Chapter

Influence of Skin-Contact Treatment on Aroma Profile of Malvasia *Aromatica* Wines in D.O. "Vinos de Madrid"

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Abstract

The effects of prefermentative cold skin-contact technique using Malvasia *aromatica* were studied as a first step to adapt to the climate change related effects in order to intensify the aroma potential of white wines of the D.O. "Vinos de Madrid" keeping the organoleptic characteristics of the region. Major volatile compounds were extracted by liquid–liquid extraction and quantified by GC-FID. Minor volatile compounds were determined by HS-SPME/GC–MS. Sensory analysis were also carried out to describe and quantify attributes of the wines. A total of 37 components were identified and quantified. Volatile components showed mixed behavior depending on the skin-contact time. Skin-contact for longer helps to enhance the floral character provided by some compounds contained in the skin, especially linalool and 2-phenyl etanol and were impact odorants of Malvasia *aromatica* wine based on odor activity values (OAVs).

Keywords: skin-contact, aroma, climate change, white wine, Malvasia *aromatica*

1. Introduction

Skin-contact treatment has been proposed as a technique to try to increase the extraction of varietal aromas from the skins in different white cultivars [1–3]. It is a technique extensively used in the production of young white wines with the aim of improving their intensity and aroma profile by transferring free and glycosidically bound aroma compounds from the grape skins to the must before fermentation begins. The compounds responsible for the varietal aromas of wines depend on grape variety, climate, and soil and will determine the quality and local character of wines. Early winemaking procedures such as skin contact and the amount of pressure applied during pressing together with temperature conditions applied, will affect the extraction of aroma compounds and their precursors into the grape juice and consequently their concentrations in the resulting wine [4–7]. In the course of maceration, the concentration of aromas may increase in the must but there are not always changes at the sensory level in the wines. The varietal characteristics of the wine may be enhanced with the skin contact, however, there is some risk of the

apparition of herbaceous aromas, bitter flavors and excessive color in the musts. For these reasons, the conditions of temperature and contact time between the skins and the juice must be carefully chosen.

The vineyard is a crop with a wide range of adaptation to different environmental and agronomic conditions whose correct development is strongly influenced by the climate. In particular, the suitability of wine-growing areas to reach optimum levels of sugar, pH, color and aromatic components, which are necessary for the production of quality wines, depends on weather conditions throughout the growing period [8, 9]. As a result, climatic fluctuations will make very difficult to produce the same kind of wine in a particular area over seasons. The wines would lose the typicity and distinction of the region being affected the local economy by the decrease of the value of the final product.

The adaptation responses to deal with climate change related effects on winemaking can be implemented at the winery level or at the vineyard level [10]. In oenology, innovations could serve to correct fluctuations in grape quality. Also, can be considered as the first strategy to protect against climate variations related effects by focusing on specific hazards in order to improve the production. These techniques include changes in winemaking practices.

Skin-contact treatment has been proposed as a first measure of adaptation to climate change related effects. This study was focused on variations skin-contact time in order to intensify the aroma potential of winemaking white wines in D.O. "Vinos de Madrid". The purpose of the present paper was to evaluate differences in white musts and wines, which would arise due to different skin-contact time using the same temperature. In particular, the aromatic and sensory characteristics of the wines. To achieve this aim we choose cv. Malvasia *aromatica*, a white grape variety of Italian origin that has been grown in Spain since the 14th century. The main characteristics of this cultivar are: from an aromatic point of view, the presence of terpenes responsible of citrus and floral aromas similar to Muscat varieties [11] and fermentation aroma compounds, mainly fatty acids and their esters, provide it with fruity aromas [3, 12, 13]. On the other hand, physical–chemical characteristics that give rise to musts with high acidity and low pH, which make it a suitable varietal for trying to improve the organoleptic quality of its white wines of D.O. "Vinos de Madrid".

2. Material and methods

2.1 Vintage

Grapes from *Vitis vinifera* L. cv. Malvasia *aromatica* were hand-collected from an experimental vineyard of the Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), located in "Finca El Socorro" in D.O. "Vinos de Madrid", Arganda del Rey, Spain (40°8'N, 3°22'W, 715 m altitude). Final harvest time was determined when berries reached 23°Brix and transported to the Experimental Winery from IMIDRA at the "Finca El Encín", in Alcalá de Henares, Spain (40°31'N, 3°17'W, 605 m altitude).

2.2 Skin-contact treatment

After harvest, grapes were divided into two batches for each assay (1 and 2). One batch was treated in the conventional way (C) without skin-contact and was used as control. In this way grapes were crushed and pressed in a hand-press and 5 g/hl of sulfur dioxide was added. The juice was then settled at 10°C for 12–18 h,

and then racked. The total acidity in the must was corrected with tartaric acid to 6 g/L. The must was racked, dividing the volume equally in three stainless steel tanks. Commercial yeast was added for its fermentation which took place at 16°C and was followed daily by measuring density. The conventional way samples (C) of each assay (1 and 2) were different from each other, they came from different grapes.

For the skin contact treatment, the grapes were destemmed and crushed. The pomace (musts and skin) was mixed 5 g/hl of sulfur dioxide, kept at 10°C for 18 h (A1) and 6 h (A2). At the end were pressed in a hand-press (M18 and M6 assays). The juice was settled, racked and divided as mentioned in the conventional way. The rest of the process was equal to the conventional way.

2.3 Physical-chemical analysis and fermentation kinetics

Oenological parameters (°Brix, free and total sulfur dioxide, pH, total acidity, volatile acidity, ethanol (% v/v) and residual sugars) were analyzed following OIV official methods [14]. Yeast assimilable nitrogen (YAN) was determined following the Sörensen method.

A daily control of temperature and density was carried out to determine the influence of pre-fermentative skin contact on the kinetics of the fermentations. Fermentation velocity (V_F) was measured checking daily the sugar percentage lost during the fermentation. On the other hand, V_{50} amount of sugar daily transformed by the yeasts when 50% of the sugar content had been used up was also evaluated [15].

2.4 Aromatic analysis of the wines

Analysis of free aroma compounds was performed by quantification of minor and mayor volatile compounds. Quantification of major volatile compounds was undertaken by GC-FID (Agilent Technologies, Santa Clara, CA, USA) with a DB-Wax column (60 mx 0.32 mm x 0.5 m) from J&W Scientific (Folsom, CA, USA) following the procedures proposed by Ortega [16]. The liquid phase extraction (LPE) of aroma compounds was performed in dichloromethane. The method conditions were: oven temperature 40°C for 5 min, then increased to 3°C/ min up to 200°C, and helium as carrier gas at 2 ml/min. Two mL of aroma extract were injected at 250°C in splitless mode. The total run time was 75 minutes per sample. Analyses were carried out in duplicate.

