Influence of Maturity and Vineyard Location on Free and Bound Aroma Compounds of Grapes from the País Cultivar

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Some of the volatile compounds present in grapes give wine is its unique and genuine characteristics. "Terroir" and berry maturity are considered to be the main influences on the expression of these characteristics. This work was undertaken to establish the specific characteristics that define *Vitis vinifera* cv. País, based on its aromatic profile and free and bound compounds (glycosides), and to assess the effects of location and maturity. Free and bound volatile compounds presented significant differences in the three locations studied. The total amount of free alcohols, acids and ketones depended on the location. During ripening, the amount of aroma precursors increased in all chemical groups in every location studied, and they were found mainly in the skins. With reference to free volatile compounds, it was found that *cis*-2-hexenol could be a good candidate to assess maturity, and that terpene content seemed to be strongly related to the vineyard location and cultivar conditions. Also, data analysis showed that the free aroma profile seemed to be influenced more by the maturity of the grapes and the bound aroma fraction more by the location.

INTRODUCTION

The colour and aroma of a wine are essential indicators of its quality and success in the market. Volatile compounds in the final product can come from the grape, the fermentation process or the ageing process, and compounds responsible for colour proceed from the grape (Ribéreau-Gayon *et al*., 2006). These secondary grape metabolites give a wine its unique and genuine features that differentiate it from other wines.

As mentioned above, wine aroma is an essential marker of the product's quality. Wine aroma is extremely complex because it comprises a large number of volatile compounds (several hundred) with different polarity properties, volatilities and perception thresholds, as well as a wide range of concentrations (Ferreira González, 2007). In grapes, these compounds can be found as free volatile compounds and also as non-volatile compounds (aroma precursors). Non-volatile compounds are mainly present as glycoconjugates formed by an aglycone (volatile compound) and sugar moiety (Winterhalter & Skouroumounis, 2006). Compounds responsible for the varietal aromas that come from the grape are of special interest and may appear in the free and bound form, or as non-volatile flavour precursors (Sefton *et al*., 1996; Segurel *et al*., 2009).

All of these secondary metabolites can be affected by several conditions, such as grape variety, climate, water stress, soil, viticultural practices and so on (Roujou de Boubee *et al*., 2000; Hernandez-Orte *et al*., 2014). In this sense, location and the degree of maturity are very influential parameters for the quality of wine produced from these grapes, and they modulate the phenolic and aromatic profiles of the grapes and the subsequent wine (De Andrés-De Prado *et al*., 2007; Jiang *et al*., 2013). The "terroir" is considered to be the main influence on the expression of the characteristics of a variety (De Andrés-De Prado *et al*., 2007), and the ripening of the grapes also has a deterministic effect that allows grapes to develop their varietal characteristics (Kalua & Boss, 2009) beta-caryophyllene, and alpha-humulene. Likewise, from a technological point of view, it is very interesting to know the distribution of these compounds in the berry (skin/pulp) to contemplate possible maceration techniques with the skin.

The determination of these molecules in grapes and wine may define their key compounds, which in turn define their genuine character and identity, essential components for the success of a product in developed societies (Liedtka, 2008). Currently, several minority grape varieties are being rescued to create new wines with characteristic organoleptic

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properties. This is the case of the *Vitis vinifera* cv. País, which was the first vine cultivated in America. This vine was introduced to the continent at the beginning of the Spanish conquest in the early sixteenth century. Its cultivation has gradually been decreasing due to the entry of French varieties (Lacoste *et al.*, 2010). This has motivated the displacement of its original exploitation and has led to a decline in the price of this grape; therefore, many winemakers have replaced centenarian País vines with other cultivars.

The aims of this work were to establish the specific characteristics that define *Vitis vinifera* cv. País based on its aromatic (free and bound aroma compounds) profile and to assess the effects of the location and maturation stage on this variety. As far as we know, this is the first work that includes maturity and location as variables determining the free and bound aromatic fractions of a grape, and also the first on the aroma features of País cv.

MATERIALS AND METHODS
Chemicals
Dichloromethane, ethyl acetate, ethanol, methanol, 4 methyl-2-pentanol, citric acid and ascorbic acid were supplied by Merck® (Darmstadt, Germany).

Volatile compound standards employed for identification purposes, *viz.* butanol, hexanol, heptanol, nonanol, cis-3-hexenol, 2-ethyl-1-hexanol, 2-phenylethanol, 2-methyl-2-butanol, hexanal, trans-2-hexenal, nonanal, benzaldehyde, 2,5-dimethyl benzaldehyde, β-linalool, citronellol, nerol, geraniol, limonene, α-terpineol, ethyl hexanoate, ethyl octanoate, 2,6-dimethyl-4-heptanone, β-damascenone, p-xylene, and acetic, hexanoic and octanoic acids, were purchased from Sigma-Aldrich Chemical Co. ® (St Louis, MO, USA).

Ultra-pure water was obtained from redistilled water by using a PURELAB ultra purification system (Elga Labwater, Arquimed, Santiago de Chile, Chile). Also, an alkane solution (C7 to C32) used to calculate the linear retention index (LRI) of each analyte was purchased from Sigma Aldrich Chemical Co® (St Louis, MO, USA).

Samples
Grape samples from unirrigated and self-rooted *Vitis vinifera* cv. País were collected in three different Chilean locations during the 2015 vintage: Hualañé [34°54'30.11'' (S) and 71°52'20.05'' (W)], Cauquenes [36°03'52.21'' (S) and 72°28'40.40'' (W)], and Itata [36°35'38.11'' (S) and 72°37'3.31'' (W)]. In this study, no comparisons were made

