

# Potential Aroma in Several Varieties of Spanish Grapes

Elvira López-Tamames,<sup>\*,†</sup> Nieves Carro-Mariño,<sup>‡</sup> Yusuf Ziya Gunata,<sup>§</sup> Claude Sapis,<sup>§</sup>  
Raymond Baumes,<sup>§</sup> and Claude Bayonove<sup>§</sup>

Institut National de la Recherche Agronomique, Institut des Produits de la Vigne, Laboratoire des Arômes et des Substances Naturelles, 9 Place Viala, F-34060 Montpellier, France, Unitat de Nutrició i Bromatologia, CERTA, Facultat de Farmàcia, Universitat de Barcelona, Avenida Joan XXIII s/n, E-08028 Barcelona, Spain, and Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Química, Universidad de Santiago de Compostela, Avenida de las Ciencias s/n, E-15706 Santiago de Compostela, Spain

Free and bound aroma compounds of eight Spanish grape cultivars (Albariño, Treixadura, Listan, Viura, Xarel.lo, Parellada, Garnacha, and Tempranillo) used in winemaking were studied. With a single-extraction procedure it was possible to provide information about the potential flavor of these varieties: bound aroma was determined directly by TFA glycoside analysis and indirectly by enzyme hydrolysis release of aglycon. An aroma data base of these varieties and a comparison between the grape flavor composition of each variety were established. Grape characteristics (sugars, acidity, polyphenols, juice yield), mainly due to climate and cultivar factors, were related with the aroma composition. Non-terpenyl compounds such as 2-phenylethanol and benzyl alcohol were the main glycosidically bound compounds of Spanish varieties. Direct determination of glycosides is better than hydrolysis of aglycon as an approach to the potential aroma of grapes.

**Keywords:** *Aroma; glycosides; Spanish grape cultivars; Albariño; Treixadura; Listan; Viura; Xarel.lo; Parellada; Garnacha; Tempranillo*

## INTRODUCTION

Quality wines have different flavor properties which depend on varietal characteristics. This typical flavor is mainly due to volatile compounds that proceed from the grape and whether they are in free volatile form (Williams *et al.*, 1981) or in bound form, usually as glycoside (Williams *et al.*, 1982a,b; Voirin *et al.*, 1992a). Bound aroma, potentially developed during winemaking, is unknown for several grape cultivars.

French varieties such as Muscat (Carro-Mariño *et al.*, 1995), Chardonnay (Sefton *et al.*, 1993; Arrhenius *et al.*, 1996), and Cabernet Sauvignon (Gómez *et al.*, 1995) and German varieties such as Riesling (Skouroumounis & Winterhalter, 1994) have been the cultivars most studied in the establishment of data bases of flavor compounds; however, the majority of Italian and Spanish varieties are unknown. Only few data exist about free aroma of winemaking products of Spanish cultivars such as Viura, Xarel.lo, Parellada (De la Presa *et al.*, 1995) and Albariño (Versini *et al.*, 1995). In the current study, the free and bound aroma of eight typical Spanish grapes (Albariño, Treixadura, Listan, Viura, Xarel.lo, Parellada, Garnacha, and Tempranillo) was screened to learn their varietal aroma. These grape varieties have world areas of cultivation estimated at <5 000, <5 000, 28 000, 60 000, 43 000, <5.000, 378 000, and 85 000 ha, respectively. Although some of them have small areas of cultivation, they are used in the production of wines of recognized high quality (with Appellation of Origin Controlled, AOC) and are known worldwide. The grapes considered in this study are from two different climate zones: Galician (northwestern Spain), with an

Atlantic climate (high humidity and rain), and Mediterranean, with a climate with high insolation and temperature.

Several studies (Gunata *et al.*, 1985; Strauss *et al.*, 1987; Voirin *et al.*, 1992a; Sefton *et al.*, 1993; Williams *et al.*, 1995; Zhou *et al.* 1996) have reported methods of analyzing wine flavor. However, few references describe a single and routine method which allows a quantification of the free and bound aroma of grapes, mainly non-Muscat varieties. In this research, the extraction and separation were carried out according to the method proposed by Gunata *et al.* (1985) and aroma compounds were analyzed using the same grape sample. Later, free compounds of grape extracts were analyzed by GC and MS. Bound compounds of grape extracts were determined with two procedures: a direct method, which determined glycosides by trifluoroacetylation (TFA), based on Voirin (1992b) but modified to eliminate the interferences of two glycoside isomers (Carro-Mariño *et al.*, 1995), and an indirect method, which determined the aglycons released before enzyme hydrolysis. Moreover, since climate and cultivar factors affect aroma composition (De la Presa *et al.*, 1995; Gómez *et al.*, 1995; García-Jarés *et al.*, 1995; Arrhenius *et al.*, 1996), these variables were also considered by including more than one sample of the same variety: a data base of grape aromas of Spanish varieties was reported taking into account the variation due to harvest and geography.

## MATERIALS AND METHODS

**Plant Material.** Eight Spanish grape varieties (Albariño, Treixadura, Listán, Viura, Xarel.lo, Parellada, Garnacha, and Tempranillo) were considered. Albariño, Treixadura, and Listán grapes were harvested in 1993 from different Galician AOC cultivars: *Rias Baixas AOC* [Albariño (A) grapes came from three zones: Valle del Salnés (As), Rosal (Ar), and Condado del Tea (Ac)] and *Ribeiro AOC* [Listán] (L) and Treixadura (Tr)]. Galician varieties came from a region with a Atlantic climate with an high humidity and rain, while the

\* Author to whom correspondence should be addressed (e-mail elopez@farmacia.far.ub.es).

† Universitat de Barcelona.

‡ Universidad de Santiago de Compostela.

§ Institut National de la Recherche Agronomique.

