JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Study of Low Molecular Weight Phenolic Compounds during the Aging of Sparkling Wines Manufactured with Red and White Grape Varieties

M. Ángeles Pozo-Bayón, M. Teresa Hernández, Pedro J. Martín-Álvarez, and M. Carmen Polo*

Instituto de Fermentaciones Industriales (CSIC), C/Juan de la Cierva 3, 28006 Madrid, Spain

Thirty-two phenolic compounds of low molecular weight were identified in 36 white, blanc de noir, and rosé sparkling wines by using HPLC with photodiode array and mass spectrometry detection. Some of the identified compounds, such as *cis*- and *trans*-ethylcaftaric, *cis*- and *trans*-ethylcaffeic, and *cis*- or *trans*-ethyl-*p*-coumaric acids, 2*R*,3*R*-dihydroquercetin, 2*R*,3*R*-dihydrokaempferol 3-*O*-β-D-glucoside, and a lignan derivative are described for the first time in sparkling wines manufactured with grapes of red varieties. This is also the first time that *cis*- *or trans*-diethylfertaric acids have been identified in wines. When cluster analysis was applied to the data of 19 of the 32 identified compounds, the greatest differences found in the low molecular weight phenolic compounds in sparkling wines were due to the grape variety from which they were manufactured, whereas aging time did not significantly influence phenolic composition. Nine phenolic compounds, that is, *trans*-*p*-coumaric and *trans*-caftaric acids, *trans*-resveratrol glucoside, *cis*-coutaric, *trans*-coutaric, *cis*-*p*-coumaric, and *cis*-caftaric acids, tryptophol, and syringic acid, permit the wines to be classified correctly in accordance with the grape variety from which they were manufactured.

KEYWORDS: White sparkling wines; blanc de noir sparkling wines; rosé sparkling wines; phenolic compounds

INTRODUCTION

Phenolic compounds are found in plant tissues, and their study in food in general and especially in wines is of great interest (*1*, *2*). These compounds are directly related to the quality of wines. Many factors can influence the phenolic composition of wines, including grape variety (*3*−*5*), the technology applied in their manufacture (*6*), and the reactions that take place during aging in wood (*7*). Some of these factors have been sufficiently studied, but few studies have been designed to establish the effect of the time during which the wine remains with the yeasts on their phenolic composition.

Wine aging with yeasts takes place in three types of great wines: the *crianza* wines from Jerez, the *sur lie* from Burgundy, and the sparkling wines produced by the *champenoise* method. Biological aging of the wines from Jerez and the *sur lie* wines from Burgundy takes place under a layer of yeasts in wooden barrels, and in the same barrel a population of viable yeasts coexists with dead yeasts. In the sparkling wines, aging takes place during at least 9 months in the bottle itself that reaches the consumer. After approximately the first month there are no viable yeasts in the wine (*8*).

Barón et al. (*9*) and Fabios et al. (*10*) found that during biological aging of wines from Jerez, there is no browning of the wine despite the fact that aging is dynamic and not static and involves a periodic transfer of a volume fraction of each barrel. To our knowledge, there is only one study in the literature designed to establish the changes occurring in low molecular weight phenolic compounds during the manufacture of sparkling wines of white varieties (*11*) and no study aimed at establishing the changes that occur in this phenolic fraction in sparkling wines manufactured with red varieties. In research by Ibern-Gómez et al. (*11*), the authors observed modifications in the phenolic composition that they attributed to the oxidation of this type of compound despite the reducing atmosphere existing within the bottle of wine.

The present work was developed because of the nonexistence of studies concerning sparkling wines manufactured with red varieties and the discrepancies between the few existing studies on the aging with yeasts of wines manufactured with white varieties. The objective is to determine the possible changes that occur in low molecular weight phenolic compounds in sparkling wines from white and red grape varieties during aging with yeasts.

MATERIAL AND METHODS

Samples. Three white base wines, four blancs de noir (red varieties fermented without skins), and one rosé (red variety partially fermented with skins) were manufactured industrially. Two of the white base wines

* Corresponding author [fax (34) 91 564 48 53; telephone (34) 91 562 29 00, ext. 398; e-mail mcpolo@ifi.csic.es].

10.1021/jf021017z CCC: $25.00 © 2003 American Chemical Society
Published on Web 02/26/2003

were obtained with grapes of the Parellada variety, and the third was a mixture of white wines of Macabeo, Parellada, and Xarel.lo varieties (58:36:6). Two of the four blancs de noir base wines were manufactured with grapes of the Trepat variety and another two with grapes of the Monastrell variety. The rosé base wine was manufactured with grapes of the Garnacha variety. From each of the eight base wines a batch of sparkling wines was manufactured industrially by the champenoise method. Samples were taken for analysis of the base wines manufactured with a mixture of wines of white varieties, of the blancs de noir, and of the rosé. In the Garnacha sparkling wines, degorging was performed after 9 and 12 months of aging with yeasts and in the rest of the sparkling wines after 9, 12, 15, and 18 months of aging with yeasts. At each degorging time, six bottles were mixed and homogenized before sampling. All analyses were conducted on wines that had been centrifuged at 5 °C and 5000*g* for 15 min.

Extraction of Phenolic Compounds from Wine. A volume of 50 mL of each wine was extracted three times with 25 mL of diethyl ether and three times with 25 mL of diethyl acetate, and the organic fractions were combined. After 30 min of drying with anhydrous Na_2SO_4, the extract was evaporated to dryness under vacuum. The residue, dissolved in 2 mL of methanol/water (1:1, v/v), was analyzed by high-performance liquid chromatography (HPLC).