with the following vintage (2016) due to the huge differences in climatic conditions in Chile, mainly due to the profuse rainfall and the need for wineries to harvest earlier in 2016 to avoid the microbiological alterations associated with the growth of *Botrytis cinerea*. Therefore, the number of days between ripening states was not the same as in 2015 and circumstances were not similar. Hualañé vineyard (HUA) is located 85 m above sea level in a sandy-loam soil. The vines are trained on a vertical trellis system arranged in rows spaced 2.5 m apart, with 1 m spacing within each row. The production yield for the vineyard in the 2015 vintage was approximately 8.7 tonnes/ha. The weather conditions for the 2015 vintage were an average minimum temperature of 8.3°C and an average maximum temperature of 21.1°C, with 776 mm of annual rainfall. Cauquenes vineyard (CAU) is located 234 m above sea level in a clay-loam soil. The vines were head trained, with a planting framework of 1 m x 1 m. The production yield for CAU in the 2015 vintage was approximately 10 tonnes/ha, and the weather conditions were an average minimum temperature of 8.4°C and an average maximum temperature of 19.6°C, with 824 mm of annual rainfall. Itata vineyard (ITA) was head trained, with a planting framework of 1 m x 1 m and located approximately 116 m above sea level, in a zone of clay-loam soil. The production yield for ITA in the 2015 vintage was approximately 10 tonnes/ha, and the weather conditions were an average minimum temperature of 8.4°C, an average maximum temperature of 19.5°C, and 1 185 mm of annual rainfall. Moreover, two different maturity degrees were harvested in each location. The first one (HUA0, CAU0, ITA0) corresponded with the véraison stage. The second one (HUA1, CAU1, ITA1) was at the optimal degree of maturation for harvesting. From each location and maturity, ten grape clusters were harvested. From these clusters, samples for analysis were composed randomly by taking grapes from all the clusters in order to obtain representative and homogeneous samples (Table 1), after which they were immediately transported to the laboratory and whole frozen (-20°C) until analysis.

General analyses
Titratable acidity (TA, expressed as sulphuric acid equivalents), pH (S220 Seven Compact pH/Ion, Mettler Toledo, Santiago, Chile), and soluble solids expressed as degrees Brix (Hand Held Refractometer, Winkler) were evaluated following the OIV official analytical methods (International Organisation of Vine and Wine, 2016).

TABLE 1
General parameters and dates of harvest of the grape samples.

	HUA_0	HUA_1	CAU_0	CAU_1	ITA_0	ITA_1
Date of harvest	28 February	19 March	11 March	31 March	5 March	25 March
SS (Brix)	16.5 ± 0.07^b	20.0 ± 0.14^c	20.0 ± 0.07^c	22.5 ± 0.04^d	15.5 ± 0.06^a	23.0 ± 0.21^e
TA (g/L)	5.60 ± 0.14^d	2.15 ± 0.07^{bc}	1.55 ± 0.78^{ab}	1.10 ± 0.14^a	2.70 ± 0.00^c	1.30 ± 0.14^a
pH	3.04 ± 0.01^a	3.29 ± 0.01^c	3.95 ± 0.02^d	4.27 ± 0.01^f	3.24 ± 0.01^b	4.10 ± 0.01^e

[a,b,c,d,e,f] Values with a different letter indicate statistically significant differences ($p < 0.05$).

Extraction and analysis of free aroma compounds

To obtain a representative sample of the free aroma compounds of the grapes, the sample preparation method employed by Salinas *et al.* (2004) was used. A total of 200 grams of grapes were crushed for 1 min with an Ultra Turrax T25 Basic (Staufen, Germany) without breaking the seeds. The must and skins were kept in contact for 2 h at 5ºC in hermetically closed containers. Afterwards, the maceration and 7.5 mL of the mash were placed in a 20 mL vial. Volatile compound extraction was performed as described by Ubeda *et al.* (2015)EG-Silicone and PDMS polymeric phases were compared and, afterwards, the most adequate was selected. The best extraction method turned out to be a sequential extraction in the headspace and by immersion using two PDMS twisters. A total of 130 compounds were determined. In traditional Chilean sparkling wines, ethyl esters were significantly higher, while acetic esters and ketones were predominant in the Charmat wines. PCA and LDA confirmed the differences in the volatile profiles between the production methods (traditional vs. Charmat. In this study, a sequential stir bar sorptive extraction (SBSE) was carried out with stir bars coated with polydimethylsiloxane (Twister, Gerstel). First, immersion assays were performed, placing the polymer in the stainless wire device and using a stir bar for one hour at 200 rpm. After one hour, the polymer was removed and stored in a glass vial. Then, another polymer was placed in the headspace for one hour in a water bath at 62ºC. The internal standard used was 4-methyl-2-pentanol.

Finally, both twisters were thermally desorbed and injected into a gas chromatograph/mass spectrometer (GC/MS) for analysis.

Gas chromatography analysis was carried out using a 7890B Agilent GC system, coupled to a quadrupole mass spectrometer Agilent 5977 inert (Agilent Technologies, Palo Alto, CA, USA) thermodesorption system and a cryo-focusing CIS-4 PTV injector (Gerstel). Thermal desorption was performed in splitless mode with a flow rate of 70 mL/min. The desorption temperature program was the following: the temperature was held at 35ºC for 0.1 min, ramped at 60ºC/min to 210 ºC, and then held for 5 min. The temperature of the CIS-4 PTV injector, with a Tenax TA inlet liner, was held at -35ºC using liquid nitrogen for the entire desorption time, and was then raised at 10ºC/s to 260ºC and held for 4 min. The solvent vent mode was used to transfer the sample to the analytical column. A DB wax capillary column with dimensions of 60 m x 0.25 mm and a 0.25 μm film thickness (J&W Scientific, Folsom CA, USA) was used, and the carrier gas was helium at a flow rate of 1 mL/min.

The oven temperature program was the following: The temperature was held at 40ºC for 3 min, raised to 110ºC at 6.5ºC/min, and then raised to 230ºC at 2.5ºC/min and held for 5 min. The quadrupole, source and transfer line temperatures were maintained at 150ºC, 230ºC and 280ºC respectively. Electron ionisation mass spectra in the full-scan mode were recorded at 70 eV with a scan range from m/z 35 to 300. All data were recorded using MS ChemStation. The samples were analysed in duplicate, and blank runs using an empty glass tube were performed before and after each analysis.

Isolation and analysis of glycoside aroma precursors

To analyse the bound aroma precursors, a solid-phase extraction (SPE) technique was applied to 100 g of grapes, followed by acid hydrolysis (Ibarz *et al.*, 2006). For the analyses of the glycosylated aroma precursors, the gas chromatograph oven temperature was programmed in the same way as for the free aroma compounds. The samples were analysed in duplicate, and blank runs were performed prior to and following each analysis.

Volatile compounds were identified based on comparisons with the linear retention indices (LRIs) of the standards and computer matching to reference mass spectra from the NIST 2011 library. When standards were not available, the compounds were identified by computer matching to the reference mass spectra from the NIST 2011 library, and by comparison of their LRIs with the LRIs obtained for standards that have been reported in the literature. LRIs were calculated based on the retention times of n-alkanes (C7 to C32) under conditions that were identical to those of the analyses.

