Phenolics in White Free Run Juices and Wines from Penedès by High-Performance Liquid Chromatography: Changes during Vinification

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A precise and sensitive HPLC method, with diode array detection, for phenolic compounds in white juices and wines is presented. Eighteen of these phenolics have been identified and quantitated in white free run juices and 19 in white wines. Identification of benzoic acids was confirmed by GC/MS. The only flavonol detected, quercetin 3-glucuronide, was identified by TLC and electrospray (ESI) techniques. Levels of the *trans*- and *cis*-hydroxycinnamic acids, flavonoids, and benzoic acids present in white juice and wine are reported. Hydroxycinnamates were the major phenols; among them, *trans*-caftaric, *trans*-coutaric, and *cis*-coutaric were the predominant. The *cis*-coutaric represented a substantial percentage in the total amount of phenols. Flavonoids were present at low concentration, and benzoic acids were the minor compounds. During white vinification, a significant decrease in the phenolic compounds present in free run juice was observed, mainly caused by the hydroxycinnamic acids.

Keywords: *Phenolics; HPLC; white free run juice; white wine; vinification*

INTRODUCTION

Phenols from white wine contribute to flavor (Baranowski and Nagel, 1981; Robichaud and Noble, 1990), and they can reflect grape variety, growing conditions, and winemaking techniques (Singleton and Trousdale, 1983). Their antioxidant properties may exert a positive health effect that is attributed to moderate wine consumption (Frankel et al., 1995). The hydroxycinnamates are major phenolic components, oxidation substrates, and browning precursors in white wines obtained from free run juices (Singleton et al., 1984; Cilliers and Singleton, 1989). These compounds increase, as do flavonoids and gallic acid, when there is pomace contact, during the winemaking process (Singleton and Trousdale, 1983).

HPLC is used for the analysis of the different groups of phenols, phenolic acids (Cartoni et al., 1991), hydroxycinnamates (Nagel et al., 1979; Singleton et al., 1984; Herrick and Nagel, 1985; Somers et al., 1987), and flavonoids (Lea, 1980; Alonso et al., 1986) in white juices or wines. Different procedures were applied when various groups of phenols were studied together: a fractionation step (Jaworski and Lee, 1987; Spanos and Wrolstad, 1990) or an extraction procedure (Oszimianski et al., 1986; Buïarelli et al., 1995). Some authors (Singleton and Trousdale, 1983; Frankel et al., 1995) have analyzed different groups of phenols in white juices or wines without any sample treatment, by direct injection in HPLC; however, the number of phenolic compounds that have been quantified is relatively low.

Here, the presence of 31 phenolic compounds was studied in white juices and wines, including phenolic acids, *trans*- and *cis*-hydroxycinnamic acids and derivatives, flavan-3-ols, flavonols, and the oligomeric procyanidins, by HPLC. Juices were analyzed by single direct run injection, and wine samples were concentrated to remove the ethanol present. The use of a diode array UV−vis detector (DAD) allowed the choice of the maximum absorbance for each group of compounds, the control of the peak purity, and moreover the identification by spectra of some phenols for which standards were not available. In addition, the identification of benzoic acids was achieved by GC/MS and of the flavonol quercetin 3-glucuronide by TLC and ESI.

This paper presents an HPLC method to profile the phenolic composition of white juices and wines. We establish the phenolic levels for the three varietal juices and wines (Macabeo, Xarel.lo, and Parellada) most widely planted in the Penedès area, using 18 juices and 31 wines produced at an industrial scale, each from 3 consecutive years. The phenolic composition changes during fermentation were also investigated.

MATERIALS AND METHODS

Juice. Eighteen samples from three Penedès varieties (Macabeo, Xarel.lo, and Parellada), from 3 years (1990, 1991, and 1992), coming from two different cellars, were all obtained from the free run juice of a pneumatic presses. Juices were treated with SO_2 (60−90 mg/L) and settled for 24 h before fermentation. Samples were centrifuged at 1800*g*, for 20 min, to eliminate large particles and filtered through a 0.45 μm poly(vinylidene) difluride (PVDF) membrane.

Wine. Thirty-one monovarietal white wines from Macabeo, Xarel.lo, and Parellada were collected from the same lots and cellars as the juices. The fermentations took place in stainless steel tanks (100 000 L, temperature 15−18 °C) where the yeast used was *Saccharomyces cerevisiae*. Juice and wine samples were stored at −18 °C in 250 mL bottles until analysis. Wine samples were centrifuged and filtered as were the juices, and then 5 mL of filtered sample was concentrated under vacuum to a volume of 3 mL, below 30 °C, and protected from light. They were concentrated to remove ethanol allowing greater sensitivity.

Standards. Phenolic standards gallic acid, protocatechuic acid, gentisic acid, vanillic acid, tyrosol, (+)-catechin, syringic acid, (−)-epicatechin, *trans*-caffeic acid, *trans*-*p*-coumaric acid, *trans*-ferulic, tryptophol, rutin, quercetin 3-glucoside, kaempferol 3-glucoside, isorhamnetin 3-glucoside, and quercetin were

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Table 1. Elution Program Used for the HPLC Analysis

time (min)	solvent A (%)	solvent B (%)
0	100	0
5	98	2
10	96	4
15	90	10
30	80	20
35	70	30
40	0	100
45	100	0

obtained from commercial sources. *trans*-Caftaric and *trans*-coutaric acids were provided by V. L. Singleton and A. L. Waterhouse, and procyanidins B1, B2, B4, and C2 by H. Peleg and A. C. Noble, all of them from the Departament of Viticulture and Enology, University of California, Davis. The *cis* isomers were obtained after the exposure of the *trans* standards to UV light (Engelsma, 1974; Singleton et al., 1978).