**Table 1. Characteristics of the Grape Juices**

sample <sup>a</sup>	variety (color)	origin	AOC (origin controlled)	sugars (g/L)	soluble solids (° Brix)	titratable acidity (g of H <sub>2</sub> SO <sub>4</sub> /L)	pH	maturity index (° Brix/titratable acidity)	poly-phenols (280 nm)	juice yield (mL of juice/kg of grape)
Ac	Albariño (white)	Galician	Rias Baixas	197	22.0	3.50	3.68	6.3	30.3	0.755
Ar	Albariño (white)	Galician	Rias Baixas	175	18.5	4.45	3.42	4.2	24.3	0.785
As	Albariño (white)	Galician	Rias Baixas	174	18.4	6.70	3.28	2.7	19.9	0.630
Gp	Grenache (red)	Mediterranean	Penedès	244	24.5	2.50	3.47	9.8	28.7	0.740
Gh	Grenache (red)	Mediterranean		153	16.5	6.05	3.15	2.7	17.2	0.640
L	Listan (white)	Galician	Ribeiro	145	15.8	2.30	3.57	6.9	24.8	0.710
P92	Parellada (white)	Mediterranean	Penedès & Cava	156	16.8	3.40	3.21	4.9	10.4	0.760
P93	Parellada (white)	Mediterranean	Penedès & Cava	146	15.9	2.30	3.49	6.9	12.3	0.810
T92	Tempranillo (red)	Mediterranean	Penedès	212	21.8	2.25	3.98	9.7	29.0	0.720
T93	Tempranillo (red)	Mediterranean	Penedès	170	18.1	2.60	3.67	7.0	24.8	0.780
Tr	Treixadura (white)	Galician	Ribeiro	128	14.3	4.35	3.58	3.3	22.2	0.845
V92	Viura (white)	Mediterranean	Penedès & Cava	149	16.2	1.95	3.43	8.3	10.4	0.770
V93	Viura (white)	Mediterranean	Penedès & Cava	163	17.4	2.05	3.70	8.5	15.1	0.820
X92	Xarel.lo (white)	Mediterranean	Penedès & Cava	148	16.1	3.15	3.33	5.1	11.6	0.790
X93	Xarel.lo (white)	Mediterranean	Penedès & Cava	175	18.5	2.25	3.62	8.2	12.1	0.835

<sup>a</sup> c, r, s, p, h: geographical origin. c: Condado del Tea. r: Rosal. s: valle del Salnés. p: Penedès. h: Hérault. 92, 93: year of harvest.

**Table 2. Free Volatile Compounds in Spanish Varieties<sup>a</sup> (in µg/L)**

	Kovats index (Carbowax phase)	identification a, retention time b, MS	Galician varieties					Mediterranean varieties									
			Ac	Ar	As	L	Tr	Gp	Gh	P92	P93	T92	T93	V92	V93	X92	X93
1 hexanal	1127	a,b	57	1032	741	199	34	416	844	257	201	294	564	139	44	479	317
2 (E)-hex-3-en-1-al	1148	a,b	2	14	16	1	1	3	2	3	3	4	5	10	3	3	2
3 (Z)-hex-2-en-1-al	1236	a,b	8	40	24	12	8	208	9	122	11	110	14	109	13	189	23
4 (E)-hex-2-en-1-al	1243	a,b	349	2373	1300	587	193	401	1624	436	1124	371	941	231	215	715	686
5 hexanol	1365	a,b	400	843	485	151	449	346	119	194	407	441	263	83	136	207	160
6 (E)-hex-3-en-1-ol	1376	a,b	8	15	13	3	6	3	7	5	10	5	6	1	1	5	4
7 (Z)-hex-3-en-1-ol	1395	a,b	26	81	81	128	35	59	21	73	125	70	78	91	69	50	10
8 (E)-hex-2-en-1-ol	1410	b	799	1153	809	352	415	162	52	318	686	273	313	65	132	327	207
9 (Z)-hex-2-en-1-ol	1416	a,b	5	4	19	3	5	9	4	9	7	1	5	3	1	5	5
10 trans-furanic linalool oxid	1449	a,b		1			1	1	1	1	1	1					
11 cis-furanic linalool oxid	1476	a,b				1		2	1	1		3					
12 benzaldehyde	1538	a,b	4	13	9	8	3	16	10	6	4	5	3	15	2	17	4
13 linalool	1558	a,b	1	2	3	5	1	2	2	1	1	1					
14 hotrienol	1680	b	2	1	1		1	1	1	1	1	1	1	1		1	
15 α-terpineol	1697	a,b	4	1	1	1	3	0	2		1	2	1		2		1
16 7-ethoxy-3,7-dimethyl-1,5-octadien-3-ol	1725	b	1	4	2	2	1	1	2	1	1	1	1	1		1	1
17 trans-pyranic linalool oxid	1755	b	7	13	17	3	1	1	1	1		1			2		2
18 cis-pyranic linalool oxid	1772	b		1	1			1	1	2	1	1	2	1	3	1	
19 citronellol	1777	a,b	1	3	2	2	1	6	2		2	2	1	4		4	1
20 nerol	1809	a,b	1	2	2	1		0	11	2		2		4	1	3	2
21 hexanoic acid	1857	b	206	199	131	26	69	138	14	144	91	43	40	88	67	135	97
22 geraniol	1858	a,b	13	24	16	11	18	14	17	10	3	6	4	2	15	5	
23 benzyl alcohol	1877	a,b	30	59	66	19	32	66	44	111	28	26	16	49	23	167	50
24 2-phenylethanol	1910	a,b	64	76	79	92	27	56	63	84	22	50	17	49	13	87	27
25 2,6-dimethyl-3,7-octadiene-2,6-diol	1958	a,b	3	16	15	6	3	49	10	64	13	17	9	11	1	7	6
26 2,6-dimethyl-7-octen-2,6-diol	2027	a,b	3	8	4	4	3	5	4	11	9	7	7	3	3	13	2
27 2,6-dimethyl-1,7-octadiene-3,6-diol	2219	b	2	2	1	2	2		29		5	1	2		1		3
28 2,6-dimethyl-7-octene-1,6-diol	2266	b	1	2	1	1	1	1	1	2	2		1				1
29 (E)-2,6-dimethyl-2,7-octadiene-1,6-diol	2298	b	2	5	2	3	3	7	13	5	23	14	6	10	6	10	15
30 (Z)-2,6-dimethyl-2,7-octadiene-1,6-diol	2343	b	11	17	10	9	8	7	3	9	5	3	2	3	1	6	7
31 geranic acid	2567	b	1	1		1		12	60	19	3	10		2		8	1
32 3-hydroxy-β-damascone	2613	b	3	3	3	4	2	10	13	4	3	4	2		3		4
33 unknown	2620	a	1		2			1	9			2		7		5	1
34 vanillin	2625	b	3	9	4	6	2	26	8	33	7	9	4	28	4	22	46
35 3-oxo-α-ionol	2637	b	2	3	1	1	2	5	1	2	1	7	1	2	1	9	