Semi-quantitative determination of volatile compounds was performed using the relative area, which was calculated as the ratio of the target ion of each compound and the internal standard.

Statistical analyses

Analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test were used for mean separation, with a significance level of 95% ($P < 0.05$), and they were carried out using the Infostat version 2012p software package (FCA-Universidad Nacional de Córdoba, Argentina). Principal component analysis (PCA) was performed using SPSS software (SPSS Inc., Chicago, IL). No pre-processing of the data was done for the statistical analyses.

RESULTS AND DISCUSSION

General parameters

Statistically significant differences were found in the general parameters for the different locations and for different stages of maturation. Thus, in general, grapes at lower maturation stages (HUA_0, CAU_0 and ITA_0) had lower values of SS and pH than HUA_1, CAU_1, and ITA_1 and higher values of TA (Table 1).

Free volatile profile

Table 2 presents the results of the free volatile aroma compounds in the País grape variety at two maturation stages and from the three locations studied. The data are expressed as the mean of the relative area with the internal standard. Important differences were observed between the different locations. Cauquenes showed the lowest total volatile amount, with Hualañé and Itata being very similar in this parameter. A total of 34 volatile compounds were determined in the grape samples. Among them, alcohols (10) constituted the most abundant group in number of compounds, followed by aldehydes (nine), terpenes (five), esters (four), acids (three), ketones (three) and benzene compounds (one). Alcohols was also the most abundant group with reference to the total amount, and C_6 alcohols clearly predominated in this group.

TABLE 2
Free volatile compounds of grape samples. Relative area (standard deviation).

Compound	First harvest			Second harvest			KI	ID
	HUA_0	CAU_0	ITA_0	HUA_1	CAU_1	ITA_1		
Alcohols								
Hexanol	105 (5)[a]	62.4 (2.7)[a]	161 (19)[b]	114 (2)	75.6 (5.4)	144 (4)	1348	A
cis-3-Hexenol	22.5 (2.5)[b]	24.3 (1.8)[b]	11.3 (1.8)[a]	7.35 (0.11)[*]	10.2 (1.6)[*]	2.2 (0.3)[*]	1394	A
cis-2-Hexenol	14.7 (2.4)[a]	12.3 (1.6)[a]	56.2 (7.1)[b]	29.6 (0.5)[*]	9.1 (1.1)[*]	24.6 (2.3)[*]	1432	B
1-Octen-3-ol	13.2 (0.6)[b]	5.54 (0.09)[a]	11.9 (0.2)[b]	14.6 (0.8)	9.01 (1.18)[*]	14.7 (1.3)	1433	B
Heptanol	7.01 (0.48)[a]	6.87 (0.67)[a]	8.5 (1)[a]	10.3 (0.4)[*]	5.71 (0.76)	9.9 (0.8)	1448	A
2-Ethyl-1-hexanol	136 (3)[c]	18.5 (2.3)[a]	82.6 (11)[b]	409 (1)[*]	33.3 (2.9)[*]	235 (14)[*]	1574	A
Nonanol	27.7 (1.6)[b]	10.2 (1.9)[a]	20.2 (2.3)[b]	51.6 (5.7)[*]	9.6 (1.4)	27.1 (2.3)	1664	A
Benzyl alcohol	15.1 (0.4)[a]	21.9 (0.3)[a]	20.2 (3.8)[a]	41 (2.1)[*]	25.8 (0.4)[*]	31.8 (0.9)	1906	B
2-Phenylethanol	19.2 (3.4)[a]	19.7 (0.1)[a]	23.3 (0.9)[a]	27.8 (1.4)	19.8 (0.2)	21.8 (1.8)	1921	A
Dodecanol	5.39 (0.74)[a]	4.51 (0.7)[a]	4.15 (0.21)[a]	15.2 (0.7)[*]	4.29 (0.05)	4.28 (0.62)	1982	B
Total	**366**[b]	**186**[a]	**399**[b]	**720**[*]	**203**[*]	**515**		
Aldehydes								
Hexanal	17.1 (0.1)[a,b]	16.2 (0.7)[a]	21.8 (3.1)[a]	17.4 (0.9)	19.5 (0.2)	25.3 (0.8)	1081	A
trans-2-Hexenal	36.1 (3.1)[a]	25.5 (4.5)[a]	44 (3.6)[b]	37.6 (4.6)	26.9 (1.2)	38.5 (2.2)	1232	A
cis-2-Heptenal	35.5 (1.2)[a]	22.9 (1.4)[a]	54.5 (6.7)[b]	38.8 (0.8)	26.2 (0.8)	49.1 (1.1)	1315	B
Nonanal	30.4 (1.8)[b]	18.5 (1.1)[a]	27.8 (4.5)[b]	33.1 (2.8)	10.3 (1.5)[*]	40.1 (5.8)[*]	1413	A
trans-2-Octenal	5.9 (0.86)[a]	4.81 (0.42)[a]	7.62 (1.04)[a]	8.86 (0.22)[*]	4.62 (0.51)	8.2 (0.16)	1416	B
Decanal	16.1 (2.1)[a]	15.6 (0.5)[a]	9.77 (1.59)[a]	13.8 (0.4)	6.31 (0.38)[*]	9.6 (0.79)	1553	C
trans-2-Nonenal	15.6 (1.1)[a]	10.7 (1.1)[a]	14.8 (1.4)[a]	21.3 (0.6)[*]	12.5 (1.7)	18.1 (0.3)	1548	B
Benzaldehyde	6.08 (0.02)[b]	5.84 (0.29)[a,b]	5.18 (0.19)[a]	5.4 (0.26)	5.93 (0.32)	7.16 (1.05)	1564	A
2,4-Nonadienal	14.2 (0.6)[a]	12 (2)[a]	16 (1.2)[a]	15.7 (1.1)	14.3 (1.5)	16.8 (0.7)	1714	B
Total	**177**[a,b]	**132**[a]	**201**[b]	**192**	**121**	**213**		
Terpenes								
β-Linalool	6.04 (0.11)[b]	3.37 (0.21)[a]	3.21 (0.52)[a]	4.11 (0.19)[*]	2.61 (0.22)	1.97 (0.13)	1580	A
Citral	4.3 (0.24)[a]	13.1 (1.8)[b]	17.1 (2.7)[b]	10.8 (1.2)[*]	10.5 (1.3)	11.6 (1.2)	1770	B
Citronellol	23.6 (0.7)[b]	11.2 (0.6)[a]	20.1 (2.4)[b]	23.1 (0.1)	11.7 (1.1)	16.7 (0.1)	1793	A
Nerol	9.97 (0.07)[c]	6.1 (0.19)[a]	9.05 (0.25)[b]	11.2 (0.7)	6.21 (0.46)	9.12 (0.69)	1840	A
Geraniol	148 (1)[b]	66.4 (8.6)[a]	129 (13)[b]	159 (12)	59.7 (7.7)	126 (1)	1868	A
Total	**193**[b]	**101**[a]	**179**[b]	**209**	**91.2**[*]	**166**		
Esters								
Ethyl hexanoate	4.07 (0.36)[a]	5.35 (0.19)[b]	3.67 (0.28)[a]	6.44 (0.26)[*]	6.57 (0.19)[*]	5.97 (0.58)[*]	1260	A
Ethyl 2-hexenoate	53 (2.6)[a]	31.5 (0.9)[a]	81.6 (9.7)[b]	58.1 (1.1)	39.3 (2.5)[*]	73.2 (2.4)	1319	B
Ethyl octanoate	15.1 (1.3)[b]	1.89 (0.25)[a]	2.4 (0.4)[a]	25.5 (3.6)	2.84 (0.05)[*]	12.3 (2.1)[*]	1453	A
3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	162 (3)[b]	92.5 (5.6)[a]	140 (17)[b]	231 (12)[*]	166 (11)[*]	172 (6)	1829	C
Total	**215**[b]	**135**[a]	**232**[b]	**231**[*]	**218**[*]	**266**		
Ketones								
2,6-Dimethyl-4-heptanone	125 (14)[a]	98.6 (11.1)[a]	76.1 (10.6)[a]	73.4 (8.2)[*]	78.3 (11.9)	110 (19)	1128	A
3,5-Octadien-2-one	6.05 (0.49)[a]	4.8 (0.33)[a]	5.89 (0.48)[a]	7.62 (0.18)[*]	6.27 (0.59)	5.93 (0.04)	1515	B
beta-Ionone	47.7 (0.7)[c]	24 (1.6)[a]	36.6 (4.2)[b]	55.6 (3.1)[*]	25.2 (2.7)	22.9 (0.4)[*]	1964	B
Total	**194**[b]	**118**[a]	**126**[a]	**181**	**137**	**133**		