HPLC-PAD Analysis. The chromatographic system consisted of a model 600E pump system controller, a U6K universal injector, and a model 991 photodiode array detector (PAD) (Waters, Milford, MA). Separations were carried out following the method described by Peña-Neira et al. (*12*). The column was a reversed-phase Nova-Pak C18 (300 mm × 3.9 mm) with 4 μm packing. Eluent A was water/acetic acid (98:2). Eluent B was water/acetonitrile/acetic acid (78:20:2). Flow rate was 1 mL/min. Gradient conditions were as follows: 0−55 min, 0−80% B; 55−85 min, 80−90% B. Chromatograms were monitored simultaneously at three wavelengths, 254, 280, and 340 nm. Spectra were obtained by scanning from 210 to 360 nm with an acquisition speed of 1 s. Waters 991 software (version 720) was used for data acquisition and processing.

HPLC-PAD-MS Analysis. HPLC-PAD-MS equipment was used to obtain qualitative information about the phenolic composition of the wines. The chromatographic system consisted of a Hewlett-Packard 1100 MSD (Hewlett-Packard, Palo Alto, CA) with a PAD detector and a single quadrupole instrument equipped with an atmospheric pressure ionization (API) source using an electrospray ionization (ESI) interface. Nitrogen was used as nebulizing and drying gas. By using HP Chem Station software (version A.07.01, Hewlett-Packard), the *m*/*z* spectral data were acquired, processed, and transformed to spectra representing mass values. A reversed-phase Nova-Pak C18 column (150 mm × 3.9 mm) with 4 μm packing was used. Flow rate was 0.6 mL/min. Eluent A was water/acetic acid (99:1) and eluent B, acetonitrile/acetic acid (99:1). The gradient profile was 0−60 min, 0−80% B; 60−70 min, 80−100% B. The chromatograms were monitored simultaneously at three wavelengths, 254, 280, and 340 nm. Spectra were obtained by scanning from 210 to 360 nm with an acquisition speed of 1 s. ESI conditions were as follows: nebulizer pressure, 40 psi; drying gas flow and temperature, 10 L/min and 320 °C, respectively; and capillary voltage, 4000 V. Mass spectra were obtained in negative-ion mode in the range *m*/*z* between 50 and 1000 uma using different programmable fragmentor voltages: 50−200 uma, 50 V; 200−350 uma, 80 V; 350−1000 uma, 200 V.

Qualitative and Quantitative Analysis. The identification of specific compounds was carried out by comparing their retention times with those of standards. Standard compounds were purchased from Aldrich (Steinheim/Albuch, Germany), syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid) and tyrosol (2- [4-hydroxyphenyl]ethyl alcohol); from Sigma (Deisenhofen, Germany), gallic (3,4,5-trihydroxybenzoic), *trans*-caffeic (3,4-dihydroxycinnamic), protocatechuic (3,4-dihydroxybenzoic), vanillic (4-hydroxy-3-methoxybenzoic), *trans*-*p*-coumaric (4-hydroxycinnamic), and *trans*-ferulic (4-hydroxy-3-methoxycinnamic) acids, *trans*-resveratrol (*trans*-3,4′,5-trihydroxy stilbene), (+)-catechin, and tryptophol; and from Fluka (Buchs, Switzerland), *p*-hydroxybenzoic acid. *cis*-*p*-Coumaric acid (*cis*-4-hydroxycinnamic acid) was obtained from the standard of *trans*-*p*-coumaric acid after exposure to UV light (340 nm) for 2 h. *cis*-Resveratrol was obtained from the standard of *trans*-resveratrol after exposure to UV light (340 nm) for 1 h. Resveratrol glucosides were identified by comparing their spectra with those of their free forms. Other compounds for which no standards were available, such as hydroxycinnamic acid derivatives, were identified by their spectral UV−vis parameters (*13*, *14*) or by MS.

Quantitative determinations were done using the external standard method. *cis*- and *trans*-caftaric acids, *cis*- and *trans*-coutaric acids, *trans*-fertaric acid, and *trans*- and *cis*-resveratrol glucosides were quantified using the calibration curves generated with the free forms, because the UV responses were expected to be similar (*15*).

Statistical Analysis. The statistical methods used for data analysis were cluster analysis (Ward's method from standardized variables), to discover natural groupings of the samples of wines in relation to the two factors of the study (type of wine and aging time); two-way analysis of variance (ANOVA), to test the effects of the two factors; Student−Newman−Keuls test for means comparisons; and stepwise discriminant analysis to select the variables most useful in differentiating the groups. STATISTICA (*16*) and SPSS (*17*) programs were used for data processing. These programs were run on a personal computer.

RESULTS AND DISCUSSION

Phenolic Compounds Identified in the Wines. A total of 32 phenolic compounds were identified in the wines. **Table 1** lists the phenolic compounds detected in the different wines studied and the number assigned to them in accordance with their elution order in the chromatogram (**Figure 1**). It is also indicated whether the identification was carried out by comparing the retention times with those of the standard compounds, whether spectral parameters were determined to assign the identity of the peaks, and whether mass spectrometry was essential for identification. The group of wines in which each of the compounds was detected is recorded. The table also includes peak 2, which was detected in all of the wines and is one of the majority peaks in the chromatograms. This elutes between gallic acid (peak 1) and protocatechuic acid (peak 3), and its UV−vis spectrum has an absorption maximum at 294 nm. Two majority ions appear in its mass spectrum with *m*/*z* ratios of 177 and 147, respectively. This compound may be an aldehyde, because *m*/*z* 147 could be the molecular ion formed after loss of a carbonyl group, as described by other authors (*18*).