<sup>a</sup> A: Albariño. L: Listán. Tr: Treixadura. G: Garnacha. P: Parellada. T: Tempranillo. V: Viura. X: Xarel.lo. 92, 93: year of harvest. c, r, s, p, h: geographical origin.

other varieties were collected from an area with a Mediterranean climate. The Garnacha grape variety (G) was harvested in 1992 from two areas: domaine du Chapitre ENSA-INRA, Hérault (Gh), France, and vineyards in the *Penedès AOC* (Gp). Viura (V), Xarel.lo (X), Parellada (P), and Tempranillo (T) grapes also came from *Penedès AOC* and were harvested in 1992 and 1993 (V92, V93, X92, X93, P92, P93,

T92, and T93, respectively). Albariño, Treixadura, Listán, Viura, Xarel.lo, and Parellada are white varieties, and Garnacha and Tempranillo are red varieties. All grape samples were collected on the cellar before winemaking. They were frosted rapidly with liquid nitrogen and stored at -20 °C until crushing.

**Juice Preparation.** Grape berry samples (1 kg) were

**Table 3. Bound Compounds (Released by Enzyme Hydrolysis and Glycoside Form) in Spanish Varieties<sup>a</sup> (in  $\mu\text{g/L}$ )**

	Galician varieties					Mediterranean varieties									
	Ac	Ar	As	L	Tr	Gp	Gh	P92	P93	T92	T93	V92	V93	X92	X93
aglycons released by hydrolysis															
36 <i>trans</i> -furanic linalool oxid	1	4	2				2		2		1		3		2
37 <i>cis</i> -furanic linalool oxid	1	1	1						1		1				
38 linalool	2	10	9		1		2	5	6		5	13	13	4	16
39 hotrienol		1	1			1	1	2	1		1		1	1	1
40 $\alpha$ -terpineol		1	2				2		1		1		1	1	1
41 <i>trans</i> -pyranic linalool oxid	1	5	2						1						
42 citronellol	1	2	1				2		1		1		1		1
43 nerol	1	3	2				2	1	1		2	1	2	1	3
44 geraniol	8	29	21	3	5	2	23	8	4	2	4	3	5	7	10
45 benzyl alcohol	16	65	58	18	18	79	193	193	67	44	24	181	108	283	129
46 2-phenylethanol	24	36	37	40	11	22	69	52	31	21	16	70	52	61	43
47 2,6-dimethyl-3,7-octadiene-2,6-diol	3	13	5	1			1	4	1			1	1	3	1
48 2,6-dimethyl-7-octen-2,6-diol	1	2	1	1		1	1		3	1	1	3	4	1	2
49 2,6-dimethyl-1,7-octadiene-3,6-diol		1	1						1						
50 2,6-dimethyl-7-octene-1,6-diol	1	3	1	1		2	3	5	1	2		11	1	7	2
51 ( <i>E</i> )-2,6-dimethyl-2,7-octadiene-1,6-diol	5	6	3	3	1	1	7	6	1	2	1	5	2	7	3
52 ( <i>Z</i> )-2,6-dimethyl-2,7-octadiene-1,6-diol	32	38	19	23	10	3	11	8	3	3	3	7	8	17	13
53 geranic acid	3	8	3	1	1	2	5	14	2	5	4	2	9	4	
54 3-hydroxy- $\beta$ -damascone		1	1			2	1	19	1	2		4		10	
glycosides															
55 benzyl $\beta$ -D-glucopyranoside	500	681	289	1419	2462	652	578	1015	1512	554	439	316	923	709	1802
56 linalyl $\beta$ -D-glucopyranoside	22	30	16	25	6	10			7	5		7	15	7	6
57 2,6-dimethyl-1,7-octadiene-3,6-diol $\beta$ -D-glucopyranoside	15									5			12	5	4
58 2-phenylethyl $\beta$ -D-glucopyranoside	448	185	130	831	180	125	281	97	134	123	67	129	82	34	291
59 neryl $\beta$ -D-glucopyranoside		34	9									9	15		9
60 geranyl $\beta$ -D-glucopyranoside	139	28	5	93	26	14			11		11	11	19		9
61 citronellyl $\beta$ -D-glucopyranoside	6	18	4	29	9	2			12		4	11	19		
62 $\alpha$ -terpineyl $\beta$ -D-glucopyranoside	53	22		22	16	20	10	7	6	6		14	11		13
63 ( <i>R</i> )-linalyl rutinoside															
64 benzyl rutinoside	332	99	32	140	139	245	183	187	455	152	137	232	413	64	288
65 ( <i>S</i> )-linalyl rutinoside	17		11	45	42	64	62	36	55	26	23	24	130	8	114
66 benzyl 6- <i>O</i> -( $\alpha$ -L-arabinofuranosyl) $\beta$ -D-glucopyranoside + neryl rutinoside	190	26	30	147	246	633	431	155	119	89	35	178	358	124	517
67 2-phenylethyl rutinoside	226	2810	439	718	571	651	2989	94	2915	247	672	805	1366	229	3108
68 neryl 6- <i>O</i> -( $\alpha$ -L-arabinofuranosyl) $\beta$ -D-glucopyranoside + $\alpha$ -terpineyl rutinoside	298	91	8	27	17	25	36		260	13		22	36	9	52
69 2-phenylethyl 6- <i>O</i> -( $\alpha$ -L-arabinofuranosyl) $\beta$ -D-glucopyranoside	735	1802	180	326	401	163	469	127	1537	203	162	316	1290	130	818
70 geranyl 6- <i>O</i> -( $\alpha$ -L-arabinofuranosyl) $\beta$ -D-glucopyranoside	211	21	20	25	12		55	13	35	9		6	13	37	65
71 $\alpha$ -terpineyl 6- <i>O</i> -( $\alpha$ -L-arabinofuranosyl) $\beta$ -D-glucopyranoside	119	3	10	64	86	43	59	10	40	22		47	253	35	140

