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Aroma enhancement in wines from different grape varieties using exogenous glycosidases

E. Sánchez Palomo, M.C. Díaz-Maroto Hidalgo, M.Á. González-Viñas, M.S. Pérez-Coello *

Área de Tecnología de los Alimentos, Facultad de Ciencias Químicas, Campus Universitario no. 10, 13071 Ciudad Real, Spain

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Abstract

Musts from Airen, Macabeo, Albillo and Chardonnay grape varieties, cultivated in Spain, were fermented using a fungal glycosidase enzyme. Aroma compound analyses by gas-chromatography showed a slight increase of some compounds, that are glycosidically bound, in enzyme-treated wines. Principal components analysis of chemical data indicated out that the effects of varietal characteristics of wines were greater than the effects of enzyme treatment. Generalized Procrustes analysis was applied to sensory descriptive data. Enzyme-treated wines showed different sensorial attributes from control wines and were judged as wines with more floral and fruity aroma and some sweet, ripened fruit notes.

Keywords: Glycosidases; Aroma compounds; GC–MS; Sensory analysis

1. Introduction

Wine aroma, widely considered to be a key aspect of quality, is the result of the interaction between components of the grapes themselves and those produced during processing, fermentation and aging, and the consumers' sense of smell.

Today there is an increasing demand for young white wines with a fresh and fruity aroma, this being a major factor determining wine character and quality.

The typical flavour of wines is mainly due to volatile compounds deriving from the grapes, and several grape varieties (e.g., Muscat, Chardonnay, Sauvignon) have been characterized in terms of their flavour composition (Flanzy, 2000; Ribereau-Gayon, Glories, Maujean, & Dubourdieu, 2000). Other factors also contribute to the complexity of wine aroma, including geographical, cultural and viticultural factors and wine-making techniques (Bureau, Razungles, & Baumes, 2000; Jackson & Lombard, 1993; Zoecklein, Wolf, Marcy, & Jasinski, 1998).

Volatile compounds deriving from the grape include monoterpenes, C_{13}-norisoprenoids, benzene derivatives, and aliphatic alcohols, most of which possess pleasant floral and fruity aromas, which have very low perception thresholds. These compounds can appear in their free form or as odourless, non-volatile glycosides (Günata, Bayonove, Baumes, & Cordonnier, 1985a; Voirin, Sapis, & Bayonove, 1992; Williams, Sefton, & Wilson, 1989; Winterhalter & Skouroumounis, 1997).

Volatile compounds from glycosides can be released by acid or enzyme hydrolysis, thus enhancing the aromatic profile of wines. Acid hydrolysis occurs very slowly during wine storage or can be accelerated by heat induction (Günata, Bayonove, Baumes, & Cordonnier, 1985b; Sefton, 1998), but both processes may prompt a deterioration in wine quality.

Enzymatic hydrolysis, due to grape or yeast glycosidases, is very limited, since these enzymes present low activity under fermentation conditions (Günata, Dugelay,

* Corresponding author.
E-mail address: Soledad.Perez@uclm.es (M.S. Pérez-Coello).

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Sapis, Baumes, & Bayonove, 1993; Lecas, Günata, Sapis, & Bayonove, 1991). Enzymes from *Aspergillus niger* are widely used in wine-making, largely because their pectinolytic activity is useful for must clarification and colour extraction. However, some of these enzymes possess considerable glycosidase activity (Günata et al., 1993; Günata, Dugelay, Vallier, Sapis, & Bayonove, 1997).

The effects of fungal enzymes, and especially of AR2000, in white and red wines have been reported for several aromatic varieties (Aldave, 1999; Baek & Cadwallader, 1999; Canal-Llauberes, 1993; Castro Vázquez, Pérez Coello, & Cabezudo, 2002; Günata, Dugelay, Sapis, Baumes, & Cordonnier, 1990) as well as for a model solution (Spagna, Barbagaló, Greco, Manenti, & Pifferi, 2002); in all cases, they produced an increase in free volatile compounds, such as terpenes, C_{13}-norisoprenoids and benzene compounds. This practice is particularly valuable for enhancing the aroma of neutral or non-floral grape varieties containing only small amounts of odour-active compounds (Cabaroglu, Selli, Canbas, Lepoutre, & Günata, 2003).