Minor volatile compounds (terpenoids and C_{13}-norisoprenoids) were determined by HS-SPME/GC–MS following the method proposed by Yuan & Qian [17]. A 50/30 μm DVB/CAR/PDMS fiber (Supelco Inc., Bellefonte, PA) was used for volatile extraction. 20 mL vials were used for chromatography (Agilent Technologies). Two mL of the wine sample were diluted with 8 mL of a citric acid solution (0.5 g/L citric acid, pH 3 saturated with sodium chloride) and 20 μL of 4-octanol (100 μg/L) was used as internal standard were added with a small magnetic stir bar. The vials were capped and equilibrated at 50°C in a thermostatic bath for 10 min. The aromatic compounds were extracted through SPME fiber for 50 min at 50°C with stirring (1000 rpm). The fiber was inserted into the injection port of the GC (230°C) to desorb the compounds. The injection into the chromatograph was manual. An Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass selective detector (Agilent, Santa Clara, CA) was used. Compound separation was achieved with a DB-WAX de J&W Scientific (Folsom, CA, USA) (60 m x 0.32 mm x 0.5 μm film thickness, Phenomenex, Torrance, CA). A constant helium column

flow rate of 1.0 mL/min was used. The chromatographic program was set at 40°C for 3 min, raised to 230°C at 5°C/min for 15 min. Splitless injection mode was used.

2.5 Sensory analysis

Descriptive sensory analyses were performed by a trained panel of 8 people (4 expert tasters and 4 habitual consumers) from the IMIDRA Institute. This panel had been previously trained in the recognition of wine flavor. Sensory descriptive analysis was performed to describe and quantify attributes of the wines based on a scale from 1 (low intensity) to 10 (high intensity). A hedonic classification was also carried out establishing the order of preference of the samples presented. The final score was obtained as the mean of the wine evaluations with their respective standard deviation and interpreted by graphical representation.

2.6 Statistical analyses

The statistical processing of the data was carried out with software SPSS ver. 20.0 (SPSS, Inc., Chicago, USA). Analysis of variance (ANOVA) was applied on oenological parameters, volatile compounds and sensory attributes of the wines. Tukey HSD post-hoc tests were used to establish the significance of differences between means to assess significance ($p < 0.05$).

3. Results and discussion

3.1 General must and wine composition

General composition of must obtained with the two skin-contact treatment and conventional way from cv. Malvasia *aromatica* are given in **Table 1**. In the skin-contact treatment assays, the total acidity of the must decreases along with a slight increase in pH. This is due to the transfer of cations from the skin to the must during the previous maceration stage, and results in a decrease of acidity in the form of potassium bitartrate together with a salification of the acids [18]. The results show an increase in Yeast assimilable nitrogen (YAN) according to the time of contact with the skin, being more notable with M18; M6 did not cause variations in YAN content. These results are in agreement with the studies carried out by other authors, where a period of contact with the skin favors the enrichment of the musts in terms of amino acid content [19, 20]. In general, the effect of skin-contact in both assays is not very pronounced, which could be related to the low temperature

	A1		A2	
	C	M18	C	M6
°Brix	23.2 ± 0.1	23.1 ± 0.1	21.4 ± 0.1	20.3 ± 0.1
pH	3.20 ± 0.0	3.23 ± 0.0	3.25 ± 0.0	3.26 ± 0.0
Total acidity [a] ($g\ l^{-1}$)	5.9 ± 0.0	5.7 ± 0.0	5.7 ± 0.0	5.4 ± 0.0
YAN [b] ($mg\ l^{-1}$)	135.0 ± 0.0	151.3 ± 0.0	109.9 ± 6.4	105.0 ± 12.0

[a] *As tartaric acid.*
[b] *Yeast assimilable nitrogen (YAN).*

Table 1.
General composition of must obtained with different treatment: Conventional (C) and skin-contact treatment: Assay 1 (A1), 18 h (M18) and assay 2 (A2), 6 h (M6).

(10°C) and the time of contact, compared to other studies on white varieties (15.5°C, 20°C and 24°C [21]).

3.2 Fermentation kinetics

Figure 1 shows the average of the fermentative kinetics evolution of Malvasia musts at 10°C. Skin contact for 6 hours does not influence the development of fermentation (a), no differences were found in terms of fermentation time and velocity between vinifications (**Table 2**). However, in the case of skin-contact for 18 hours (b), there are differences in the time and velocity of fermentation compared to the conventional one. The macerated must concludes its fermentation almost a week before the conventional one. This fact may be related to the YAN content and its high content of nutrients and fermentation activators, which seem to have a strong influence on the process (see **Table 1**).

General composition of wines obtained with skin-contact treatment and conventional way from cv. Malvasia *aromatica* are given in **Table 3**. Wines from skin contact treatments had lower values for total acidity. There was no significant difference for any quality parameter that is in accordance with research published