The largest number of compounds detected belongs to the group of hydroxycinnamic acids and their esters. These compounds have been detected both in the wines manufactured with white grape varieties and in those manufactured with red grape varieties except for peak 17 (*cis*-caffeic), which was identified in only the rosé sparkling wine. The identity of peak 31 has been tentatively assigned, by its spectral UV−vis and MS characteristics, to *cis*- or *trans*-diethylfertaric acid. Its UV−vis characteristics are similar to those described by Sommers et al. (*19*) for *trans*-fertaric acid, but its MS spectrum with a majority ion of *m*/*z* 381 may correspond to the molecular ion of the *cis*- or *trans*-diethylfertaric acid. This is the first time this compound has been identified in wines. Five other compounds of the hydroxycinnamic group have been described in the literature in wines manufactured only with the Riesling white grape variety (*19*, *20*), and this is the first time they are described in wines manufactured with red grape varieties. These compounds were *cis*- and *trans*-ethylcaftaric acids (peaks 23 and 24), *cis*- and *trans*-ethylcaffeic acids (peaks 29 and 30), and one of the two possible isomers of ethyl-*p*-coumaric acid (peak 32).

Figure 1 shows, by way of example, the chromatogram corresponding to the phenolic compounds determined in the rosé sparkling wine manufactured from the Garnacha variety. The figure also shows the chemical structures and the UV−vis and

Table 1. Phenolic Compounds Identified in the Wines, Detection Methods Used, and Group of Wines in Which Each of the Compounds Was Detected

peak no.	compound	detection method: standard[a]	detection method: UV–vis spectral parameters[b]	detection method: MS[c]	white wine: Parellada	white wine: MPX[d]	blanc de noir wine: Trepat	blanc de noir wine: Monastrell	rosé wine: Garnacha
	hydroxybenzoic acids								
1	gallic	+			+	+	+	+	+
3	protocatechuic	+			+	+	+	+	+
7	*p*-hydroxybenzoic	+			+	+	+	+	+
12	vanillic	+			–	–	+	+	+
16	syringic	+			–	–	+	+	+
	hydroxycinnamic acids								
15	*trans*-caffeic	+			+	+	+	+	+
17	*cis*-caffeic			+	–	–	–	–	+
18	*trans*-*p*-coumaric	+			+	+	+	+	+
19	*cis*-*p*-coumaric	+			+	+	+	+	+
	hydroxycinnamates								
4	*cis*-caftaric			+	+	+	+	+	+
6	*trans*-caftaric		+		+	+	+	+	+
8	*cis*-coutaric		+		+	+	+	+	+
10	*trans*-coutaric		+		+	+	+	+	+
13	*cis*-fertaric			+	+	+	+	+	+
14	*trans*-fertaric			+	+	+	+	+	+
23	*cis*- or *trans*-ethylcaftaric[f]			+	+	–	+	–	+
24	*cis*- or *trans*-ethylcaftaric[f]			+	+	-	+	–	+
29	*cis*- or *trans*-ethylcaffeic[f]			+	-	+	–	–	+
30	*cis*- or *trans*-ethylcaffeic[f]			+	+	–	–	–	+
31	*cis*- or *trans*-diethylfertaric[g]			+	+	–	–	–	–
32	*cis*- or *trans*-ethyl-*p*-coumaric[f]			+	+	+	+	+	+
	stilbenes								
22	*trans*-resveratrol glucoside		+		+	–	+	–	–
27	*cis*-resveratrol glucoside		+		+	+	+	+	–
28	*trans*-resveratrol	+			–	–	+	+	–
33	*cis*-resveratrol	+			+	+	+	+	+
	flavonoids								
11	catechin	+			–	–	+	+	+
20	2*R*,3*R* dihydroquercetin[f]			+	–	–	-	+	+
21	2*R*,3*R*-dihydrokaempferol 3-*O*-β-D-glucoside[f]			+	+	+	+	+	+
	other compounds								
2	unknown compound				+	+	+	+	+
5	2-furoic			+	+	+	+	+	+
9	tyrosol	+			+	+	+	+	+
25	lignan derivative[e,f]			+	–	–	–	+	+
26	tryptophol	+			+	+	+	+	+

[a] Standard compounds were available. [b] UV–vis spectral parameters were essential for the identification of these compounds. [c] Mass spectrometry detection were essential for the identification of these compounds. [d] Wines made with a coupage of three varietal white wines: Macabeo (58%), Parellada (36%), and Xarel.lo (6%). [e] 2*R*,2*R*-2,3-Dihydro-2-(4′-hydroxy-3′-methoxyphenyl)-3-(glucosyloxymethyl)-7-hydroxy-5-benzofuranpropanol. [f] Compounds identified for the first time in sparkling wines manufactured with red grapes. [g] Compound identified for the first time in wines.