TABLE 2 (CONTINUED)

Compound	First harvest			Second harvest			KI	ID
	HUA_0	CAU_0	ITA_0	HUA_1	CAU_1	ITA_1		
Acids								
Acetic acid	17.2 (0.6)[b]	29.1 (2)[c]	11 (1.5)[a]	24.2 (1.9)	34.3 (3.5)	21.8 (1.1)*	1523	A
Hexanoic acid	10.6 (0.7)[a]	12.7 (1.3)[a,b]	15.6 (0.3)[b]	14.4 (0.4)*	14.7 (2.4)	17.6 (3.3)	1907	A
Octanoic acid	22.1 (2.1)[a]	30.9 (1.8)[a,b]	23.8 (3.4)[b]	16.8 (0.9)	13.8 (1.1)	18.8 (0.2)	2090	A
Total	**49.9**[a]	**72.7**[b]	**50.4**[a]	**55.4**	**62.8**	**58.2**		
Total volatile amount	**1145**[b]	**669**[a]	**1133**[b]	**1533***	**676**	**1291**		

[a,b,c] Different letters in different columns indicate significant differences ($p < 0.05$) between locations. *Significant differences according to Tukey's test ($p < 0.05$) with the first maturation stage of every location.
ID: reliability of identification: A, mass spectrum and LRI agreed with standards; B, mass spectrum agreed with mass spectral database and LRI agreed with the literature data: [14, 17, 27, 39]; C, tentatively identified, mass spectrum agreed with mass spectral data base.
KI: Kovats Index.

With reference to the location, significant differences were found between them, since the major alcohol in Hualañé was 2-ethyl-hexanol, which is described as having a green, flowery, cucumber odour, while in contrast it was hexanol (green, grass and fresh) in Cauquenes and Itata. In other grape varieties, such as Cabernet Sauvignon and Pinot noir, the major alcohols are *cis*-3-hexenol and *trans*-2-hexenol respectively (Kalua & Boss, 2009)beta-caryophyllene, and alpha-humulene. There also was a remarkable difference in the total amount of alcohols, with Cauquenes presenting a lower total than Hualañé and Itata.

With respect to aldehydes, the major aldehyde was *trans*-2-hexenal in Hualañé and Cauquenes and *cis*-2-heptenal in the case of Itata. Among the terpenes, geraniol reached the highest amounts, followed by citronellol. This is in agreement with previously published data (Coelho *et al*., 2006; Fenoll *et al*., 2009), which described these two terpenes as the most abundant in Muscat and Baga cv. grapes. Geraniol could be an important aroma contributor in wine due to its low sensory threshold, and it is one of the main contributors to the floral character of Muscat varieties (Lund & Bohlmann, 2006).

Regarding the esters that were determined, 3-hydroxy-2,4,4-trimethylpentyl-2-methylpropanoate was the major one, representing 75% (Hualañé), 69% (Cauquenes) and 60% (Itata) of the total group of esters. This volatile compound has not been identified in grapes, but has been found in strawberries and in cantaloupes, for example (Obando-Ulloa *et al*., 2010; Negri *et al*., 2015). This compound has a mild characteristic odour and may be a varietal marker of the País grape, since it was the major ester in all locations and yet has not previously been described in grapes.

On the other hand, the ripening stage had a significant influence on several free volatile compounds. The total composition of alcohols increased during ripening in all the samples, with 2-ethyl-1-hexanol presenting the highest significant increases in all samples. In contrast, other volatile compounds underwent significant decreases in all samples, such as *cis*-3-hexenol. This was also observed by Salinas *et al*. (2004) in Monastrell grapes. As observed by other authors, several compounds formed before véraison and decreased after it (Hashizume & Samuta, 1999; Belancic &

Agosin, 2007), and their concentrations may even fluctuate during maturation (Salinas *et al*., 2004).

The main trend in the aldehyde group was augmentation of the total amount, except in the Cauquenes grapes, where it remained the same, but with little significance.

