36^{b}	0.00^{a}	0.048	*
Honey	1.20^{a}	0.60^{a}	8.46^{b}	0.000	**
Raisin	1.00^{a}	1.13^{a}	26.73^{b}	0.000	**
Mint	1.53^{a}	1.10^{a}	1.76^{a}	0.839	NS
Cooked vegetable	7.90^{a}	15.73^{b}	1.03^{a}	0.001	**
Dried fruit	1.86^{a}	6.50^{a}	2.93^{a}	0.176	NS
Sweet	35.43^{b}	33.83^{b}	26.93^{a}	0.035	*
Sour	40.36^{a}	43.76^{a}	52.50^{b}	0.005	**
Bitter	30.36^{a}	31.16^{a}	46.70^{b}	0.000	**
Astringent	25.76^{a}	26.00^{a}	41.33^{b}	0.010	*

Different letters mean significant differences ($p < 0.05$) between the average values for the LSD Fisher test. Control: wine without pre-fermentative maceration; ScBF: skin contact before fermentation; FoS: fermentation on skins.
NS: non-significant
* Significance at $p < 0.05$
** Significance at $p < 0.01$

the differences between the wines. The concentration of the volatile thiol 3MH probably played an important role in the overall aroma of the ScBF wines, which, together with some higher alcohols (3-ethoxy-1-propanol, methanol, propanol, isoamyl alcohol, 2-phenylethanol), was conferring riper fruit attributes on these wines. The perception of fruity attributes such as stone fruit (non-significant) and dried fruit or yellow fruit (significant) has also been observed by other authors when studying the effect of skin contact treatment in Albillo wines (Sanchez-Palomo *et al*., 2007). These attributes were not perceived in the control wines in our study. Conversely, the control wines had higher intensities of pineapple and passion fruit notes. The compounds related to these flavours might be esters (isoamyl acetate, ethyl hexanoate and 2-phenylethyl acetate), terpenes (nerol and geraniol) or the volatile thiol 3MHA, which have a lower sensory threshold than 3MH (Coetzee & Du Toit, 2012). Moreover, banana and citrus descriptors were also significantly higher in the ScBF and Control wines compared to the FoS wines.

CONCLUSIONS

The duration of the skin contact treatment seemed to change the aromatic profile of Chenin blanc wines, with the appearance of riper attributes. On the other hand, the control wines without skin contact can be described as fresher, with tropical fruit notes. A pronounced decrease in volatile levels was observed as the length of the skin contact increased. Fermentation differences or adsorption due to the skin contact treatments may lead to these changes. However, the hypothesis proposed in this study must be confirmed by further studies in order to fully understand the mechanisms that influence the aromatic composition of Chenin blanc wines elaborated using different skin contact techniques.

In addition, and taking into account that a high standard of quality needs to be maintained or even improved, we have demonstrated in this study that new styles of Chenin blanc wines can be obtained using alternative winemaking techniques. A prolonged skin contact period may contribute to increased wine complexity, with the appearance of interesting new sensory attributes. Moreover, changes in mouthfeel properties, such as astringency, sourness and bitterness, are to be expected due to changes in wine composition. Finally, an increased wine ageing potential might have been achieved, which also needs further investigation.

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