At the same time, it is essential to bear in mind the effect of other activities, such as pectinolytic or cinnamate esterase activity, in the enzyme preparation used, since these might prompt the release of colour compounds or vinylphenols which have a negative effect on wine quality (Chatonnet, Dubourdieu, Boidron, & LaVigne, 1993; Dugelay, Günata, Sapis, Baumes, & Bayonnove, 1993). Moreover, changes taking place due to enzyme treatment may differ from one wine to another, depending on the chemical composition of the grapes and on the wine-making techniques used. Some volatile compounds arising from fermentation, such as esters and acetates, play major roles in the overall aroma of wines with low varietal contribution (Cabaroglu, Canbas, Lepoutre, & Günata, 2002a; Ferreira, Fernandez, Peña, Escudero, & Cacho, 1995; López, Ortín, Pérez-Trujillo, Cacho, & Ferreira, 2003).

The present study was designed to investigate the efficacy of treatment with a fungal enzyme rich in glycosidase activity in enhancing the potential aroma of wines produced from three grape varieties (Airén, Macabeo, Chardonnay) grown in the La Mancha region of Spain, and one variety (Albillo) not usually used for wine production. The aroma composition and sensory characteristics of wines were studied in order to determine the influence of the glycosidase enzyme on each grape variety.

2. Materials and methods

2.1. Samples

Grapes from *Vitis vinifera* vars. Airen, Macabeo, Chardonnay, and Albillo cultivated in the La Mancha region (Spain), were harvested at optimum ripeness.

Laboratory fermentations using 3 l vessels were carried out in triplicate, after inoculation with *Saccharomyces cerevisiae* race *cerevisiae* yeasts (CECT No. 10835). Following fermentation at 18 °C, a commercial enzyme preparation (AR-2000, Gist Brocades) was added to three vessels for each grape variety. The remaining vessels were used as controls.

2.2. Analysis of volatile compounds

Varietal compounds were extracted using the method developed by Günata, Bayonove, Baumes, and Cordonnier (1985c). Two hundred millilitres of must or wine were fractionated on preconditioned styrenedivinylbenzene cartridges (Bond Elut, Varian, 1 g of phase) using 4-nonanol as internal standard, with subsequent elution using 50 ml of pentane-dichloromethane (2:1). Extracts were concentrated on a Vigreux column to a volume of 200 μl.

Fermentation compounds were isolated by continuous liquid–liquid extraction using pentane-dichloromethane (60:40) as solvent, and 4-nonanol as internal standard. Extracts were desiccated over anhydrous sodium sulphate, filtered and concentrated by evaporation on a Vigreux column. Finally, the concentrates were frozen at −20 °C prior to GC analysis.

2.3. Gas chromatography conditions

A Hewlett–Packard model 4890 gas chromatograph equipped with a flame ionisation detector (280 °C) was used with a BP-21 capillary column (50 m × 0.32 mm i.d.; 0.32 μm film thickness). Injector temperature was 250 °C and oven temperature was set at 70 °C (5 min) than 1 °C/min to 95 °C (10 min), and 2 °C/min to 190 °C (40 min). Carrier gas: was He (0.7 ml/min); injection volume was 1 μl.

Identification was achieved by comparison of GC retention times with those of authentic standards from Sigma–Aldrich, and by GC–MS. For quantification purposes, calibration curves were used when standards were available; otherwise semi-quantitative analysis was performed, assuming a response factor equal to one.

2.4. Colour compounds

Total polyphenol index of wines was determined by measuring absorbance at 280 nm after dilution with ethanol (1/10).

3-Flavanols were measured using the method described by Amerine and Ough (1980). Values for CIELAB parameters L^*, C^* and h^*, were calculated from the absorbances measured at 450, 520, 570 and 630 nm, after sample filtration through a 0.45 μm mesh, following the simplified method proposed by Pérez Caballero, Ayala, Echávarri, and Negueruela (2003).

2.5. Descriptive sensory analysis

Wines were evaluated in duplicate by a panel of 8 experienced wine-testers. Assessment took place in a standard sensory-analysis chamber (ISO 8589, 1998) equipped with separate booths. Wines were only sniffed and only aroma attributes were considered.

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

Physical standards were used to help define attributes (Noble et al., 1984). The panellists used a 10 cm unstructured scale from 0 to 10 to rate the intensity of each attribute previously selected. The left-hand end of the scale was "attribute not perceptible" and the right-hand end was "attribute strongly perceptible".