HPLC Analysis. A Hewlett-Packard (HP) 1050 gradient liquid chromatograph with a DAD 1040M coupled to a ChemStation HP 79995A was used. The column, a Nucleosil 120 C_{18} (250 mm × 4 mm), 5 μm particle size, was kept at 40 °C. Injection was by means of a Rheodyne injection valve (Model 7125) with a 100 μL fixed loop. Volume injected was 100 μL.

A constant flow rate of 1.5 mL/min was used with two solvents: solvent A, glacial acetic acid in water at pH 2.65; solvent B, 20% solvent A mixed with 80% acetonitrile. All the solvents used were of HPLC grade. The elution program is shown in Table 1.

Identification. The chromatogram was monitored simultaneously at three wavelengths: 280, 320, and 365 nm. Each wavelength was suitable for each group of compounds: 280 nm was used for benzoic acids, tyrosol, flavan-3-ols, and the oligomers procyanidins; 320 nm was used for cinnamic acids and their tartaric esters; and 365 nm was used for flavonols.

Isolation and Identification Techniques. *Extraction and Derivatization of Benzoic Acids.* A 3 mL SAX quaternary amine cartridge (Varian, Harbor City, CA) was conditioned by sequentially passing dropwise 3 mL each of methanol and distilled water; 5 mL of run juice sample was adjusted to pH 9.0 with 0.01 N NaOH and passed trough the cartridge to adsorb the phenolic acids. The adsorbed fraction was eluted with acidulated methanol. The eluted fraction was evaporated to dryness under vacuum, and then the dry extract was allowed to react with 1 mL of BSTFA in an incubator at 150 °C for 20 min.

Gas Chromatography/Mass Selectivity Detector Analysis of Benzoic Acids. They were identified by gas chromatography (HP5890 Series II) coupled with mass spectrometry (HP 5970). The derivatized extract (1 μL) was injected on to a splitless injector heated at 300 °C. The transfer line temperature was maintained at 290 °C. The GC oven program was initially set at 110 °C and increased at a rate of 8 °C/min to 310 °C. The column used was a glass capillary HP-5 column, 25 m long, 0.25 mm i.d., and 0.25 μm film thickness. Data were acquired on the scan mode (50−500 au) for gallic and protocatechuic acids and on selective ion monitoring (SIM) mode using ions 327 and 342 for syringic acid.

Isolation of Quercetin 3-Glucuronide. The juice sample was adsorbed on to a preconditioned 60 mL C-18 cartridge (Varian); this was eluted with water to clean the sample and then subsequently eluted with methanol, for the more apolar phenolic compounds. The eluted fraction was concentrated under vacuum to a final volume of 3.0 mL, at a temperature lower than 40 °C; 100 μL of this extract was injected in the analytical HPLC in the same conditions described before, at the wavelength of 365 nm, to collect the peak with $t_R = 31.8$ min. The recollection was repeated 10 times to have enough substance, and all the collected fractions were grouped and concentrated under vacuum to a final volume of 0.5 mL.

Thin Layer Chromatography (TLC) Analysis. TLC using activated (at 120 °C for 20 min) silica gel plates (60 F_{254}) was carried out for the concentrated methanolic fraction. Developing solvents were a mixture of ethyl acetate:formic acid:glacial acetic acid:water (100:11:11:27) (v:v:v:v). R_f values were compared with the standards of isoquercitrin and rutin. The plate was sprayed with 1% methanolic diphenylboric acid β-ethylamino ester followed by 5% ethanolic poly(ethylene glycol) 4000 (PEG) (5 and 6 mL, respectively). The spots were examined under UV at 365 nm.

For electrospray ion identification (ESI), a VG Quattro instrument was used in the negative mode; the cone voltage was 55 V. The flow rate of nebulizer nitrogen was 10 L/h and of drying nitrogen was 450 L/h. The source temperature was 80 °C. The carrier solution was methanol with a flow rate of 5 μL/min. The injection loop was 10 μL.

Statistical Treatment. The STATGRAPHICS 7.0. program was used to calculate the average and the confidence interval (95% CI) of each compound, either juices or wines (n = 18 for juices, and 31 for wines). Moreover, the significance of phenolic variations between juices and wines was evaluated by the Student's t-test ($p < 0.05$).