<sup>a</sup> See Table 2 footnotes.

defrosted (4 °C overnight) and crushed. Cloudy juices were centrifuged (10 min at 7500g) at the same temperature and then filtered. Clear juice volumes were measured to calculate the juice yield (Table 1), expressed as mL of juice/kg of grape. Moreover, juice characteristics [sugar content (g/L), soluble solids (°Brix), titratable acidity (g of H<sub>2</sub>SO<sub>4</sub>/L), maturity index (°Brix/titratable acidity), pH, and total polyphenols (absorbance at 280 nm)] were established.

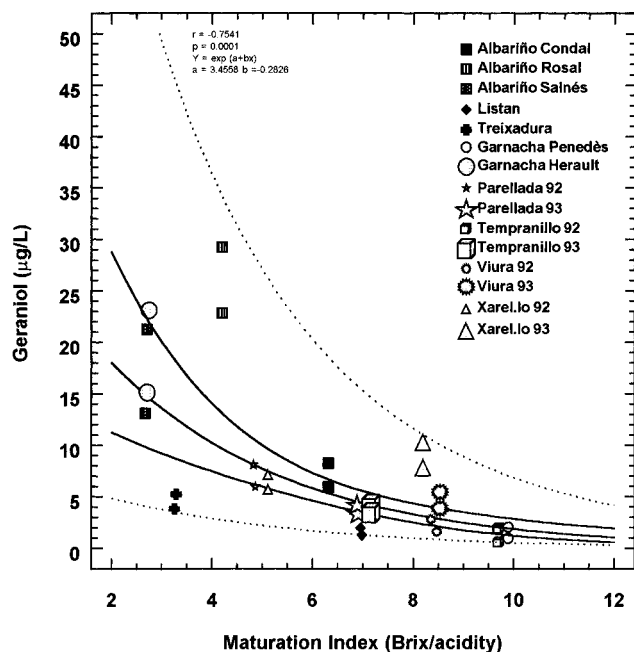
**Extraction of Free and Bound Flavor.** 200 mL of must [to which 10  $\mu\text{L}$  of 4-nonanol (Fluka 98%) solution (3.6 mg/mL of methanol) was added as internal standard] was eluted through an Amberlite XAD-2 column (120 mm  $\times$  7 mm i.d.) with 1.5 mL/min as flow rate. Free compounds were collected by elution with 50 mL of pentane/dichloromethane (2:1, v/v), and the bound fraction was obtained by elution with 50 mL of ethyl acetate. The free fraction was dried over sodium sulfate and then concentrated to 500  $\mu\text{L}$  by Dufton columns. Extracts were stored at -20 °C until analysis by GC. The bound fraction was diluted with ethyl acetate at 100.0 mL, and then two 50.0 mL portions were obtained. All extractions were performed in triplicate.

**Enzyme Hydrolyses.** One 50.0 mL portion of the ethyl acetate fraction was dried over sodium sulfate and evaporated at 30 °C under vacuum, then redissolved in 100  $\mu\text{L}$  of 0.1 M citrate-phosphate buffer (pH 5.0) and washed five times with 1 mL of pentane/dichloromethane (2:1, v/v) to remove traces of free compounds. 100  $\mu\text{L}$  of Pektolase 3PA (Grindsted) solution was added [1.2 mg of Pektolase 3PA in 100  $\mu\text{L}$  of 0.1

M citrate-phosphate buffer (pH 5.0)], and the mixture was incubated at 40 °C for 16 h. After incubation, released aglycons were extracted four times with 200  $\mu\text{L}$  of pentane/dichloromethane (2:1, v/v) and 10  $\mu\text{L}$  of 4-nonanol solution (3.6 mg/mL of methanol) was added as internal standard. Aglycons were dried over sodium sulfate and then concentrated to 500  $\mu\text{L}$  by Dufton columns. Extracts were stored at -20 °C until analysis by GC.

