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# Phenolic Composition of Champagnes from Chardonnay and Pinot Noir Vintages

MOHAMED CHAMKHA,† BERNARD CATHALA,† VÉRONIQUE CHEYNIER,‡ AND ROGER DOUILLARD†

UMR INRA/URCA Fractionnement des Agro-Ressources et Emballage, CRA, 2 Espl. R. Garros, BP 224, 51686 Reims Cedex 2, France, and UMR Sciences pour l'Oenologie, 2 Place Viala, 34060 Montpellier, France

Nineteen phenolic compounds including hydroxybenzoic acids, hydroxycinnamic acids, flavonoids, phenolic alcohols, and phenolic aldehydes have been identified and quantified in two monovarietal champagnes, Chardonnay and Pinot Noir, by using a reverse-phase high-performance liquid chromatography (HPLC) system coupled with diode array detection. The identification of four hydroxycinnamic tartaric esters (caftaric, coutaric, fertaric, and 2-*S*-glutathionylcaftaric acids), two flavanonols (astilbin and engeletin), and some other compounds was confirmed by HPLC coupled with mass spectrometry. Caftaric acid and tyrosol were the major phenols. Hydroxybenzoic acids and flavonoids were present at low concentrations. The phenolic compositions of 2000 and 2001 Chardonnay and Pinot Noir vary quantitatively according to the year and the variety, but the chemical natures of the molecules are the same. The total phenolic content determined by colorimetric measurement ranges from 176 to 195 mg/L of gallic acid equivalent and is similar to that described in white wines.

**KEYWORDS: Phenolics; Champagne; Chardonnay; Pinot Noir; HPLC analysis**

## INTRODUCTION

The phenolic compounds of wine are very important, as they contribute to sensory characteristics, particularly color (*1*), astringency, and bitterness (*2*), and as they are also involved in biochemical and pharmacological effects, including antimicrobial, anticarcinogenic, and antioxidant properties (*3*−*5*). The phenolic composition varies with a wide range of factors, including species, variety, season, growing conditions, and processing practices (*6*, *7*).

Most studies on phenolic composition were focused on red wine, and less information is available on white wine. Many studies reported the phenolic composition of Spanish "cavas", a kind of natural sparkling wines very similar to the champagnes, also obtained by the "champenois" method (*8*, *9*); but there is little information in the literature on phenolic compounds in champagnes. Only one publication concerning the estimation of must oxidation during pressing in champagne was reported (*10*). The major phenolics present in white wine are the hydroxycinnamic acids and, especially, caftaric acid. The total phenolic content ranges from 50 to 350 mg/L of gallic acid equivalent in white wines and is lower than that described in red wines (800 mg/L to 4 g/L) (*11*).

Champagnes are made using three grape varieties: Chardonnay, Pinot Noir, and Pinot Meunier. A "Blanc de Blancs" champagne is made by using only the Chardonnay grape and it is characterized by its finesse. A "Blanc de Noirs" champagne uses Pinot Noir and/or Pinot Meunier grapes, and it is characterized by power and/or fruitiness, sometimes both together. This paper presents a high-performance liquid chromatography (HPLC) method used to determine some phenolic compounds occurring in champagne and involving no sample pretreatment. The identification of four hydroxycinnamic tartaric esters and two flavanonols was confirmed by HPLC coupled with mass spectrometry. It permits the evaluation of the phenolic contents of two monovarietal champagnes (Chardonnay and Pinot Noir) widely planted in the Champagne region, each for two consecutive years (2000 and 2001).

## MATERIALS AND METHODS

**Champagne Samples.** Bottles of monovarietal champagnes prepared from both Pinot Noir and Chardonnay varieties and from 2 vintages (2000 and 2001) were obtained from the Comité Interprofessionnel du Vin de Champagne (CIVC), Epernay, France. For determination of phenolics, samples of champagne were filtered through a Millipore 0.45-$\mu$m filter (type HA).