Table 2. Retention Time and Identification of the Phenolic Compounds

compound	t_R (min)	free run juice[a]	wine
gallic acid	4.2	+	+
cis-caftaric acid	7.1	+	+
protocatechuic acid	7.4	+	+
trans-caftaric acid	8.7	+	+
gentisic acid	9.9	−	−
2-*S*-glutathionylcaftaric acid	11.4	+	+
cis-coutaric acid	11.8	+	+
tyrosol	12.4	−	+
trans-coutaric acid	12.8	+	+
procyanidin B3	15.4	+	+
procyanidin C2	16.1	−	−
(+)-catechin	16.3	+	+
cis-caffeic acid	16.5	+	+
vanillic acid	17.0	−	−
trans-caffeic acid	17.7	+	+
syringic acid	19.2	+	+
procyanidin B2	19.6	+	+[b]
procyanidin B4	19.8	−	−
fertaric acid	20.0	+	+
(−)-epicatechin	21.6	+	+
cis-coumaric acid	22.0	−	−
trans-coumaric acid	22.9	+	+
cis-ferulic acid	25.2	−	−
trans-ferulic acid	26.1	+[c]	+
tryptophol	28.5	−	−
quercetin 3-glucuronide	31.8	+	+
rutin	31.9	−	−
isoquercetin	32.3	−	−
kaempferol 3-glucoside	34.9	−	−
isorhamnetin 3-glucoside	35.6	−	−
quercetin	38.5	−	−

[a] −, not detected; +, detected. [b] Detected in 64% of the samples. [c] Detected in 94% of the samples.

RESULTS AND DISCUSSION

HPLC Identification. Thirty-one phenolic compounds were assessed for their presence in two kinds of samples: juices and wines (Table 2). The identification of the peaks was carried out by their spectra, by their retention time in comparison with standards, and by the method of standard addition to the samples. However, those hydroxycinnamic acids for which standards were not available, 2-*S*-glutathionylcaftaric and *trans*-fertaric acids, were identified using their spectra and retention time, as described by other authors (Baranowski and Nagel, 1981; Spanos and Wrolstad, 1990).

Of the 31 phenolic compounds considered, 18 were identified in juices and 19 in wines (see Table 2). This numerical difference between juice and wine is caused by the tyrosol, which is produced during fermentation by the deamination of the amino acid tyrosine (Sapis and Ribéreau-Gayon, 1969; Singleton and Trousdale, 1983). Some phenolic compounds, which have been identified by other authors in white grape juice or wine,

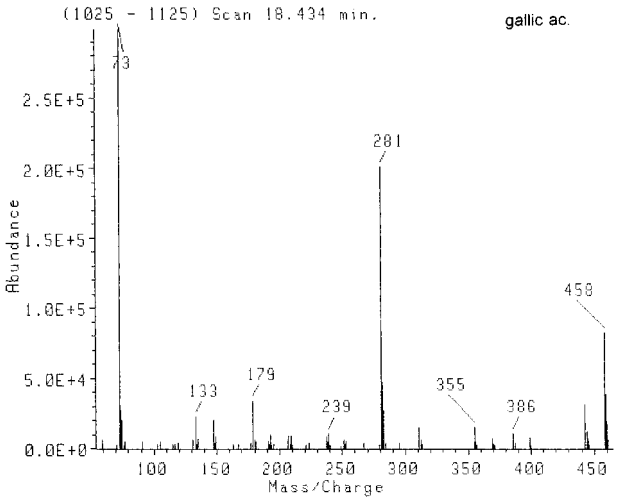

Figure 1. Mass ion spectrum of gallic acid.

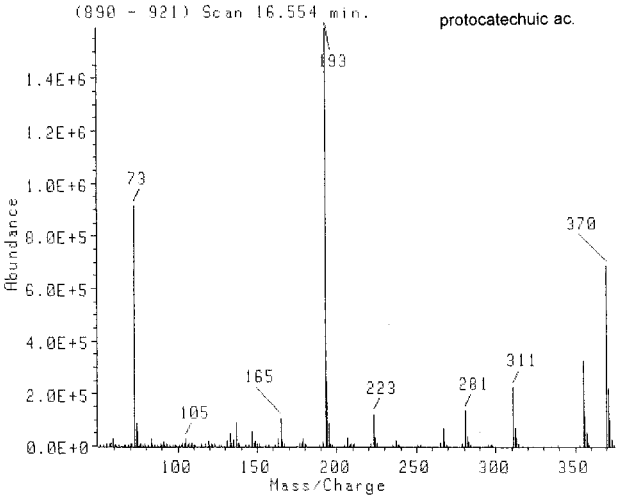

Figure 2. Mass ion spectrum of protocatechuic acid.

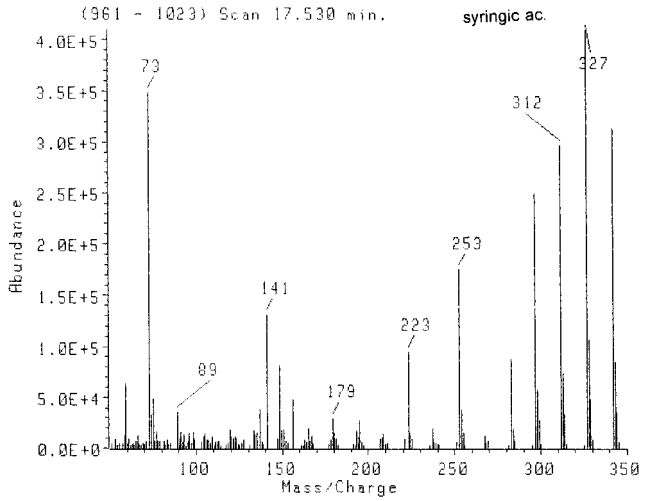

Figure 3. Mass ion spectrum of syringic acid.