**Glycoside Determination.** The other 50.0 mL portion of the ethyl acetate fraction was dried over sodium sulfate and evaporated at 30 °C under vacuum and then redissolved in 2.0 mL of bidistilled water and treated with 150 mg of polyvinylpyrrolidone (PVPP) (Polyclar AT, GAF) to remove probable polyphenol interferences; this mixture was magnetically stirred for 1 h at 4 °C with, and then PVPP was eliminated by cellulose filtration. The extract was dried on a Réacti-vial of 2 mL capacity at 60 °C with a nitrogen stream. Trifluoroacetylation was carried out at 60 °C (20 min) using 20  $\mu\text{L}$  of anhydrous pyridine and 20  $\mu\text{L}$  of MBTFA reagent [*N*-methylbis(trifluoroacetamide)] (Sigma). Just before GC analysis, 10  $\mu\text{L}$  of phenyl  $\beta$ -D-glucopyranoside (1 g/L of methanol) (Sigma 98%) was added as internal standard.

**Chromatographic Method of Free Volatiles and Aglycons.** A Varian model 6000 provided with a CP-Wax 52 CB, fused-silica capillary column (Chrompack) (25 m  $\times$  0.3 mm i.d.; 1.2  $\mu\text{m}$  bonded phase) was used to perform the on-column GC analysis: injector temperature was programmed from 20 to 250 °C with a rate of 180 °C/min; column temperature was



**Figure 1.** Exponential regression between the content of bound geraniol and the maturation index. \*Upper and lower limits of 95% confidence interval for the three analytical replicates were considered for the samples values.

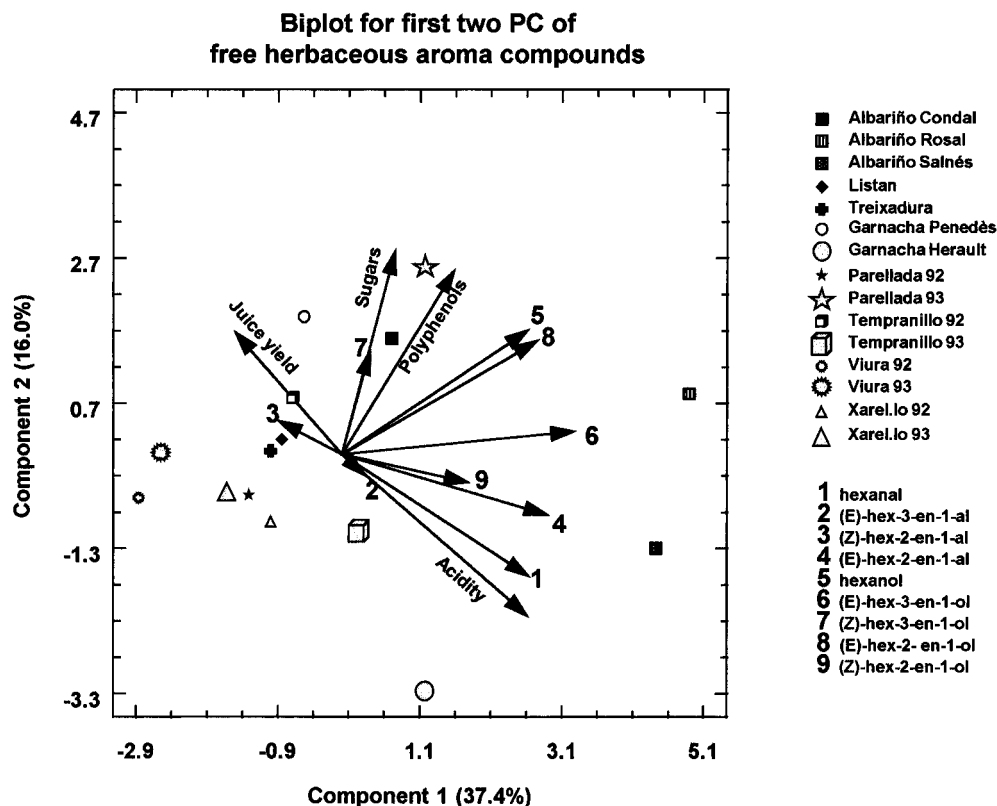
programmed at 2 °C/min from 60 °C (3 min isothermal) to 220 °C and at 3 °C/min from 220 °C to 245 °C; hydrogen at 2.5 mL/min was used as carrier gas; FID temperature was 250 °C. Electron impact mass spectra were obtained by coupling the CP-Wax 52 CB, fused-silica capillary column to a Finnigan MAT ITD 700. The transfer line, heated at 240 °C, consisted of an open-split GC-ITD interface at atmospheric pressure and a flow restrictor which was a DB-5 fused-silica capillary column (1.15 m × 0.15 mm i.d.; bonded phase). The temperature program used was the same as for the GC analysis. The

source temperature was 220 °C. Mass spectra were scanned between 50 and 80 eV in the range  $m/z$  41–250 at 2-s intervals. Identification was also performed by comparison with Kovats Index of standards (Table 2).

**Chromatographic Method of Glycosides.** Equipment used was a Varian model 3300 with DB 5 fused-silica capillary column (30 m × 0.32 mm) and an on-column injector. Injector temperature was programmed at 60 °C/min from 90 to 280 °C. Column temperature was programmed from 125 to 220 °C at 3 °C/min and from 220 to 280 °C at 2 °C/min with hydrogen as carrier gas at 1.4 mL/min. Flame ionization detector temperature was 300 °C. Identification was carried out by GC coupling to MS (Hewlett-Packard 5989 MS Engine) with the chromatographic conditions described previously but with helium (1 mL/min) as carrier gas. Source temperature was 250 °C, mass spectra were scanned at 70 eV in the range  $m/z$  29–850 at 2-s intervals and by retention times comparison of standards (Voirin, 1992a).

**Quantitation.** Concentrations of free fraction (Table 2) and aglycones enzymatically hydrolyzed from glycosides (Table 3) were calculated using 4-nonanol as internal standard and they were expressed by average of three repeated analytical assays in µg/L. Glycosides (Table 3) were quantified by phenyl β-D-glucopyranoside as internal standard, taking into account the extraction efficiency calculated with a reference solution fixed on Amberlite resin. The average of three repeated analytical assays was considered.