**Standards.** Phenolic standards tested included: benzoic acids (gallic, protocatechuic, *p*-hydroxybenzoic, gentisic, vanillic, and syringic acids), cinnamic acids (caffeic, *p*-coumaric, ferulic, and sinapic acids), flavonoids ((+)-catechin, (−)-epicatechin, and quercetin), phenolic alcohols (vanillic alcohol, guaiacol, tryptophol, *trans*-resveratrol, and

* To whom correspondence should be addressed. E-mail: douillar@reims.inra.fr.
† UMR INRA/URCA Fractionnement des Agro-Ressources et Emballage.
‡ UMR Sciences pour l'Oenologie.

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tyrosol), *p*-hydroxybenzaldehyde, vanillin, and 3,4-dihydroxyphenylacetic acid. These phenolic compounds were obtained from commercial sources (Sigma-Aldrich and Fluka).

**HPLC Analysis.** A high-pressure liquid chromatography apparatus (Waters, model 2690) equipped with a diode array detector (Waters, model 996) was used. Separation of phenolics was carried out on a C18 Symmetry column 4.6 × 150 mm, 5 μm particle size (Waters Chromatography) at 30 °C. The volume injected was 10 μL. A constant flow rate of 1.2 mL/min was used with two solvents: solvent A, 0.7% glacial acetic acid in water; solvent B, 20% solvent A mixed with 80% acetonitrile. All the solvents used were of HPLC grade. For the elution program, the following proportions of solvent B were used: 0−5 min, 2%; 5−10 min, 6%; 10−15 min, 12%; 15−30 min, 22%; 30−35 min, 34%; 35−40 min, 100%; and 40−45 min, 0%. The mean and the standard deviation were calculated from three independent injections. The chromatograms were monitored between 220 and 400 nm and routinely checked at three wavelengths: 280, 320, and 365 nm (*12*).

**Concentration of the Phenolic Compounds.** A 50 mL volume of champagne sample was concentrated under vacuum to 23 mL at a temperature below 30 °C to remove ethanol. This solution was passed through a Sep-Pack C18 cartridge (Waters) which had previously been washed with 25 mL of 2% (v/v) formic acid in water (pH 3) and subsequently with 50 mL of water and 25 mL of methanol, then the cartridge was washed with 10 mL of 2% (v/v) formic acid in water (pH 3). The phenolics were recovered by elution with 10 mL of methanol. The solvent was evaporated (below 30 °C), and the residue was dissolved in 630 μL of methanol. The concentrated fraction was used for analyses by HPLC coupled with mass spectrometry.

**HPLC/ESI-MS.** Chromatographic separation was achieved on a C18 Symmetry column 4.6 × 150 mm, 5 μm particle size (Waters Chromatography) at 30 °C. The solvent system used was a gradient of solvent A (formic acid, 0.2% v/v in water) and solvent B (acetonitrile, solvent A, 80/20, v/v). The elution conditions were as follows: flow rate 1.2 mL/min, temperature 30 °C, volume injection 50 μL, gradient as described in HPLC analysis. Split was done with 250 μL/min going through the mass detector and 950 μL/min into the diode array detector. Negative-ion mode electrospray ionization mass spectrometry (ESI-MS) spectra were recorded on a Sciex API I plus (Sciex, Thornhill, Ontario, Canada) simple quadrupole mass spectrometer with a nominal mass range up to *m*/*z* 2400, equipped with an ion spray source. ESI-MS was performed using the following conditions: −4 kV were applied to the electrospray needle and −60 V to the orifice under normal operating conditions. The mass was scanned from *m*/*z* 80 to 1000, in steps of 0.3 u and with a dwell time of 0.9 ms.

**Colorimetric Determination of Total Phenolics.** Colorimetric determination of total phenolics was based on the procedure of Singleton and Rossi (*13*). Champagne (50 μL) filtered through a 0.45 μm Millipore (type HA) membrane was mixed with 450 μL of distilled water and 2.5 mL of 0.2 N Folin−Ciocalteu reagent (Sigma). Two milliliters of saturated sodium carbonate (75 g/L) were added and the mixture was shaken. The absorbance of the solution at 765 nm was measured after 2 h with a Perkin-Elmer Lambda 14 spectrophotometer. Quantification was based on the standard curve of 50, 100, 150, 200, 250, 300, 350, 400, and 500 mg/L of gallic acid prepared at the same time.