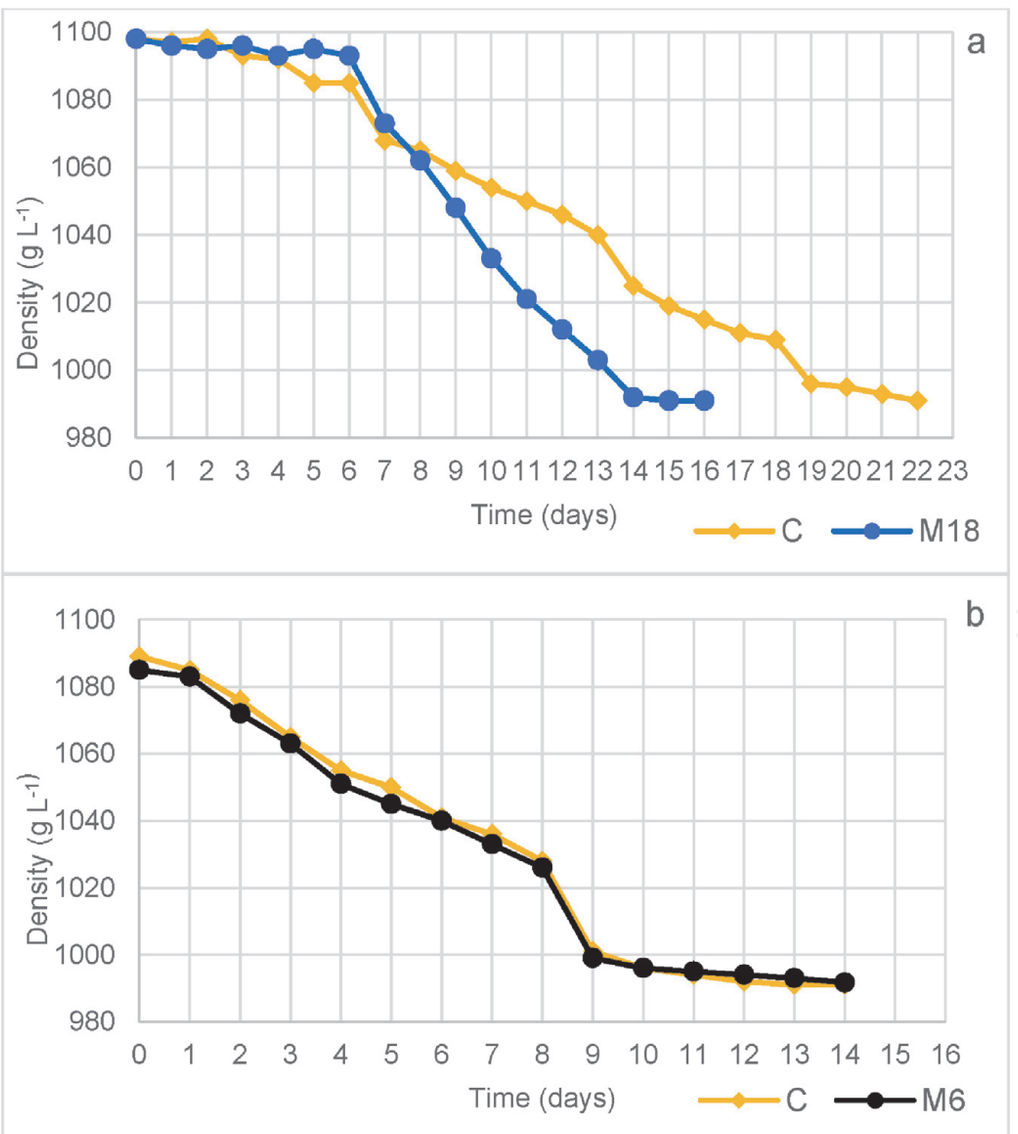


Figure 1.
Fermentative kinetics evolution of Malvasia musts. (a) Assay 1, skin-contact 18 h (M18). (b) Assay 2, skin-contact 6 h (M6).

	Treatment	V_{50} (%)	V_f (%)
A1	C	8.3	4.5
	M18	11.1	6.7
A2	C	16.7	7.7
	M6	16.7	7.1

V_{50}: amount of sugar daily transformed when 50% of the sugar content had been used up; V_f: Fermentation velocity (daily sugar % lost).

Table 2.
Influence of skin-contact on fermentation velocity.

	A1		A2	
	C	M18	C	M6
Ethanol (% v/v)	13.0 ± 0.1	12.9 ± 0.1	13.8 ± 0.1	13.0 ± 0.1
pH	3.20 ± 0.0	3.18 ± 0.0	2.95 ± 0.0	2.90 ± 0.0
Total acidity [a] ($g\ l^{-1}$)	7.1 ± 0.0	6.6 ± 0.0	6.3 ± 0.0	6.4 ± 0.0
Volatile acidity [b] ($mg\ l^{-1}$)	—	0.2 ± 0.0	0.5 ± 0.1	0.5 ± 0.0
Residual sugar ($g\ l^{-1}$)	2.8 ± 0.3	2.8 ± 0.0	1.3 ± 0.0	1.1 ± 0.1

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

Table 3.
General composition of wines obtained with different treatment: Conventional (C) and skin-contact treatment: Assay 1 (A1), 18 h (M18) and assay 2, 6 h (M6).

studies [22–24]. As explained in point 2.2, the conventional way samples were different from each other, hence the difference in ethanol content.

3.3 Influence on aroma compounds

Varietal aromas from grapes, terpenols and C-13 and those from fermentation were determined. The aromatic compounds have been grouped by aromatic families: terpenols, C13, alcohols, lactones, acids, esters, aldehydes and ketones (**Table 4**). These were 37 aromatic compounds studied from the three processing methods together with an analysis of variance to determine the influence of two maceration times (18 hours and 6 hours) on the total volatile content. In addition, the real contribution of each compound to the aroma of the wine was measured by the corresponding perception thresholds.

Table 5 shows the odor threshold values (OTH) and their sensory descriptors for those compounds with odor activity values (OAVs) >1, which actively contribute to the aroma of the wines.

In both assays, skin contact treatment increased the total concentration of volatiles in wines compared to the control wine. From the A1, the control and M18 wines contained 303.9 and 413.9 mg/L and from A2, the control and M6 309.9 and 318.1 mg/L of volatiles, respectively. Similar results were found by other authors [6, 28] on different varieties. Also, in a study carried out using a period of contact between the skins and the must of the Narince grape variety resulted in an increase of the aromatic content of the wines subjected to maceration [29].

Higher alcohols were the most abundant family of volatile compounds in the four winemaking processes, contributing more than 90% of the total volatile

	A1			A2		
Compounds	**C**	**M18**	**Sig.[a]**	**C**	**M6**	**Sig.[a]**
Terpenols (μg l^{-1})						
β-Myrcene	1.26 ± 0.04	1.49 ± 0.18	Ns	1.03 ± 0.19	0.75 ± 0.09	*
α-Terpinene	0.22 ± 0.01	0.17 ± 0.01	Ns	0.13 ± 0.04	0.12 ± 0.03	Ns
Limonene	0.52 ± 0.03	0.36 ± 0.07	Ns	0.34 ± 0.04	0.24 ± 0.03	*
γ-Terpinene	1.59 ± 0.09	1.46 ± 0.14	Ns	1.04 ± 0.20	0.76 ± 0.11	Ns
Linalool	78.75 ± 2.25	98.12 ± 11.59	*	39.77 ± 8.67	46.83 ± 9.30	Ns
α-Terpineol	15.42 ± 1.38	17.37 ± 1.96	Ns	10.83 ± 2.43	8.83 ± 1.94	Ns
β-Citronellol	6.07 ± 0.45	26.54 ± 5.31	**	2.60 ± 0.27	4.04 ± 0.76	*
Geraniol	9.83 ± 0.25	16.03 ± 3.01	*	5.60 ± 1.15	6.00 ± 1.05	Ns
Total	*113.66 ± 2.48*	*161.55 ± 21.53*		*61.35 ± 12.13*	*67.57 ± 12.17*	
C_{13}-norisoprenoids (μg l^{-1})						
β-Damascenone	1.76 ± 0.10	0.94 ± 0.04	***	1.38 ± 0.22	1.28 ± 0.24	Ns
Total	*1.76 ± 0.10*	*0.94 ± 0.04*		*1.38 ± 0.22*	*1.28 ± 0.24*	
Alcohols (mg l^{-1})						
Isobutanol	26.81 ± 1.12	25.81 ± 2.94	Ns	14.66 ± 1.33	13.92 ± 2.03	Ns
1-Butanol	0.69 ± 0.01	0.60 ± 0.04	*	0.38 ± 0.04	0.31 ± 0.06	Ns
Isoamyl alcohol	225.17 ± 7.34	288.80 ± 29.51	*	212.63 ± 8.85	213.56 ± 25.11	Ns
1-Hexanol	1.06 ± 0.05	0.62 ± 0.24	*	0.65 ± 0.02	0.84 ± 0.08	Ns
Cis-3-hexen-1-ol	0.47 ± 0.03	0.28 ± 0.11	*	Tr	Tr	
Methionol	1.23 ± 0.16	3.28 ± 0.66	**	0.80 ± 0.06	0.81 ± 0.13	Ns
Bencylalcohol	0.29 ± 0.03	0.03 ± 0.00	Ns	0.00 ± 0.00	0.00 ± 0.00	Ns
2-Phenylethyl alcohol	33.49 ± 5.20	75.14 ± 26.35	*	56.30 ± 6.99	60.55 ± 7.36	Ns
Total	*289.20 ± 5.06*	*394.56 ± 51.10*		*285.42 ± 6.51*	*289.99 ± 34.74*	
Lactones (mg l^{-1})						
y-Butyrolactone	0.49 ± 0.10	0.91 ± 0.14	*	1.67 ± 0.22	2.53 ± 0.37	*
Total	*0.49 ± 0.10*	*0.91 ± 0.14*		*1.67 ± 0.22*	*2.53 ± 0.37*	
Fatty acids (mg l^{-1})						
Isobutyric acid	0.63 ± 0.03	0.27 ± 0.01	*	2.26 ± 0.10	1.99 ± 0.20	Ns
Butyric acid	Tr	Tr		0.24 ± 0.01	0.26 ± 0.02	Ns
Isovaleric acid	0.89 ± 0.04	2.06 ± 0.99	Ns	2.88 ± 0.16	2.81 ± 0.23	Ns
Hexanoic acid	2.11 ± 0.39	1.49 ± 0.47	Ns	2.95 ± 0.21	3.68 ± 0.43	*
Octanoic acid	1.85 ± 0.30	1.27 ± 0.25	*	4.35 ± 0.25	6.34 ± 0.99	*
Decanoic acid	0.14 ± 0.01	0.14 ± 0.00	Ns	0.43 ± 0.02	0.63 ± 0.02	*
Total	*5.62 ± 0.67*	*5.22 ± 0.64*		*13.11 ± 0.60*	*15.70 ± 1.85*	
Esters (mg l^{-1})						
Ethyl butirate	0.19 ± 0.02	0.10 ± 0.00	**	0.67 ± 0.04	0.53 ± 0.07	Ns
Ethyl isovalerate	0.46 ± 0.02	0.38 ± 0.08	Ns	0.33 ± 0.04	0.41 ± 0.08	Ns
Isoamyl acetate	0.76 ± 0.07	0.54 ± 0.10	*	4.11 ± 0.68	4.58 ± 0.44	Ns
Ethyl hexanoate	0.29 ± 0.02	0.74 ± 0.13	*	0.40 ± 0.04	0.47 ± 0.06	Ns

