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Comparison of the Suitability of Different Hydrolytic Strategies To Predict Aroma Potential of Different Grape Varieties

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Precursor extracts obtained from different grape varieties were submitted to harsh acid hydrolysis (pH 2.5, 100 °C, 1 h) and enzymatic hydrolysis (AR2000, pH 5, 40 °C, 16 h) and were also added to a synthetic must (200 g L^{-1} glucose), which was fermented (yeast strain Stellevin NT 116), to compare the "natural hydrolysis" carried by yeast with alternative "fast" hydrolytic strategies. In all cases, released volatile compounds were extracted by SPE and determined by GC-MS. Leaving aside Muscat, differences between varieties were not relevant, although Grenache and Chardonnay presented some key peculiarities. In general, alcoholic fermentation showed the lowest potential to release volatile compounds from aromatic precursors, whereas enzymatic hydrolysis was the most efficient but also the most different. Practically, this implies that the predictive ability of this hydrolytic strategy is rather poor. In contrast, harsh acid hydrolysis can be considered to much more adequately measure the aroma potential of grapes for winemaking, which suggests that transformations taking place during fermentation include relevant chemical rearrangements in acid media that are better predicted by acid hydrolysis.

KEYWORDS: Glycosides; flavor; acid hydrolysis; enzymatic hydrolysis; alcoholic fermentation

INTRODUCTION

Studies carried out in recent decades have demonstrated that most flavor compounds in nonfloral grapes are in glycosylated form (*1, 2*). It has been proved that some important aromas in wine come from these flavor precursors. For example, β-damascenone, vitispirane, Riesling acetal, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), and *tert*-1-(2,3,6-trimethylphenyl)but-1,3-diene (TPB) are present as free molecules at low or even null levels in grapes, whereas they can be found at higher concentrations in aged wines (*3*−*5*). Some important monoterpenes, such as α-terpineol, linalool, and geraniol, are also formed from glycoside precursors during fermentation (*6*−*8*), although in this case other sources coexist, such as de novo synthesis (*9*) or the chemical transformation of free monoterpenes by wine yeasts (*10, 11*). In general, volatile compounds from glycoside precursors can be released or formed during the winemaking process by endogenous and exogenous glycosidases (*12*−*15*), by the action of wine yeasts (*11, 16*−*18*) and lactic bacteria (*19*−*21*), or by acid hydrolysis (*22*−*24*). In any case, it can be stated that there is strong evidence supporting the existence of a connection between the aromatic quality of wine and the grape content in aroma precursors (*25, 26*). This fact has encouraged extensive research both for the chemical characterization of this pool of precursors and of the biogenetic pathways involved (*2, 27*−*31*), and for the development of quantitative methods for the global or detailed evaluation of the fractions of precursors present in grapes (*32*−*34*).

The chemical complexity of the precursor fractions has required most studies to be based on the analysis of hydrolysates obtained via acid or enzymatic hydrolysis from these fractions and, consequently, there is today extensive information about the differential characteristics of both types of hydrolysis. In general, enzymatic hydrolysis is preferred for the chemical characterization of the precursor fraction because it induces fewer transformations than acid hydrolysis (*32, 35*). In this last case, acid-catalyzed cyclations, dehydrations, and rearrangements (*22, 36*), accelerated at high temperature (*37*), have been described. However, acid hydrolysis is particularly effective at releasing norisoprenoids (*36, 38*−*40*), and the sensory properties of acid hydrolysates are much more intense than those obtained by enzymatic hydrolysis (*26, 38*).

As for the assessment of the aroma potential of grapes, different strategies have been proposed. The first proposal was based on the colorimetric determination of terpenols released by distillation of acidified must (*41*). Other global strategies are based on the measurement of the glucose released after acid hydrolysis of the precursor fraction (*42*−*44*), whereas a recent proposal makes use of Fourier-transform infrared spectrometry

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and chemometrics (*45*) for the evaluation of the total levels of C_{13}-norisoprenoid and monoterpene glycoconjugates.

There are not many data, however, about how the general or detailed quantitative composition of the grape precursor hydrolysates will be related to the aroma composition of the wine. So far, although there are some works comparing acid and enzymatic hydrolyses with the hydrolysis carried out by yeasts (*18*, *46*), only a report by Kotseridis et al. (*47*) shows that the levels of β-damascenone in wine can be predicted by determining the β-damascenone formed by fast acid hydrolysis of the precursor fraction of grapes. A report by Winterhalter et al. (*5*) suggests that the potential levels of TDN upon aging may be predicted by analysis of the corresponding aglycone released by steam distillation at acid pH. Apart from these works, and to the best of our knowledge, there is not more information about this specific issue. Because of this, the main goal of the present work is to evaluate whether the quantitative composition of grape precursor hydrolysates obtained by two fast hydrolytic procedures can be used to predict the quantitative aroma composition of wine. To do that, fractions of precursors from grapes from different varieties have been isolated, analyzed by fast analytical procedures, and fermented in synthetic media in controlled standard conditions.

MATERIALS AND METHODS

Samples. Grapes from *Vitis vinifera* vars. Muscat (MU), Chardonnay (CH), Grenache (G), Tempranillo (T), Merlot (ME), Cabernet Sauvignon (CA), and Verdejo (V) (from D. O. Somontano, D. O. Borja, and D.O. Rueda, 2007 vintage season) were harvested by hand and were stored frozen at −30 °C in the laboratory.

Preparation of Precursor Extract. The precursors were extracted from the different grape samples following the procedure described by Loscos et al. (*7*). Grapes (1.5 kg of grapes per sample) were destemmed and homogenized, and then must and skins were separated by centrifugation. The mashes of skins obtained were suspended in a buffer solution of 0.1 M Na_2HPO_4/NaH_2PO_4 at pH 7 and containing 13% (v/v) ethanol (475 mL of buffer solution per 100 g of skins) and allowed to macerate in the dark (36 h, 20 °C, and nitrogen atmosphere) to extract the precursors. The precursors from both macerate and must were extracted using LiChrolut EN resins (Merck, Darmstadt, Germany). After percolation of the samples, resins were first washed with water to remove high-polar compounds and then with a pentane/dichloromethane (2:1 v/v) mixture to remove free volatile compounds. The retained precursors were finally eluted with an ethyl acetate/methanol (9:1 v/v) mixture. The extracts were evaporated under vacuum to dryness and then reconstituted in 20 mL of a 50% ethanol solution (from around 900 mL of must or around 240 g of skins). Finally, the macerate and must extracts were mixed.

Precursor extracts were submitted to harsh acid hydrolysis, enzymatic hydrolysis, and alcoholic fermentation.

Harsh Acid Hydrolysis. Two milliliters of the precursor extract was diluted with 8 mL of a 0.2 M citric acid buffer solution (pH 2.5) to reach 10% of EtOH. Harsh acid hydrolysis was carried out in triplicate at 100 °C for 1 h following the procedure described by Ibarz et al. (*48*). Ten milliliter SPME vials fitted with silicone/PTFE septa sealed with a steel magnetic cap (Varian, Sunnyvale, CA) were used. The vial was purged with nitrogen after sealing. The control sample (ACO) was composed of 10 mL of the citric acid buffer solution without precursor extract addition, which was heated at 100 °C for 1 h like all of the samples.

Enzymatic Hydrolysis. The procedure was adapted from that reported by Schneider et al. (*49*). Two milliliters of the precursor extract was evaporated under vacuum until ethanol was removed. The obtained precursor extract was diluted with 8.2 mL of a 0.1 M citrate/0.2 M phosphate buffer solution (pH 5), and then 800 μL of a 120 mg mL^{-1} solution in the citrate/phosphate buffer solution of AR 2000 pectinase enzyme preparation (DMS Food Specialties Beverages Ingredients, Delft, The Netherlands) was added. Enzymatic hydrolysis was carried out in triplicate at 40 °C for 16 h. Ten milliliter SPME vials fitted with silicone/PTFE septa sealed with a steel magnetic cap (Varian) were used. The vial was purged with nitrogen after sealing. The control sample (ECO) was an AR 2000 enzyme preparation solution (9.6 mg mL^{-1}, in 10 mL of the citrate/phosphate solution) without precursor extract addition, which was also heated at 40 °C for 16 h. The amount of enzyme was a compromise between that reported in the work by Schneider (*49*) and the amount recommend by the enzyme supplier. The glycosidase activity of this preparation has been reported elsewhere (*50*, *51*).

Alcoholic Fermentations. Laboratory fermentations were carried out in a synthetic nutrient medium consisting of 100 g L^{-1} glucose, 100 g L^{-1} fructose, 3 g L^{-1} tartaric acid, 0.1 g L^{-1} $CaCl_2$, 0.1 g L^{-1} NaCl, 1 g L^{-1} KH_2PO_4, 0.5 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.94 g L^{-1} $(NH_4)_2SO_4$, 0.94 g L^{-1} $(NH_4)_2HPO_4$, 198 μg L^{-1} $MnCl_2 \cdot 4H_2O$, 135 μg L^{-1} $ZnCl_2$, 29 μg L^{-1} $Co(NO_3)_2 \cdot 6H_2O$, 24 μg L^{-1} $NaMoO_4 \cdot 2H_2O$, 14 μg L^{-1} $CuCl_2$, 11 μg L^{-1} KIO_3, 5.7 μg L^{-1} H_3BO_3, 2 mg L^{-1} pyridoxine−HCl, 2 mg L^{-1} nicotinic acid, 1 mg L^{-1} calcium pantothenate, 1 mg L^{-1} thiamin−HCl, 0.2 mg L^{-1} *p*-aminobenzoic acid, 0.2 mg L^{-1} riboflavin, 0.2 mg L^{-1} folic acid, and 0.125 mg L^{-1} biotin. The medium was sterilized by filtration through 0.45 μm sterile membranes (Schleicher & Schull, Dassel, Germany). Yeast strain Stellevin NT 116 (Anchor Bio-Technologies, Cape Town, South Africa) was grown from 0.5 g of active dry yeast rehydrated in 30 mL of sterile water at 35 °C for 30 min. Fermentation was carried out in triplicate using 350 mL bottles filled with 200 mL of sterile synthetic medium. Precursor extracts were added to reach nearly the same concentration of precursors in must (25 mL of the precursor extract per liter of must). Samples were inoculated at 20 °C with 2 mL of the activated yeast solution. The inoculated synthetic medium without precursor extract addition was used as control sample (FCO). The fermentation process was monitored by weight. All fermentations were completed after 54 days. After fermentation was completed, samples were centrifuged to remove yeast lees.