## RESULTS AND DISCUSSION

**Identification and Quantification.** The tentative identification of the peaks in two champagne varieties (Chardonnay and Pinot Noir) was based on their spectra, on their retention time in comparison with phenolic standards tested under the same conditions, and on the method of standard addition to the samples. Many peaks are not identified but well separated on these profiles. Moreover, the tartaric esters of hydroxycinnamic acids (caftaric, coutaric, fertaric, and 2-*S*-glutathionylcaftaric acids) and flavanonols (astilbin and engeletin), for which standards were not available, were tentatively identified by HPLC coupled with mass spectrometry. Moreover, previously reported spectra and retention time were also used to confirm our identification (*10*, *14*−*16*). In the course of the mass spectrometric experiments, the identity of several phenolics was also confirmed (**Table 1**). Under the conditions of this study, it was not possible to determine the mass of the other compounds.

**Table 1.** Phenolic Compounds Identified[a]

| compound | index number | retention time (min) | absorption spectrum | standard addition | mass spectrometry |
|---|---|---|---|---|---|
| gallic acid | 1 | 4.3–4.4 | + | + | + |
| protocatechuic acid | 2 | 9.6–9.7 | + | + | + |
| 2-*S*-glutathionylcaftaric acid | 3 | 15.4–15.6 | + | | + |
| *p*-hydroxybenzoic acid | 4 | 15.8–16.0 | + | + | |
| tyrosol | 5 | 16.6–16.7 | + | + | |
| caftaric acid | 6 | 17.0–17.4 | + | | + |
| (+)-catechin | 7 | 19.0–19.2 | + | + | |
| coutaric acid | 8 | 19.9–20.0 | + | | + |
| caffeic acid | 9 | 20.6–20.7 | + | + | + |
| (−)-epicatechin | 10 | 23.7–24.0 | + | + | |
| vanillin | 11 | 24.0–24.2 | + | + | |
| fertaric acid | 12 | 24.3–24.6 | + | | + |
| *p*-coumaric acid | 13 | 26.1–26.3 | + | + | |
| ferulic acid | 14 | 29.3–29.4 | + | + | + |
| astilbin | 15 | 33.2–33.3 | + | | + |
| tryptophol | 16 | 35.0–35.1 | + | + | + |
| engeletin | 17 | 36.1–36.2 | + | | + |
| *trans*-resveratrol | 18 | 37.7–37.9 | + | + | |
| quercetin | 19 | 38.6–38.7 | + | + | |

[a] Identification was founded on retention time, absorption spectrum, coelution with standards, and mass spectrometry.

**Figure 1** shows the chromatograms at 280, 320, and 365 nm, of phenolic compounds in Chardonnay champagne from the 2001 vintage. Each wavelength was suitable for each group of compounds: 280 nm was used for benzoic acids, tyrosol, flavanols, and flavanonols; 320 nm was used for cinnamic acids and their tartaric esters; and 365 nm was used for flavonols (*12*). The chromatographic profiles from the 2000 Chardonnay, 2000 and 2001 Pinot Noir are similar to the 2001 Chardonnay chromatogram.

**Table 1** lists each identified peak in elution order, with its identification number and retention time. Nineteen phenolic compounds were identified in the two varieties Chardonnay and Pinot Noir from both season 2000 and 2001. These included classes of phenolic compounds, such as hydroxybenzoic acids, hydroxycinnamic acids, flavonoids, phenolic alcohols, and phenolic aldehydes.

The external standard method was used to measure the concentrations. Three calibration curves were performed for each compound over the range of concentrations observed. The hydroxycinnamic tartaric esters: caftaric, coutaric, and fertaric acids, were quantified using the free cinnamic form as standards, caffeic acid, *p*-coumaric acid, and ferulic acid, respectively. Quantification of the two flavanonols astilbin, and engeletin was not reported, because standards were not available. The concentrations of phenolic compounds identified in Chardonnay and Pinot Noir from the 2000 and 2001 vintage seasons are presented in **Table 2**. The concentration and percentage of each phenolic class and the total phenolics quantified by HPLC and by colorimetric assay are presented in **Table 3**.