MS spectra of peaks 20−22. Peak 20 is a compound that seems to coincide with that described by Badeshneider and Winterhalter (*20*) as flavonol 2*R*,3*R*-dihydroquercetin. It has been detected in the wines manufactured with the red varieties Monastrell and Garnacha. The spectral UV−vis characteristics of peak 21 with a maximum in UV at 286 nm and the MS spectrum with an *m*/z ratio of the majority ion of 449 coincide with the compound described by Badershneider and Winterhalter (*20*) in wines of the Riesling white grape variety as 2*R*,3*R*-dihydrokaempferol 3-*O*-β-D-glucoside. This compound has been detected in wines manufactured from white and red grape varieties. Peak 25 has been detected only in the sparkling wines manufactured from the Trepat and Garnacha varieties. Its UV−vis and MS spectra coincide with a lignan derivative (2*R*,2*R*-2,3-dihydro-2-[4′-hydroxy-3′-metoxyphenyl]-3-[glucosyloxymethyl]-7-hydroxy-5-benzofuranpropanol) described in wines of the Riesling variety by the above authors.

Therefore, in this study the phenolic compounds *cis*- and *trans*-ethylcaftaric acids, *cis*- and *trans*-ethylcaffeic acids, *cis*- or *trans*-ethyl-*p*-coumaric acid, 2*R*,3*R*-dihydroquercetin, 2*R*,3*R*-dihydrokaempferol 3-*O*-β-D-glucoside, and a lignan derivative are described for the first time in wines manufactured with red grape varieties. Moreover, the compound described as *cis*- or *trans*-diethylfertaric acid has been identified for the first time in wines.

Influence of Variety and Aging Time with Yeasts on Phenolic Compounds of Sparkling Wines. In an attempt to obtain a preliminary view of the main causes for the variation in phenolic compounds, cluster analysis was carried out on the data of the quantified compounds of the 36 wines studied. **Figure 2** shows the dendrogram obtained. The squared Euclidean distance was taken as a measure of proximity between two samples, and Ward's method was used as the linkage rule. The variables were standardized previously. As can be observed in this figure, there are two large groups of wines, one corresponding to wines manufactured with grapes of white varieties and the other to wines manufactured with grapes of red varieties. Within the group of wines from red varieties, two new groups could be distinguished: one of wines vinified in white, blanc de noirs, and one of rosé wines. In each of the groups, the

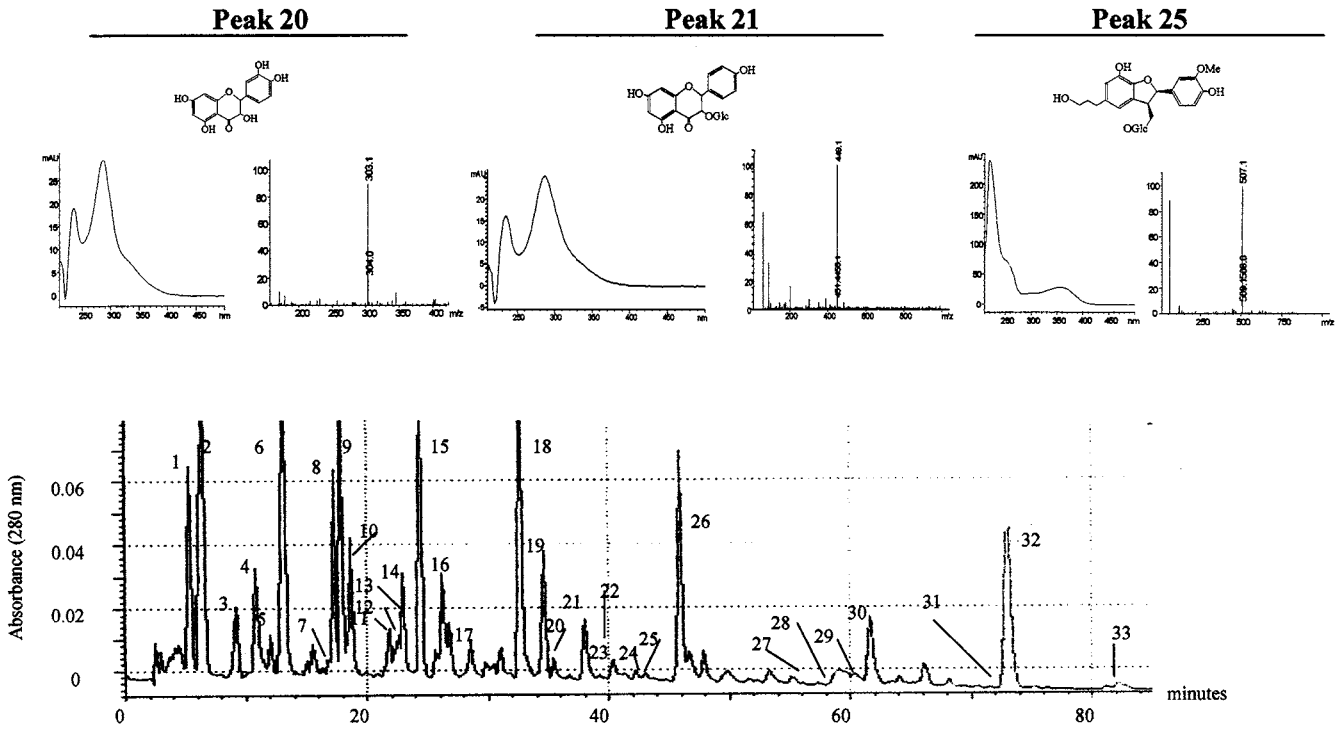

Figure 1. Chromatographic profile of the phenolic compounds determined in a wine produced with the Garnacha red grape variety. The retention times of compounds not detected in this wine are also indicated in the chromatogram. Chemical structures and UV–vis and MS spectra of peaks 20, 21, and 25 are also shown. For peak identification, see **Table 1**.