2.6. Statistical analysis

The "*t*" test, analysis of variance (ANOVA) and principal component analysis (PCA) were applied to discriminate among the means of chemical measurements of volatile compounds in the samples. Statistical processing was carried out using the SPSS 11.0 for Windows statistical package.

Sensory data were analysed by means of generalized Procrustes analysis (PSA-System Version 2.2.; Oliemans, Punter and Partners, P.O. Box 14167, 3508 SG Utrecht, The Netherlands). Average configuration plot dimensions were interpreted, taking into account the descriptors used by each of the assessors, which were most highly correlated with each dimension (Costell, Trujillo, Damasio, & Duran, 1995; Grains & Thomson, 1990).

3. Results and discussion

3.1. Chemical analysis of samples

Volatile compound concentrations in the musts of the four grape varieties studied are shown in Table 1. There was a predominance of C_6 alcohols. The (*E*) isomer of 2-hexen-1-ol predominated in the Albillo and Chardonnay varieties whereas, in Airén and Macabeo musts, it was the (*Z*)-3-hexen-1-ol (Castro-Vázquez et al., 2002). These compounds are formed from long-chain fatty acids in the grape, and increase during berry ripening; they are also increased by berry breakdown mechanisms and skin contact before fermentation (Baumes, Bayonove, Barrillere, Samson, & Cordonnier, 1989). Since all grapes used were at the same stage of ripening, and since similar pre-fermentation processes were used, differences are attributable to the grape variety.

Of the benzene compounds, benzyl alcohol and 2-phenylethanol were most abundant in Albillo and Chardonnay musts, a finding reported by other authors for Chardonnay (Castro-Vázquez et al., 2002). Geraniol was the only terpene detected in low amounts in all four varieties.

Concentrations of varietal compounds in glycosidase-treated wines from each grape variety and in untreated control wines are shown in Table 2. All these compounds may present a sugar-bound fraction – varying in significance depending on the compound type and the grape variety – which will be released by enzyme action (Günata et al., 1993, 1990). The bound fraction in six-carbon alcohols is reportedly insignificant, so glycosidase enzyme treatment is unlikely to increase their concentration (Aldave, 1999; Cabaroglu et al., 2003). In fact, a little increase was observed for six-carbon alcohols in Macabeo and Chardonnay wines, but the "*t*" test revealed that differences between control and enzyme treated wines were not significant ($p < 0.05$).

However, these compounds undergo major transformations from the must to the wine stage. Reduction of six-carbon aldehydes to alcohols due to yeast action has been reported (Etievant, 1993), although this would not account for the increase in *cis*- and *trans*-3-hexen-1-ol observed in wines, probably at the expense of *trans*-2-hexen-1-ol which has not been detected in wines. There may also be some residual glycosidase activity by grape or yeast enzymes during fermentation (Delcroix, Günata, Sapis, Salmon, & Bayonove, 1994; Delfini et al., 2001).

Of the benzene compounds, benzaldehyde was not present in quantifiable amounts in any of the varieties. There was, however, a sharp increase of benzylalcohol in enzyme treated wines, mainly in Airen, Macabeo and Albillo wines. This increase highlights the significance of the bound fraction of this compound in musts (Aldave, 1999; Carro Mariño, López Tamames, & Garcıa Jares, 1995; Castro-Vázquez et al., 2002; Schneider, Razungles, Augier, & Baumes, 2001).

Concentrations of 2-phenylethanol are shown in mg/l in Table 2; since this compound is produced by yeast in large amounts, differences due to enzyme treatment will be masked by the greater effect of fermentation.

Geraniol, present in trace amounts in all musts, was quantified only in Macabeo and Chardonnay control wines, but was increased by glycosidase in both of these, as well as in Albillo wine (Aldave, 1999; Baek & Cadwallader, 1999; Bayonove, 1993; Mateo & Jiménez, 2000). In all cases, differences were significant ($p < 0.05$) according to the "*t*" test (Table 2).