**Statistical Analysis.** Principal component analysis (PCA) was carried out using STATGRAPHICS 7.0. PCA considers as quantitative variables 71 aroma compounds, expressed in µg/L. In order to consider the relationship between aroma composition and ripening, sugars, titratable acidity, polyphenols, and juice yield were also included as a quantitative parameters. Qualitative variables were variety (A, G, L, Tr, P, T, V, and X), year of harvest (92 and 93), and geographical origin in Spain (Galician and Mediterranean). Moreover, multiple correlation was performed to establish the relationship between the free and bound aroma components related to grape characteristics.



**Figure 2.** PCA of herbaceous compounds in some Spanish varieties.

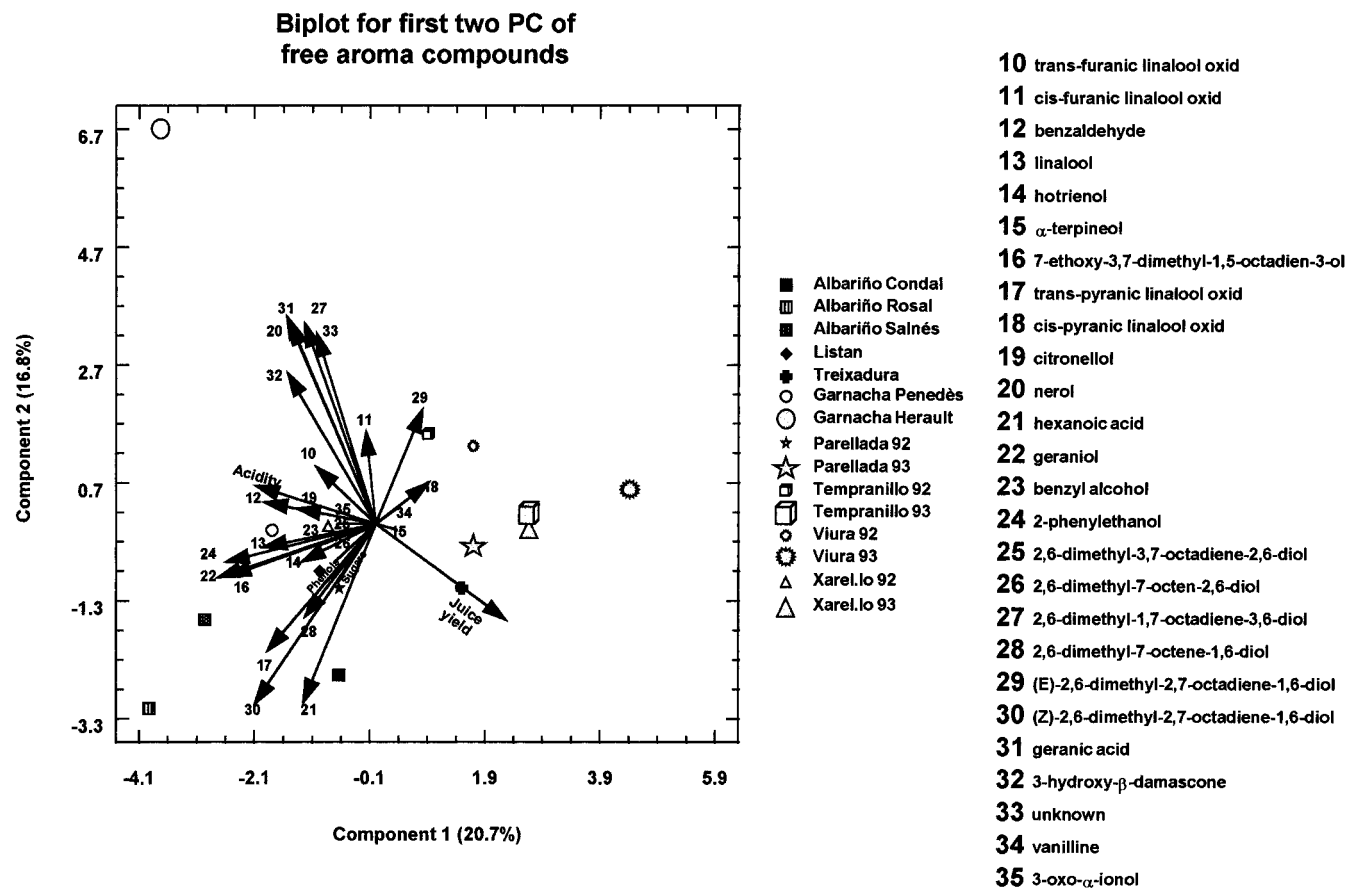


Figure 3. PCA of free aroma compounds in some Spanish varieties.