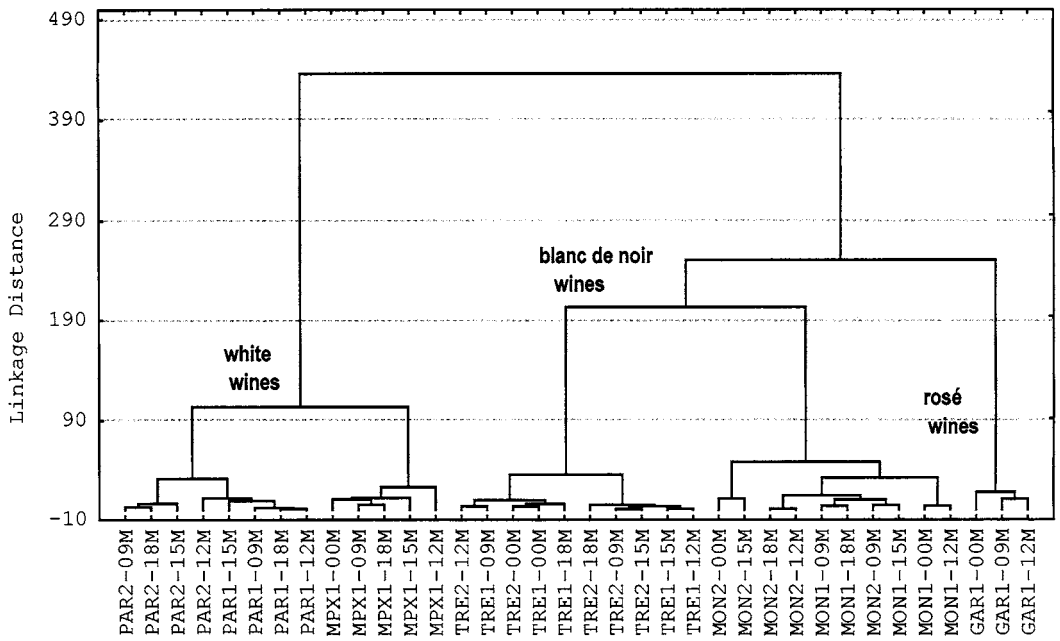

Figure 2. Dendrogram resulting from applying cluster analysis to the data corresponding to the concentration of phenolic compounds determined in the base wines and in the sparkling wines. Wines are identified by a code formed by the first letters of the variety (PAR = Parellada; MPX = Macabeo 58%, Parellada 36%, and Xarel.lo 6%; TRE = Trepat; MON = Monastrell; GAR = Garnacha) followed by the number of the wine (1 or 2) and the aging time (00, 09, 12, 15, and 18 months; 00 corresponds to the base wine).

wines were in turn grouped according to the grape variety. As can be observed in **Figure 2**, there was no grouping according to aging time with yeasts.

To test the effects of the type of wine and the aging time factors on the phenolic composition of the wines, two-way analysis of variance was carried out (the interaction and the within-error terms were pooled). This analysis was applied to the data of wines for which a sample was available with the same aging time, that is, the base wines and the sparkling wines manufactured from the Trepat and Monastrell varieties, and those obtained with mixtures of white varieties. The results of this analysis indicated that aging time with yeasts did not significantly influence the concentration of phenolic compounds. The phenolic compounds of the wines can mainly be modified by oxidation while in the bottle. In the case of sparkling wines, the atmosphere inside the bottle is highly reducing due to the high concentration of CO_2. This explains why this phenomenon does not occur and why the concentrations of the phenolic

Table 2. Mean ± Standard Deviation Values of the Phenolic Compounds Quantified in Base Wines and Sparkling Wines[a]