Extraction and Analysis of Minor Volatile Compounds. Volatile compounds released by harsh acid hydrolysis and enzymatic hydrolysis were extracted by SPE using LiChrolut EN resins (50 mg) following the procedure described by Ibarz et al. (*48*). Samples were entirely loaded in the cartridge, and analytes were recovered by elution with 700 μL of dichloromethane. An internal standard solution (4-methyl-4-pentanol, 4-hydroxy-4-methyl-2-pentanone, and 2-octanol in dichloromethane, at concentrations of 350, 450, and 500 μg g^{-1}, respectively) was added to the eluted sample. Finally, extracts were concentrated to 100 μL under nitrogen, and 4 μL was injected into the GC-MS system under the conditions described below.

Volatile compounds released after alcoholic fermentation were extracted by SPE with LiChrolut EN resins (50 mg) following the method proposed and validated by López et al. (*52*) with the modifications proposed by Loscos et al. (*7*). Fifteen milliliters of wine was loaded in the cartridge, and analytes were recovered by elution with 600 μL of dichloromethane. An internal standard solution (4-methyl-4-pentanol, 4-hydroxy-4-methyl-2-pentanone, and 2-octanol, at concentrations of 350, 450, and 500 μg g^{-1} in dichloromethane, respectively) was added to the eluted sample. Finally, 4 μL of the extract was injected into the GC-MS system under the conditions described below. A standard addition experiment was carried out to validate this procedure. Results (data not shown) indicate that recoveries are >85% for all analyzed compounds with the exceptions of guaiacol, 4-vinylguaiacol, and 2,6-dimethoxyphenol (recoveries of 35, 51, and 55%, respectively).

Gas Chromatography−Mass Spectrometry Conditions. Gas chromatographic analysis was performed with a CP-3800 chromatograph coupled to a Saturn 2200 ion trap mass spectrometric detection system from Varian. A DB-WAXetr capillary column (J&W Scientific, Folsom, CA) (60 m × 0.25 mm i.d., film thickness = 0.5 μm) preceded by a 3 m × 0.25 mm uncoated (deactivated, intermediate polarity) precolumn from Supelco (Bellefonte, PA) was used. Helium was the carrier gas at a flow rate of 1 mL min^{-1}. The oven temperature program was 3 min at 40 °C, 10 °C min^{-1} to 90 °C, 2 °C min^{-1} to 230 °C, and finally held at this temperature for 37 min. Initially the injector was kept at 35 °C during 0.3 min, and a pressure pulse of 25 psi during

Table 1. Concentration (in Micrograms per Kilogram of Grapes, Except Where Indicated[a]) of the Volatile Compounds Released after Harsh Acid Hydrolysis of the Precursor Extract from Each Grape Variety (Data Are the Average of Three Replicate Samples)

	RI[c]	source, purity		ACO	AV	AT	ACH	ACA	AME	AMU	AG
			terpenes								
1	1355	Fluka, 99%	(*Z*)-rose oxide	nd	nd	nd	nd	nd	nd	0.04 ± 0.01	0.01 ± 0.004
2	1447	tentatively identified	(*Z*)-linalool oxide[a]	nd	3.1 ± 0.6	1.9 ± 0.2	6.7 ± 0.9	3.3 ± 0.3	4.2 ± 0.3	31 ± 3	4.5 ± 0.6
3	1476	tentatively identified	(*E*)-linalool oxide[a]	nd	2.7 ± 0.6	1.2 ± 0.1	3.6 ± 0.6	2.2 ± 0.1	3.1 ± 0.1	26 ± 4	3.1 ± 0.5
4	1478	tentatively identified	nerol oxide[a]	nd	nd	nd	3.0 ± 0.5	1.6 ± 0.1	1.4 ± 0.2	115 ± 12	6.7 ± 0.7
5	1556	Fluka, 98.5%	linalool	0.19 ± 0.03	0.51 ± 0.01	0.61 ± 0.04	1.8 ± 0.2	0.52 ± 0.03	0.64 ± 0.02	29 ± 4	1.2 ± 0.02
6	1565	tentatively identified	linalyl acetate[a]	0.44 ± 0.09	0.58 ± 0.07	0.56 ± 0.04	0.80 ± 0.05	0.79 ± 0.13	0.67 ± 0.06	0.76 ± 0.12	0.78 ± 0.06
7	1608	tentatively identified	terpinen-4-ol[a]	nd	0.37 ± 0.02	0.11 ± 0.02	0.46 ± 0.06	0.34 ± 0.07	0.62 ± 0.03	4.6 ± 0.8	0.60 ± 0.01
8	1613	tentatively identified	2,6-dimethyl-1,7-octadiene-2,6-diol[a]	0.24 ± 0.06	3.3 ± 0.3	0.43 ± 0.05	1.7 ± 0.3	0.74 ± 0.07	2.2 ± 0.1	93 ± 4	8.4 ± 0.4
9	1664	tentatively identified	δ-terpineol[a]	nd	nd	nd	nd	1.2 ± 0.2	3.5 ± 0.4	18 ± 1	4.4 ± 1.1
10	1705	Fluka, 97%	α-terpineol	0.18 ± 0.04	1.3 ± 0.3	1.5 ± 0.2	9.5 ± 0.7	2.9 ± 0.2	6.2 ± 0.5	172 ± 28	7.5 ± 1.2
11	1775	Fluka, 90−95%	β-citronellol	nd	nd	nd	nd	nd	0.06 ± 0.02	0.77 ± 0.20	0.21 ± 0.06
12	1811	Fluka, 90−95%	nerol	nd	0.23 ± 0.11	nd	0.76 ± 0.11	nd	nd	6.2 ± 0.8	nd
13	1858	Fluka, 99.5%	geraniol	0.24 ± 0.04	0.48 ± 0.06	0.40 ± 0.02	1.1 ± 0.1	0.36 ± 0.06	0.43 ± 0.04	15 ± 1	0.79 ± 0.004
14	2366	tentatively identified	neric acid[a]	nd	3.8 ± 0.9	11 ± 3	15 ± 1	5.0 ± 0.8	21 ± 2	152 ± 9	28 ± 2
			total [e]	2.4	22	24	81	30	66	1325	95
			norisoprenoids								
15	1526	tentatively identified	vitispirane A[a,d]	nd	16 ± 3	40 ± 6	14 ± 1	13 ± 0.3	10 ± 1	11 ± 2	77 ± 8
16	1529	tentatively identified	vitispirane B[a,d]	nd	20 ± 3	39 ± 6	19 ± 2	13 ± 0.2	12 ± 1	14 ± 2	63 ± 8
17	1637	tentatively identified	Riesling acetal[a,d]	nd	2.2 ± 0.3	9.6 ± 3.0	2.7 ± 0.2	2.9 ± 0.3	2.8 ± 0.1	3.5 ± 0.5	20 ± 3
18	1748	tentatively identified	1,1,6-trimethyl-1,2-dihydronaphthalene (TDN)[a]	nd	9.9 ± 2.8	41 ± 8	16 ± 3	12 ± 0.3	18 ± 2	8.4 ± 0.8	89 ± 10
19	1832	tentatively identified	*tert*-1-(2,3,6-trimethylphenyl) but-1,3-diene (TPB)[a]	nd	7.5 ± 2.2	2.4 ± 0.6	23 ± 4	4.3 ± 0.6	7.5 ± 0.7	7.7 ± 2.5	2.7 ± 0.7
20	1829	Firmenich, 90%	β-damascenone	nd	4.5 ± 0.3	2.0 ± 0.4	4.4 ± 0.3	3.4 ± 0.4	4.2 ± 0.2	2.0 ± 0.1	4.4 ± 0.5
21	1939	tentatively identified	3-oxo-β-ionone[a]	nd	47 ± 11	14 ± 2	77 ± 12	56 ± 9	62 ± 3	44 ± 10	14 ± 2
22	1950	Sigma, 98%	β-ionone	0.07 ± 0.01	nd	nd	nd	nd	nd	nd	nd
23	1952	tentatively identified	actinidols[a,d]	nd	65 ± 15	18 ± 3	105 ± 14	75 ± 12	82 ± 4	63 ± 13	19 ± 3
24	2657	tentatively identified	3-oxo-α-ionol[a]	nd	1.1 ± 0.4	2.7 ± 0.8	7.3 ± 1.0	6.6 ± 0.2	4.0 ± 0.1	6.5 ± 0.2	1.9 ± 0.03
			total[e]	0.13	187	174	276	192	214	160	312
			volatile phenols								
25	1876	Aldrich, 98%	guaiacol	nd	0.05 ± 0.01	0.31 ± 0.04	0.06 ± 0.01	0.35 ± 0.04	0.61 ± 0.05	0.06 ± 0.02	0.51 ± 0.08
26	2030	Aldrich, 99%	*o*-cresol	nd	nd	nd	nd	nd	nd	nd	nd
27	2068	Lancaster, 98%	4-ethylguaiacol	nd	nd	nd	nd	nd	nd	nd	nd
28	2157	Aldrich 99%	*m*-cresol	nd	nd	0.05 ± 0.01	nd	0.04 ± 0.01	nd	nd	0.04 ± 0.01
29	2237	Aldrich, 99%	eugenol	nd	0.04 ± 0.01	0.36 ± 0.06	0.18 ± 0.01	0.12 ± 0.03	0.15 ± 0.02	0.18 ± 0.04	0.05 ± 0.004
30	2244	Aldrich, 99%	4-ethylphenol	nd	nd	0.10 ± 0.01	nd	nd	0.02 ± 0.003	nd	0.18 ± 0.02
31	2262	Aldrich, 98%	4-vinylguaiacol	nd	13 ± 2	10 ± 1	11 ± 1	9.6 ± 0.9	11 ± 1	6.7 ± 1.3	38 ± 6
32	2317	Aldrich, 99%	2,6-dimethoxyphenol	nd	nd	2.6 ± 0.7	nd	4.3 ± 0.7	5.5 ± 0.9	nd	4.6 ± 0.9
33	2279	Lancaster, 97%	(*E*)-isoeugenol	nd	0.40 ± 0.03	0.34 ± 0.01	0.44 ± 0.04	0.40 ± 0.09	0.33 ± 0.03	0.58 ± 0.09	0.32 ± 0.02
34	2404	Lancaster, 10% soln.	4-vinylphenol	nd	9.6 ± 1.5	16 ± 1	21 ± 2	13 ± 0.5	12 ± 1	4.8 ± 0.4	15 ± 1
35	2563	Aldrich 90%	4-allyl-2,6-dimethoxyphenol	nd	nd	nd	nd	nd	nd	nd	nd
			total volatile phenols (I) [e]	nd	1.6	5.7	2.4	4.3	6.2	3.0	7.4
			total volatile phenols (II) [e]	nd	164	156	196	142	168	65	545
			vanillin derivatives								