	A1			A2		
Compounds	**C**	**M18**	**Sig.[a]**	**C**	**M6**	**Sig.[a]**
Hexyl acetate	0.08 ± 0.02	0.03 ± 0.00	*	0.10 ± 0.01	0.12 ± 0.02	Ns
Ethyl lactate	1.24 ± 0.03	0.03 ± 0.00	Ns	1.46 ± 0.05	1.56 ± 0.14	Ns
Ethyl octanoate	0.15 ± 0.00	0.15 ± 0.00	Ns	0.72 ± 0.09	0.73 ± 0.13	Ns
Ethyl 3-Hidroxy-butirate	0.11 ± 0.01	0.17 ± 0.03	*	0.18 ± 0.02	0.19 ± 0.02	Ns
Diethyl succinate	0.24 ± 0.05	0.18 ± 0.00	Ns	0.11 ± 0.01	0.08 ± 0.02	Ns
2-Phenylethyl acetate	1.55 ± 0.17	1.72 ± 0.32	Ns	0.79 ± 0.07	0.89 ± 0.11	Ns
Total	*5.07 ± 0.18*	*4.03 ± 0.31*		*8.87 ± 0.87*	*9.56 ± 0.69*	
Carbonyl compounds (mg l^{-1})						
Diacetyl	Tr	Nd		0.32 ± 0.02	0.23 ± 0.02	**
Acetoín	3.42 ± 0.20	8.73 ± 2.03	*	Tr	Tr	
Benzaldehíde	Tr	0.29 ± 0.01	***	Tr	Tr	
Total	*3.42 ± 0.20*	*9.02 ± 2.03*		*0.32 ± 0.02*	*0.23 ± 0.02*	
Total (mg l^{-1})	***303.91 ± 5.72***	***413.91 ± 49.25***		***309.45 ± 32.54***	***318.07 ± 37.61***	

*[a]Significance at which means differ as shown by analysis of variance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Ns: not significant; Nd: non detected; Tr: traces.*

Table 4.
Effect of skin contact on the aroma compound levels of Malvasia aromatica *wines.*

content analyzed. Higher alcohols, in quantities below 300 mg/L can contribute to improving the aromatic complexity of white wines, however are considered to be a negative factor in terms of aromatic quality when they exceed 400 mg/l [30]. Isobutanol, isoamyl alcohol and 2-phenylethanol were the most abundant in the four wines analyzed. Among the higher alcohols, M18 has increased the levels of 2-phenylethanol being 5.3 (**Table 5**). This compound is related to floral aromas with attributes of roses and is considered to contribute positively to wine aroma [31]. There has been a significant decrease of 1-hexanol y cis-3-hexen-1-ol in M18 wines in comparison to the control. These compounds are related to herbaceous aromas and bitter taste so are unfavorable to wine quality. Skin contact treatment for 18 h resulted in significant increase in the concentration of the esters ethyl 3-hydroxybutyrate and ethyl hexanoate esters, however, the concentrations of ethyl butyrate, isoamyl acetate and hexyl acetate decreased with the maceration time. Esters are very important for the aroma of wine, they are related to fruity aromas [32]. Due to their high OAVs (**Table 5**), ethyl butyrate (apple), ethyl isovalerate (orange), isoamyl acetate (banana), ethyl hexanoate (green apple) and 2-phenylethyl acetate (flowers) should be considered as important contributors to the typical aroma of Malvasia wines. In the case of M6 no differences were found on any of the esters studied so we can conclude that maceration for a reduced period of time has not affected the ester content of the resulting wines.

Eight terpenes were identified in the wines, among them, linalool, β-citronelol and geraniol increased significantly with M18 while with M6 only β-citronelol increased significantly. Ninety percent of geraniol is in the skins, while linalool is distributed 50% between the skin and 50% in the pulp [33, 34]. Other authors [35] reported high concentrations of geraniol and its derived products throughout the ripening process in Malvasia grapes. Only linalool reached concentrations above its odor threshold in all wines, with the highest significant extraction in M18 wines.

	Sensory descriptor	OTH*	[a] OAV			
			C	M18	C	M6
Linalool	Floral, citric	25[b]	3.15	3.92	1.59	1.87
β-damascenone	Floral, lilac	0.05[c]	35.20	18.70	27.50	25.60
Isoamyl alcohol	Bitter	30[b]	7.50	9.63	7.09	7.12
Cis-3-hexen-1-ol	Herbaceus	0.4[b]	1.10			
Methionol	Onion, cauliflower	1[b]	1.20	3.30		
2-Phenylethyl alcohol	Roses	14[b]	2.40	5.30	4.02	4.32
Butyric acid	Cheese	0.17[b]				1.50
Isovaleric acid	Blue cheese	0.03[b]	29.60	68.60	96.00	93.60
Hexanoic acid	Cheese	0.42[b]	5.00	3.50	7.00	8.80
Octanoic acid	Butter, sour	0.50[b]	3.70	2.50	8.70	12.70
Ethyl butirate	Acid fruit, apple	0.02[b]	9.47	1.90	33.57	26.62
Ethyl isovalerato	Sweet fruit, orange, blackberry	0.003[b]	152.11	127.01	109.63	135.77
Isoamyl acetate	Banana	0.03[b]	25.46	18.00	136.85	152.58
Ethyl hexanoate	Fruit, Green apple	0.01[b]	29.37	73.71	39.67	46.91
Ethyl octanoate	Fruit, grapefruit	0.58[c]			1.24	1.25
2-Phenylethyl acetate	Floral, honey	0.25[b]	6.20	6.89	3.18	3.58
Diacetyl	Butter	0.10[c]			3.18	2.26

**OTH: Odor threshold values.*
[a]OAV: Odor activity values calculated by dividing concentration by odor threshold value of the compound. OTH and OAV are given in mg l^{-1} except linalool and β-damascenone which are in μg l^{-1}. Sensory descriptor according to:
[b][25, 26].
[c][27].