	white wine		blanc de noir wine		rosé wine
	Parellada ($n = 8$)	MPX[b] ($n = 5$)	Trepat ($n = 10$)	Monastrell ($n = 10$)	Garnacha ($n = 3$)
hydroxybenzoic acids					
gallic	0.29a ± 0.11	0.69b ± 0.09	0.57b ± 0.10	0.72b ± 0.28	1.30c ± 0.16
protocatechuic	0.50a ± 0.18	0.84ab ± 0.13	0.64ab ± 0.20	0.93b ± 0.38	0.58ab ± 0.16
p-hydroxybenzoic	0.14b ± 0.06	0.22c ± 0.04	0.16b ± 0.04	0.13b ± 0.03	0.02a ± 0.01
vanillic	0.00a ± 0.00	0.00a ± 0.00	0.43a ± 0.012	1.02b ± 0.62	0.48a ± 0.07
syringic	0.00a ± 0.00	0.00a ± 0.00	0.70b ± 0.05	0.84b ± 0.23	0.88b ± 0.10
hydroxycinnamic acids					
trans-caffeic	0.74b ± 0.18	1.21c ± 0.20	0.93bc ± 0.27	0.20a ± 0.13	3.00d ± 0.48
trans-*p*-coumaric	0.22b ± 0.06	0.24b ± 0.06	0.33c ± 0.06	0.13a ± 0.03	1.57d ± 0.20
cis-*p*-coumaric	0.27b ± 0.08	0.35b ± 0.11	0.35b ± 0.07	0.16a ± 0.06	0.52c ± 0.07
hydroxycinnamates					
cis-caftaric	1.57b ± 0.43	1.42b ± 0.60	0.16a ± 0.05	0.28a ± 0.15	0.39a ± 0.13
trans-caftaric	33.96d ± 5.54	20.25c ± 2.75	2.17b ± 0.72	2.57b ± 0.75	9.07b ± 1.99
cis-coutaric	5.02b ± 1.16	7.26c ± 1.21	1.79a ± 0.48	1.96a ± 0.43	1.86a ± 0.91
trans-coutaric	2.31c ± 0.55	2.63c ± 0.43	0.32a ± 0.06	0.59a ± 0.17	1.02b ± 0.56
trans-fertaric	0.31b ± 0.05	0.68c ± 0.26	0.10a ± 0.04	0.17ab ± 0.09	0.31b ± 0.17
stilbenes					
trans-resveratrol glucoside	0.22b ± 0.09	0.00a ± 0.00	0.34c ± 0.05	0.00a ± 0.00	0.00a ± 0.00
cis-resveratrol glucoside	0.23b ± 0.07	0.45c ± 0.08	0.52c ± 0.14	0.23b ± 0.04	0.00a ± 0.00
cis-resveratrol	0.10b ± 0.06	0.18cd ± 0.01	0.23d ± 0.06	0.00a ± 0.00	0.16c ± 0.07
flavonoids					
catechin	0.00a ± 0.00	0.00a ± 0.00	1.05b ± 0.33	0.90b ± 0.45	0.97b ± 0.84
other compounds					
tyrosol	10.72ab ± 2.35	9.54a ± 1.37	15.16b ± 3.18	15.51b ± 4.59	11.04ab ± 1.52
tryptophol	1.54b ± 0.48	0.24a ± 0.54	1.30b ± 0.48	0.14a ± 0.31	2.55c ± 1.13

[a] Mean values in the same row with the same letter indicate that there are no significant differences between them. [b] Wines made with mix of three varietal white wines: Macabeo (58%), Parellada (36%), and Xarel.lo (6%).

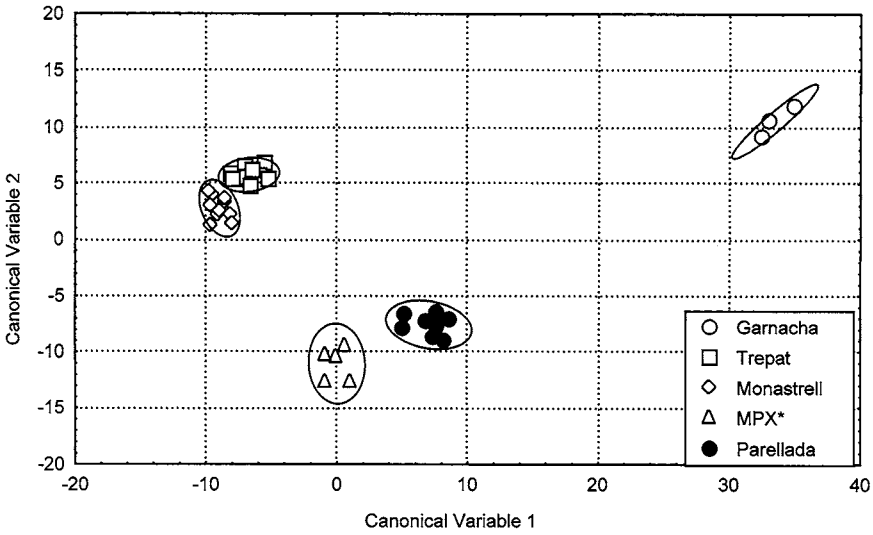

Figure 3. Representation of the 36 samples of wines on the plane defined by the first two canonical variables obtained with the nine phenolic compounds selected by stepwise discriminant analysis and 95% confidence ellipses. * Wines made with a base wine from Macabeo, Parellada, and Xarel.lo (58:36:6) varieties.

compounds do not vary greatly. On the other hand, in accordance with Ribéreau-Gayon et al. (*21*), the deposit of lees protects the wine by acting as an oxidation−reduction buffer.

To summarize the results obtained from the individual analyses of the phenolic compounds and because, as mentioned above, aging time has been shown not to influence the phenolic composition of sparkling wines, **Table 2** shows the average values ± the standard deviations of the data grouped according to the grape variety used in the preparation. The results of the application of the Student−Newman−Keuls test to compare the means for each variety are also included in the table.

As can be observed in **Table 2**, wines manufactured from the Parellada grape variety, in general, had a lower concentration of compounds belonging to the hydroxybenzoic acid group. Vanillic and syringic acids were detected only in the wines manufactured from red grape varieties. Although higher concentrations of these compounds tend to occur in the wines manufactured with red varieties, some authors also detected them in wines manufactured with white varieties. Servilli et al. (*22*), for example, detected vanillic acid in white wines manufactured from the Trebbiano and Moscato varieties, and Betés-Saura et al. (*23*) detected syringic acid in white wines manufactured from a mixture of the Macabeo, Xarel.lo, and Parellada varieties. However, Ibern-Gómez et al. (*11*) did not detect this compound when they analyzed base wines and sparkling wines manufactured with grapes of Macabeo, Xarel.lo, and Parellada varieties.