Terpenes are involved in the sensorial differentiation of wines because they are characteristic of the grape variety employed in winemaking. In this study, all terpene free volatile compounds tended to diminish or remain without significant differences during ripening, except for the increase of citral in the Hualañé grapes during ripening. It must be noted that the viticultural practices and vine and vineyard characteristics of Hualañé differ from Cauquenes and Itata, as described in the "Samples" section. The decrease or no change in this varietal compound group after véraison was an expected behaviour (Gunata *et al*., 1985; Salinas *et al*., 2004; Fenoll *et al*., 2009; Kalua & Boss, 2009).

The global trend in the ester group was to increase in all locations. This was also observed in Monastrell grapes, in which the total ester content increased significantly during the fifth week after the berries began to change from green to red; however, the total ester content remained constant until the end of the maturation period in Cabernet Sauvignon grapes (Salinas *et al*., 2004; Kalua & Boss, 2009). Thirty-four compounds were determined, among which geranyl acetone, geranyl butyrate, farnesol, and a bicycloterpene, tentatively identified as 6-methylene[3.1.0]byciclohexane, were recorded for the first time in this variety. The highest terpene and norisoprenoid contents together with the lowest values of C6 compounds were observed during the fifth week of ripening, meaning that this would be the optimal time for harvesting in terms of volatile compounds.

Among the grape volatile constituents mentioned before, the group of C_6 compounds (aldehydes and alcohols) is responsible, to some extent, for the green, herbaceous and vegetable aromas perceived in wines, which are a negative attribute regarding its quality. Hexanal, *trans*-2-hexenal, hexanol, *cis*-3-hexenol and *cis*-2-hexenol are found in this group. As can be observed in Table 2, the total contents of these compounds tended to increase in the Hualañé and Itata locations and to decrease in the southernmost location, Itata. In all cases, total C_6 alcohols decreased during maturation,

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which is consistent with previous reports. These compounds are formed from linoleic and linolenic acid when grapes come into contact with air, and are catalysed by lipoxygenase, peroxidase and alcohol dehydrogenase enzymes (Crouzet, 1999; Garcia *et al*., 2003). The decreasing trend of their presence in the grape has been related to the greatest activity of the enzyme at the beginning of véraison (Salinas *et al*., 2004), and also to the decrease in polyunsaturated fatty acids during ripening, which are precursors of these compounds.

Glycosidically bound volatile compounds

After acid hydrolysis, 23 aglycones from glycosidically bound compounds were determined in the País cv. grape samples (Table 3). The method employed implies harsh acid hydrolysis. This methodology has been tested by other authors and is considered to be much more adequate for measuring the aroma potential of grapes for winemaking towards enzymatic hydrolysis (Salinas *et al*., 2004). Among them, nine were also found in the samples as free aroma compounds. The major aroma precursors belonged to the alcohol group. Among all samples in both chemical groups, the most abundant aroma precursors were benzaldehyde and 2-ethyl-1-hexanol. Conversely, in other grape varieties such as Merlot, the major precursors are terpenes (Pedroza *et al*., 2010). In all cases, the Hualañé location produced the lowest number and amount of aroma precursors. This could be attributed partially to the different viticultural practices, such as the trellis system (Hernandez-Orte *et al*., 2014), which is different in the Hualañé location from the other two zones. For example, 2-phenylethanol was the second major alcohol, but was not present in Hualañé grapes. This compound is a derivative of phenyl propanoid metabolism and has a floral/rose-like aroma. It is mainly formed during fermentation, but some grape varieties present this compound in a glycosylated form. Another of the remarkable differences is that the amount of terpenes (quantitative and qualitative) in this location represents one-third of the amount in Cauquenes and Itata. This is of special interest because terpenes account for the highest number of precursors, and because of their importance in the aroma of wine. Because of their low threshold, they contribute greatly to the final aroma of wine and are associated with citric and floral aromas (Étievant, 1991; Park & Noble, 1993). They are varietal aromas and, as demonstrated by Rapp (1998), only a selected number of wine terpenes can be used to distinguish between grape varieties and even to determine the region of origin.

At all locations, the total amount of aroma precursors increased during ripening. This is consistent with previous studies on different *Vitis vinifera* grape varieties (Fenoll *et al*., 2009; Vilanova *et al*., 2012). This behaviour is common because most active aroma compounds are lipophilic; however, high, localised concentrations of lipophilic molecules can be toxic to the plant by disrupting cellular membranes, or can induce cell death (Sikkema *et al*., 1995; Hjelmeland & Ebeler, 2015).

With respect to the aldehyde group, the major aroma precursor was benzaldehyde, a bitter, almond-like volatile compound. It can be transformed by *Saccharomyces cerevisiae* strains into benzoic acid, benzyl alcohol and other major compounds characteristic of wine (Delfini

et al., 1991). Benzaldehyde increased significantly during ripening, which is in agreement with the results obtained by Fenoll *et al*. (2009).

The alcoholic non-volatile precursors had the same trend as the other chemical groups, increasing during ripening; even 2-methyl-2-butanol, which was not present in the early stage of ripening of the grapes, was found in mature samples from Hualañé.

As mentioned above, the terpenes, C_6 compounds and C_{13} norisoprenoids were the glycoside aroma precursors that contributed most to the final aroma of the wine and, in this case, were the most numerous. All the terpenes increased in all locations during ripening. An interesting result was that, despite geraniol being found as the most abundant terpene in the free volatile fraction, this was not observed in the case of the glycosylated terpenes. This method is effective for the determination of geraniol (Ubeda *et al*., 2012; Hernandez-Orte *et al*., 2014). Therefore, geraniol can be transformed into other terpenols, such as α-terpineol. Moreover, geraniol can be transformed into its isomer nerol, and further, by acid-catalysed rearrangement, into a mutual carbo-cation, which can be stabilised to α-terpineol and limonene (Ribéreau-Gayon *et al*., 1975).

Another result to highlight is the significant augmentation of TDN (1,1,6-trimethyl-1,2-dihydronapthalene), an important C_{13}-norisoprenoid in wine associated with kerosene- and petrol-like odours. This compound is found in grapes mainly in its glycosylated form and is released by enzymatic and non-enzymatic (acid-catalysed) mechanisms during winemaking or storage (Ibarz *et al*., 2006).