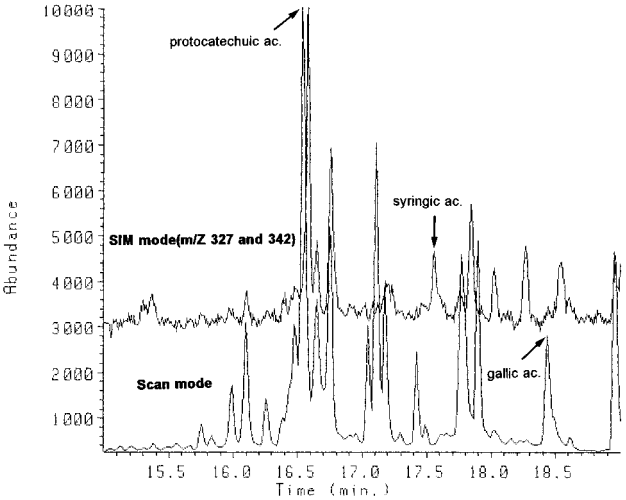

Figure 4. GC chromatogram of the derivatized extract on scan mode for gallic and protocatechuic acids and on SIM mode using m/z 327 and 342 for syringic acid.

such as gentisic acid (Ribereau-Gayon et al., 1972), procyanidin C2 (Spanos and Wrolstad, 1990), vanillic acid (Macheix et al., 1990), procyanidin B4 (Oszmianski et al., 1986; Spanos and Wrolstad, 1990), traces of tryptophol in some white wines (Sapis and Ribéreau-Gayon, 1969), rutin (Alonso and Estrella, 1986), and quercetin (Ribéreau-Gayon et al., 1972; Spanos and Wrolstad, 1990), were not detected in any of the 49 samples analyzed (see Table 2).

Cis forms are formed for the *trans* isomerization under UV light exposition. *cis*-Caftaric, *cis*-caffeic, and *cis*-coutaric were identified in juice and wines; however, *cis*-coumaric and *cis*-ferulic were not detected.

Benzoic acids were identified by GC/MS, since protocatechuic and syringic acids, as far as we know, were not described previously in white wines. The mass spectrum obtained from the GC/MS analysis of the pure standards revealed two fragments suitable to identify the presence of each compound: gallic acid (Figure 1), $m/z = 281$ and 458, protocatechuic acid (Figure 2), $m/z = 193$ and 370, and syringic acid (Figure 3), $m/z = 327$ and 342. Gallic and protocatechuic acids were identified on the scan mode (see Figure 4). Syringic acid was detected when selecting the ion mass at $m/z = 327$ and 342 (see Figure 4) in juice extract. Retention times of the three benzoic acids were consistent with the pure standards.

Cheynier and Rigaud (1986) observed that the major peak of the flavonols present in red grape skins is quercetin 3-glucuronide; however, this compound has similar polarity and the same spectrum as rutin, so it can be easily identified as rutin (Alonso and Estrella, 1986; Lamuela-Raventós and Waterhouse, 1994; Goldberg et al., 1996). For this reason, the identity of this compound was verified by TLC and electrospray techniques. The TLC of the concentrated juice showed an intense orange zone ($R_f = 0.53$) with the same R_f described for quercetin 3-glucuronide (Wagner et al., 1984) between rutin ($R_f = 0.4$) and isoquercitrin ($R_f = 0.7$), which were not present in the samples. For electrospray, an aliquot of the isolated fraction (10 μL) was injected into the electrospray. The result obtained (see Figure 5) confirmed the presence of quercetin 3-glucuronide (MW = 478).

Quantitation. The external standard method was used to measure the concentrations. Three calibration curves (standard area vs concentration in mg/L) were performed for each compound over the range of concentrations observed. The *cis* and *trans* isomers of the hydoxycinnamic tartaric esters of caffeic acid and *trans*-fertaric acid were quantified as their *trans* free cinnamic form, *trans*-caffeic acid, and *trans*-ferulic acid, respectively; *trans*-caftaric was quantitated as *trans*-caffeic since the standard was not totally pure, and for *cis*-coutaric, the *trans*-coutaric curve was used. Procyanidins were quantified as (+)-catechin equivalents, and quercetin 3-glucuronide was quantified as quercetin.