The rosé wine manufactured from the Garnacha variety contained a higher concentration of the hydroxycinnamic acid compounds, mainly of *trans*-caffeic acid. The concentrations of each of the cis or trans isomers of the *p*-coumaric acid were practically the same in wines belonging to the same variety, with the exception of those manufactured from the Garnacha grape variety. Nevertheless, Betés-Saura et al. (*23*) and Ibern-Gómez et al. (*11*) did not detect the cis isomer of the *p*-coumaric acid in the base wines or in sparkling wines manufactured from the white grape varieties Macabeo, Xarel.lo, and Parellada. The major differences between the concentrations of phenolic compounds manufactured with white and red grape varieties were in the hydroxycinnamate group. Wines manufactured with white grape varieties had a higher concentration than those of red varieties, as can be seen in **Table 2**. In general, the values of the stilbenes detected in the wines studied, both as the free forms and as the glucosylated forms, were within the concentration range determined by other authors in still wines manufactured from white grape varieties and in rosé (*24*−*27*). The highest concentration of stilbenes was found in wines manufactured from grapes of the Trepat variety. From **Table 2** it can be observed that the concentrations of other compounds such as tryptophol and tyrosol were similar in the wines manufactured from different varieties, except in wines manufactured from wine mixtures which had the lowest concentration of these two compounds.

To select the phenolic compounds most useful to differentiate the wines by variety, a stepwise discriminant analysis was used. Values of 4.0 and 3.9 were used for *F* statistics to enter and to remove variables, respectively. Nine phenolic compounds of those quantified (see **Table 2**) were selected: *trans*-*p*-coumaric and *trans*-caftaric acids, *trans*-resveratrol glucoside, *cis*-coutaric, *trans*-coutaric, *cis*-*p*-coumaric, and *cis*-caftaric acids, tryptophol, and syringic acid. A 100% correct assignment of the wines was obtained either by the standard or by leave-one-out cross-validation procedures with these selected compounds. **Figure 3** shows the wines on the plane defined by the first two canonical variables, obtained with the nine selected phenolic compounds. The population canonical ellipses for the five types of wines for 95% confidence are also represented in the figure. As can be observed, the blanc de noir wines elaborated with the Monastrell and Trepat red grape varieties were very close to each other and separated from the whites manufactured with the Parellada grape variety and the wines produced from the mixture of white grape varieties, which were also very close. The last group, which was very separate from the rest, corresponds to the rosé wines. From the factor structure matrix, the first canonical variable with 60.2% of variance extracted was positively correlated with *trans*-*p*-coumaric acid and permitted the rosé wines manufactured with the Garnacha variety to be separated from the rest. The second canonical variable with 21.7% of the variance extracted was negatively correlated with the *trans*-caftaric and *trans*-coutaric acids, which had high values in the wines produced with the Parellada variety and in the wines manufactured with mixtures of white varieties and low values in the wines produced from the Monastrell, Trepat, and Garnacha varieties. This second canonical variable was positively correlated with syringic acid, which had high values in wines produced with the Trepat, Monastrell, and Garnacha varieties and low values in white wines. Therefore, with this second canonical variable, the white wines could be separated from the rest. Canonical variable 3 with 15.4% of variance extracted was positively correlated with the *trans*-resveratrol glucoside and had high values in the Trepat and Parellada wines and low values in the remaining groups of wines.

In conclusion, we found that low molecular weight phenolic compounds of sparkling wines do not change significantly during aging with yeasts. The phenolic composition depends on the grape variety from which the wines were produced as well as the manufacturing technique, in white or in rosé.

ACKNOWLEDGMENT

We are grateful to Castellblanch S.A. (Sant Sadurní d'Anoia, Spain) for preparing the wines for the purpose of this research.

LITERATURE CITED

(1) Singleton, V. L.; Esau, P. Phenolic substances in grape and wine and their significance. *Adv. Food Res.* **1969**, *1*, 1−282.

(2) Fernández de Simón, B.; Pérez-Ilzarbe, J.; Hernández, T.; Gómez-Cordovés, C.; Estrella, I. Importance of phenolic compounds for the characterization of fruit juices. *J. Agric. Food Chem.* **1992**, *40*, 1531−1535.

(3) Nagel, C. W.; Wulf, L. W. Changes in the anthocyanins, flavonoids and hydroxycinnamic acid esters during fermentation and aging of Merlot and Cabernet Sauvignon. *Am. J. Enol. Vitic.* **1979**, *30*, 111−116.

(4) Presa-Owens, C.; Lamuela-Raventós, R. M.; Buxaderas, S.; Torre-Boronat, M. C. Differentiation and grouping characteristics of varietal grape musts from Penedes region. I. *Am. J. Enol. Vitic*. **1995**, *46*, 283−291.

(5) Presa-Owens, C.; Lamuela-Raventós, R. M.; Buxaderas, S.; Torre-Boronat, M. C. Characterization of Macabeo, Xarel.lo and Parellada white wines from the Penedés region. II. *Am. J. Enol. Vitic*. **1995**, *46*, 529−541.

(6) Singleton, V. L.; Trousdale, E. White wine phenolics: varietal and processing differences as shown by HPLC. *Am. J. Enol. Vitic*. **1983**, *34*, 27−34.

(7) Cadahía, E.; Muñoz, L.; Fernández de Simón, B.; García-Vallejo, C. Changes in low molecular weight phenolic compounds in Spanish, French, and American oak woods during natural seasoning and toasting. *J. Agric. Food Chem*. **2001**, *49*, 1790−1798.

(8) Martínez-Rodríguez, A.; Carrascosa, A. V.; Martín-Álvarez, P. J.; Moreno-Arribas, M. V.; Polo, M. C. Influence of the yeast strain on the changes of the amino acids, peptides and proteins during sparkling wine production by the traditional method. *J. Ind. Microbiol. Biotechnol*. **2002**, in press.