The amount of non-volatile precursors in the skin and pulp were analysed to determine the proportion of these molecules located in each part of the grape. Fig. 1 presents the total sum of the relative area of each chemical group per 100 g of sample divided into the amount that comes from the pulp and the quantity that is contained in the skins in each sample. Taking into account the weight of the skin and pulp per grape, we can conclude that non-volatile compounds were mainly located in the grape skin, which is in accordance with previous studies (Gunata *et al*., 1985; Park *et al*., 1991; Gomez *et al*., 1994). As mentioned above, the total amount of aroma precursors increased in all chemical groups during ripening. Comparing the evolution of the distribution of glycosidically bound aroma compounds during ripening, it was observed that, depending on the location and chemical group, the increases were produced in the skin or in the pulp. Beginning with the northernmost location, Hualañé, significant differences in the total amount of aroma precursors between the two harvest dates were found for terpenes and C_{13}-norisoprenoids, both due to an increase in the skin. However, the southernmost location, Itata, presented significant differences in the total amount for all chemical groups. Increases in terpenes and ketones were attributed to the augmentation of precursors placed in the pulp, and the increase in the rest of the chemical groups was attributed to bound aromas located in both the pulp and the skin. In addition, in the location situated in the middle, Cauquenes, significant differences between harvest dates were found in all groups, although all of the increases were attributed to increased aroma precursors in the skin and, in

the case of C_{13}-norisoprenoids and acids, also to an increase in the pulp-bound aroma fraction. These results note that the distribution of the bound aroma fraction in the same grape variety is different in different locations. However, additional research is needed to confirm these results.

Finally, it is important to highlight that some of the compounds are present in very different amounts in the pulp and skin in all locations. Terpenes, in general, were mainly present in the skin, while norisoprenoids showed different trends. TDN was primarily present in the skin, and β-damascenone and α-ionone were primarily present in the pulp. Other remarkable compounds present in much higher amounts in the skin were the aldehydes hexanal and benzaldehyde, which has also been described in the case of hexanal (Gomez *et al.*, 1994).

Multivariate analysis

Principal component analysis (PCA) was performed using free and glycosidically bound volatile compounds to minimise the number of variables and to better understand the results

obtained. The analysis selected five principal components (PC) with an eigenvalue greater than 1, which explained 95.6% of the total variance. Fig. 2 shows the distribution of the different grape samples in the plane defined by PC1 and PC2, which accounted for 71% of the cumulative variance and enabled significant separation of the samples.

Component 1 seems to explain the variance among locations, especially for the Hualañe grapes, whereas unripe Cauquenes (CAU_0) grapes and mature Itata (ITA_1) grapes show similar scores for PC1. The high similarity of the cultivar conditions in the Itata and Cauquenes vineyards (*i.e.* vine training, planting framework, soil type, etc.) could explain the similarity among the studied variables and therefore the similar scores for PC1.

By contrast, component 2 seems to explain the variance between grape maturity levels, since the mature grapes reached higher scores for PC2 than the unripe grapes from all locations. However, the Itata grapes reached the highest scores for PC2 for both unripe and mature grapes compared with the same maturity at the other locations. Table 4 shows
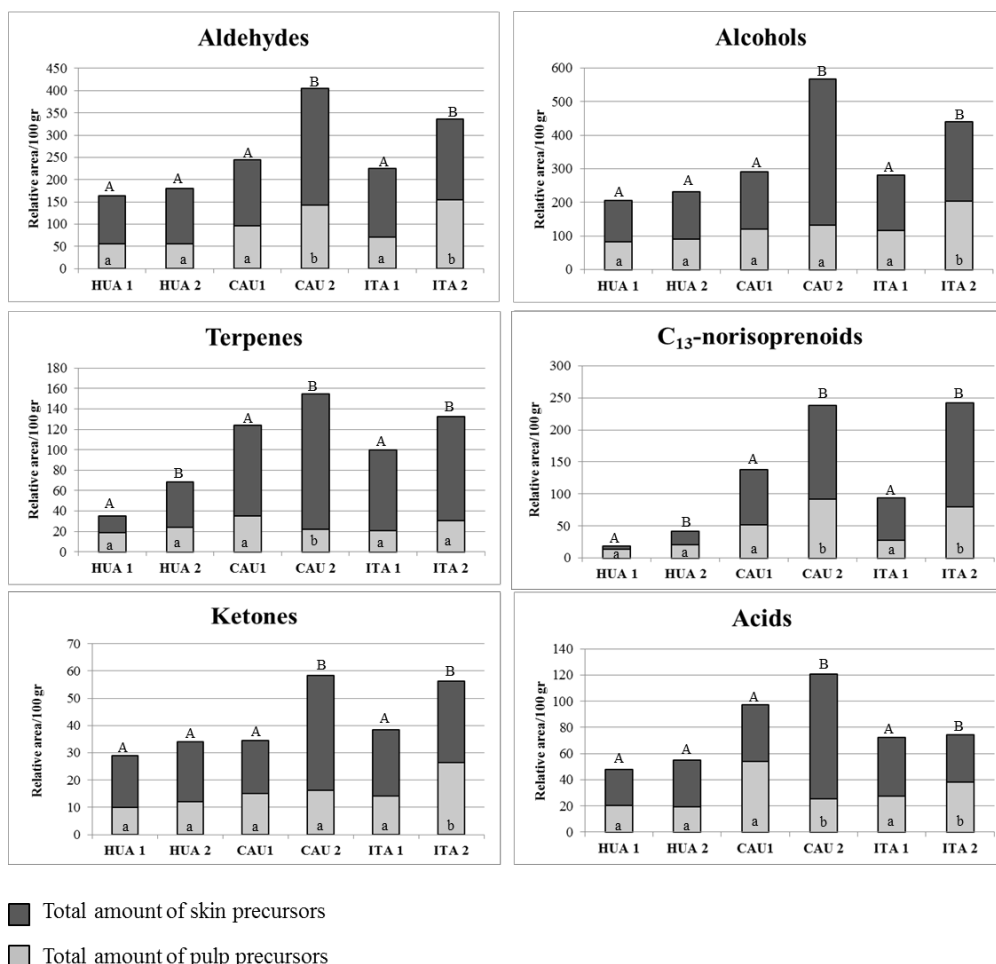

FIGURE 1
Distribution of aromatic precursors (relative area) of 100 g of grapes.
[a] Different letters in different columns indicate significant differences according to Tukey's test ($p < 0.05$) between ripening degrees (1 and 2) of the same location (HUA, CAU and ITA) in pulp. [A] Different letters in different columns indicate significant differences according to Tukey's test ($p < 0.05$) between ripening degrees (1 and 2) of the same location (HUA, CAU and ITA) in the skins.