**Phenolic Composition.** The same phenolic compounds are found in Chardonnay and Pinot Noir for the vintage 2000 and 2001 (**Table 2**). However, the amount of these compounds varies significantly.

The hydroxycinnamic acids are the major class of phenolics present. This phenolic class represents 48% and 50% of the total phenol quantified by HPLC in 2000 and 2001 Chardonnay, respectively and 62% and 52% in 2000 and 2001 Pinot Noir, respectively (**Table 3**). These values compare well with those

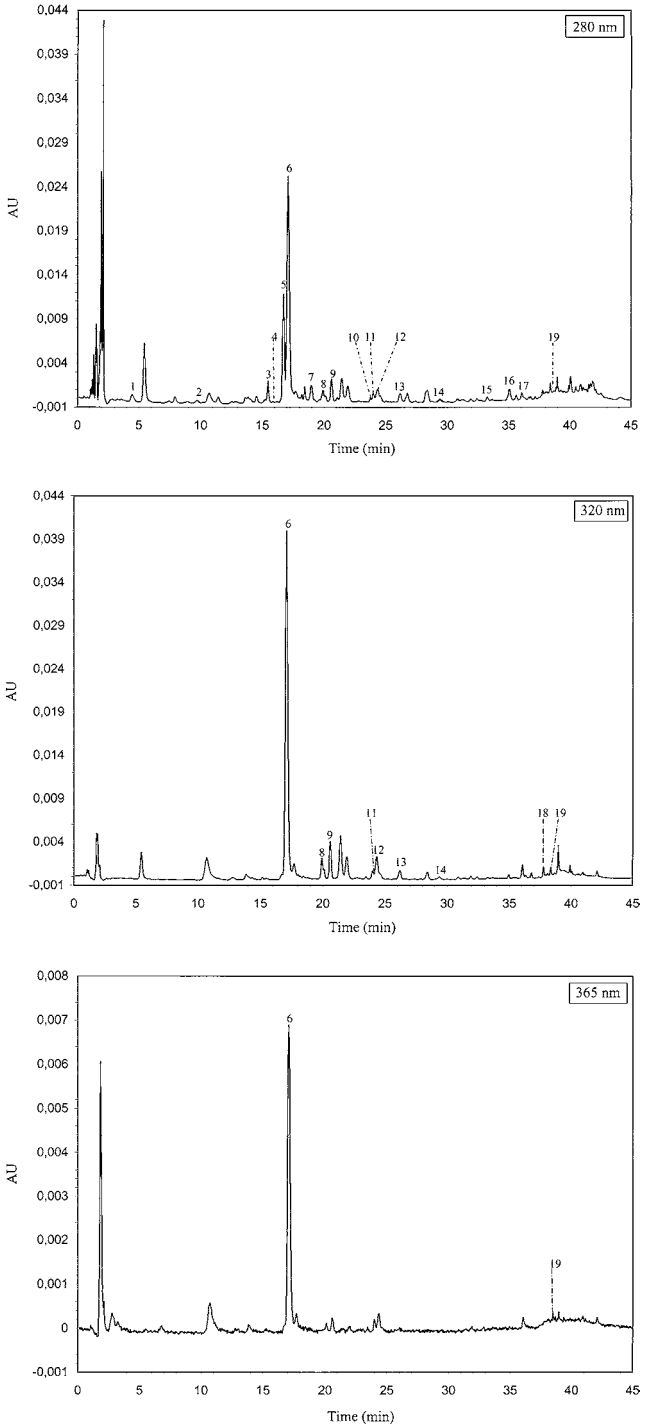


**Figure 1.** Chromatogram of a champagne wine (Chardonnay 2001) at 280, 320, and 365 nm.

reported previously for other white wines (*12*, *17*−*18*). In the sparkling wines (cavas), the hydroxycinnamic acids are the predominant phenols present (*9*). Caftaric acid is the most abundant hydroxycinnamic acid derivative with concentrations ranging from 11 to 31 mg/L in 2001 Pinot Noir and 2000 Chardonnay, respectively (**Table 2**). These values are similar to those reported in white wine from Penedès (10.54−13.36 mg/L) (*12*), Thompson Seedless grape juice (14 mg/L) (*15*), and Gewürztraminer in musts from Washington state (29.9 mg/L) (*19*). However, some other varietal wines had higher levels of caftaric acid: 73 mg/L for white Riesling wine, and 79 mg/L for Chardonnay from Washington state (*19*). These values are not exceptionally high, because caftaric acid can reach 225 mg/L in some white wines (*20*). 2-*S*-Glutathionylcaftaric acid, a product of the caftaric acid oxidation catalyzed by polyphenol oxidase (*10*), is the second most abundant hydroxycinnamic acid, with levels ranging from 2.3 to 7.9 mg/L in 2000 Chardonnay and 2001 Pinot Noir, respectively (**Table 2**). These levels are similar to those reported in white Penedès wines (2.86−3.49 mg/L) (*12*), and Thompson Seedless grape juice (7.6 mg/L) (*15*). Coutaric and fertaric acids are the hydroxycinnamic esters present at the lowest concentrations (**Table 2**). Coutaric acid (0.11−1.12 