Table 1. Continued

	RI[c]	source, purity		ACO	AV	AT	ACH	ACA	AME	AMU	AG
36	2592	Panreac, 99%	vanillin	0.21 ± 0.01	0.60 ± 0.09	1.1 ± 0.1	0.81 ± 0.07	0.74 ± 0.10	1.1 ± 0.03	0.98 ± 0.01	1.5 ± 0.1
37	2629	Aldrich, 99%	methyl vanillate	nd	0.44 ± 0.04	1.3 ± 0.1	0.39 ± 0.02	1.6 ± 0.1	1.4 ± 0.01	0.27 ± 0.01	3.4 ± 0.2
38	2654	Lancaster, 97%	ethyl vanillate	nd	0.17 ± 0.004	1.1 ± 0.2	0.17 ± 0.01	2.1 ± 0.4	3.1 ± 0.2	0.18 ± 0.01	2.7 ± 0.3
39	2664	Aldrich, 98%	acetovanillone	nd	1.4 ± 0.1	1.6 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.9 ± 0.1	1.3 ± 0.1	2.5 ± 0.2
40	2829	Aldrich, 96%	zingerone	nd	tr	0.15 ± 0.06	tr	tr	0.05 ± 0.01	tr	1.8 ± 0.2
41	2892	Aldrich, 99%	homovanillyl alcohol	nd	0.51 ± 0.01	1.0 ± 0.11	1.7 ± 0.1	0.44 ± 0.10	2.3 ± 0.2	1.4 ± 0.2	1.5 ± 0.1
42	3040	Aldrich, 98%	syringaldehyde	nd	1.1 ± 0.2	2.7 ± 0.1	1.6 ± 0.1	2.0 ± 0.3	1.2 ± 0.1	1.3 ± 0.2	6.9 ± 0.2
43	3099	tentatively identified	homovanillic acid[a]	nd	17 ± 2	13 ± 1	16 ± 1	42 ± 5	19 ± 1	29 ± 4	32 ± 1
44	3123	Aldrich, 97%	acetosyringone	nd	0.64 ± 0.13	1.2 ± 0.1	0.26 ± 0.02	0.88 ± 0.16	2.5 ± 0.2	0.42 ± 0.07	1.7 ± 0.2
			total [e]	0.44	45	88	47	117	129	60	220
			benzenes								
45	1520	Fluka, 99%	benzaldehyde	0.27 ± 0.02	1.1 ± 0.1	1.3 ± 0.1	3.4 ± 0.3	0.85 ± 0.05	0.84 ± 0.13	0.72 ± 0.04	1.8 ± 0.1
46	1659	Aldrich, 90%	phenylacetaldehyde	nd	3.2 ± 0.1	2.2 ± 0.2	14 ± 1	3.3 ± 0.4	2.5 ± 0.4	3.7 ± 0.02	4.4 ± 0.6
47	1891	Aldrich, 99%	benzyl alcohol	0.33 ± 0.04	2.2 ± 0.3	1.8 ± 0.2	3.0 ± 0.5	2.4 ± 0.3	3.3 ± 0.3	2.0 ± 0.3	2.6 ± 0.5
48	1908	Aldrich, 99%	ethyl dihydrocinnamate	nd	nd	nd	nd	nd	nd	nd	nd
49	1926	Fluka, 99%	β-phenylethanol	1.9 ± 0.04	2.7 ± 0.2	3.2 ± 0.3	3.1 ± 0.3	2.8 ± 0.2	3.9 ± 0.2	3.0 ± 0.2	2.5 ± 0.1
50	2081	Aldrich, 99%	ethyl cinnamate	nd	nd	nd	0.11 ± 0.01	nd	nd	nd	nd
51	2219	Fluka, 98%	2-phenoxyethanol	0.16 ± 0.03	0.20 ± 0.004	0.40 ± 0.06	0.29 ± 0.03	0.33 ± 0.06	0.35 ± 0.03	0.34 ± 0.06	0.31 ± 0.04
52	2725	tentatively identified	1,2-dimethoxy-4-propylbenzene[a]	nd	nd	0.47 ± 0.11	nd	0.96 ± 0.14	1.3 ± 0.1	0.23 ± 0.05	0.54 ± 0.09
			total benzenes (I) [e]	2.8	31	31	102	31	37	34	40
			total benzenes (II) [e]	0.53	4.9	4.4	6.7	6.1	8.5	4.6	6.2
			lactones								
53	1988	Lancaster, 98%	δ-octalactone	nd	nd	nd	0.04 ± 0.003	nd	nd	1.6 ± 0.3	nd
54	2068	Aldrich, 97%	γ-nonalactone	nd	0.04 ± 0.002	0.11 ± 0.04	0.23 ± 0.03	0.14 ± 0.04	0.14 ± 0.01	0.13 ± 0.05	0.13 ± 0.03
55	2154	Aldrich, 98%	δ-nonalactone	nd	nd	nd	nd	nd	nd	nd	nd
56	2141	Aldrich, 98%	γ-decalactone	0.05 ± 0.002	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.02	0.06 ± 0.01	0.09 ± 0.02	0.07 ± 0.01
57	2260	Lancaster, 98%	δ-decalactone	nd	nd	nd	nd	nd	nd	nd	nd
			total [e]	0.23	0.78	1.1	1.9	1.3	1.2	5.6	1.2
			miscellaneous								
58	1390	Aldrich, 98%	(*Z*)-3-hexen-1-ol	nd	nd	1.1 ± 0.03	nd	0.89 ± 0.01	1.0 ± 0.03	nd	0.87 ± 0.01
59	1413	Aldrich, 98%	(*E*)-2-hexen-1-ol	87 ± 1	85 ± 3	88 ± 0.2	83 ± 2	86 ± 3	83 ± 3	86 ± 1	83 ± 4
60	1672	Lancaster, 98%	3-methylbutyric acid	tr	0.30 ± 0.05	1.1 ± 0.1	tr	tr	tr	1.3 ± 0.1	0.04 ± 0.01
61	1677	Aldrich, 98%	2-methylbutyric acid	tr	0.27 ± 0.04	0.22 ± 0.02	1.3 ± 0.1	0.79 ± 0.06	0.91 ± 0.04	0.23 ± 0.02	0.13 ± 0.01

[a] Chemical standard not available. Tentatively identified. Data are the relative areas (to 4-hydroxy-4-methyl-2-pentanone × 1000). [b] For the calculation of the concentrations 4-methyl-4-pentanol has been used as internal standard of the miscellaneous compounds; 2-octanol of β-damascenone, *m*-cresol, 4-ethylphenol, (*E*)-isoeugenol, methyl vanillate, benzaldehyde, phenylacetaldehyde, and benzyl alcohol; and 4-hydroxy-4-methyl-2-pentanone has been used for the rest. [c] Retention index calculated in a DB-WAXetr column. [d] Actinidols, 2,2,6-trimethyl-8-(1-hydroxy)ethyl-7-oxabicyclo[4.3.0]nona-4,9-dienes; Riesling acetal, 2,2,6,8-tetramethyl-7,11-dioxatricyclo[6.2.1.0(1,6)]undec-4-ene; vitispirane, 2,10,10-trimethyl-6-methylen-1-oxaspiro-[4,5]dec-7-ene. [e] Sum of relative areas. Data corresponding to **Figure 2**: norisoprenoids do not include 3-oxo-α-ionol; volatile phenols (I) do not include 2,6-dimethoxyphenol; volatile phenols (II) include only vinylphenols; benzenes (II) include only 1,2-dimethoxy-4-propylbenzene and benzyl alcohol. [f] Control sample (CO), Verdejo (V), Tempranillo (T), Muscat (MU), Chardonnay (CH), Cabernet Sauvignon (CA), Merlot (ME), and Grenache (G). [g] nd, not detected; tr, traces.

2.60 min was applied. The injector was then heated to 250 °C at rate of 200 °C min^{-1}. The splitless time was 2.60 min. CarboFrit plugs (Restek, Bellefonte, PA) were used as a packing material in the insert. The global run time was recorded in full-scan mode (*m*/*z* 40−220 mass range). The chromatographic data were analyzed by Varian Saturn GC-MS version 6.3 software. Volatile compound identification was carried out using commercial references. The chemical standards were supplied by Aldrich (Gillingham, U.K.), Sigma (St. Louis, MO), Chemservice (West Chester, PA), Polyscience (Miles, IL), Firmenich (Geneva, Switzerland), Panreac, Merck, Fluka, and Lancaster (Strasbourg, France). When the chemical standards were not available, the identification was carried out by comparison of gas chromatographic retention and mass spectrometric data reported in the literature.

Statistical Analysis. The quantitative data were analyzed by analysis of variance (ANOVA). The analyses were carried out using SPSS (SPSS Inc., Chicago, IL) for Windows, version 11.5. Principal component analysis (PCA) was performed using The Unscrambler (CAMO ASA, Oslo, Norway) for Windows, version 7.5.