Vinylguaiacol is a cinnamic acid derivative that may be formed by fermentation yeasts. Increased levels, following enzyme treatment in Airen, Macabeo and Chardonnay wines, may be due either to the hydrolysis of

Table 1
Concentrations of varietal volatile compounds (μg/l) in musts

Compound	Albillo must		Airen must		Macabeo must		Chardonnay must	
	Mean (n = 3)	RSD[a](%)	Mean (n = 2)	RSD (%)	Mean (n = 3)	RSD (%)	Mean (n = 3)	RSD (%)
2-Hexenal	45.2	2.6	6.4	5.6	29.6	0.9	36.2	0.1
1-Hexanol	118.9	7.9	72.5	1.3	83.2	1.0	92.9	2.6
(E)-3-Hexen-1-ol	7.2	15.2	10.2	5.4	5.1	5.1	6.4	4.9
(Z)-3-Hexen-1-ol	10.8	6.4	185.1	0.7	169.8	2.7	8.0	7.8
(E)-2-Hexen-1-ol	301.7	1.3	74.2	16.1	154.6	15.7	180.7	9.1
Benzaldehyde	Tr	–	Tr	–	Tr	–	Tr	–
Benzyl alcohol	25.1	9.4	10.4	7.6	10.4	10.6	28.7	4.6
2-Phenylethanol	16.4	5.9	7.3	2.5	9.0	7.9	9.8	2.3
Geraniol	Tr	–	Tr	–	Tr	–	Tr	–

Tr: concentrations <0.05 μg/l.
[a] RSD, relative standard deviation.

glycosylated forms in the wine, or to the additional cinnamate esterase activity of the enzyme preparation used (Cabaroglu et al., 2003). This vinyl phenol can enhance overall wine flavour, provided that concentrations do not exceed a critical level, beyond which phenolic aromas are observed; the critical level of 725 μg/l proposed by Chatonnet et al. (1993) was not exceeded in any of the wines tested here.

Table 3 shows colour-related variables: optical density at 280 nm, total polyphenols and catechins, and values for CIELAB parameters (L, C, h, a and b). Addition of a glycosidase may prompt the release of sugar-bound polyphenols, leading to colour increase in treated wines (Wightman et al., 1997). However, as Table 3 shows, enzyme treated white wines displayed no increase in optical density at 280 nm or in total polyphenols.

While varietal compounds make a major contribution to the aromatic profile of wines, other compounds, such as acetates and short-chain or medium-chain esters, can play a significant role in the sensory properties of young white wines made from less aromatic grape varieties (Etievant, 1993; Ferreira et al., 1995). Fermentation compound concentrations for the studied wines are shown in Table 4. Results for enzyme-treated wines are included, although enzyme treatment took place after fermentation and was therefore not expected to affect fermentation compounds.

Fermentation compounds are influenced by must composition, yeast effects and wine-making techniques. Since the yeast and the fermentation conditions used here were the same for all varieties, differences in compound concentrations may be ascribed to the initial must, i.e., the grape variety.

To highlight differences between varieties and the effect of glycosidase enzyme treatment, experimental data were subjected to principal component analysis. The first three principal components accounted for 75% of total variance. Sample distribution in the space formed by these three components is shown in Fig. 1. The main sample cluster was associated with the grape variety. Clustering of fermentation replicates is also appreciable. Glycosidase-treated wines lie close to controls, except in the case of Airen.

The variables displaying the best correlation with each principal component are shown in Table 5, together with their coefficients. Principal component 1 separated Albillo wines from the others, due to a higher concentration of 2-phenylethanol, γ-butyrolactone, 4-OH-ethyl butyrate and 1-hexanol and lower levels of isoamyl and 2-phenylethyl acetates, and of medium-chain fatty acids. Principal component 2 distinguished Airen wines from the rest, due to higher levels of *cis*- and *trans*-3-hexen-1-ol, absence of geraniol and higher concentrations of catechins and total polyphenols. Principal component 3 separated Chardonnay wines, due to their greater benzyl alcohol concentration.

Studies of aroma compounds in wines made from the Airen grape variety widely grown in the La Mancha region of Spain have highlighted their low terpene content and their greater concentration of six-carbon alcohols (Castro-Vázquez et al., 2002; Pérez-Coello, Gonzalez-Viñas, García-Romero, Diaz-Maroto, & Cabezudo, 2003). Little information is available on Albillo wines, since this variety is not widely grown and is mostly destined for eating. An earlier study of Albillo wines yielded results similar to those reported for Chardonnay wines; levels of C6 alcohols were lower than those found for Airen and Macabeo, while benzyl alcohol and 4-vinylguaiacol levels were higher (Jurado, Pinilla, Ballesteros, Pérez-Coello, & Cabezudo, 2002). Research on musts and wines made from the Chardonnay grape in other countries has shown the low terpene content of this variety, norisoprenoids and benzenic compounds being the most abundant (Sefton, Francis, & Williams, 1993). Other authors underline the influence of the production area, and the contribution of certain fermentation compounds to the flavour of Chardonnay wines (Arrhenius, McCloskey, & Sylvan, 1996).