Method Validation. The parameters considerated for the validation were selectivity (specificity), precision,

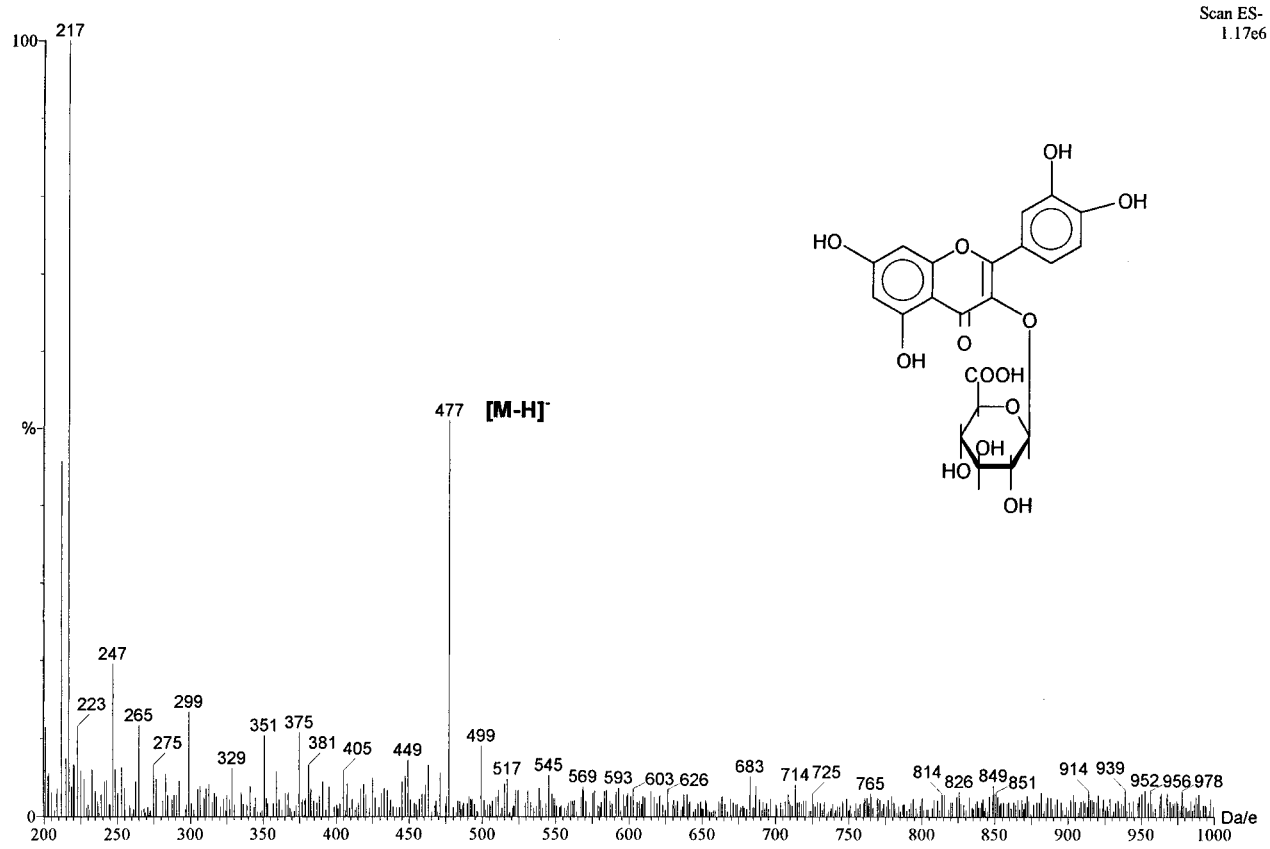

Figure 5. Negative ion mass spectra of the collected fraction. $[M - H]^- = 477$ corresponding to quercetin 3-glucuronide.

linearity, and sensitivity (limit of detection, LD; limit of quantification, LQ).

Selectivity. To improve the selectivity, each group of compounds was quantified at their maximum absorbance (280, 320, and 365 nm). An example of the chromatogram at each wavelength is shown in Figure 6 where good resolution is observed. The use of DAD revealed peak purity; all the peaks quantified had a good peak purity level, except for two compounds: *cis*-caffeic and epicatechin. The *cis*-caffeic acid peak contained two different spectra, the main spectrum belonging to *cis*-caffeic; however, the second spectrum was similar to those described by Spanos and Wrolstad (1990) and Cilliers and Singleton (1991) as oxidized hydroxycinnamic acid. Epicatechin, in some wine samples, had two different spectra.

Precision. This was studied in two ways (see Table 3): precision in retention times and precision in peaks areas. Six aliquots of the same sample were analyzed on 6 days; the values are expressed by the coefficient of variation (CV). The CV for retention time of all peaks was <0.54%. The precision in peaks areas was measured using the concentrations found in juice and wine samples. The variations were lower than those acceptable according to the CV established by Horwitz for intralaboratory analysis (Horwitz, 1982).

Linearity. Linearity of the standard curves (see Table 3) was studied for those standards available, except for the procyanidins and *trans*-caftaric acid, the purity of which was not known. Three-point standard curves were generated for each of the phenols. The linearity is expressed in terms of the correlation coefficient (r), from plots of the integrated peak area vs concentration of the standard; $r > 0.9990$, except for the *trans*-coutaric acid, which was 0.998.

Sensitivity. The limit of detection (LD) and limit of quantitation (LQ) (Table 3) were calculated according to the method approved by the American Chemical Society (1980) by running 10 blanks using the maximum sensitivity allowed by the integration system. The LD for the phenol varied from 0.003 mg/L for *cis*-caftaric acid to 0.051 mg/L for tyrosol and the LQ from 0.006 mg/L for *cis*-caftaric acid to 0.127 mg/L for tyrosol.