RESULTS AND DISCUSSION

In this work, extracts of flavor precursors obtained from seven different grape varieties (Verdejo, Tempranillo, Chardonnay, Cabernet Sauvignon, Merlot, Muscat, and Grenache) were hydrolyzed by two fast methods (harsh acid and enzymatic), and wines were made from a synthetic must supplemented with the different precursor fractions. The aroma compositions of both wines and hydrolysates were analyzed by GC-MS. Results of the analysis are shown in **Tables 1** (harsh acid hydrolysis), **2** (enzymatic hydrolysis), and **3** (alcoholic fermentation). A total of 61 volatile compounds, classified into seven categories (terpenes, norisoprenoids, volatile phenols, vanillin derivatives, benzenes, lactones, and miscellaneous) were determined. Data were studied by two-way ANOVA, the factors being the hydrolytic procedure and the grape variety. Results (data not shown) indicate that both factors exert a significant effect in

Table 2. Concentrations (in Micrograms per Kilogramof Grapes, Except Where Indicated[a]) of the Volatile Compounds Released after Enzymatic Hydrolysis of the Precursor Extract from Each Grape Variety (Data Are the Average of Three Replicate Samples)

	RI[c]		ECO	EV	ET	ECH	ECA	EME	EMU	EG
		terpenes								
1	1355	(*Z*)-rose oxide	nd	0.02 ± 0.003	0.05 ± 0.001	0.02 ± 0.002	0.02 ± 0.003	nd	0.62 ± 0.10	0.10 ± 0.004
2	1447	(*Z*)-linalool oxide[a]	nd	7.4 ± 1.3	4.2 ± 0.1	6.9 ± 1.1	4.6 ± 0.2	1.5 ± 0.2	46 ± 2	4.7 ± 0.2
3	1476	(*E*)-linalool oxide[a]	nd	14 ± 3	1.5 ± 0.1	1.9 ± 0.5	2.9 ± 1.0	2.2 ± 0.2	20 ± 1	2.3 ± 0.1
4	1478	nerol oxide[a]	nd	10 ± 2	0.86 ± 0.10	nd	1.9 ± 0.1	1.1 ± 0.1	8.2 ± 0.9	1.2 ± 0.1
5	1556	linalool	0.19 ± 0.04	0.81 ± 0.05	4.9 ± 0.3	8.4 ± 0.5	2.0 ± 0.3	0.54 ± 0.13	60 ± 8	3.1 ± 0.3
6	1565	linalyl acetate[a]	0.20 ± 0.04	1.2 ± 0.4	0.58 ± 0.15	1.1 ± 0.2	2.8 ± 0.1	1.1 ± 0.7	1.1 ± 0.3	1.9 ± 0.5
7	1608	terpinen-4-ol[a]	nd	nd	nd	nd	0.08 ± 0.02	0.12 ± 0.01	nd	nd
8	1613	2,6-dimethyl-1,7-octadiene-2,6-diol[a]	nd	2.4 ± 0.3	17 ± 6	2.1 ± 0.5	4.2 ± 1.2	1.3 ± 0.3	106 ± 4	5.6 ± 0.2
9	1664	δ-terpineol[a]	nd	0.26 ± 0.08	1.1 ± 0.1	nd	0.64 ± 0.04	0.21 ± 0.04	2.9 ± 0.4	0.43 ± 0.09
10	1705	α-terpineol	nd	0.71 ± 0.12	0.78 ± 0.01	0.61 ± 0.11	1.6 ± 0.4	2.3 ± 0.3	8.6 ± 1.3	1.9 ± 0.2
11	1775	β-citronellol	nd	0.20 ± 0.01	0.53 ± 0.05	0.39 ± 0.05	0.28 ± 0.03	0.73 ± 0.05	5.5 ± 0.8	3.1 ± 0.4
12	1811	nerol	nd	1.9 ± 0.1	7.2 ± 0.5	4.5 ± 0.5	3.1 ± 0.4	3.3 ± 0.4	18 ± 3	4.8 ± 0.6
13	1858	geraniol	nd	5.1 ± 0.4	13 ± 0.5	7.5 ± 0.8	6.3 ± 0.5	6.1 ± 0.5	42 ± 5	11 ± 1
14	2366	neric acid[a]	nd	33 ± 3	108 ± 14	57 ± 11	45 ± 7	48 ± 6	332 ± 75	71 ± 10
		total[e]	0.56	96	218	137	105	97	963	162
		norisoprenoids								
15	1526	vitispirane A[a,d]	nd	nd	6.7 ± 1.1	9.9 ± 2.5	4.1 ± 0.6	2.8 ± 0.7	3.8 ± 0.4	13 ± 2
16	1529	vitispirane B[a,d]	nd	10 ± 2	7.0 ± 1.2	15 ± 4	4.1 ± 0.5	3.9 ± 0.9	8.1 ± 1.2	9.3 ± 0.9
17	1637	Riesling acetal[a,d]	nd	nd	nd	nd	nd	nd	nd	nd
18	1748	1,1,6-trimethyl-1,2-dihydronaphthalene (TDN)[a]	nd	5.7 ± 1.1	2.3 ± 0.1	6.2 ± 1.6	5.2 ± 0.3	1.2 ± 0.3	3.1 ± 0.2	3.3 ± 0.5
19	1829	β-damascenone	nd	0.52 ± 0.05	0.28 ± 0.07	0.33 ± 0.07	0.49 ± 0.04	0.38 ± 0.08	0.17 ± 0.02	0.42 ± 0.06
20	1832	*tert*-1-(2,3,6-trimethylphenyl) but-1,3-diene (TPB)[a]	nd	2.0 ± 0.4	0.39 ± 0.16	3.6 ± 1.0	0.64 ± 0.04	0.31 ± 0.05	1.9 ± 0.1	0.22 ± 0.01
21	1939	3-oxo-β-ionone[a]	nd	2.3 ± 0.3	0.47 ± 0.04	2.9 ± 0.7	2.0 ± 0.1	1.1 ± 0.1	1.5 ± 0.2	0.51 ± 0.07
22	1950	β-ionone	nd	0.08 ± 0.02	0.07 ± 0.01	nd	0.11 ± 0.01	0.07 ± 0.002	0.08 ± 0.01	0.08 ± 0.01
23	1952	actinidols[a,d]	0.13 ± 0.02	3.1 ± 0.6	0.64 ± 0.06	4.3 ± 1.1	2.8 ± 0.4	1.8 ± 0.1	2.2 ± 0.3	1.0 ± 0.2
24	2657	3-oxo-α-ionol[a]	nd	63 ± 10	60 ± 12	242 ± 42	133 ± 11	72 ± 7	102 ± 18	42 ± 5
		total[e]	0.13	26	19	44	21	13	21	30
		volatile phenols								
25	1876	guaiacol	nd	0.44 ± 0.08	2.3 ± 0.4	0.35 ± 0.07	1.9 ± 0.1	0.67 ± 0.01	0.39 ± 0.07	1.2 ± 0.2
26	2030	*o*-cresol	nd	0.60 ± 0.09	0.60 ± 0.08	0.66 ± 0.13	1.1 ± 0.1	0.30 ± 0.03	0.36 ± 0.07	0.88 ± 0.08
27	2068	4-ethylguaiacol	nd	0.42 ± 0.09	0.17 ± 0.05	0.24 ± 0.03	0.25 ± 0.02	0.14 ± 0.02	0.21 ± 0.01	0.38 ± 0.06
28	2157	*m*-cresol	nd	0.26 ± 0.02	0.18 ± 0.02	0.20 ± 0.03	0.24 ± 0.02	0.18 ± 0.01	0.13 ± 0.03	0.21 ± 0.02
29	2237	eugenol	nd	0.43 ± 0.04	7.6 ± 1.2	4.0 ± 0.8	2.2 ± 0.4	1.3 ± 0.3	2.3 ± 0.5	0.72 ± 0.12
30	2244	4-ethylphenol	nd	0.48 ± 0.11	1.9 ± 0.4	0.57 ± 0.10	1.4 ± 0.3	0.62 ± 0.01	0.17 ± 0.02	0.94 ± 0.13
31	2262	4-vinylguaiacol	3.8 ± 0.2	162 ± 35	52 ± 14	81 ± 22	65 ± 21	39 ± 4	42 ± 6	176 ± 26
32	2317	2,6-dimethoxyphenol	nd	0.28 ± 0.06	2.2 ± 0.7	0.25 ± 0.08	13 ± 0.4	3.0 ± 0.3	nd	6.5 ± 1.1
33	2279	(*E*)-isoeugenol	nd	1.8 ± 0.4	1.6 ± 0.1	4.6 ± 1.1	0.96 ± 0.003	1.2 ± 0.1	4.8 ± 0.3	1.8 ± 0.2
34	2404	4-vinylphenol	3.2 ± 0.5	423 ± 108	1739 ± 137	388 ± 69	1487 ± 371	497 ± 28	121 ± 40	1258 ± 6
35	2563	4-allyl-2,6-dimethoxyphenol	nd	1.3 ± 0.2	3.7 ± 1.0	3.9 ± 1.1	2.2 ± 0.2	1.4 ± 0.4	2.7 ± 0.6	1.4 ± 0.2
		total volatile phenols (I)[e]	nd	25	90	61	52	28	45	37
		total volatile phenols (II)[e]	18	3737	8118	2609	7128	2538	997	7451
		vanillin derivatives								
36	2592	vanillin	1.4 ± 0.1	2.1 ± 0.3	2.0 ± 0.2	3.0 ± 0.5	1.7 ± 0.02	3.1 ± 0.7	2.8 ± 0.6	4.1 ± 0.7
37	2629	methyl vanillate	nd	10 ± 1	2.1 ± 0.4	6.3 ± 1.6	3.0 ± 0.9	8.4 ± 1.7	1.2 ± 0.3	18 ± 3
38	2654	ethyl vanillate	nd	1.2 ± 0.1	5.7 ± 0.9	0.68 ± 0.14	7.9 ± 2.1	12 ± 3	1.0 ± 0.2	10 ± 2
39	2664	acetovanillone	1.1 ± 0.03	18 ± 2	19 ± 3	8.4 ± 1.7	13 ± 3	17 ± 3	13 ± 3	34 ± 6
40	2829	zingerone	nd	9.9 ± 1.5	8.5 ± 1.3	2.6 ± 0.8	5.6 ± 1.4	7.7 ± 1.7	3.0 ± 1.0	39 ± 7
41	2892	homovanillyl alcohol	nd	14 ± 3	60 ± 6	34 ± 9	23 ± 7	86 ± 19	35 ± 7	74 ± 13
42	3040	syringaldehyde	0.02 ± 0.005	nd	1.5 ± 0.5	0.89 ± 0.05	0.95 ± 0.11	1.5 ± 0.4	0.85 ± 0.14	4.9 ± 1.1
43	3099	homovanillic acid[a]	nd	440 ± 120	208 ± 101	544 ± 122	281 ± 24	108 ± 14	347 ± 54	427 ± 71
44	3123	acetosyringone	nd	3.4 ± 0.5	2.6 ± 0.7	1.0 ± 0.3	2.2 ± 0.7	4.7 ± 1.2	1.5 ± 0.4	3.7 ± 0.7
		total[e]	12	1001	898	929	743	1048	730	1966
		benzenes								
45	1520	benzaldehyde	0.30 ± 0.03	85 ± 9	0.80 ± 0.08	45 ± 3	1.1 ± 0.2	0.71 ± 0.07	2.3 ± 0.3	0.99 ± 0.06