Table 2
Concentrations of varietal volatile compounds (μg/l) in wines

Compound	Albillo control wine		Albillo wine with enzyme		Airén control wine		Airén wine with enzyme		Macabeo control wine		Macabeo wine with enzyme		Chardonnay control wine		Chardonnay wine with enzyme	
	Mean ($n = 3$)	RSD (%)	Mean ($n = 3$)	RSD (%)	Mean ($n = 2$)	RSD (%)	Mean ($n = 2$)	RSD (%)	Mean ($n = 3$)	RSD (%)	Mean ($n = 3$)	RSD (%)	Mean ($n = 3$)	RSD (%)	Mean ($n = 3$)	RSD (%)
1-Hexanol	881.4	7.7	848.4	4.1	451.2	2.4	476.3	6.2	499.1	6.5	580.4	9.7	502.3	0.8	608.2	2.6
(*E*)-3-Hexen-1-ol	44.9	3.7	43.9	3.7	119.7[b]	3.5	92.4	0.4	47.6	9.4	53.2	6.5	56.8	5.7	55.9	2.3
(*Z*)-3-Hexen-1-ol	87.5	8.9	81.1	5.6	1110.3[b]	3.5	945.8	3.6	479.3	5.8	513.6	1.0	108.4	15.8	103.0	10.5
Benzaldehyde	Tr	–	Tr	–	Tr	–	Tr	–	Tr	–	Tr	–	Tr	–	Tr	–
Benzyl Alcohol	141.1[b]	3.0	247.6	1.3	156.7[b]	3.4	315.6	7.2	108.5[b]	11.8	361.7	12.7	218.0[b]	4.9	298.6	16.9
2-Phenylethanol[a]	20.0	2.6	19.7	4.5	11.8[b]	2.0	12.9	2.1	8.4	4.0	8.8	6.5	9.8 [b]	2.4	9.3	1.6
Geraniol	Nd[b]	–	11.0	7.0	nd	–	nd	–	7.9[b]	4.7	9.1	7.7	7.0[b]	–	8.7	5.9
4-Vinylguaiacol	529.8[b]	8.4	310.8	8.4	260.4[b]	0.2	424.3	1.7	178.6	5.7	335.9	1.9	555.7	4.4	619.8	7.5

nd: not detected.
Tr: concentration <0.05 μg/l.
[a] Concentration (mg/l).
[b] Compounds that show significant differences between control and enzyme treated wines according to the "*t*" test.

Table 3
Phenolic compounds and chromatic characteristics of wines

	Albillo control wine		Albillo wine with enzyme		Airén control wine		Airén wine with enzyme		Macabeo control wine		Macabeo wine with enzyme		Chardonnay control wine		Chardonnay wine with enzyme	
	Mean ($n = 3$)	RSD (%)	Mean ($n = 3$)	RSD (%)	Mean ($n = 2$)	RSD (%)	Mean ($n = 2$)	RSD (%)	Mean ($n = 3$)	RSD (%)	Mean ($n = 3$)	RSD (%)	Mean ($n = 3$)	RSD (%)	Mean ($n = 3$)	RSD (%)
D.O.280 nm.	5.8	3.8	5.7	1.3	6.1	1.2	6.1	1.9	5.9	1.6	5.8	1.2	6.6	1.9	6.6	4.2
Total polyphenols [a]	202.1	0.4	198.2	2.3	278.5	2.1	275.9	7.5	253.8[c]	1.9	196.1	5.0	253.8[c]	4.8	219.8	1.5
Catechins [b]	19.5[c]	8.9	22.6	4.2	66.4	4.8	61.4	2.4	14.3	18.7	19.3	3.5	22.1	0.7	19.4	6.4
L	89.7	7.1	95.3	0.3	96.3	0.4	96.4	0.6	95.7	1.5	95.7	0.3	91.9	2.6	95.4	0.3
C	8.8	1.3	7.1	0.8	5.4	0.0	5.4	0.0	4.4	0.2	5.5	0.2	7.6	0.5	7.5	0.6
h	96.2	3.3	100.3	1.3	98.9[c]	0.4	88.5	0.4	103.4	1.8	107.0	0.8	86.2	12.7	95.4	0.5
a	−0.9	0.4	−1.3	0.2	−0.8[c]	0.0	0.2	0.0	−1.0	0.0	−1.6	0.4	0.6	1.7	−0.7	0.1
b	8.7	1.4	7.0	0.8	5.3	0.0	5.4	0.0	4.2	0.3	5.3	0.2	7.4	0.3	7.5	0.6

[a] meq. gallic acid/l.
[b] meq. (+) Catechin/l.
[c] Compounds that show significant differences between control and enzyme treated wines according to the "*t*" test.