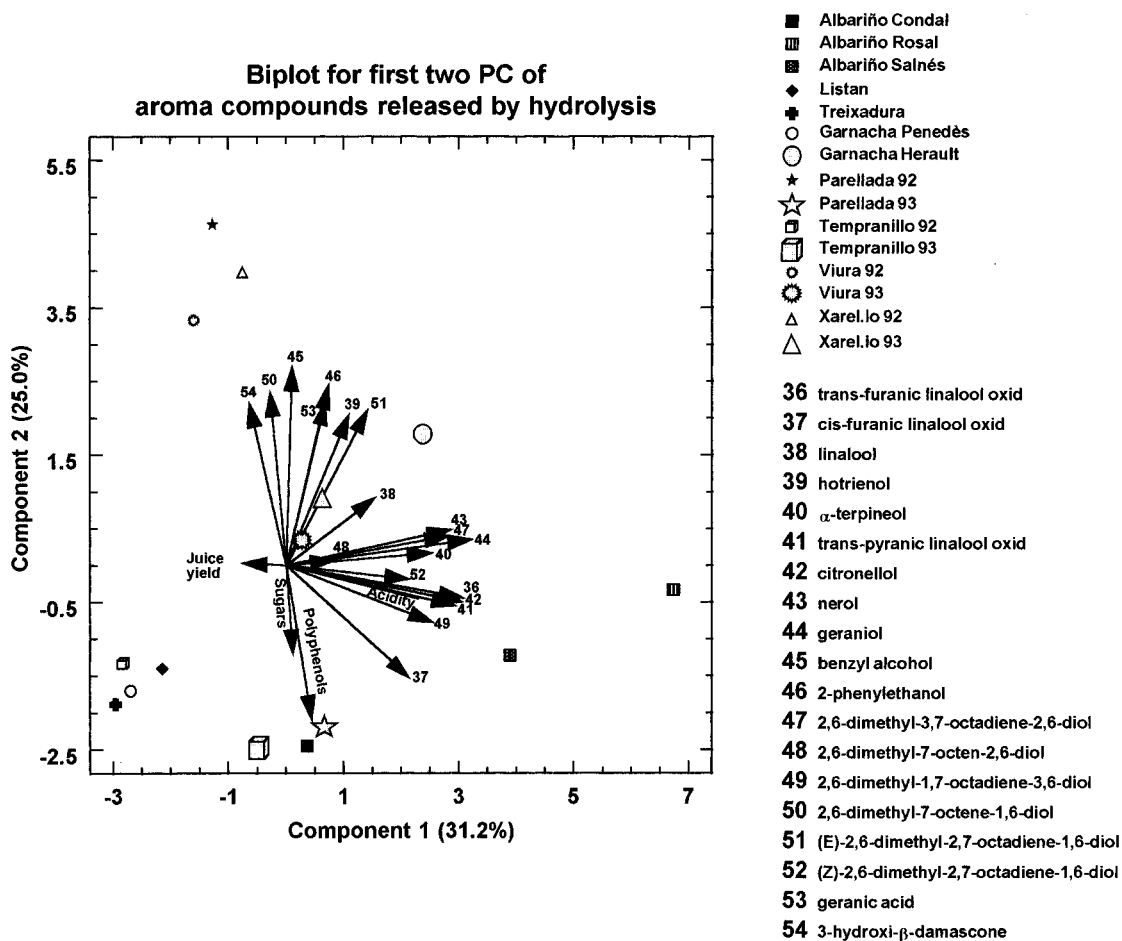
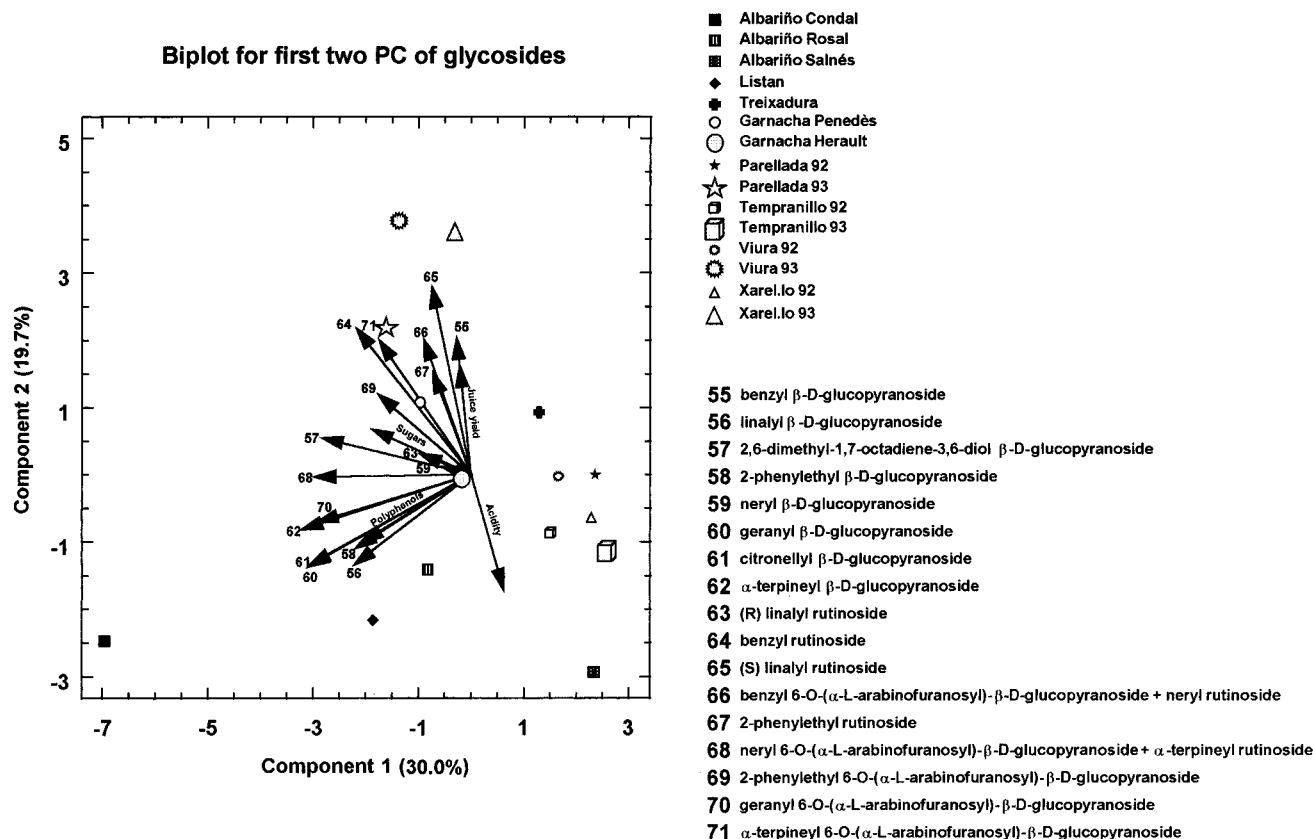


Figure 4. PCA of the bound compounds released by enzymic hydrolysis in some Spanish varieties.