Table 2. Continued

	RI[c]		ECO	EV	ET	ECH	ECA	EME	EMU	EG
46	1659	phenylacetaldehyde	1.4 ± 0.2	0.84 ± 0.07	1.0 ± 0.1	2.2 ± 0.04	0.84 ± 0.03	0.98 ± 0.13	1.7 ± 0.2	1.4 ± 0.2
47	1891	benzyl alcohol	0.27 ± 0.06	996 ± 77	401 ± 72	954 ± 152	1086 ± 185	712 ± 86	433 ± 0.5	927 ± 96
48	1908	ethyl dihydrocinnamate	nd	nd	nd	nd	nd	nd	nd	nd
49	1926	β-phenylethanol	2.0 ± 0.1	160 ± 12	126 ± 23	188 ± 31	149 ± 25	164 ± 21	148 ± 28	105 ± 13
50	2081	ethyl cinnamate	nd	0.40 ± 0.03	0.15 ± 0.01	0.76 ± 0.14	0.07 ± 0.01	0.17 ± 0.03	0.13 ± 0.02	0.16 ± 0.03
51	2219	2-phenoxyethanol	0.18 ± 0.06	0.63 ± 0.07	0.49 ± 0.12	nd	0.71 ± 0.10	nd	0.51 ± 0.08	nd
52	2725	1,2-dimethoxy-4-propylbenzene[a]	nd	10 ± 1	11 ± 2	22 ± 6	23 ± 6	31 ± 8	4.5 ± 0.3	10 ± 2
		total benzenes (I)[e]	10	1784	1057	1814	1252	1373	1251	882
		total benzenes (II)[e]	0.40	2284	925	2198	2502	1656	993	2126
		lactones								
53	1988	δ-octalactone	nd	0.14 ± 0.02	0.10 ± 0.03	0.06 ± 0.02	0.09 ± 0.02	0.07 ± 0.01	nd	0.12 ± 0.02
54	2068	γ-nonalactone	0.13 ± 0.02	0.18 ± 0.04	0.18 ± 0.06	0.38 ± 0.08	0.35 ± 0.02	0.13 ± 0.02	0.30 ± 0.03	0.20 ± 0.03
55	2154	δ-nonalactone	nd	0.09 ± 0.02	nd	nd	nd	nd	nd	nd
56	2141	γ-decalactone	nd	0.08 ± 0.005	0.09 ± 0.01	0.14 ± 0.03	0.07 ± 0.02	0.08 ± 0.02	0.10 ± 0.02	0.04 ± 0.004
57	2260	δ-decalactone	nd	nd	nd	nd	nd	nd	nd	nd
		total[e]	0.87	2.2	1.8	3.1	2.6	1.5	2.3	1.7
		miscellaneous								
58	1390	(*Z*)-3-hexen-1-ol	nd	7.6 ± 0.3	20 ± 1	4.3 ± 0.1	15 ± 0.34	29 ± 0.3	9.4 ± 1.1	17 ± 1
59	1413	(*E*)-2-hexen-1-ol	nd	nd	nd	nd	nd	1.9 ± 0.1	nd	nd
60	1672	3-methylbutyric acid	tr	0.44 ± 0.16	0.38 ± 0.51	tr	tr	tr	1.1 ± 0.2	0.13 ± 0.15
61	1677	2-methylbutyric acid	0.28 ± 0.05	0.96 ± 0.05	0.21 ± 0.05	1.4 ± 0.1	1.2 ± 0.2	1.1 ± 0.04	0.53 ± 0.08	0.28 ± 0.02

[a] Chemical standard not available. Tentatively identified. Data are the relative areas (to 4-hydroxy-4-methyl-2-pentanone × 1000). [b] For the calculation of the concentrations 4-methyl-4-pentanol has been used as internal standard of the miscellaneous compounds; 2-octanol of β-damascenone, *m*-cresol, 4-ethylphenol, (*E*)-isoeugenol, methyl vanillate, benzaldehyde, phenylacetaldehyde, and benzyl alcohol; and 4-hydroxy-4-methyl-2-pentanone has been used for the rest. [c] Retention index calculated in a DBWAXetr column. [d] Actinidols, 2,2,6-trimethyl-8-(1-hydroxy)ethyl-7-oxabicyclo[4.3.0]nona-4,9-dienes; Riesling acetal, 2,2,6,8-tetramethyl-7,11-dioxatricyclo[6.2.1.0(1,6)]undec-4-ene; vitispirane, 2,10,10-trimethyl-6-methylen-1-oxaspiro-[4,5]dec-7-ene. [e] Sum of relative areas. Data corresponding to **Figure 2**: norisoprenoids do not include 3-oxo-α-ionol; volatile phenols (I) do not include 2,6-dimethoxyphenol; volatile phenols (II) include only vinylphenols; benzenes (II) include only 1,2-dimethoxy-4-propylbenzene and benzyl alcohol. [f] Control sample (CO), Verdejo (V), Tempranillo (T), Muscat (MU), Chardonnay (CH), Cabernet Sauvignon (CA), Merlot (ME), and Grenache (G). [g] nd, not detected; tr, traces.

nearly all cases but that by far the strongest influence is exerted by the hydrolytic procedure. This behavior was corroborated by a principal component analysis (PCA) study, partially shown in **Figure 1**, which shows the projection of samples (scores) and variables (loadings) on the plane formed by the two first principal components (PCs). Notwithstanding the major role of the hydrolytic procedure, variety also plays a role, and although the sample design does not allow the extraction of anything but preliminary and rough conclusions, because samples from each variety come from a single vineyard and vintage, some observations about the role of the variety should be made.

Role of Variety. As expected, Muscat contained the highest concentration of terpenes whatever the hydrolytic procedures, except for linalyl acetate. This variety presented, in some cases, levels of terpenes up to 200 times higher than those found in the other varieties, which certainly can be considered a major difference. On the other hand, the levels of vinylphenols of Muscat were the lowest, although this difference cannot be observed in fermented samples. An observation of general validity is that varietal differences in the volatile composition of hydrolysates tend to be smaller between fermented samples, as can also be seen from **Figure 1**. This can be attributed to the comparatively low efficiency of yeast to release volatile compounds from grape precursors, as will be discussed later.

Leaving aside Muscat, differences between varieties were not outstanding, although as it can be seen on the PC plot (**Figure 1**), Chardonnay and Grenache produce hydrolysates with rather particular profiles. A look at **Tables 1−3** confirms that hydrolysates from Grenache were richest in some norisoprenoids such as vitispirane A (up to 8 times higher), vitispirane B, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), and Riesling acetal; in 4-vinylguaiacol (up to 6 times higher); and in some vanillin derivatives such as methyl vanillate (up to 15 times higher), acetovanillone (up to 4 times higher), zingerone (not detected in some varieties), and syringaldehyde (up to 6 times higher). As was aforementioned, these differences were most important after harsh acid hydrolysis, were also evident after enzymatic hydrolysis, and became weaker in the case of the samples obtained by fermentation.

Hydrolysates from Chardonnay were richest in some norisoprenoids such as *tert*-1-(2,3,6-trimethylphenyl)but-1,3-diene (TPB) (up to 12 times higher), 3-oxo-α-ionol (up to 7 times higher), 3-oxo-β-ionone (up to 5 times higher), and actinidols (up to 7 times higher); in some benzene compounds such as phenylacetaldehyde, ethyl dihydrocinnamate, and ethyl cinnamate; and in γ-nonalactone. For norisoprenoids and phenylacetaldehyde, as observed in the case of Grenache, differences were most important in acid hydrolysates and were less evident in samples obtained by fermentation. The case of cinnamates and γ-nonalactone is different, because these compounds were mostly obtained in samples obtained by fermentation. Although the levels of these compounds are very low, it should be noted that ethyl dihydrocinnamate and ethyl cinnamate seem to be quite specific of Chardonnay because only in those wines (**Table 3**) are these compounds significantly present. This observation

Table 3. Concentration (in Micrograms per Kilogram of Grapes, Except Where Indicated[a]) of the Volatile Compounds Released after Alcoholic Fermentation of a Synthetic Must Added with Precursor Extract from Each Grape Variety (Data Are the Average of Three Replicate Samples)