Table 5.
Odor threshold values and odor activity values of the volatile compounds with the greatest influence on the aroma of Malvasia wines from the two skin contact treatment (A1: C-M18; A2: C-M6).

This terpene gives the wine floral and citrus notes (**Table 5**) typical of Muscat because it is one of the main compounds involved in the typical aromas of this variety [36]. Similar results were found by other authors in wines from white varieties for this family of compounds [23, 37].

β-damascenone was the only compound from the C13-norisoprenoid family found in Malvasia wines. The concentration of this compound decreases with skin-contact time, showing a significant decrease in M18 wine. C13 come from the carotenoids degradation and the hydrolysis of their glycosylated forms. In young wines they are usually present in the form of glycoconjugates [38, 39]. According to the OAVs, in all wines β-damascenone is above its perception threshold and should be considered as an important compound in the aroma of Malvasia wines (**Table 5**). Provides floral aromas with lilac attributes [17]. Other authors agree with these results for this variety [40].

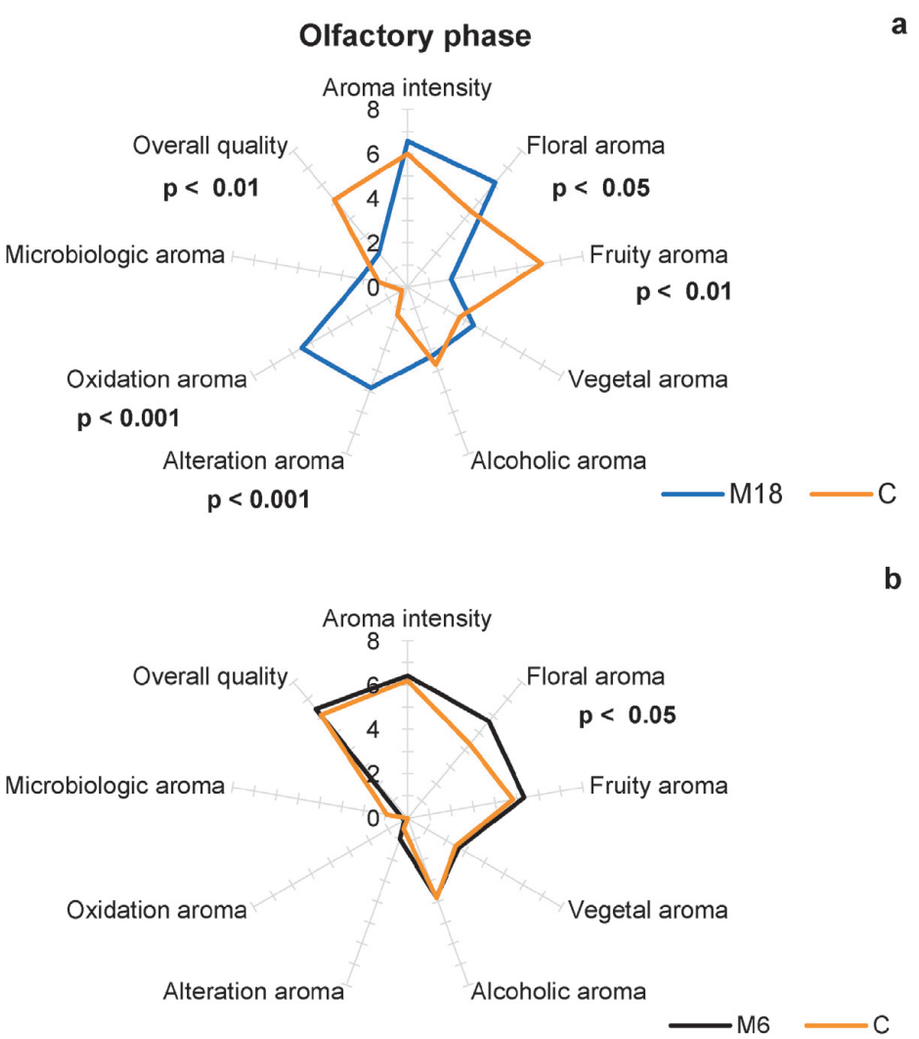
The most abundant fatty acids in the wines were hexanoic and octanoic acid (**Table 4**). These results are in agreement with those found by other authors [3, 41, 42]. The maceration seems to have different effects depending on the compound and the contact time between the skin and the must. In the case of M18 wines, the total concentration of fatty acids decreases, being particularly significant in octanoic acid. In M6 wines the total concentration of fatty acids increased significantly for hexanoic,

octanoic and decanoic acid regards to the conventional way. In all wines, regardless of the increase or decrease produced as a result of skin-contact, isovaleric, hexanoic and octanoic acids have OAVs >1 so must to be accounted in the aroma of Malvasia wines (**Table 5**). Regarding the group of aldehydes and ketones, it is known that alterations due to oxidation processes, imply the appearance of unpleasant aromas (cooked vegetables) related to the presence of compounds such as benzaldehyde, acetoin, hexanal, methional etc. [43]. Acetoin and benzaldehyde were detected in the control and M18 wines, with a significant increase in both with the maceration process ($p < 0.05$ and $p < 0.001$ respectively). According to [44] on the Verdejo grape variety, the presence of acetoin in white wines is considered negative for the flavor. In both cases, acetoin and benzaldehyde concentrations are below their perception threshold 150 mg/L [45] and 5 mg/L [46].

The two treatments (M18 and M6) significantly increased the concentration of γ-butyrolactone respect to the conventional way but in all cases it was far from its OTH (35 mg/L [47]).