Table 4
Concentrations of fermentation volatile compounds (mg/l)

Compound	Albillo control wine		Albillo wine with enzyme		Airén control wine		Airén wine with enzyme		Macabeo control wine		Macabeo wine with enzyme		Chardonnay control wine		Chardonnay wine with enzyme	
	Mean ($n = 3$)	RSD (%)	Mean ($n = 3$)	RSD (%)	Mean ($n = 2$)	RSD (%)	Mean ($n = 2$)	RSD (%)	Mean ($n = 3$)	RSD (%)	Mean ($n = 3$)	RSD (%)	Mean ($n = 3$)	RSD (%)	Mean ($n = 3$)	RSD (%)
Ethyl lactate	0.80	6.24	0.81	3.80	1.29	5.20	1.20	8.61	1.19	17.45	1.49	11.44	0.35	2.14	0.38	9.20
Ethyl hexanoate	0.98	3.45	0.96	1.59	1.31[a]	3.86	1.11	1.45	1.33	2.63	1.34	7.68	1.24[a]	1.50	1.12	5.26
Ethyl octanoate	0.87[a]	3.89	0.76	3.94	0.85	4.45	0.86	1.59	0.93	8.09	0.81	8.83	0.96[a]	1.18	0.94	0.69
Ethyl decanoate+ isovaleric acid	1.03[a]	3.12	1.07	8.92	1.12	3.40	1.12	0.10	0.90	5.74	0.91	6.04	0.89	7.35	0.76	7.04
Diethyl succinate	0.38[a]	5.75	0.45	5.69	0.29	7.02	0.34	5.36	0.19	4.58	0.22	5.73	0.18[a]	8.94	0.28	8.23
Ethyl 4-hydroxybutyrate	16.87	2.71	15.97	4.75	9.94	9.04	7.76	8.12	3.31	6.56	3.92	4.35	6.65[a]	6.32	5.35	5.24
Ethyl acetate	45.61	2.30	45.71	5.60	66.51	2.08	58.91	4.50	67.24	2.39	69.43	2.84	65.44	2.86	65.12	2.59
Isoamyl acetate	2.12	5.45	2.04	4.84	6.87[a]	1.29	5.23	3.03	4.38	2.67	4.49	9.53	6.13	5.94	5.70	8.24
Hexyl acetate	0.02[a]	2.02	0.03	4.58	0.05[a]	3.81	0.10	4.94	0.04	2.51	0.04	9.10	0.05[a]	9.01	0.04	3.34
Ethyl phenylacetate	0.10	1.44	0.11	9.70	0.12[a]	4.64	0.31	2.98	0.34[a]	7.10	0.22	13.78	0.33[a]	2.65	0.29	4.10
Isobutiric acid	1.55	2.27	1.41	3.83	1.26	9.79	1.07	8.81	0.78	4.28	0.84	6.56	0.76	2.87	0.88	1.74
Butanoic acid	0.38	1.10	0.37	5.41	0.36	3.04	0.43	4.85	0.42[a]	3.27	0.36	5.44	0.37	7.40	0.37	7.18
Hexanoic acid	3.87[a]	0.46	4.12	2.43	5.24	1.19	5.26	1.44	6.24	7.03	6.21	7.40	5.47	3.93	5.22	3.49
Octanoic acid	5.26	1.69	5.34	7.56	6.73	4.17	7.52	3.39	9.05	6.70	8.87	10.13	7.61	2.92	7.03	1.65
Decanoic acid	2.17	19.20	2.09	4.53	2.38	14.20	2.15	2.40	2.29	5.25	3.05	9.49	3.69	2.35	2.70	3.60
Methanol	17.59	2.49	17.84	2.59	25.30	9.44	27.81	5.59	23.43	0.53	25.12	4.57	20.64	1.67	20.12	5.93
1-Propanol	15.18	1.62	15.59	1.86	25.44	5.13	24.13	2.85	27.59	2.49	29.83	7.38	19.73	2.85	25.35	2.01
2-Methyl-1-propanol	17.40	2.89	18.90	4.50	17.86	4.36	18.36	4.57	14.78	1.63	14.64	1.72	13.90	5.45	65.74	6.43
2-Methyl-1-butanol	25.01	1.60	26.38	1.35	21.34	5.37	21.85	7.46	16.65	2.90	17.48	2.80	17.79	4.51	17.87	5.78
3-Methyl-1-butanol	95.83	4.59	99.67	3.04	97.59	9.69	95.37	2.10	81.63	4.34	87.86	1.04	87.54	2.09	86.44	1.24
3-(Methylthio)-1-propanol	0.52	5.34	0.48	2.91	0.69	1.03	0.61	5.91	0.26	10.20	0.32	1.87	0.26	6.38	0.22	23.12
Acetaldehyde	40.64	1.86	38.50	5.87	44.93	5.64	52.60	8.37	25.43	8.27	23.12	2.65	25.64	6.24	24.55	2.87
γ-Butyrolactone	6.50	1.89	6.92	3.52	4.84	2.02	4.91	8.03	2.61	2.83	3.10	18.89	4.99	12.10	4.37	7.93
3-OH Butanone	0.23	4.10	0.27	4.8	0.16	7.04	0.25	17.54	0.09[a]	3.10	0.16	4.20	0.14	5.94	0.11	11.24

