Role of Hydroxycinnamic Acids and Flavanols in the Oxidation and Browning of White Wines

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A study of the changes in phenolic compounds during the oxidation of eight white wines reveals that the content of hydroxycinnamic acids and esters decreases significantly during the process; however, the decrease is seemingly unrelated to the extent of browning of the wine. On the other hand, the flavanol contents of the wines are found to be correlated with the degree of browning at the end of the oxidation process. The behaviors of both types of phenol compound at the two temperatures studied are quite similar.

Keywords: *Browning; oxidation; hydroxycinnamic compounds; flavanols; wine*

INTRODUCTION

Some oxidation reactions are facilitated by such enzymes as polyphenol oxidase or through chemical oxidation processes. The latter is the case if the enzyme concerned is inactive or eliminated during the processing of foods such as white wines (Singleton, 1972, 1987; Cilliers and Singleton, 1991). The oxidation is known to give rise to wine browning, which originates virtually exclusively from phenol compounds in the wine (Rossi and Singleton, 1966; Singleton and Kramling, 1976). The main step leading to browning is the conversion of *o*-diphenols to their corresponding quinones, which takes place at different rates depending on whether the process is enzymatically or chemically mediated. After these highly reactive *o*-quinones have been formed, they can oxidize other polyphenols in the medium and hence give rise to coupled reactions; the end result is the formation of brown pigments (Singleton, 1987).

Not every phenol in wine is autoxidizable; also, not all phenols contribute identically to browning (Rossi and Singleton, 1966; Singleton, 1969; Singleton et al., 1979; Simpson, 1982). Thus, according to Romeyer et al. (1985), the browning potential is unrelated to the content of hydroxycinnamic acids; although these compounds possess the lowest redox potentials among those of wine components (Cheynier et al., 1988; Richard-Forget, 1992) and hence are the first to undergo nonenzymatic oxidation, they seemingly take no part in forming brown pigments. In this respect, we found the content of hydroxycinnamic acids in the wines to be poorly correlated with browning (Fernández-Zurbano et al., 1995). This seems to hold particularily in the case of wine, since experiments involving model solutions containing caffeic or caftaric acid (Cilliers and Singleton, 1989; Cheynier and Moutounet, 1992) were found to brown to an extent proportional to the decrease in the content of many such acids. These seemingly differential roles of some compounds in wine oxidation appear to arise from the fact that wine contains other phenol compounds that take part in coupled oxidation reactions, the end products of which are the brown pigments.

Some authors have suggested that flavanols may play a prominent role in the formation of brown pigments (Simpson, 1982; Cheynier et al., 1989; Fernández-Zurbano et al., 1995). Also, the experiments of Cheynier et al. (1988a, 1989) with synthetic solutions revealed that catechin oligomers are formed more rapidly than those of caftaric acid oligomers.

The aim of this work was to elucidate the role and significance of hydroxycinnamic acids and flavanols in the oxidation of white wines, as well as to check for any differences in their behavior between ambient temperature and a higher temperature.

MATERIALS AND METHODS

Samples. All of the wines considered in this study were young, dry white wines, made by following standard winemaking procedures using grapes harvested at the so-called industrial maturity (maximum ratio of sugar/total acidity). In a preliminary study (Fernández-Zurbano et al., 1995), the browning tendency of 32 wines was measured according to the Singleton−Kramling accelerated browning test, and 8 of the 32 wines were chosen so that the whole natural range of browning tendency was represented. The selected wines had been produced from *Vitis vinifera* var. Macabeo (wines 1−6) and Chardonnay (wines 7 and 8) and belong to three different Denominations of Origin from the Aragonese region in Spain, viz. Borja, Cariñena, and Somontano (Table 1). They were all harvested in 1994, and the experiment was carried out between April and June 1995.

The wines were cleared with a 1 g/L concentration of bentonite (Singleton and Kramling, 1976). Two hours after the addition, the wines were stored frozen at −23 °C and kept for 2 days to ensure correct and fast clarification. They were then defrosted, decanted, and passed under CO_2 pressure through a filter of 0.65 μm pore size and then through one of 0.45 μm pore size.

Oxidation of Samples. All of the wines were analyzed before oxidation. They were then subjected to two different oxidation processes, namely, accelerated oxidation at 55 °C (Singleton and Kramling, 1976) and oxidation under milder temperature conditions (20 °C).