mg/L in 2001 Pinot Noir and 2000 Chardonnay, respectively) was at a concentration smaller than in white Penedès wines (8.57 mg/L) (*12*) and other varietal wines from the Pacific cost (average 2.9 mg/L) (*17*, *19*). The levels of fertaric acid range from 0.54 to 0.90 mg/L in 2000 Pinot Noir and 2001 Chardonnay, respectively (**Table 2**). That fertaric acid concentration was similar to that observed in white Penedès wines (0.15 mg/L) (*12*) but much lower than the levels observed in white Riesling (11.7 mg/L) and Chardonnay (3 mg/L) wines (*19*). Among free hydroxycinnamic acids, which probably originate from hydroxycinnamic tartaric esters hydrolysis during the fermentation process (*17*, *21*), caffeic acid has the highest concentration, ranging from 1.3 to 2 mg/L in 2001 Chardonnay and 2000 Pinot Noir, respectively (**Table 2**). These levels are similar to those described in white Penedès wines (1.61 mg/L) (*12*), Thompson Seedless juice (2.4 mg/L) (*15*), fortified Portugal wines (1.1−1.5 mg/L) (*22*), Tokay wines from Hungary (0.9−1.1 mg/L) (*22*), and other white wines (0.4−8 mg/L) (*20*). *p*-coumaric acid (0.16−0.62 mg/L in 2001 Pinot Noir and 2000 Chardonnay, respectively) and ferulic acid (0.07−0.41 mg/L in 2000 Chardonnay and 2001 Pinot Noir, respectively) account for lower concentrations (**Table 2**), as in white Penedès wines (0.15 mg/L for *p*-coumaric acid and 0.11 mg/L for ferulic acid) (*12*).

Tyrosol, a product of tyrosine transformation in the fermentation process (*17*), is the second most abundant phenolic compound after the hydroxycinnamic acid derivatives (**Table 3**). The tyrosol concentration ranges from 12 to 36 mg/L in 2001 Pinot Noir and 2000 Chardonnay, respectively (**Table 2**). Similarly, tyrosol is the second major phenolic compound, next to caftaric acid, in sparkling wines (14 mg/L) (*9*), with white Penedès wines levels ranging from 10.56 to 12.70 mg/L (*12*). Its concentration in some white wines from Bordeaux ranges from 25 to 29 mg/L (*23*).