(9) Barón, R.; Mayén, M.; Mérida, J.; Medina, M. Changes in phenolic compounds and browning during biological aging of Sherry-type wine. *J. Agric. Food Chem*. **1997**, *45*, 1682−1685.

(10) Fabios, M.; López-Toledano, A.; Mayen, M.; Mérida, J.; Medina, M. Phenolic compounds and browing in Sherry wines subjected to oxidative and biological aging. *J. Agric. Food Chem*. **2000**, *48*, 2155−2159.

(11) Ibern-Gómez, M.; Andrés-Lacueva, C.; Lamuela-Raventós, R. M.; Buxaderas, S.; Singleton, V. L.; de la Torre-Boronat, M. C. Browning of cava (sparkling wine) during aging in contact with lees due to phenolic composition. *Am. J. Enol. Vitic*. **2000**, *51*, 29−36.

(12) Peña-Neira, A.; Hernández, T.; García-Vallejo, C.; Estrella, I.; Suárez, J. A. A survey of phenolic compounds in Spanish wines of different geographical origin. *Eur. Food Res. Technol*. **2000**, *210*, 445−448.

(13) Bengoechea, L.; Hernández, T.; Quesada, C.; Bartolomé, B.; Estrella, I.; Gómez-Cordovés, C. Structure of hydroxycinnamic acid derivatives established by high performance liquid chromatography with photodiode array detection. *Chromatographia* **1995**, *41*, 94−98.

(14) Bartolomé, B.; Bengoechea, M. L.; Gálvez, M. C.; Pérez-Ilzarbe, F. J.; Hernández, T.; Estrella, I.; Gómez-Cordovés, C. Photodiode array detection for elucidation of the structure of phenolic compounds. *J. Chromatogr. A* **1993**, *665*, 119−125.

(15) Möller, B.; Herrmann, K. Analysis of quinic acid ester of hydroxycinnamic acids in plant material by capillary gas chromatography and high-performance liquid chromatography. *J. Chromatogr. A* **1982**, *241*, 371−379.
(16) STATISTICA program for Windows release 5.1; Statsoft Inc., Tulsa, OK, 1998.
(17) SPSS program for Windows, release 11.01; SPSS Inc., Chicago, IL, 2001.
(18) Pérez-Magariño; Revilla, I.; González-SanJosé, M. L.; Beltrán, S. Various applications of liquid chromatography−mass spectrometry to the analysis of phenolic compounds. *J. Chromatogr. A* **1999**, *847*, 75−81.
(19) Somers, T. C.; Vérette, E.; Pocock, K. F. Hydroxycinnamate esters of *Vitis vinifera*: changes during white vinification, and effects of exogenous enzymic hydrolysis. *J. Sci. Food Agric.* **1987**, *40*, 67−78.
(20) Baderschneider, B.; Winterhalter, P. Isolation and characterization of novel benzoates, cinnamates, flavonoids, and lignans from Riesling wine and screening for antioxidant activity. *J. Agric. Food Chem.* **2001**, *49*, 2788−2798.
(21) Ribéreau-Gayon, P.; Glories, Y.; Maujean, A.; Dubourdieu, D. Other winemaking methods. In *Handbook of Enology. The Microbiology of Wine and Vinifications*; Wiley: Chichester, U.K., 2000; Vol. I, pp 129−185.
(22) Servili, M.; Di Stefano, G.; Piacquadio, P.; Sciancalepore, V. A novel method for removing phenols from grape must. *Am. J. Enol. Vitic.* **2000**, *51*, 357−361.
(23) Betés-Saura, C.; Andrés-Laueva, C.; Lamuela-Raventós, R. M. Phenolics in white free run juices and wines from Penedés by high-performance liquid chromatography: changes during vinification. *J. Agric. Food Chem.* **1996**, *44*, 3040−3046.
(24) Goldberg, D. M.; Yan, J.; Ng, E.; Diamandis, A.; Karumanchiri, A.; Soleas, G.; Waterhouse, A. L. A global survey of trans-resveratrol concentrations in commercial wines. *Am. J. Enol. Vitic.* **1995**, *46*, 156−165.
(25) Romero-Pérez, A. I.; Lamuela-Raventós, R. M.; Waterhouse, A. L.; de la Torre-Boronat, M. C. Levels of *cis*- and *trans*-resveratrol and their glucosides in white and rosé *Vitis vinifera* wines from Spain. *J. Agric. Food Chem.* **1996**, *44*, 2124−2128.
(26) Lamuela-Raventós, R. M.; Romero-Pérez, A. I.; Waterhouse, A. L.; de la Torre-Boronat, M. C. Direct HPLC analysis of *cis*- and *trans*-resveratrol and piceid isomers in spanish red *Vitis vinifera* wines. *J. Agric. Food Chem.* **1995**, *43*, 281−283.
(27) Romero-Pérez, A. I.; Lamuela-Raventós, R. M.; Buxaderas, S.; de la Torre-Boronat, M. C. Resveratrol and piceid as varietal markers of white wines. *J. Agric. Food Chem.* **1996**, *44*, 1975−1978.

Received for review October 7, 2002. Revised manuscript received January 16, 2003. Accepted January 18, 2003. This study was supported by the Spanish Comisión Interministerial de Ciencia y Tecnología (Project ALI97-0396-CO2-02).

JF021017Z