**Figure 5.** PCA of glycosides in some Spanish varieties.

## RESULTS AND DISCUSSION

Table 2 shows the free aroma of Spanish grapes, expressed by mean ( $\mu\text{g/L}$ ), which correspond to the three analytical replicates. The bound aroma (released by enzyme hydrolysis and in glycoside form) of the Spanish grapes is expressed also by mean ( $\mu\text{g/L}$ ) and displayed in Table 3. Penedès grape varieties have a higher ( $p < 0.05$ ) maturation index and more juice yield than Galician cultivars (mainly Albariño variety). The different ripening stages (Table 1) among varieties and within the same variety affect the level of several aroma compounds: for example, in Figure 1 the exponential regression between maturation index and the concentration of geraniol released by enzymic hydrolysis shows that the greater the ripening of the grape, the lower the amount of bound geraniol.

**Free Aroma Fraction.** Non-terpenyl compounds were the most abundant substances in the aroma-free fraction of Spanish varieties (Table 2): alcohols and aldehydes of six carbon atoms (benzyl alcohol, 2-phenylethanol, benzaldehyde, and hexanoic acid). Geraniol and several diols were the terpenes that appeared in higher concentration. Norisoprenoids such as 3-hydroxy- $\beta$ -damascone and 3- $\alpha$ -oxoionol are present in several cultivars. This free aroma composition indicates that these cultivars have a nonfloral aroma profile with respect to the Muscat cultivars.

Alcohols and aldehydes of six carbon atoms (Figure 2) have a greasy odor, and their origin is mainly in the lipoxygenase activity of the grape (Cordonnier, 1989). PCA shows that these compounds were correlated ( $p < 0.05$ ). Hexanal ( $p < 0.05$ ), (*E*)-hex-2-en-1-al ( $p < 0.04$ ), and (*E*)-hex-3-en-1-ol ( $p < 0.01$ ) were herbaceous compounds that seem related to the maturation index of the grape: the lower the acidity, the lower the level of these substances. The concentration of greasy alcohols and

aldehydes depends on the grape cultivar ( $p < 0.01$ ): Albariño had the greatest levels, although there were variations due to the geographical origin or to the ripening stage; Listan, Xarel.lo, and Viura have the smallest quantities of greasy compounds. No differences in these alcohols and aldehydes were found between white and red cultivars.

Terpenes [linalool with their *trans*-furanic linalool oxide ( $p < 0.001$ )] and non-terpenyl substances (benzyl alcohol, 2-phenylethanol, benzaldehyde, norisoprenoids, geranic acid) were also correlated ( $p < 0.05$ ). Galician cultivars, mainly Albariño, had more terpenyl compounds (*trans*-pyranic linalool oxid and several diols) than the Mediterranean varieties (Figure 3). Free aroma composition depended on grape cultivar: Listan had more linalool, Garnacha had more 3-hydroxy- $\beta$ -damascone, Xarel.lo had more vanillin, and Parellada had more (*E*)-2,6-dimethyl-2,7-octadiene-1,6-diol. Furthermore, the year of harvest changed free aroma composition: 1993 grapes had fewer aroma substances than 1992 grapes; this could be explained by the different maturation index of the grapes between years: terpenyl compounds, mainly geraniol, were less when acidity was lower ( $p < 0.005$ ) (Figure 1).

**Bound Aroma Fraction.** Quantitatively, bound aroma released by enzyme hydrolysis was lower than the direct glycoside composition (Table 3). A possible explanation of this observation could be a partial hydrolysis of the enzyme. The aglycons released in the highest level are non-terpene: benzyl alcohol and 2-phenylethanol. Moreover, glycosides of benzyl alcohol and 2-phenylethanol were the highest glycosides. Voirin (1992b) indicates that the varieties richest on this non-terpene glycosides are neutral cultivars. PCA of bound aroma compounds released by enzyme hydrolysis (Figure 4) shows two groups of bound substances: terpenyl,

linalool oxide, and diol aglycones and other aglycon groups with benzyl alcohol, 2-phenylethanol, and geranic acid. Quantitatively, differences in the released aglycons were found when the variety (Albariño was richest in bound terpenyl aroma) and the year of harvest were considered. However, only the content of bound 2-phenylethanol and  $\alpha$ -terpineol seems related to the maturation index: relationships between these compounds and their acidity ( $r = 0.8137$  and  $r = 0.9017$ , respectively) were found for the grapes of the same Albariño variety.

PCA of grape glycosides is displayed in Figure 5. Two groups of glycosides are shown: glucopyranosides (except the benzyl  $\beta$ -D-glucopiranoside) and arabinosides with rutosides; multiple correlations between both groups were found ( $p < 0.05$ ). Furthermore, glycoside composition was highly variable and depended on the geographical origin and/or the year; for example, Viura, Xarel.lo, and Parellada harvested in 1993 have arabinoside and rutoside values higher than those found in 1992; this difference could be explained by the degree of ripening of the grapes.

## CONCLUSIONS

Free and potential aroma of eight Spanish varieties of grapes were established using a single extraction. The potential aroma was determined by two procedures: direct analysis of glycosides and indirect analysis of aglycons released by hydrolysis; indirect analysis was not representative of aroma precursors. Non-terpenyl compounds were the most abundant aroma substances in the considered Spanish grapes. Moreover, aroma components were quantitatively affected by the maturation index. Differences due to varietal and climate characteristics were shown; Galician grapes, mainly Albariño, were richest in varietal aroma.

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