Flavonoids detected in the champagne samples include flavanols, (+)-catechin (0.31−4.9 mg/L in 2000 and 2001 Pinot Noir, respectively), and (−)-epicatechin (0.36−1.92 mg/L in 2001 and 2000 Pinot Noir, respectively), flavanonols, astilbin and engeletin, and only the flavonol, quercetin (0.06−0.17 mg/L in 2000 and 2001 Pinot Noir, respectively) (**Table 2**). The flavonoids represent low levels, only 2.6−13% of the total phenolics quantified by HPLC (**Table 3**). This is less than in most white wines, in which flavonoids attain approximately 20% of the phenolic content, whereas in red wines, they constitute more than 85% of the phenolic compounds (*24*). The flavanols, (+)-catechin and (−)-epicatechin, were also detected in other white wines with similar levels: 6.6 and 2 mg/L in Thompson Seedless grape juice (*15*), 2.49 and 4.14 mg/L in white Penedès wines (*12*), 3.4 and 6.6 mg/L in some white wines from Bordeaux (*23*), and 2.4−6.2 and 0.6−3.1 mg/L in wines from Chardonnay grapes produced in five different regions (Australia, California, Canada, France, and Italy) (*25*). In other instances, a wide range of concentrations of these two flavanols was reported in white wines: 1−46 mg/L for catechin, and 0.1−60 mg/L for epicatechin (*20*). The two flavanonols, astilbin and

**Table 2.** Concentration of Phenolic Compounds Identified in 2000 and 2001 Chardonnay and Pinot Noir Champagnes[a]

| compound | index number | concentration (mg/L) | | | |
|---|---|---|---|---|---|
| | | Chardonnay | | Pinot Noir | |
| | | 2000 | 2001 | 2000 | 2001 |
| gallic acid | 1 | 0.42 ± 0.03 | 0.83 ± 0.01 | 0.19 ± 0.02 | 0.73 ± 0.02 |
| protocatechuic acid | 2 | 0.15 ± 0.03 | 0.32 ± 0.04 | 0.26 ± 0.02 | 0.56 ± 0.03 |
| 2-*S*-glutathionylcaftaric acid [c] | 3 | 2.3 ± 0.2 | 3.3 ± 0.4 | 3.42 ± 0.3 | 7.9 ± 0.4 |
| *p*-hydroxybenzoic acid | 4 | 0.10 ± 0.03 | 0.07 ± 0.02 | 0.05 ± 0.01 | 0.18 ± 0.02 |
| tyrosol | 5 | 36 ± 3 | 31 ± 3 | 18 ± 3 | 12 ± 2 |
| caftaric acid [c] | 6 | 31 ± 2 | 29 ± 2 | 29 ± 2 | 11 ± 2 |
| (+)-catechin | 7 | 0.71 ± 0.11 | 2.2 ± 0.1 | 0.31 ± 0.09 | 4.9 ± 0.2 |
| coutaric acid [c] | 8 | 1.12 ± 0.05 | 0.36 ± 0.06 | 0.46 ± 0.08 | 0.11 ± 0.03 |
| caffeic acid | 9 | 1.6 ± 0.1 | 1.3 ± 0.1 | 2.0 ± 0.1 | 1.71 ± 0.07 |
| (−)-epicatechin | 10 | 1.15 ± 0.05 | 0.5 ± 0.1 | 1.92 ± 0.06 | 0.36 ± 0.08 |
| vanillin | 11 | 0.33 ± 0.04 | 0.22 ± 0.03 | 0.21 ± 0.03 | 0.23 ± 0.04 |
| fertaric acid [c] | 12 | 0.7 ± 0.1 | 0.9 ± 0.1 | 0.54 ± 0.05 | 0.7 ± 0.1 |
| *p*-coumaric acid | 13 | 0.62 ± 0.05 | 0.34 ± 0.04 | 0.54 ± 0.03 | 0.16 ± 0.04 |
| ferulic acid | 14 | 0.07 ± 0.01 | 0.40 ± 0.03 | 0.36 ± 0.03 | 0.41 ± 0.02 |
| astilbin | 15 | *b* | *b* | *b* | *b* |
| tryptophol | 16 | 0.68 ± 0.04 | 1.07 ± 0.05 | 0.42 ± 0.03 | 0.51 ± 0.04 |
| engeletin | 17 | *b* | *b* | *b* | *b* |
| *trans*-resveratrol | 18 | 0.08 ± 0.03 | 0.10 ± 0.02 | 0.16 ± 0.02 | 0.08 ± 0.03 |
| quercetin | 19 | 0.12 ± 0.03 | 0.08 ± 0.02 | 0.06 ± 0.01 | 0.17 ± 0.04 |

[a] The mean and standard deviation were calculated from three independent injections. [b] The concentration is not determined because standard is not available. [c] The concentration was calculated using the equivalent of the corresponding cinnamic acid derivative.