Phenols in Juices. The hydroxycinnamates were the major phenols (79.71% of the total phenol quantified), as reported previously (Singleton and Trousdale, 1983; Lee and Jaworski,1987). *trans*-Caftaric acid was found at highest level (see Table 4), ranging from 14.87 to 19.58 mg/L, similar to those values reported in Thompson Seedless juice (14.0 mg/L) (Spanos and Wrosland, 1990). However, higher levels were observed in some other varietal juices: 181 mg/L in juices from grapes grown in New York state (Lee and Jaworski, 1987) and 79.0 mg/L for Chardonnay, 110.8 mg/L for White Riesling, and 29.9 mg/L for Gewürztraminer in musts from Washington state (Nagel et al., 1979); Singleton et al. (1986) found an average of 127 mg/L in grape juices from California.

trans-Coutaric acid was in the second highest concentration, and its levels ranged from 9.75 to 12.4 mg/L. Curiously, the large variability noticed in the *trans*-caftaric acid levels were not observed for *trans*-coutaric acid; Lee and Jaworski (1987) found 10 mg/L in the juices from white grape grown in New York, and Nagel et al. (1979) found 20.5 mg/L for Chardonnay, 19.3 mg/L for White Riesling, and 8.6 mg/L for Gewürztraminer. Singleton et al. (1986) found an average of 14 mg/L when they analyzed 23 grape juices of different varieties.

Instead of *cis*-caftaric acid, the next plentiful phenolic was *cis*-coutaric acid, averaging 10.05 mg/L. Singleton et al. (1986) and Lee and Jaworski (1987) also noted that *trans*-caftaric acid, *trans*-coutaric acid, and *cis*-coutaric acid were the major hydroxycinnamic acids.

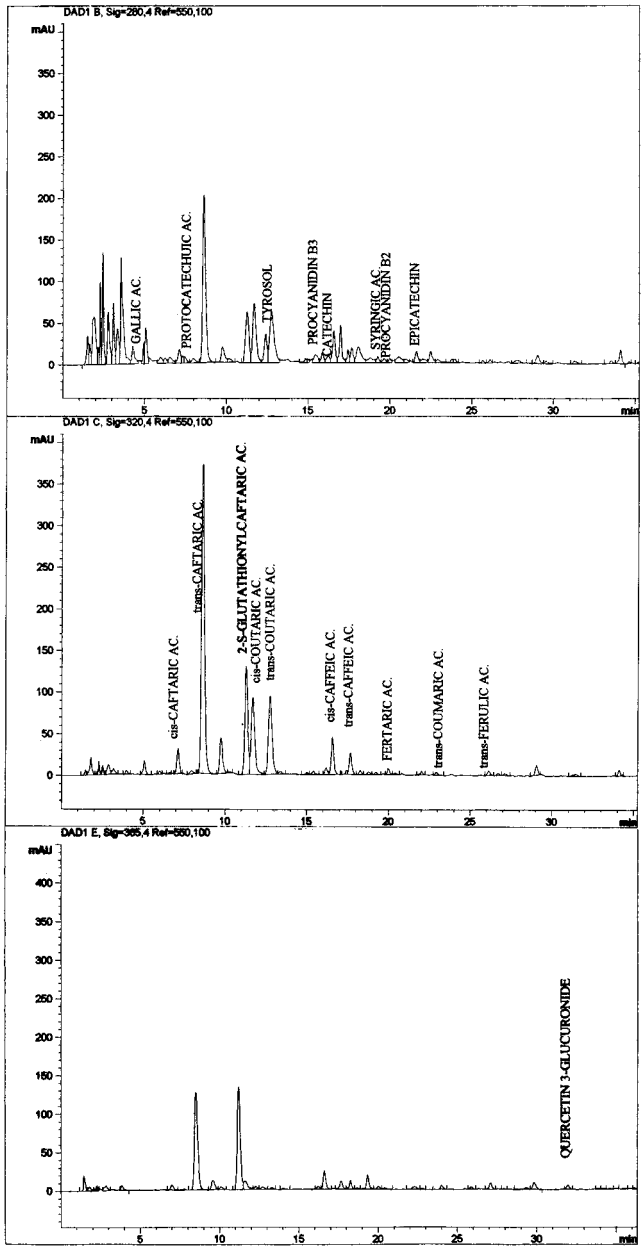

Figure 6. Chromatogram of a white wine at 280, 320, and 365 nm.

trans-Coutaric acid seems to be more sensitive to isomerization to the cis form than *trans*-caftaric acid.

2-*S*-Glutathionylcaftaric acid (a product of the caftaric acid oxidation) was present at similar levels (from 4.97−7.16 mg/L) as those described in other varieties, such as Thompson Seedless, in which reported levels are 6.0−7.8 mg/L (Spanos and Wrolstad, 1990); however, its presence depends on the glutathione amount of the grapes (Cheyner et al., 1989).

The isomers *cis* and *trans* of caffeic acid were the free cinnamic acids present in juice at larger concentration, since they are formed from the hydrolysis of the tartrate ester, *trans*-caftaric, that is the major phenol.

trans-Fertaric acid (average 0.31 mg/L), *trans*-coumaric acid (average 0.25 mg/L), and *trans*-ferulic acid (average 0.12 mg/L) were the phenolic acid compounds present at the lowest concentrations. These three *trans*-cinnamic acids are precursors of volatile phenols, important to the formation of unpleasant off-flavors in wine (Dugelay et al., 1993). Moreover, *trans*-ferulic acid levels were lower than the LQ in some Macabeo juices.