[a] Compounds that show significant differences between control and enzyme treated wines according to the "*t*" test.

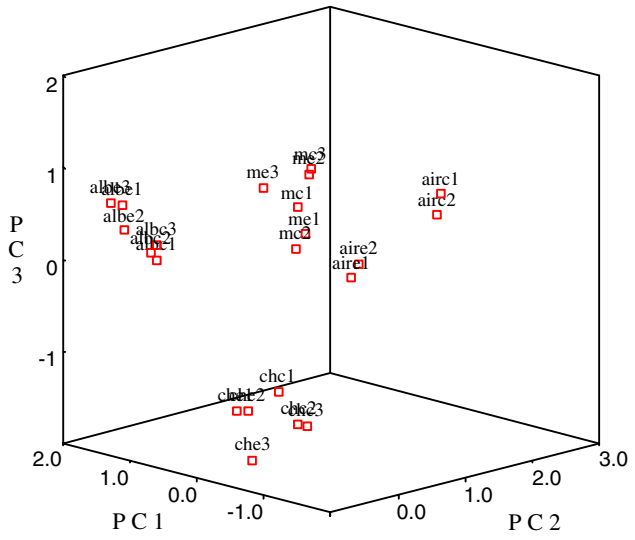

Fig. 1. Plot of wines with and without enzyme treatment on the space defined by the first three principal components: Albe, albillo with enzyme treatment; albc, albillo control; aire, airen with enzyme treatment; airc, airen control; me, macabeo with enzyme treatment; mc, macabeo control; che, chardonnay with enzyme treatment; chc, chardonnay control.

3.2. Sensory analysis of samples

Generalized Procrustes analysis applied to sensory descriptive data allows discrimination between the samples, providing information on the attributes responsible for the differences identified. The method also yields information on the evaluation behaviour of each of the assessors.

All wines were assessed by skilled tasters, using attributes previously agreed upon as best for describing sensorial characteristics and capable of distinguishing one from another.

Table 5
Correlation coefficients for wine volatile components against principal components 1, 2 and 3

Principal component	Compound[a]	Correlation coefficient
PC1	2-Phenylethanol	0.97
	γ-Butyrolactone	0.96
	Ethyl 4-OH butyrate	0.95
	Hexanoic acid	−0.94
	Octanoic acid	−0.90
	1-Hexanol	0.84
	Decanoic acid	−0.79
	Isoamyl acetate	−0.74
	Ethyl phenyl acetate	−0.72
PC2	Catechins	0.96
	trans-3-Hexen-1-ol	0.83
	cis-3-Hexen-1-ol	0.77
	Geraniol	−0.72
	Total polyphenols	0.70
PC 3	Benzyl alcohol	−0.88

[a] Only those compounds with absolute correlation coefficients greater than 0.70 have been included.