**Table 3.** Phenolic Class Percentages, Total HPLC Phenolics, and Total Phenolics by the Colorimetric Assay

| phenolics | concentration (mg/L) | | | |
|---|---|---|---|---|
| | Chardonnay | | Pinot Noir | |
| | 2000 | 2001 | 2000 | 2001 |
| total hydroxycinnamics | 37 (48%)[a] | 36 (50%) | 36 (62%) | 22 (52%) |
| total flavonoids | 2 (2.6%) | 2.8 (3.9%) | 2.3 (4%) | 5.4 (13%) |
| total benzoic acids | 0.7 (0.9%) | 1.2 (1.7%) | 0.5 (0.9%) | 1.5 (3.6%) |
| other phenolics | 1.1 (1.4%) | 1.4 (1.9%) | 0.8 (1.4%) | 0.8 (1.9%) |
| tyrosol | 36 (47%) | 31 (43%) | 18 (31%) | 12 (29%) |
| total phenolics by HPLC | 77 | 72 | 58 | 42 |
| total phenolics by HPLC in mg/L of gallic acid | 68 | 63 | 45 | 32 |
| total phenolics by colorimetric assay in mg/L of gallic acid | 195 | 176 | 193 | 188 |

[a] Percentage of the total phenolics quantified by HPLC.

engeletin, were identified in some white wines in the ranges of 0.1−2.3 mg/L and 0.06−2 mg/L, respectively (*20*). The flavonol, quercetin was detected at low concentration (0.2 mg/L) in some white wines from Bordeaux (*23*). Other quercetin derivatives, quercetin 3-glucoside and quercetin 3-glucuronide, were detected in white grape varieties (*26*) and spanish white wines (*12*), respectively.

Benzoic acids represent the class with the lowest level, 0.9−3.6%, of the total phenolics quantified by HPLC (**Table 3**). Gallic acid is the major benzoic acid (0.19−0.83 mg/L in 2000 Pinot Noir and 2001 Chardonnay, respectively), followed by protocatechuic acid (0.15−0.56 mg/L in 2000 Chardonnay and 2001 Pinot Noir, respectively), and finally, *p*-hydoxybenzoic acid (0.05−0.18 mg/L in 2000 and 2001 Pinot Noir, respectively). The three benzoic acids have also been described in some white wines (*20*). Gallic acid is present at similar levels in white Penedès wines (0.95 mg/L) and Thompson Seedless grape juice (1.2 mg/L) (*12*, *15*). However, protocatechuic acid is the major benzoic acid in sparkling wines (1.6 mg/L) (*9*) and white Penedès wines (1.23 mg/L) (*12*). Some benzoic acids, which have been described previously in white grape juice or wine such as gentisic acid (*24*, *27*), vanillic acid (*28*), and syringic acid (*12*, *24*), were not detected in any of the champagne samples.

Other phenolic compounds, including vanillin and tryptophol, previously identified in Chardonnay wine (*29*), as well as resveratrol, a biologically active compound that is produced by plants in response to fungal infection or abiotic stress (*30*), were identified in the two monovarietal champagnes. Vanillin is present at concentrations ranging from 0.21 to 0.33 mg/L in 2000 Pinot Noir and 2000 Chardonnay, respectively. This amount is lower than the detection limit of vanilla flavor in white wine (0.4 mg/L) (*31*, *32*). Tryptophol, which results from tryptophan transformation in the fermentation process, has a concentration ranging from 0.42 to 1.07 mg/L in 2000 Pinot Noir and 2001 Chardonnay, respectively. *trans*-Resveratrol was identified in all champagne samples analyzed with levels ranging from 0.08 to 0.16 mg/L (**Table 2**). The resveratrol concentration was similar to that reported in wines from Chardonnay grapes (0.05−0.26 mg/L) (*25*), Pinot wines (0.05−0.32) (*25*), and some other white wines (0.01−1.1 mg/L) (*20*).