3.4 Influence on sensory profile of wines

Wines were evaluated using descriptive and preference tests. The olfactory phase of the Malvasia wines from assay 1 (A) and assay 2 (B) is shown in **Figure 2**. The macerated wine (a) 18 hours had a higher score in the descriptors of altered



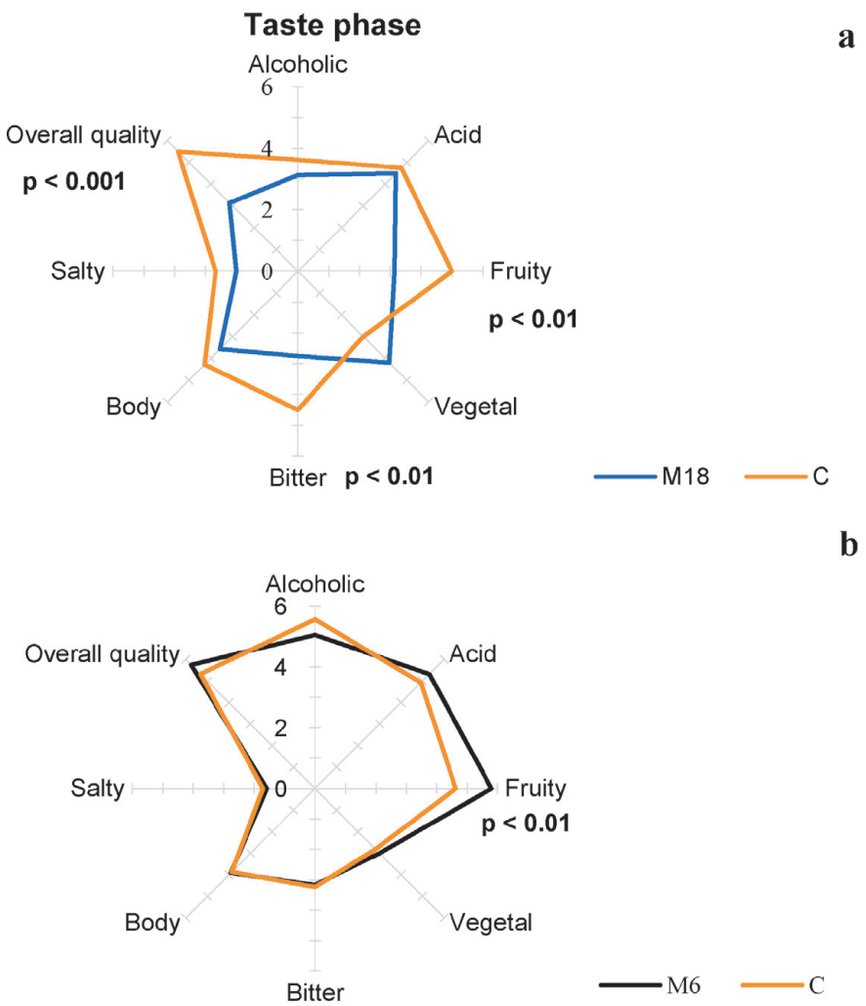
Tukey test with significance at which means differ as shown by analysis of variance: $p < 0.05$; $p < 0.01$; $p < 0.001$.

Figure 2.
Olfactory phase for the sensory analysis of the Malvasia wines from assay 1 (a) and assay 2 (b).

aroma due to problems during the conservation process of the M18 wine. Tasters also indicated oxidation aromas in M18 sample with a significance level of $p < 0.001$. The conventional wine in assay 1 was scored positively on overall aroma quality and fruity character ($p < 0.01$). In spite of the above-mentioned defects, the M18 wine received the highest score in floral character, being significantly superior to the control wine. This fact is in consonance with the results obtained in the aroma profile of these wines (see **Table 4**). In **Figure 2(b)**, M6 wines score higher in terms of fruit and floral aromatic intensity ($p < 0.05$). The rest of the parameters obtained similar scores regarding their control.

Figure 3 contain graphs of the taste using different winemaking methods. The results of the taste evaluation of in assay 1 (a), show significant differences in favor of C wine in overall taste quality ($p < 0.001$), bitterness ($p < 0.01$) and fruity character ($p < 0.01$). This could be related to the oxidation suffered by the M18. In case of assay 2, M6 wine (b) received the highest score in the fruity character with respect to the control ($p < 0.01$). This fact may be related to the release of varietal aromas through the hydrolysis of aromatic precursors by the enzymatic activity over the period of conservation in the bottle.

In the preference test, in A1 the preferences were shared between the M18 and C wines. The most preferred wine was the one produced with a 6 h skin contact treatment in the A2.



Tukey test with significance at which means differ as shown by analysis of variance: $p < 0.05$; $p < 0.01$; $p < 0.001$.

Figure 3.
Taste phase for the sensory analysis of the Malvasia wines from assay 1 (a) and assay 2 (b).

4. Conclusions

This first study in order to combat climate change related effects, the aromatic profile of Malvasia wines winemaking with different skin-contact time shows some relevant conclusions. Volatile components showed mixed behavior depending on the skin-contact time. Some compounds increased in concentration with time, while others decreased. Skin-contact for longer helps to enhance the floral character provided by the terpenols contained in the skin, especially linalool, major alcohols such a 2-phenylethanol. It also helps the increase of some esters (ethyl 3-hydroxy butyrate, ethyl hexanoate and 2-phenylethyl acetate) and the loss of others (isoamyl acetate, ethyl isovalerate and ethyl butyrate), all related to the fruity character of the wines. Short skin-contact does not cause significant effect on the content of terpenols, or ester content. The β-damascenone remains constant during M6 period, on the contrary, decreases significantly in case of M18. In general, the results of the sensory analysis show a preference for wines macerated for 6 hours. The wines macerated for 18 hours highlighted their floral character. The skin-contact process needs more studies at different time periods to optimize the aromatic potential of the grape and wine and oenological and conservation conditions of the wine.

Acknowledgements

Juan M. Cabellos acknowledges the INIA for the pre-doctoral fellowships FPI-INIA 2012. Authors acknowledge the IMIDRA, Finca El Socorro for providing material of Malvasia *aromatica* used in this work and Ministry of Science and Innovation, State Research Agency (EI) and FEDER for financial support for the RTI2018-101085-R-C31 project.

Conflict of interest

The authors declare no conflict of interest.

Acronyms and abbreviations

D.O.	Origin Appellation
YAN	Yeast assimilable nitrogen
DVB/CAR/PDMS	divinylbenzene/carboxen polydimethylsiloxane
GC-FID	gas chromatography with flame-ionization detection
GC/MS	gas chromatography–mass spectrometry
LPE	liquid phase extraction
HS-SPME	Headspace-solid phase microextraction
C13	C13-norisoprenoids

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References

[1] Castro Vázquez L, Pérez-Coello MS, Cabezudo MD. Effects of enzyme treatment and skin extraction on varietal volatiles in Spanish wines made from Chardonnay, Muscat, Airén, and Macabeo grapes. Anal Chim Acta. 2002; 458(1):39–44.

[2] Sánchez-Palomo E, Pérez-Coello MS, Díaz-Maroto MC, González-Viñas MA, Cabezudo MD. Contribution of free and glycosidically-bound volatile compounds to the aroma of muscat "a petit grains" wines and effect of skin contact. Food Chem. 2006;95(2):279–89.