	RI[c]		FCO	FV	FT	FCH	FCA	FME	FMU	FG
		terpenes								
1	1355	(*Z*)-rose oxide	nd	nd	nd	nd	nd	nd	0.13 ± 0.02	nd
2	1447	(*Z*)-linalool oxide[a]	nd	nd	nd	nd	nd	nd	0.30 ± 0.02	nd
3	1476	(*E*)-linalool oxide[a]	nd	nd	nd	nd	nd	nd	0.16 ± 0.02	nd
4	1478	nerol oxide[a]	nd	nd	nd	nd	nd	nd	0.59 ± 0.04	nd
5	1556	linalool	3.6 ± 0.3	4.4 ± 0.1	4.4 ± 0.2	6.7 ± 0.3	4.2 ± 0.2	5.0 ± 0.7	24 ± 1	4.6 ± 0.3
6	1565	linalyl acetate[a]	4.4 ± 1.1	8.7 ± 1.2	3.9 ± 0.9	12 ± 0.4	6.0 ± 0.5	13 ± 2	12 ± 1	3.3 ± 0.3
7	1608	terpinen-4-ol[a]	nd	nd	nd	nd	nd	nd	nd	nd
8	1613	2,6-dimethyl-1,7-octadiene-2,6-diol[a]	nd	nd	nd	nd	nd	nd	36 ± 2	nd
9	1664	δ-terpineol[a]	nd	nd	nd	nd	nd	nd	nd	nd
10	1705	α-terpineol	1.2 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	2.3 ± 0.2	1.2 ± 0.1	1.8 ± 0.3	10 ± 1	1.4 ± 0.2
11	1775	β-citronellol	1.8 ± 0.3	1.8 ± 0.1	2.1 ± 0.2	2.2 ± 0.1	1.8 ± 0.02	2.0 ± 0.1	4.0 ± 0.2	2.0 ± 0.2
12	1811	nerol	nd	nd	nd	nd	nd	nd	2.0 ± 0.2	nd
13	1858	geraniol	1.4 ± 0.1	1.6 ± 0.2	2.0 ± 0.1	2.2 ± 0.3	1.9 ± 0.1	1.7 ± 0.1	3.7 ± 0.3	2.1 ± 0.2
14	2366	neric acid[a]	nd	nd	nd	3.5 ± 0.4	nd	nd	74 ± 5	3.7 ± 0.4
		total[e]	27	35	32	54	31	47	266	36
		norisoprenoids								
15	1526	vitispirane A[a,d]	nd	nd	nd	nd	nd	nd	nd	nd
16	1529	vitispirane B[a,d]	nd	nd	nd	nd	nd	nd	nd	nd
17	1637	Riesling acetal[a,d]	nd	nd	0.90 ± 0.19	nd	nd	nd	nd	0.94 ± 0.26
18	1748	1,1,6-trimethyl-1,2-dihydronaphthalene (TDN)[a]	nd	nd	nd	nd	nd	nd	nd	nd
19	1829	β-damascenone	nd	1.2 ± 0.05	0.77 ± 0.06	1.2 ± 0.1	1.2 ± 0.04	1.0 ± 0.01	0.63 ± 0.04	1.3 ± 0.04
20	1832	*tert*-1-(2,3,6-trimethylphenyl)but-1,3-diene (TPB)[a]	nd	nd	nd	nd	nd	nd	nd	nd
21	1939	3-oxo-β-ionone[a]	nd	3.5 ± 0.5	1.0 ± 0.2	3.4 ± 0.1	2.3 ± 0.2	1.9 ± 0.1	2.6 ± 0.1	nd
22	1950	β-ionone	0.39 ± 0.07	0.38 ± 0.04	0.37 ± 0.02	0.42 ± 0.04	0.46 ± 0.05	0.46 ± 0.05	0.44 ± 0.02	0.38 ± 0.02
23	1952	actinidols[a,d]	nd	3.3 ± 0.5	1.0 ± 0.2	3.4 ± 0.1	2.3 ± 0.2	2.0 ± 0.1	2.6 ± 0.3	nd
24	2657	3-oxo-α-ionol[a]	nd	5.8 ± 0.6	12 ± 0.2	20 ± 0.6	17 ± 2	19 ± 1	16 ± 2	4.2 ± 0.2
		total[e]	1.2	13	6.6	13	11	9.4	8.7	7.2
		volatile phenols								
25	1876	guaiacol	nd	0.18 ± 0.02	0.29 ± 0.09	0.21 ± 0.01	0.28 ± 0.02	0.29 ± 0.04	0.20 ± 0.02	0.42 ± 0.05
26	2030	*o*-cresol	nd	nd	nd	nd	nd	nd	nd	nd
27	2068	4-ethylguaiacol	nd	nd	nd	nd	nd	nd	nd	nd
28	2157	*m*-cresol	nd	nd	nd	nd	nd	nd	nd	nd
29	2237	eugenol	nd	0.20 ± 0.06	0.29 ± 0.02	0.21 ± 0.03	0.16 ± 0.02	0.19 ± 0.03	0.32 ± 0.06	0.20 ± 0.02
30	2244	4-ethylphenol	nd	0.11 ± 0.02	nd	0.10 ± 0.02	nd	0.12 ± 0.02	0.09 ± 0.01	0.13 ± 0.02
31	2262	4-vinylguaiacol	nd	18 ± 2	15 ± 0.2	17 ± 0.2	16 ± 0.2	14 ± 0.1	16 ± 0.4	22 ± 1
32	2317	2,6-dimethoxyphenol	nd	nd	0.38 ± 0.04	nd	0.62 ± 0.08	0.42 ± 0.08	nd	0.48 ± 0.05
33	2279	(*E*)-isoeugenol	nd	0.92 ± 0.08	1.0 ± 0.1	0.90 ± 0.02	0.89 ± 0.02	0.86 ± 0.02	1.0 ± 0.03	nd
34	2404	4-vinylphenol	13 ± 0.02	17 ± 1	16 ± 0.3	21 ± 1	16 ± 0.4	15 ± 0.2	15 ± 0.1	18 ± 0.2
35	2563	4-allyl-2,6-dimethoxyphenol	nd	nd	nd	nd	nd	nd	nd	nd
		total volatile phenols (I)[e]	*nd*	3.7	4.4	3.7	3.3	4.3	4.5	4.5
		total volatile phenols (II)[e]	2.5	94	52	87	55	38	60	132
		vanillin derivatives								
36	2592	vanillin	nd	0.81 ± 0.02	nd	0.85 ± 0.02	0.87 ± 0.03	0.83 ± 0.01	1.0 ± 0.1	0.84 ± 0.01
37	2629	methyl vanillate	nd	4.1 ± 0.4	2.0 ± 0.1	2.9 ± 0.1	2.2 ± 0.02	4.4 ± 0.03	1.2 ± 0.01	9.9 ± 0.1
38	2654	ethyl vanillate	nd	nd	2.4 ± 0.2	nd	2.7 ± 0.3	3.3 ± 0.1	nd	3.7 ± 0.3
39	2664	acetovanillone	4.7 ± 0.03	10 ± 1	14 ± 1	7.1 ± 0.1	10 ± 0.4	12 ± 0.21	10 ± 0.3	21 ± 0.4

Table 3. Continued

	RI[c]		FCO	FV	FT	FCH	FCA	FME	FMU	FG
40	2829	zingerone	nd	tr	0.10 ± 0.01	tr	tr	tr	tr	6.7 ± 1.2
41	2892	homovanillyl alcohol	nd	nd	nd	1.1 ± 0.1	nd	1.2 ± 0.1	1.0 ± 0.03	nd
42	3040	syringaldehyde	nd	nd	nd	nd	nd	nd	nd	nd
43	3123	acetosyringone	nd	1.6 ± 0.1	2.0 ± 0.3	0.70 ± 0.02	1.3 ± 0.1	3.0 ± 0.1	1.1 ± 0.02	2.6 ± 0.1
44	3099	homovanillic acid[a]	nd	31 ± 4	36 ± 3	22 ± 2	31 ± 2	29 ± 2	31 ± 3	34 ± 3
		total [e]	4.0	166	219	88	159	226	116	481
		benzenes								
45	1520	benzaldehyde	1.8 ± 0.4	1.2 ± 0.03	1.2 ± 0.2	1.4 ± 0.03	1.7 ± 0.1	1.4 ± 0.1	1.4 ± 0.03	1.2 ± 0.1
46	1659	phenylacetaldehyde	nd	nd	nd	nd	nd	nd	nd	nd
47	1891	benzyl alcohol	1.8 ± 0.2	1.1 ± 0.1	1.2 ± 0.2	1.5 ± 0.02	1.7 ± 0.05	1.3 ± 0.05	1.2 ± 0.1	1.2 ± 0.2
48	1908	ethyl dihydrocinnamate	nd	0.18 ± 0.04	0.19 ± 0.02	1.6 ± 0.3	0.10 ± 0.03	nd	nd	nd
49	1926	β-phenylethanol	1127 ± 45	850 ± 111	1071 ± 55	941 ± 19	895 ± 10	847 ± 46	974 ± 52	950 ± 35
50	2081	ethyl cinnamate	nd	nd	nd	1.1 ± 0.1	nd	nd	nd	nd
51	2219	2-phenoxyethanol	16 ± 3	12 ± 1	18 ± 2	14 ± 2	14 ± 1	12 ± 0.1	14 ± 1	16 ± 2
52	2725	1,2-dimethoxy-4-propylbenzene[a]	3,1 ± 0,3	7,0 ± 0,8	9,7 ± 0,8	12 ± 0,5	14 ± 0,5	17 ± 1	6,4 ± 0,2	9,0 ± 0,3
		total benzenes (I)[e]	9402	7077	8927	7864	7453	7065	8127	7911
		total benzenes (II)[e]	6.2	8.6	11	14	17	19	8.2	11
		lactones								
53	1988	δ-octalactone	nd	0.20 ± 0.06	0.27 ± 0.03	0.23 ± 0.02	0.15 ± 0.04	0.19 ± 0.01	0.23 ± 0.02	0.21 ± 0.01
54	2068	γ-nonalactone	0.11 ± 0.04	0.18 ± 0.04	0.27 ± 0.07	1.0 ± 0.02	0.39 ± 0.10	0.24 ± 0.05	0.18 ± 0.02	0.14 ± 0.07
55	2154	δ-nonalactone	nd	nd	nd	nd	nd	nd	nd	nd
56	2141	γ-decalactone	0.36 ± 0.05	0.29 ± 0.03	0.31 ± 0.05	0.37 ± 0.03	0.28 ± 0.03	0.28 ± 0.01	0.32 ± 0.01	0.28 ± 0.04
57	2260	δ-decalactone	3.0 ± 0.4	2.7 ± 0.1	3.3 ± 0.4	3.1 ± 0.2	3.0 ± 0.1	2.6 ± 0.1	2.7 ± 0.1	3.0 ± 0.2
		total[e]	5.5	5.7	7.0	11	6.9	5.9	6.0	5.8
		miscellaneous								
58	1390	(*Z*)-3-hexen-1-ol	nd	nd	3.9 ± 0.01	nd	nd	3.8 ± 0.03	nd	nd
59	1413	(*E*)-2-hexen-1-ol	nd	nd	nd	nd	nd	nd	nd	nd
60	1672	3-methylbutyric acid	nd	nd	nd	nd	nd	nd	nd	nd
61	1677	2-methylbutyric acid	4.5 ± 1.1	4.9 ± 1.5	3.6 ± 0.1	5.5 ± 1.1	4.4 ± 1.1	3.9 ± 0.2	4.3 ± 0.3	3.7 ± 0.4