TABLE 3
Glycosidically bound compounds of grape samples. Relative area (standard deviation).

Compound	First harvest date			Second harvest date			KI	ID
	HUA_0	CAU_0	ITA_0	HUA_1	CAU_1	ITA_1		
Alcohols								
2-Methyl-2-butanol	20.3 (1.1)[a]	22.3 (1.9)[a]	21.5 (1.2)[a]	19.1 (0.2)	24.8 (0.7)	30.3 (0.3)[*]	1005	A
Butanol	3.21 (0.31)[b]	1.89 (0.21)[a]	4.99 (0.02)[c]	3.02 (0.26)	8.63 (0.06)[*]	3.28 (0.3)[*]	1170	A
2-Ethyl-1-hexanol	169 (2)[a]	204 (8)[a]	204 (21)[a]	178 (26)	338 (4)[*]	298 (32)[*]	1574	A
Benzyl alcohol	12.6 (1.5)[a]	19.8 (1.2)[a,b]	27.1 (4.1)[b]	30.8 (4.2)[*]	42.9 (1.8)[*]	40.7 (3.1)[*]	1906	B
2-phenylethanol	ND[a]	43.8 (1.5)[c]	24.3 (2.9)[b]	ND	134 (19)[*]	66.8 (2.8)[*]	1921	A
Total	**205**[a]	**292**[b]	**282**[b]	**231**	**557**[*]	**439**[*]		
Aldehydes								
Hexanal	17.1 (0.4)[a]	20.4 (1.2)[a]	17.7 (1.8)[a]	14.2 (1.2)	19.8 (2.8)	17.1 (1.2)	1081	A
Nonanal	13.6 (0.3)[a]	13.5 (0.5)[a]	18.4 (0.4)[b]	13.2 (0.8)	23.5 (5.9)	18.4 (0.5)	1413	A
Benzaldehyde	113 (1)[a]	168 (14)[b]	144 (9)[a,b]	125 (6)[*]	265 (17)[*]	255 (7)[*]	1564	A
2,5-Dimethyl benzaldehyde	20.5 (1.6)[a]	42.5 (8.8)[a]	45.9 (8.4)[a]	28.1 (2.6)	102 (12)[*]	45.2 (6.9)	1930	A
Total	**164**[a]	**244**[b]	**226**[b]	**181**	**410**[*]	**336**[*]		
Terpenes								
Limonene	15.8 (0.5)[a]	67.2 (3.6)[b]	27.8 (3.8)[a]	21.6 (0.5)[*]	60.4 (5.3)	21.4 (0.4)	1230	A
β-Linalool	ND[a]	4.1 (0.63)[b]	3.42 (0.03)[b]	1.97 (0.04)[*]	6.66 (0.74)	5.22 (0.04)[*]	1580	A
Myrcenol	ND[a]	2.91 (0.34)[b]	2.72 (0.19)[b]	ND	4.84 (0.14)[*]	4.93 (0.71)	1610	B
Ocimenol	1.05 (0.04)[a]	9.39 (0.57)[b]	9.21 (0.94)[b]	4.37 (0.71)[*]	14.1 (1.4)	18.85 (1.82)[*]	1750	C
α-terpineol	9.75 (0.01)[a]	16.3 (0.5)[b]	16.8 (1.9)[b]	11.3 (1.3)	25.8 (3.1)	25.2 (3.4)	1730	A
6,7-Dihydro-7-hydroxylinalool	2.87 (0.23)[a]	15.2 (0.8)[b]	26.5 (2.6)[c]	21.4 (3.1)[*]	16.5 (1.4)	43.9 (5.1)[*]	2025	C
Total	**29.5**[a]	**115**[c]	**85**[b]	**60.6**[*]	**137**	**120**[*]		
Ketones								
Cyclohexanone	14.3 (0.1)[a]	15.8 (1)[a,b]	18.4 (0.9)[b]	16.9 (0.3)[*]	26.7 (5.1)	26.8 (2.2)	1360	C
Acetophenone	14.6 (0.1)[a]	18.7 (0.6)[a]	20.1 (2.6)[a]	17.1 (2.9)	34.2 (2.2)[*]	29.4 (2.5)	1695	B
Total	**28.9**[a]	**34.5**[b]	**38.5**[b]	**34.0**	**59.1**[*]	**56.2**[*]		
Acids								
Acetic acid	33.2 (3.2)[a]	97.4 (18.7)[b]	53.2 (0.2)[a,b]	35.7 (4.6)	99.5 (13.1)	53.2 (2.8)	1523	A
Hexanoic acid	14.9 (1.7)[b]	ND[a]	19.2 (1.2)[b]	19.4 (2)	28.9 (0.1)[*]	21.3 (2.5)[*]	1907	A
Total	**48.1**[a]	**97.4**[b]	**72.4**[a,b]	**55.1**	**119**	**74.5**		
C_{13}-norisoprenoids								
α-Ionene	6.29 (0.7)[a]	26.9 (0.1)[c]	22.8 (0.9)[b]	9.07 (1.36)	52.6 (3.2)[*]	51.1 (3.1)[*]	1552	B
TDN	4.44 (0.21)[a]	98.7 (2.2)[c]	71.1 (10.5)[b]	23.7 (2.6)[*]	177 (4)[*]	191 (33)[*]	1733	B
β-Damascenone	7.45 (0.95)[b]	12.4 (1.4)[c]	ND[a]	8.52 (1.17)	15.5 (0.6)	ND	1845	A
Total	**18.8**[a]	**138**[c]	**94**[b]	**41.2**[*]	**244**[*]	**242**[*]		
Benzenes								
p-Xylene	2.54 (0.14)[b]	0.942 (0.123)[a]	3.19 (0.41)[b]	2.42 (0.04)	3.85 (0.03)[*]	2.45 (0.09)[*]	1220	A