[3] Chacon J, Martínez J, García E. Comportamiento de la variedad Malvasía Aromática en el centro de la península ibérica. In: III Simposio Internacional "Malvasías" [Internet]. La Palma, Islas Canarias, España; 2009 [cited 2017 Jan 22]. Available from: http://pagina.jccm.es/ivicam/investigacion/congresos/2009/lapalma.htm

[4] Ramey D, Bertrand A, Ough C. Effects of skin contact temperature on Chardonnay must and wine composition. Am J Enol Vitic [Internet]. 1986 [cited 2017 Feb 6];37(2):99–106. Available from: http://www.ajevonline.org/content/37/2/99.short

[5] Marais J, Rapp A. Effect of skin-contact time and temperature on juice and wine composition and wine quality. South African J Enol Vitic [Internet]. 1988 [cited 2017 Feb 6];9(1):22–30. Available from: https://dialnet.unirioja.es/servlet/articulo?codigo=5607506

[6] Falqué E, Fernández E. Effects of different skin contact times on Treixadura wine composition. Am J Enol Vitic [Internet]. 1996 [cited 2017 Feb 6];47(3):309–12. Available from: http://www.ajevonline.org/content/47/3/309.short

[7] Garcia-Romero E, Perez-Coello M, Cabezudo MD, Sanchez-Munoz G, Martin-Alvarez PJ. Fruity flavor increase of Spanish Airen white wines made by brief fermentation skin contact / Aumento del aroma afrutado de los vinos blancos Airen fermentados en presencia de hollejos. Food Sci Technol Int [Internet]. 1999 Jan 1 [cited 2017 Feb 6];5(2):149–57. Available from: http://fst.sagepub.com/cgi/doi/10.1177/108201329900500204

[8] Adams D. Phenolics and ripening in grape berries. Am J Enol Vitic [Internet]. 2006 [cited 2017 Jan 21];57: 249–56. Available from: http://www.ajevonline.org/content/57/3/249.short

[9] Orduña R. Climate change associated effects on grape and wine quality and production. Food Res Int [Internet]. 2010 [cited 2017 Jan 22];43(7):1844–55. Available from: http://www.sciencedirect.com/science/article/pii/S0963996910001535

[10] Nicholas K, Durham W. Farm-scale adaptation and vulnerability to environmental stresses: Insights from winegrowing in Northern California. Glob Environ Chang [Internet]. 2012 [cited 2017 Feb 6];22(2): 483–94. Available from: http://www.sciencedirect.com/science/article/pii/S0959378012000131

[11] Cabello F, Ortiz J, Muñoz G, Rodríguez I, Benito A. Variedades de vid en España [Internet]. 1st ed. Madrid, España: Agrícola Española; 2012 [cited 2017 May 14]. 504 p. Available from: https://scholar.google.es/scholar?q=Cabello%2C+F.%2C+Ortiz%2C+J.M.%2C+Muñoz%2C+G.%2C+Rodriguez%2C+I.%2C+Benito%2C+A.%2C+Rubio+de+Miguel%2C+C.%2C+García%2C+S.%2C+Sáiz%2C+R.+%282011%29.Variedades+de+vid+en+España.+Editorial+Agrícola

[12] Amerine M, Roessler E. Composition of wines. In: M.A. Amerine

and E.B. Roessler, editor. Wines Their sensory evaluation [Internet]. New York: W.H Freeman; 1976 [cited 2017 May 14]. p. 72–7. Available from: https://scholar.google.es/scholar?q=Amerine%2C+M.A.%2C+and+E.B.+Roessler.+%281976%29.+Composition+of+wines.+In+Wines-Their+Sensory+Evaluation%2C+M.A.+Amerine+and+E.B.+Roessler+%28Eds.%29%2C+pp.+72-77.+W.H.+Freeman%2C+New+York.&btnG=&hl=es&as_sdt=0%2C5

[13] Flanzy C, Bayonove C, Günata Z, Baumes RL, Crouzet J. Aromas. In: Ediciones AV, editor. Enología: fundamentos científicos y tecnológicos [Internet]. 1a. ed. Madrid, España: Mundi Prensa; 2000 [cited 2017 May 14]. p. 137–76. Available from: https://dialnet.unirioja.es/servlet/articulo?codigo=589744

[14] OIV. Official methods for the analysis of musts and wines of the international organisation of vine and wine (OIV). Methods of analysis of wines and musts. (OIV-MA-INT-00-2012). [Internet]. 2012. Available from: http://www.oiv.int/oiv/infoenmethodesinternationalesvinenmethodesinternationalesvin

[15] Cordero-Bueso G, Esteve-Zarzoso B, Gil-Díaz M, García M, Cabellos J, Arroyo T. Improvement of Malvar Wine Quality by Use of Locally-Selected *Saccharomyces cerevisiae* Strains. Fermentation [Internet]. 2016 Mar 14 [cited 2017 Mar 8];2(1):7. Available from: http://www.mdpi.com/2311-5637/2/1/7

[16] Ortega C, López R, Cacho J, Ferreira V. Fast analysis of important wine volatile compounds: Development and validation of a new method based on gas chromatographic–flame ionisation detection analysis. J Chromatogr A [Internet]. 2001 [cited 2017 Feb 6];923(1):205–14. Available from: http://www.sciencedirect.com/science/article/pii/S0021967301009724

[17] Yuan F, Qian MC. Development of C13-norisoprenoids, carotenoids and other volatile compounds in *Vitis vinifera* L. Cv. Pinot noir grapes. Food Chem [Internet]. 2016 Feb;192:633–41. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0308814615010602

[18] Flanzy C. Enología: Fundamentos científicos y tecnológicos [Internet]. 1st ed. Ediciones AV, editor. Madrid, España: Mundi Prensa; 2000 [cited 2017 Jan 27]. 708 p. Available from: https://dialnet.unirioja.es/servlet/libro?codigo=1670

[19] Aldave L. Effects de la macération pelliculaire sur cépages Vermentino et Semillon. Rev française d'oenologie [Internet]. 1992 [cited 2017 Mar 4];32(135):9–15. Available from: http://cat.inist.fr/?aModele=afficheN&cpsidt=5247453

[20] Sánchez-Palomo E, González-Viñas MA, Díaz-Maroto MC, Soriano-Pérez A, Pérez-Coello MS. Aroma potential of Albillo wines and effect of skin-contact treatment. Food Chem [Internet]. 2007 Jan;103(2):631–40. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0308814606007072

[21] Lateyron C. Vinification et etude de la maceration des moQts blancs dans une cave Californienne [Internet]. 1983 [cited 2017 Mar 4]. Available from: https://scholar.google.es/scholar?hl=es&q=Lateyron%2C+C.+Vinification+et+etude+de+la+maceration+des+moQts+blancs+dans+une+cave+Californienne.+Thesis%2C+Institute+of+Enology%2C+University+of+Bordeaux+II+%281983%29.+&btnG=&lr=

[22] Peinado RA, Moreno J, Bueno JE, Moreno JA, Mauricio JC. Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. Food Chem. 2004;84(4):585–90.

[23] Cabaroglu T, Canbas A, Baumes R, Bayonove C, Lepoutre JP, Gunata Z. Aroma Composition of a White Wine of *Vitis vinifera* L. cv. Emir as Affected by Skin Contact. J Food Sci [Internet]. 1997 Jul [cited 2017 Feb 27];62(4):680–3. Available from: http://doi.wiley.com/10.1111/j.1365-2621.1997.tb15434.x

[24] Korenika AMJ, Prusina T, Ivić S. Influence of Cold Maceration Treatment on Aromatic and Sensory Properties of Vugava Wine (*Vitis vinifera* L.). J Microbiol Biotechnol Food Sci. 2020;10 (1):49–53.