**Total Phenolics by HPLC and by the Colorimetric Assay.** The total phenolic composition of Chardonnay and Pinot Noir varieties (2000 and 2001), as determined by HPLC and by the colorimetric assay, are presented in **Table 3**. The total phenolics quantified by HPLC varies from 42 to 77 mg/L (32−68 mg/L of equivalent gallic acid). The value obtained by colorimetric assay varies from 176 to 195 mg/L of equivalent gallic acid and was similar to that described in some white wines (50−350 mg/L of gallic acid) (*11*). The lowest amount determined by HPLC is due to (i) a lack of quantification of all phenolic compounds detected in the chromatograms, (ii) the fact that many phenolics are not detected by HPLC in our experimental conditions, and (iii) the presence of interactions between Folin reagent and hydroxyl groups of other compounds, such as proteins and sugars. Finally, with HPLC, it is possible to

determine, very accurately, the amounts of individual compounds, though it is not possible to determine the total phenolics, even by summation.

**Effect of Season and Variety.** The two methods, HPLC and colorimetric assay, show a total phenolic content in Chardonnay and Pinot Noir champagnes higher in 2000 than in 2001 (**Table 3**). The variations of concentration are noticed as a function of the variety (Chardonnay and Pinot Noir) and of the vintage (2000 and 2001) (**Tables 2** and **3**). The amounts of caftaric acid and tyrosol, for example, the major phenolic compounds identified, decreased in 2001 in the two varieties Chardonnay and Pinot Noir (**Table 2**). However, both the 2001 Chardonnay and Pinot Noir have higher proportions of catechin and 2-*S*-glutathionylcaftaric acid than their 2000 counterparts. These results can be related to the weather conditions, as rain was more abundant in 2001 than in 2000 in the Champagne region, so the dilution of many compounds present in champagne was more important in 2001 than in 2000 (*33*). Similarly, it has been reported that the concentration and composition of phenolic compounds varies with variety, vintage, and a wide range of environmental and management factors (*10*, *34*).

## GENERAL DISCUSSION AND CONCLUSIONS

The reverse-phase HPLC system coupled with diode array detection for characterization of phenolic compounds in champagne demonstrates its suitability to analyze various classes of phenolics of champagne samples in a single chromatogram. The procedure avoids the time-consuming prepurification steps and makes possible the determination of the phenolic composition of a large number of champagne samples to investigate the effects of biological parameters and cultural and processing practices.

Nineteen phenolic compounds, including hydroxybenzoic acids, hydroxycinnamic acids, flavonoids, phenolic alcohols, and phenolic aldehydes, have been identified and quantified in two monovarietal champagnes: Chardonnay and Pinot Noir. Identification of four hydroxycinnamic tartaric esters (caftaric, coutaric, fertaric, and 2-*S*-glutathionylcaftaric acids) and two flavanonols (astilbin and engeletin) is confirmed by HPLC coupled with mass spectrometry. Caftaric acid and tyrosol are the major phenols. Hydroxybenzoic acids and flavonoids are present at low concentrations. For the first time, the phenolic composition of the Chardonnay and Pinot Noir champagnes is reported and is found to vary in terms of concentration of individual compounds but not in terms of class and number. Although only four different wines have been analyzed in this study, it seems that these concentration variations may depend on variety (Chardonnay and Pinot Noir) and on season (2000 and 2001). Moreover, the results show that the phenolic composition of champagnes is not very different from that of other white wines, although some qualitative peculiarities and quantitative trends may be noticed. The effect of many cultural and processing practices, environmental factors, and growing conditions on phenolic composition of champagne are under investigation.

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