[a] Different letters in different columns indicate significant differences ($p < 0.05$) between locations. [*]Significant differences according to Tukey's test ($p < 0.05$) with the first maturation stage of every location.
ID: reliability of identification: A, mass spectrum and LRI agreed with standards; B, mass spectrum agreed with mass spectral data base and LRI agreed with the literature data: [14, 17, 27, 39]; C, tentatively identified, mass spectrum agreed with mass spectral database.
KI: Kovats Index
ND: not detected

the loading values for the variables with an absolute value higher than 0.9, which contributes most significantly to the explanatory meaning of the components. As seen, most of these variables are associated with PC1, which in general allowed for the separation of the samples according to their location. Several of these variables are related to terpenes, both in their free and glycosylated forms. Thus, it seems that the terpene content of grapes is strongly related to vineyard location and cultivar conditions. On the other hand, since component 2 separates grapes with different maturities, and the loading value for *cis*-2-hexenol is negative (-0.962; Table 4), it seems clear that *cis*-2-hexenol was higher in the unripe grapes and that its amount decreased throughout maturation. This compound corresponds to a family of compounds that are related to green, herbaceous and vegetable aromas in wines, so it is quite logical that unripe grapes contain higher amounts of *cis*-2-hexenol than mature grapes. Thus, *cis*-2-hexenol could be a good candidate to assess the maturity of País cv. grapes. Also, the positive loadings are aldehydes and terpenes, therefore the higher the degree of maturity, the higher the amount of these compounds in the grape.

Finally, since most of the variables with the loading absolute value above 0.8 in the PC1 are from the glycosidically bound volatile fraction, it seems that the composition of aroma precursors in the grapes could be related more to the location. In the same way, most of the variables with the loading absolute value above 0.8 in the PC2 are from the free volatile fraction, and therefore may be related more to the degree of maturity.

CONCLUSIONS

The aromatic profile of *Vitis vinifera* cv. País was strongly affected by the location of the vineyard and the stage of maturity of the grape. Among the free volatile compounds described in the País cv., 3-hydroxy-2,4,4-trimethylpentyl-2-methylpropanoate may be a varietal marker of this grape and could also appear in wines from this grape, giving them a varietal character. The ripening stage has a significant influence on several free volatile compounds. The main trend in terpenes was to decrease or remain the same and, in the case of esters and aldehydes, to increase or remain unchanged. The aldehyde trend was also confirmed with the PCA loading values. Finally, the amounts of total alcohols, acids and ketones depended on the location. Differences between locations presented significant differences, with Hualañé having the lowest amount of all the chemical groups studied, with this being attributed partially to the different viticultural practices in this location. Otherwise, the amount of glycosidically bound aroma compounds of all chemical groups increased during ripening. These non-volatile molecules were found mainly in the skins. Comparing the evolution of the distribution of the precursors in the grapes, it was observed that, depending on the location and on the chemical group, the increases were produced in the skin or in the pulp. It was observed that the terpene content of grapes seemed to be strongly related to vineyard location and cultivar conditions, and that *cis*-2-hexenol could be a good candidate to assess the maturity of País cv. grapes. Finally, the free aroma profile of the grapes seemed to be influenced more by the degree of maturity, while the bound aroma fraction was influenced more by the location.
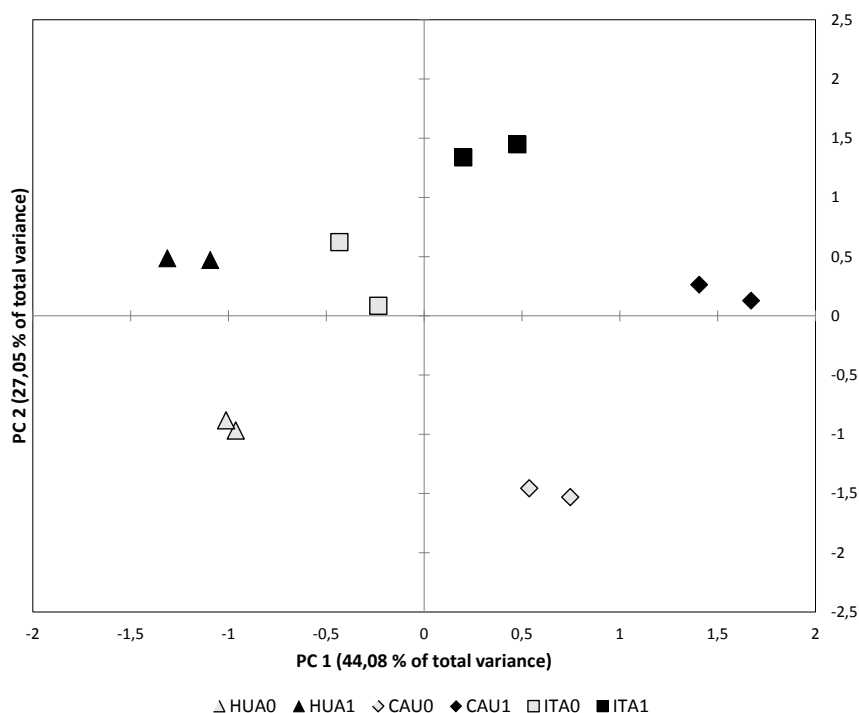

FIGURE 2

Plot of data scores on the plane made up of the first two principal components (PC1 against PC2) for all the grape samples.

TABLE 4
Loadings with absolute value > 0.8

Fraction	Variable	PC 1	PC 2
F	Nerol	-0.937	-
G	Total alcohols	0.935	-
F	Ethyl hexanoate	-0.931	-
F	Total terpenes	-0.925	-
G	β -Damascenone	0.924	-
G	Hexanal	0.909	-
F	Geraniol	-0.907	-
G	Cyclohexanone	0.905	-
G	Myrcenol	0.896	-
F	Total ketones	-0.889	-
G	Hexanoic acid	0.883	-
G	Ocimenol	0.875	-
G	Linalool	0.871	-
G	Limonene	0.870	-
G	2,5-Dimethylbenzaldehyde	0.852	-
G	Total aldehydes	0.848	-
G	Total C_{13} norisoprenoids	0.846	-
G	6,7-Dihydro-7-hydroxylinalool	0.837	-
G	Benzyl alcohol	0.827	-
G	Total ketones	0.811	-
F	Benzyl alcohol	-0.808	-
F	*cis*-2-Hexen-1-ol	-	-0.962
F	Total aldehydes	-	0.840
F	*trans*-2-Hexenal	-	0.824
G	Total terpenes	-	0.815

F: Fraction of free volatile compounds; G: Glycosidically bound aroma fraction.

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