Flavonoids analyzed were present at low concentrations; only 15.85% of the total phenols quantified were flavonoids because these samples were obtained from free run juice. Juices were richer in (+)-catechin (3.69 mg/L) than in (−)-epicatechin (1.19 mg/L). Quercetin 3-glucuronide was the only flavonol detected, at an average level of 0.5 mg/L. The dimers, procyanidin B3 and B2, were also present at low concentration: 2.41 and 1.31 mg/L, respectively.

Finally, benzoic acids were the least abundant class: only 4.43% of the phenols observed in this study. The major one was gallic acid (1.09 mg/L), with similar levels to those reported by Spanos and Wrolstad (1990) for white grape juice. Protocatechuic and syringic acid levels were lower than 1 mg/L in all the samples analyzed. Protocatechuic varied from 0.41 to 0.82 mg/L, and syringic acid ranged from 0.40 to 0.61 mg/L.

Wines. Vinification resulted in a marked decrease in the phenolic content: 20.73% (tyrosol was not considered in the total amount). Almost all the phenolic compounds studied varied significantly ($p < 0.001$), except for the following phenolic acids: gallic acid, syringic acid, and *trans*-ferulic acid and procyanidin B2, in which differences between free run juice and wine were not significant.

Considering the three groups of phenols (hydroxycinnamic acids, flavonoids, and benzoic acids), the main variation was observed in the hydroxycinnamic acid content (averaging 46.76 mg/L in juice and 34.07 mg/L in wines); approximately 27% of the hydroxycinnamates were lost in the vinification process. Nevertheless, the dominant compounds in wines were also the hydroxycinnamates (73.26% of the total phenols analyzed in wines). As in juice, the major phenol was *trans*-caftaric acid (see Table 4), with levels ranging from 10.54 to 13.36 mg/L. The large difference observed for *trans*-caftaric among free run juices, from grapes grown in different regions, was not maintained in wines, except for White Riesling wines, with 72.7 mg/L (Nagel et al., 1979).

Penedès wines had higher levels of *trans*-coutaric (ca. 8.57 mg/L) compared to other varietal wines obtained from grapes grown in the Pacific Coast, with an average level of 2.9 mg/L (Nagel et al., 1979; Singleton and Trousdale, 1983). *trans*-Fertaric acid was the hydroxycinnamic ester present at the lowest concentration (0.15 mg/L), much lower levels than those observed by Nagel et al. (1979) in Chardonnay (3.0 mg/L) and White Riesling (11.7) wines, who did not observe any present in Gewürztraminer wines.

The levels of the *cis*-hydroxycinnamates present in white wines ranged from 0.59 to 0.71 mg/L for *cis*-caftaric and from 7.16 to 8.26 mg/L for *cis*-coutaric. The quantitation of the latter compound represents aproximately the 15% of the total wine phenolic content measured here. The hydroxycinnamate tartaric acid esters may have been hydrolyzed in the fermentation process, giving as a result free cinnamic acids, which can be also oxidized (Cilliers and Singleton, 1989). They can be transformed into volatile phenols by decarboxylase activity provided by yeast (Chatonnet et al., 1993; Dugelay et al., 1993) or presumably adsorbed by interaction with yeasts (Somers et al., 1987). However, the only cinnamic acid that increased in the fermentation was *trans*-caffeic acid (ca. 64% of an increase), likely due to this hydrolysis by yeast esterase, since caftaric acid decreased. The second most abundant phenolic compound was a noncarboxylic phenol, tyrosol, not present in grapes or juices, which is a result of tyrosine

Table 3. Precision, Linearity, and Sensitivity (LD, LQ) of the HPLC Method

		precision, CV (%) ($n = 6$)			linearity ($n = 6$)		sensitivity (mg/L) ($n = 10$)	
compound	t_R (min)	t_R	juice	wine	range (mg/L)	r	LD	LQ
gallic acid	4.2	0.50	4.17	2.45	0.52−10.51	0.999	0.018	0.052
cis-caftaric acid	7.1	0.50	3.32	1.19	0.55−10.88	*a*	0.003	0.006
protocatechuic acid	7.4	0.41	3.24	3.99	0.25−8.10	0.999	0.015	0.038
trans-caftaric acid	8.7	0.35	0.45	1.26	0.27−21.76	*a*	0.004	0.010
2-*S*-glutathionylcaftaric acid	11.4	0.30	0.93	6.96	0.27−21.76	*b*	0.005	0.013
cis-coutaric acid	11.8	0.28	1.25	1.68	5.25−52.50	*a*	0.020	0.055
tyrosol	12.4	0.30		2.01	2.32−46.40	0.999	0.051	0.127
trans-coutaric acid	12.8	0.23	1.86	1.60	5.25−52.50	0.998	0.011	0.029
procyanidin-B3	15.4	0.50	8.16	9.59	0.60−11.98	*a*	0.034	0.083
(+)-catechin	16.3	0.16	6.10	5.06	0.60−11.98	0.999	0.035	0.086
cis-caffeic acid	16.5	0.14	2.68	2.87	0.27−21.76	*a*	0.005	0.011
trans-caffeic acid	17.7	0.18	3.28	3.95	0.27−21.76	0.999	0.004	0.010
syringic acid	19.2	0.25	4.49	7.95	0.25−7.96	0.999	0.009	0.023
procyanidin-B2	19.6	0.54	3.99	1.36	0.60−11.98	*a*	0.035	0.087
fertaric acid	20.0	0.41	2.10	1.80	0.04−4.58	*b*	0.008	0.022
(−)-epicatechin	21.6	0.11	6.20	3.85	0.51−16.42	0.999	0.035	0.087
trans-coumaric acid	22.9	0.15	3.90	4.01	0.14−4.43	0.999	0.005	0.012
trans-ferulic acid	26.1	0.12	2.81	13.49	0.04−4.58	0.999	0.005	0.013
quercetin 3-glucuronide	31.8	0.16	0.26	2.50	0.05−1.51	0.999	0.013	0.035