[a] Chemical standard not available. Tentatively identified. Data are the relative areas (to 4-hydroxy-4-methyl-2-pentanone × 1000). [b] For the calculation of the concentrations 4-methyl-4-pentanol has been used as internal standard of the miscellaneous compounds; 2-octanol of β-damascenone, *m*-cresol, 4-ethylphenol, (*E*)-isoeugenol, methyl vanillate, benzaldehyde, phenylacetaldehyde, and benzyl alcohol; and 4-hydroxy-4-methyl-2-pentanone has been used for the rest. [c] Retention index calculated in a DBWAXetr column. [d] Actinidols, 2,2,6-trimethyl-8-(1-hydroxy)ethyl-7-oxabicyclo[4.3.0]nona-4,9-dienes; Riesling acetal, 2,2,6,8-tetramethyl-7,11-dioxatricyclo[6.2.1.0(1,6)]undec-4-ene; vitispirane, 2,10,10-trimethyl-6-methylen-1-oxaspiro-[4,5]dec-7-ene. [e] Sum of relative areas. Data corresponding to **Figure 2**: norisoprenoids do not include 3-oxo-α-ionol; volatile phenols (I) not include 2,6-dimethoxyphenol; volatile phenols (II) include only vinylphenols; benzenes (II) include only 1,2-dimethoxy-4-propylbenzene and benzyl alcohol. [f] Control sample (CO), Verdejo (V), Tempranillo (T), Muscat (MU), Chardonnay (CH), Cabernet Sauvignon (CA), Merlot (ME), and Grenache (G). [g] nd, not detected; tr, traces.

corroborates previous findings (*53*, *54*) about the importance of these compounds in wines made from Chardonnay grapes.

Apart from these previous observations, differences between grape varieties seem to be quite small, and most of the compounds, including some that are relevant from the aromatic point of view such as β-damascenone and β-ionone, were present at levels relatively similar in the hydrolysates from the different varieties. Only in the enzyme hydrolysates did benzaldehyde, eugenol, 4-vinylphenol, and 4-ethylphenol show relevant differences between varieties. In the case of benzaldehyde, Verdejo and Chardonnay were much richer than the others; Tempranillo hydrolysates contained the highest concentrations of eugenol, and together with Cabernet Sauvignon and Grenache, also of 4-vinylphenol. The presence of significantly higher levels of eugenol in wines made with Tempranillo has been previously observed (*55*). In the case of δ-octalactone, a higher concentration was observed in the acid hydrolysates of Muscat. All of these results suggest that grape variety is certainly, and leaving aside the aforementioned cases of Muscat, Grenache, and Chardonnay, not a major factor determining the aroma composition of glycosidic flavor precursors. A secondary but interesting observation is the fact that the levels of some volatile phenols, such as guaiacol, 2,6-dimethoxyphenol, and 4-ethylphenol, of ethyl vanillate and (*Z*)-3-hexenol were higher in red grape varieties.

Comparison between Hydrolytic Procedures. As can be seen in the tables and in **Figure 2**, it can be stated that alcoholic fermentation has a rather low potential to release volatile compounds from aromatic precursors, at least in comparison with acid and enzymatic hydrolysis. This result is not surprising because hydrolytic conditions in fermentation are far from optimal and confirms the known fact that a major part of the aroma potential of grapes remains not expressed after conventional winemaking (*56*, *57*).

The distribution of the different volatile compounds in the plane formed by the two first principal components (**Figure 1**)

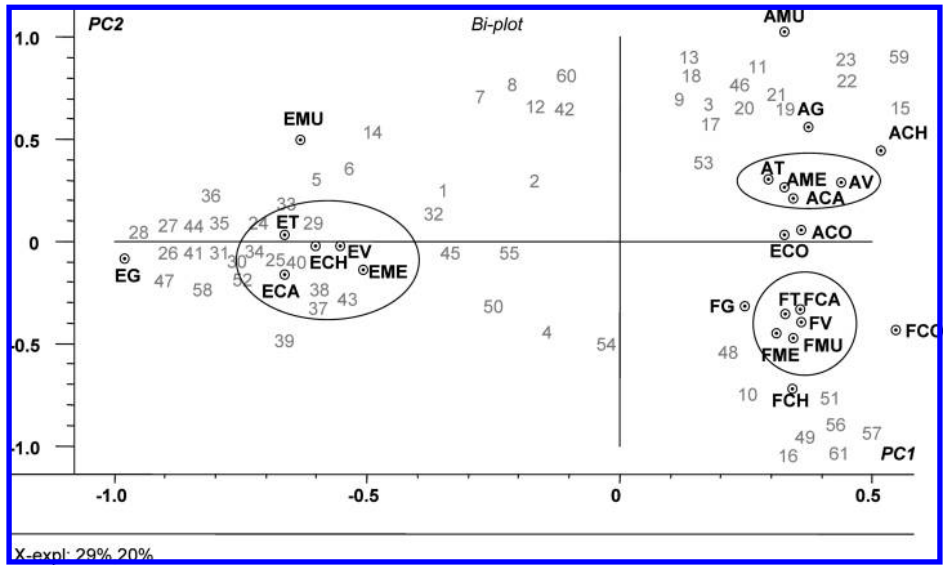

Figure 1. Principal component plot with sample loadings and variable scores for PC1 and PC2. Samples labeled E, A, and F are samples from enzymatic hydrolysis, harsh acid hydrolysis, and alcoholic fermentation, respectively. The averages of the three replicate samples were used for the study. Control samples (CO), Muscat (MU), Chardonnay (CH), Grenache (G), Tempranillo (T), Merlot (ME), Cabernet Sauvignon (CA), and Verdejo (V).

gives us some ideas about the nature of the differences introduced by the hydrolytic procedure. As the figure shows, PC1 separates samples from enzymatic hydrolysis from the others, and it is highly correlated with most volatile phenols, vanillin derivatives, 3-oxo-α-ionol, and benzyl alcohol. Samples from harsh acid hydrolysis are found in the upper right region of the plane, where are also found most of the norisoprenoids, some terpenes, such as α-terpineol, phenylacetaldehyde, and (*Z*)-2-hexen-1-ol. Finally, samples from alcoholic fermentation are found in the bottom right part of the plane, near compounds such as *β*-ionone, 2-methylbutyric acid, some lactones, and some benzene derivatives. Control samples from enzymatic and harsh acid hydrolysis are both grouped in the right-center part of the plane separated of the rest of samples, whereas the control sample from fermentation has a composition closer to the rest of the fermentation samples.

Terpenes were mainly released or formed by both harsh acid and enzymatic hydrolysis, as can be seen in **Figure 2**. However, and as expected (*22*), nearly 40% of the total terpenes formed by harsh acid hydrolysis was α-terpineol, enzymatic hydrolysis being clearly more efficient at releasing most terpenes (*38*). Norisoprenoids, except 3-oxo-α-ionol and *β*-ionone, were mainly hydrolyzed by harsh acid hydrolysis in accordance with previous reports (*38, 39, 46*). The small amount of *β*-damascenone present in the enzymatic hydrolysates could have been formed by unwanted acid hydrolysis during sample manipulation. As expected from earlier observations (*4, 58, 59*), vitispiranes, TDN, and TPB were not even detected after alcoholic fermentation. Riesling acetal was not detected by enzymatic hydrolysis (*38, 40, 49*).

Volatile phenols were much more efficiently released by enzymatic hydrolysis in accordance with the paper by Dugelay et al. (*60*). Some phenols, such as *o*-cresol, 4-ethylguaiacol, and 4-allyl-2,6-dimethoxyphenol, were detected only by this hydrolytic procedure, whereas the levels of 4-vinylphenol released by this procedure are up to 100 times higher than those observed in acid hydrolysates or in fermented samples.

Vanillin derivatives were also found at highest levels by enzymatic hydrolysis, except syringaldehyde, mainly released by harsh acid hydrolysis, and acetovanillone, released at similar levels in both procedures. In the case of homovanillic acid, the concentrations after enzymatic hydrolysis were up to 100 times higher than those found in the other samples. Finally, the concentrations of some benzenes, such as *β*-phenylethanol and phenoxyethanol, were, as expected, highest in fermented samples. Ethyl dihydrocinnamate was found in only these samples. Enzymatic hydrolysis seems to be quite efficient at releasing benzyl alcohol, in accordance with a previous study (*46*), reaching levels up to 100 times higher than those observed in the other hydrolytic strategies. On the contrary, phenylacetaldehyde was mainly found by harsh acid hydrolysis.

Correlations between Hydrolytic Procedures. A correlation study has been carried out to determine whether the levels of some relevant volatile compounds present in the samples after fermentation can be predicted by the levels of the compounds found in the acid or enzyme hydrolysates. Results are shown in **Table 4**.

In the case of terpenes, Muscat samples were not included in the correlation study because the high levels of these compounds in such samples carried too much weight in the linear regression. As shown in **Table 4**, the sum of the levels of some important terpenes present in the fermented samples, such as linalool, α-terpineol, geraniol, nerol, and *β*-citronellol, is significantly correlated with the sum of those formed by acid hydrolysis ($R^2 = 0.630$), but not with those released by enzymatic hydrolysis. Similar results are obtained when the sum of the levels of those important terpenes in fermented samples is compared with the levels of α-terpineol and geraniol found in the acid or enzyme hydrolysates.