[25] Ferreira V, Ortín N, Escudero A, López R. Chemical characterization of the aroma of Grenache rose wines: Aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. J Agric Food Chem [Internet]. 2002 [cited 2017 Jan 27];50(14):4048–54. Available from: http://pubs.acs.org/doi/abs/10.1021/jf0115645

[26] Etievant P. Wine. In: Maarse H, editor. Volatile compounds in foods and beverages [Internet]. New York: Marcel Dekker; 1991 [cited 2017 Feb 27]. p. 483–546. Available from: https://scholar.google.es/scholar?hl=es&as_sdt=0,5&q=Etievant,+P.+X.+(1991).+Wine.+In+H.+Maarse+(Ed.),+Volatile+Compounds+in+Food+and+Beverages+(pp.+483–546).+Newyork%3A+Marcel+Dekker.

[27] Guth H. Quantitation and Sensory Studies of Character Impact Odorants of Different White Wine Varieties. J Agric Food Chem. 1997;45(8):3027–32.

[28] Cabaroglu T, Canbas A. Effects of skin-contact on aromatic composition of the white wine of *V. vinifera* L. cv. Muscat of Alexandria grown in Southern Anatolia. Acta Aliment [Internet]. 2002 Feb [cited 2017 Feb 27]; 31(1):45–55. Available from: http://www.akademiai.com/doi/abs/10.1556/AAlim.31.2002.1.5

[29] Selli S, Canbas A, Cabaroglu T, Erten H, Lepoutre J-P, Gunata Z. Effect of skin contact on the free and bound aroma compounds of the white wine of *Vitis vinifera* L. cv Narince. Food Control [Internet]. 2006 Jan;17(1):75–82. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0956713504002014

[30] Rapp A, Mandery H. Wine aroma. Cell Mol life Sci [Internet]. 1986 [cited 2017 Jan 22];42(8):873–84. Available from: http://www.springerlink.com/index/P53P13710907903L.pdf

[31] Gil M, Cabellos JM, Arroyo T, Prodanov M. Characterization of the volatile fraction of young wines from the Denomination of Origin "Vinos de Madrid" (Spain). Anal Chim Acta. 2006;563(1):145–53.

[32] Etievant P. Wine. In: H. Maarse, editor. Volatile compounds in foods and beverages [Internet]. New York: Marcel Dekker; 1991 [cited 2017 Mar 13]. p. 456–83. Available from: https://scholar.google.es/scholar?hl=es&q=%5D+P.X.+Etievant%2C+in%3A+H.+Marse+%28Ed.%29%2C+Volatile+Compounds+in+Foods+and+Bev-+´+erages%2C+Marcel+Dekker+Inc.%2C+New+York%2C+USA%2C+1991%2C+pp.+483–587&btnG=&lr=

[33] Gunata YZ, Bayonove CL, Baumes RL, Cordonnier RE. The aroma of grapes. Localisation and evolution of free and bound fractions of some grape aroma components c.v. Muscat during first development and maturation. J Sci Food Agric [Internet]. 1985 Sep [cited 2017 Jan 30];36(9):857–62. Available from: http://doi.wiley.com/10.1002/jsfa.2740360915

[34] Wilson B, Strauss CR, Williams PJ. The distribution of free and glicosidally bound monoterpenes among skin, juice and pulp fractions of some white grape varieties. J Enol Vitic. 1986;37: 107–11.

[35] D'Onofrio C, Matarese F, Cuzzola A. Study of the terpene profile at harvest and during berry development of *Vitis vinifera* L. aromatic varieties Aleatico, Brachetto, Malvasia di Candia aromatica and Moscato bianco. J Sci Food Agric [Internet]. 2016 Nov [cited 2017 May 11];97(9):2898–907. Available from: http://doi.wiley.com/10.1002/jsfa.8126

[36] Marais J. Terpenes in the aroma of grapes and wines: a review. South African J Enol Vitic [Internet]. 1983 [cited 2017 Jan 30];4(2):49–60. Available from: http://www.sawislibrary.co.za/dbtextimages/MaraisJ.pdf

[37] Selli S, Canbas A, Cabaroglu T, Erten H, Günata Z. Aroma components of cv. Muscat of Bornova wines and influence of skin contact treatment. Food Chem [Internet]. 2006 [cited 2017 Feb 20];94(3):319–26. Available from: http://www.sciencedirect.com/science/article/pii/S0308814604008507

[38] Schneider R, Razungles A, Augier C, Baumes R. Monoterpenic and norisoprenoidic glycoconjugates of *Vitis vinifera* L. cv. Melon B. as precursors of odorants in Muscadet wines. J Chromatogr A. 2001;936(1-2):145–57.

[39] Schneider R, Razungles A, Charrier F, Baumes R. Effet du site, de la maturité et de l'éclairement des grappes sur la composition aromatique des baies de Vitis vinifera L. cv. Melon B. dans le vignoble du Muscadet. Bull l'OIV. 2002; 75(855-56):269–82.

[40] Câmara J., Herbert P, Marques J., Alves M. Varietal flavour compounds of four grape varieties producing Madeira wines. Anal Chim Acta [Internet]. 2004 Jun;513(1):203–7. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0003267004000637

[41] Falqué E, Fernández E, Dubourdieu D. Differentiation of white wines by their aromatic index. Talanta. 2001;54(2):271–81.

[42] Selli S, Cabaroglu T, Canbas A, Erten H, Nurgel C, Lepoutre JP, et al. Volatile composition of red wine from cv. Kalecik Karası grown in central Anatolia. Food Chem [Internet]. 2004 Apr [cited 2017 Jan 23];85(2):207–13. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0308814603003388

[43] Escudero A, Cacho J, Ferreira V. Isolation and identification of odorants generated in wine during its oxidation: a gas chromatography-olfactometric study. Eur Food Res Technol [Internet]. 2000 Jul 4 [cited 2017 Mar 3];211(2):105–10. Available from: http://link.springer.com/10.1007/s002179900128

[44] Herraiz T, Reglero G, Martin-Alvarez PJ, Herraiz M, Cabezudo MD. Identification of aroma components of Spanish "Verdejo" wine. J Sci Food Agric [Internet]. 1991 [cited 2017 Feb 27];55(1):103–16. Available from: http://doi.wiley.com/10.1002/jsfa.2740550111

[45] Ferreira V, Lopez R, Cacho JF. Quantitative determination of the odorants of young red wines from different grape varieties. J Sci Food Agric [Internet]. 2000 Sep 1 [cited 2017 Feb 27];80(11):1659–67. Available from: http://doi.wiley.com/10.1002/1097-0010%2820000901%2980%3A11%3C1659%3A%3AAID-JSFA693%3E3.0.CO%3B2-6

[46] Zea L, Moyano L, Medina M. Changes in aroma profile of sherry wines during the oxidative ageing. Int J Food Sci Technol [Internet]. 2010 Nov [cited 2017 Feb 27];45(11):2425–32. Available from: http://doi.wiley.com/10.1111/j.1365-2621.2010.02422.x

[47] Aznar M, López R, Cacho J, Ferreira V. Prediction of Aged Red Wine Aroma Properties from Aroma Chemical Composition. Partial Least Squares Regression Models. J Agric Food Chem. 2003;51(9):2700–7.