[a] The amount of standard is unknown. [b] There was no standard available.

Table 4. Average and Confidence Interval of Phenolic Levels in White Free Run Juice and Wine

	average (mg/L)		confidence interval (mg/L) (mean: 95%)	
compound	free run juice ($n = 18$)	wine ($n = 31$)	free run juice ($n = 18$)	wine(mg/L) ($n = 31$)
gallic acid	1.09	0.95	0.66−1.53	0.87−1.04
cis-caftaric acid	1.00	0.65	0.90−1.09	0.59−0.71
protocatechuic acid	0.62	1.23	0.41−0.82	1.13−1.33
trans-caftaric acid	17.23	11.95	14.87−19.58	10.54−13.36
2-*S*-glutathionylcaftaric acid	6.07	3.17	4.97−7.16	2.86−3.49
cis-coutaric acid	10.05	7.71	9.22−10.87	7.16−8.26
tyrosol		11.63		10.56−12.70
trans-coutaric acid	11.08	8.57	9.75−12.4	7.95−9.18
procyanidin-B3	2.41	1.16	1.90−2.91	0.92−1.41
(+)-catechin	3.69	2.49	3.03−4.36	2.24−2.73
cis-caffeic acid	1.29	1.00	1.18−1.39	0.93−1.07
trans-caffeic acid	0.39	0.61	0.30−0.48	0.55−0.67
syringic acid	0.51	0.43	0.40−0.61	0.37−0.49
procyanidin-B2	1.31	1.39	1.01−1.61	0.82−1.96
fertaric acid	0.31	0.15	0.21−0.39	0.13−0.17
(-)-epicatechin	1.19	4.14	0.85−1.52	3.60−4.69
trans-coumaric acid	0.25	0.15	0.19−0.30	0.09−0.21
trans-ferulic acid	0.12	0.11	0.08−0.15	0.09−0.13
quercetin 3-glucuronide	0.50	0.25	0.35−0.64	0.20−0.31
total phenolics	58.66	46.50[a]		
total hydroxycinnamics	46.76	34.07		
total flavonoids	7.91	9.43		
total benzoic acids	2.60	2.61		

[a] Data obtained without tyrosol value.

transformation in the juice fermentation (Singleton and Trousdale, 1983).

The low flavonoid levels confirm that these white wines were made from the free run juice. The average level of catechin (2.49 mg/L) was about 60% of the epicatechin level (4.14 mg/L). Singleton and Trousdale (1983) and Frankel et al. (1995) describe higher levels for these two flavonoids in white wine. The epicatechin level was higher in wines than in free run juice, probably from hydrolysis of epicatechin gallate (Singleton and Trousdale, 1983); however, gallic acid did not increase. Querectin 3-glucuronide was present in these wines at a low level (0.25 mg/L). Procyanidins B3 and B2 were both present in wine; procyanidin B3 had suffered a significant decrease during vinification, aproximately 50%, but the procyanidin B2 level was in average the same as in the must.

The benzoic acid levels were the same as in juice: 2.61% of the total phenol in wine. Protocatechuic acid was the major benzoic acid (1.23 mg/L) followed by gallic acid (0.95 mg/L) and, finally, syringic acid (0.43 mg/L).

CONCLUSIONS

The presence of 31 phenolic compounds was studied in 18 white juices and 31 wines produced at industrial scale. For this, an HPLC method that allows the separation of 31 phenolic compounds is presented. The method was validated considering precision in t_R and peak areas, linearity, and sensitivity. Eighteen of these have been identified and quantitated in white free run juices and 19 in white wine. Identification of benzoic acids was confirmed by GC/MS. The only flavonol detected, quercetin 3-glucuronide, was identified by TLC and electrospray techniques. Levels of the *trans*- and *cis*-hydroxycinnamic acids, flavonoids, and benzoic acids present in white juice and wine are reported. Hydroxycinnamates were the major phenols; among them, *trans*-caftaric, *trans*-coutaric, and *cis*-coutaric were the predominant. The *cis*-coutaric represented a substantial percentage in the total amount of phenols. Flavonoids were present at low concentration, and benzoic acids were the minor compounds.

Vinification resulted in a significant drop of almost all the phenolic compounds observed in free run juices. The main variation was observed in the hydroxycinnamic acid content.

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