In the case of linalool, the predictive ability of acid hydrolysis is also better than that of enzymatic hydrolysis. This could be due to the low activity of glycosidase enzymes toward tertiary alcohol glycosides (*12*). However, a closer look at data reveals that it is the likely presence of different precursors for linalool in the different varieties, the probable cause of such lack of predictive ability. In fact, if samples are split by varieties, two clear relationships appear: one for Cabernet Sauvignon, Grenache, Tempranillo, and Chardonnay (shown in the table) and another one for Merlot and Verdejo (data not shown). Precursors of linalool from these last two varieties are less efficiently

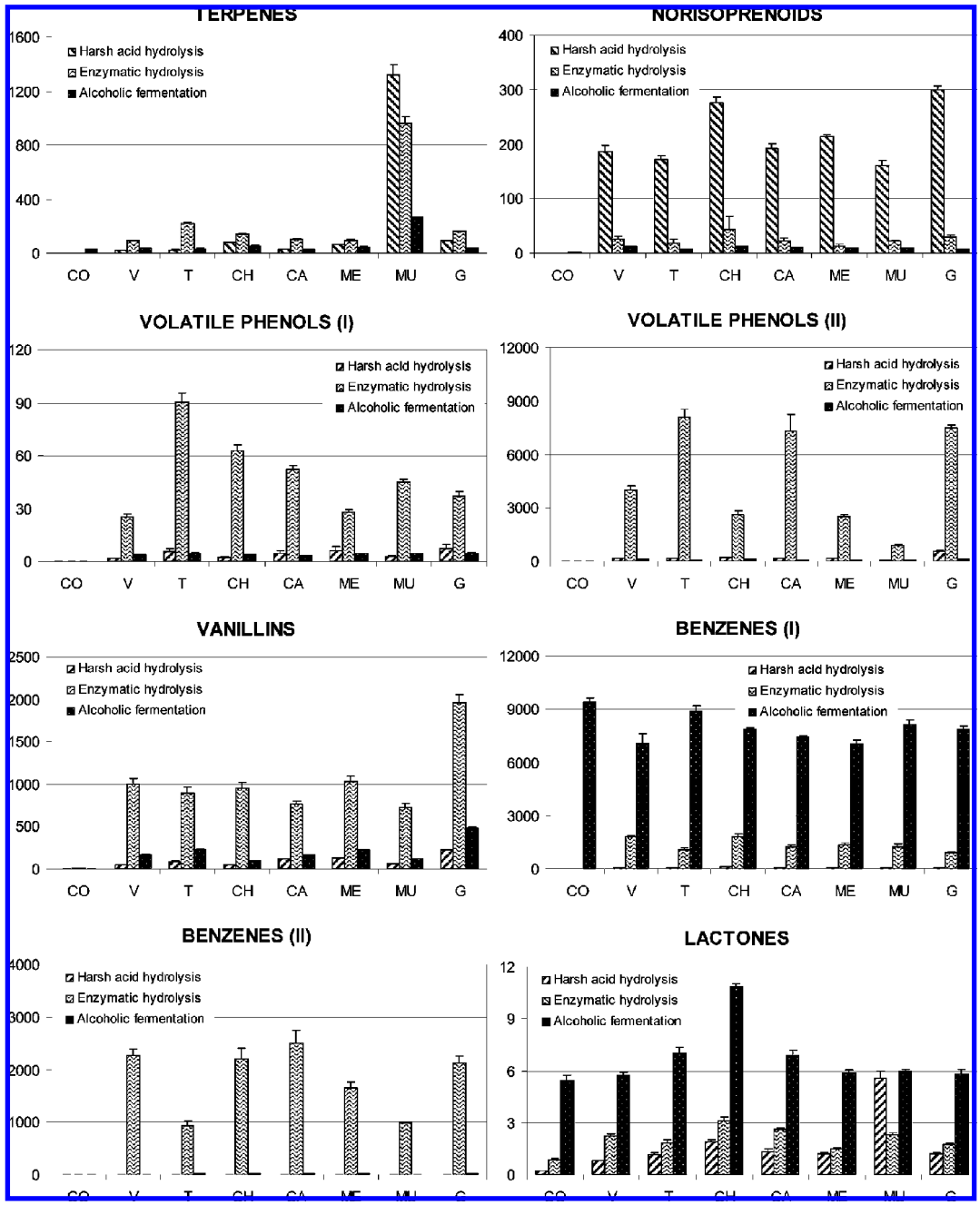

Figure 2. Graphical comparison of the three hydrolytic procedures for each grape variety. Data are the sum of relative areas (to 4-hydroxy-4-methyl-2-pentanone × 1000). In the case of alcoholic fermentation, data are multiplied by a factor (4.57) to take into account the amount of hydrolysate treated and the amount of precursor extract used. Compound groups correspond to those shown in **Tables 1−3** with the following remarks: norisoprenoids do not include data from 3-oxo-α-ionol; volatile phenols and benzenes have been split into two groups, volatile phenols (I) do not include 2,6-dimethoxyphenol, volatile phenols (II) include only vinylphenols, and benzenes (II) include only 1,2-dimethoxy-4-propylbenzene and benzyl alcohol.

transformed by enzymatic hydrolysis, which may be related to a major presence of polyols or of polyol-glycosides in the precursor fraction (*29*, *61*−*63*) of these varieties. In the case of α-terpineol, as expected, only acid hydrolysis can predict the levels found in fermented samples. This fact proves that acid hydrolysis reproduces better the terpene rearrangements taking place during alcoholic fermentation (*39*).

Among norisoprenoids, only the levels of β-damascenone in fermented samples were found to be correlated with the levels found in the hydrolysates. Again, the predictive ability of harsh acid hydrolysis ($R^2 = 0.741$) was better, in accordance with previous results (*47*). In addition, and as commented earlier, the small amount of β-damascenone found by enzymatic hydrolysis could have been formed by acid hydrolysis. It is remarkable that the levels of β-damascenone in fermented samples are not well correlated with the total amount of norisoprenoids formed by both acid and enzymatic hydrolyses. The absence of vitispiranes, TDN, and TPB in the fermented samples makes it impossible to establish correlations for these compounds.

The levels of vinylphenols in fermented samples are only poorly predicted by acid hydrolysis and are not predicted at all by enzymatic hydrolysis. Such lack of predictive ability is due to the differential behavior of samples of Cabernet Sauvignon and Tempranillo. Enzyme hydrolysates of these grapes had maximum levels of vinylphenol, but this is not observed in fermented samples. This could be due to the ability of the enzyme preparations to form vinylphenols from ferulic and

Table 4. Coefficients of Determination (R^2), Slopes, and Significance of the Linear Regression between Alcoholic Fermentation and Acid and Enzymatic Hydrolyses (Only the Best Correlations for the Most Important Compounds Are Shown)

	enzymatic hydrolysis		acid hydrolysis	
	R^2	slope	R^2	slope
terpenes[b]				
sum of "important terpenes"[c]	0.084 (ns)	0.07	0.630***	0.25
sum of "important terpenes" with α-terpineol	0.028 (ns)	−0.32	0.599***	0.30
sum of "important terpenes" with geraniol	0.001 (ns)	−0.02	0.589***	3.7
linalool	0.380**	0.19	0.617***	1.3
linalool (CA, G, T, CH)[d]	0.787***	0.34	0.792***	1.5
linalool with "important terpenes"	0.029 (ns)	0.02	0.587***	0.13
linalool with α-terpineol	0.042 (ns)	−0.21	0.551***	0.16
linalool with geraniol	0.023 (ns)	−0.04	0.556***	2.0
α-terpineol	0.017 (ns)	−0.08	0.544***	0.09
α-terpineol with "important terpenes"	0.021 (ns)	0.01	0.561***	0.08
norisoprenoids				
β-damascenone	0.594***	1.5	0.741***	0.19
β-damascenone with the sum of norisoprenoids	0.001 (ns)	0.0005	0.357**	0.01
volatile phenols				
sum of vinylphenols	0.019 (ns)	0.002	0.525***	0.15
sum of vinylphenols (without T, CA)	0.542***	0.01	0.560***	0.15
guaiacol	0.285*	0.06	0.521***	0.28
guaiacol (without T, CA)	0.905***	0.26	0.593***	0.28
vanillin derivatives				
sum of vanillins[e]	0.835***	2.6	0.845***	0.29
lactones[b]				
sum of lactones	0.470**	2.9	0.482**	1.7

[a] Results of the ANOVA analysis: ns, not significant; *, significant at $P > 0.95$; **, significant at $P > 0.99$; ***, significant at $P > 0.999$. [b] Muscat variety is not included. [c] "Important terpenes" are linalool, α-terpineol, geraniol, nerol, and β-citronellol. [d] Only Cabernet Sauvignon, Grenache, Tempranillo, and Chardonnay were included. [e] Syringaldehyde, acetosyringone, and homovanillic acid were not included.

coumaric acids (*60*), whereas the yeast selected in this study is not able to carry out this transformation (*64*). Ferulic and coumaric acids can be present in higher concentration in these grape varieties and can be also present in the precursor fraction (*6*). If samples from those varieties are not included, results significantly improve.

The case of guaiacol is quite similar, although there is not an obvious explanation. The sum of vanillin derivatives present in fermented samples can be predicted by both harsh acid and enzymatic hydrolyses (**Table 4**), whereas the levels of lactones found in fermented samples are just loosely correlated to those found in acid or enzymatic hydrolysates. Finally, it should be noted that the summation of all the volatiles released by both enzymatic and acid hydrolyses was not significantly correlated with the level of any volatile compound found after fermentation (data not shown), which suggests that the applicability of global indices should be further studied.

In conclusion, it has been shown that leaving aside Muscat, and to a lesser extent Grenache and Chardonnay, differences in the composition of hydrolysates from different grape varieties were not very high, which suggests that leaving aside those aforementioned cases, grape variety is not a major factor determining the composition of the precursor fraction of the studied varieties. In general, alcoholic fermentation shows a low potential to release volatile compounds from aromatic precursors, harsh acid hydrolysis has an intermediate releasing potential, and enzymatic hydrolysis is the most efficient hydrolytic procedure. However, it is also the most different, and the levels of most volatile compounds found in enzyme hydrolysates are poorly correlated with those found after alcoholic fermentation. On the other hand, the composition of harsh acid hydrolysates can be used to predict the levels of some of the relevant varietal wine aroma compounds and, therefore, the detailed GC analysis of acid hydrolysates can be considered to be more adequate for measuring the aroma potential of grapes for winemaking. This suggests that transformations taking place during fermentation include relevant chemical rearrangements in acid media that are better predicted by acid hydrolysis.

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