The residual variance for each assessor provides information on the ratio between the configuration plot of the individual samples for that assessor and the configuration plot of the samples for the panel as a whole, the best fits corresponding to the lowest residual variance values. The residual variance was small (<0.5%) for all assessors. It would, therefore, seem safe to assume that the taste panel did comprise a homogeneous grouping, in that none of the assessors was an outlier distinctly separate from the group as a whole.

The distribution of the samples in the consensus space (Fig. 2) is indicative of the differences between wines. The first two dimensions explained most of the variance among the samples, with the remaining

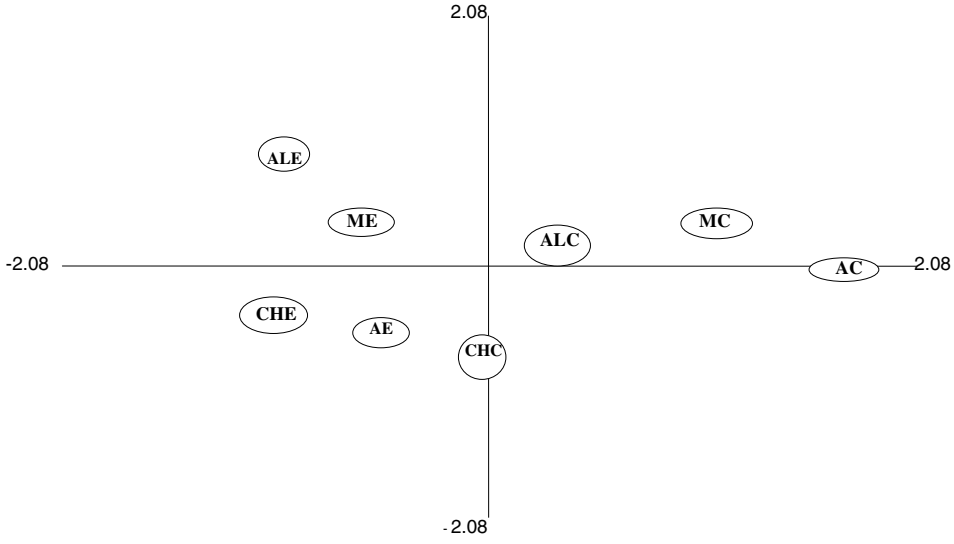

Fig. 2. Two dimensional average space plot of the wines with and without enzyme treatment: ALC, Albillo control; ALE, Albillo enzyme; AC, Airen control; AE, Airen enzyme; CHC, Chardonnay control; CHE, Chardonnay enzyme; MC, Macabeo control; ME, Macabeo enzyme.

Table 6
Descriptors most closely correlated (>0.7) with the first two GPA dimensions and mean data of the loading

Dimension 1	Dimension 2
Fresh (0.95)	Grape notes (0.73)
Citric (0.91)	Fruity (0.73)
Green apple (0.86)	
Floral (−0.81)	
Ripened fruit (−0.77)	
Sweet (−0.74)	

Only those descriptors with absolute correlation coefficients greater than 0.70 have been included.

dimensions explaining only a small proportion of the variance (<6%). Dimension 1 explained 57.06% of the total variance, while dimension 2 explained 17.67%. These two dimensions may be interpreted according to the content of Table 6, which list the descriptors most closely correlated with both dimensions.

Control wines from different grape varieties were grouped according with dimension 1. Airén and Macabeo control wines were judged as fresh, with citric and green apple notes, attributes well known for these varieties (García Romero, Pérez Coello, Cabezudo, Sánchez-Muñoz, & Martín-Alvarez, 1999; Pérez-Coello et al., 2003). Based on the sensory results, enzyme treated wine presented a more floral aroma and some sweet and ripened fruit notes. The same characteristics were found in wines treated with exogenous fungal glycosidases winemaking from other grape varieties (Cabaroglu et al., 2003). Acid hydrolysated from juice of different grape varieties were found to be more intense in attributes such as honey, dried fig and lime (Francis, Kassara, Noble, & Williams, 1998; Francis, Sefton, & Williams, 1992).

Dimension 2 explains a low percentage of variance; according to this dimension, Albillo and Macabeo wines treated with enzymes presented more higher fruity aroma and grape notes than did control wines.

Liberation of aglycones by using exogenous glycosidases can enhance the floral and fruity aroma of wines, but glycosidases they can also generate new compounds that influence wine aroma.

In conclusion, control wines from different grape varieties showed differences in aroma composition and intensity of sensorial attributes; however, the effect of the glycosidase was more clear in the sensorial perception.

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