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Table 1. Categories in Which Wines Were Classified

wine	grape	region	absorbance data showed for the wine 55 °C	absorbance data showed for the wine 20 °C	browning category
1	Macabeo	Borja	1.13	1.05	intense
2	Macabeo	Borja	0.48	0.26	moderate
3	Macabeo	Cariñena	0.25	0.09	slight
4	Macabeo	Cariñena	3.17	1.60	intense
5	Macabeo	Cariñena	0.68	0.38	moderate
6	Macabeo	Cariñena	0.28	0.13	slight
7	Chardonnay	Somontano	0.65	0.33	moderate
8	Chardonnay	Somontano	0.49	0.20	slight

The experiment took place with 500 mL of each wine placed in sterile amber bottles in duplicate. The bottles were saturated with oxygen to ensure an excess throughout the whole experiment and then sealed and stored.

The wines undergoing accelerated oxidation were kept at 55 °C in a heating unit; the samples were drawn aseptically after 3, 5, and 8 days of storage in these conditions.

The wines undergoing oxidation at 20 °C were kept for a 3 month period; the samples were drawn aseptically after 3, 8, and 13 weeks. In both cases microbial activity was assessed by examination with a microscope.

Sample Analyses. The wines studied were subjected to the following determinations:

(1) Hydroxycinnamic acids and esters and 2-*S*-glutathionylcaffeoyltartaric acid (GRP) were all determined by HPLC. Musts and wines were filtered through a membrane of 0.45 μm pore size and injected directly into the chromatograph (Baranowski et al., 1981; Singleton et al., 1984). The chromatographic conditions used were as follows: eluent A, H_2O/acetic acid at pH 2.6; eluent B, CH_3CN; flow rate, 1.2 mL/min; gradient, 3−10% B over the first 10 min, 10−24% in 14 min, and 14−40% in 20 min. A Nucleosil C-120 C18 25 × 4.6 cm reversed-phase column of 5 μm particle size was used. Detection was performed at 320 nm (Fernández, 1994).

The chromatographic responses of hydroxycinnamic acids and esters, and that of GRP, were calibrated from solutions containing known concentrations of caffeic, coumaric, and ferulic acid. The calibration slope obtained for the caffeic acid was used to quantify the *cis*- and *trans*-caftaric acids and the GRP and was also used for the quantification of the esters of the coumaric and ferulic acids. To do this, it was supposed that the ester's adsorption at 320 nm is similar to that of the corresponding acid. In each case, the different molecular weights of the esters and acids were taken into account.

(2) Neutral phenol compounds were extracted according to the methods of Nagel and Wulf (1979) and Salagoïty-Auguste et al. (1984). The extracts were analyzed by HPLC, using the following conditions: eluent A, H_2O/acetic acid at pH 4; eluent B, 80% CH_3CN; flow rate, 0.9 mL/min; gradient, 3−10% B over the first 10 min, 10−17% in 10 min, and 17−25% in 35 min. The column used was of the same type as the previous one but was 30 cm in length. Also, detection was carried out at 280 nm. Catechin and epicatechin were quantified from their respective standards. The lack of standards for the other neutral phenols led us to designate their chromatographic peaks generically as F-1, F-2, F-3, etc. These peaks were identified in the chromatograms (Figure 1) from their retention times and UV−vis spectra.

(3) The determination of the wine browning was carried out by measuring the color change at 420 nm.

(4) Both the starting (unoxidized wines) samples and those resulting from the accelerated and normal oxidation processes were subjected to a spectral scan from 380 to 730 nm; this was used to calculate the CIELab coordinates (Castells, 1990).

Instrumentation. The chromatographic setup used consisted of a Waters automatic gradient controller furnished with two model 510 pumps, a model 712 Wips autoinjector, and a model 991 photodiode array detector, all also from Waters. Signals were acquired and processed by an NEC PC computer running Waters PDA-991 v. 3.3 software. Tracer Nucleosil 120 C18 columns of 0.46 cm i.d. and 25 or 30 cm length from Teknokroma (Barcelona, Spain) were used.

The absorbance of the samples at 420 nm was measured on a Hewlett-Packard 8452A diode array spectrophotometer interfaced to an HP Vectra C5 computer.

Statistical Analyses. The data were analyzed using repeated-measures ANOVA (Ferreira et al., 1997). The statistical program used was Statiew 4.0 for Macintosh.

RESULTS AND DISCUSSION

This paper focuses on the chemical changes that take place in the composition of phenolic compounds in young white wines during accelerated oxidation and oxidative storage, to establish, on the one hand, whether these changes are similar and, on the other hand, if there are any links between these changes of the phenolic compounds and the different browning tendencies of the wines. Consequently, the strategy chosen was to study the behavior of a set of wines with rather different browning tendencies. The data were analyzed using repeated-measures ANOVA to establish whether there were any statistically significant differences.

The extents of browning observed in the wines at the two temperatures studied were similar; that is, there was a linear relationship (0.988) between the browning slopes obtained from the accelerated oxidation and from the oxidative storage (Fernández-Zurbano et al., 1995). Therefore, the wine browning was divided into three categories as a function of the absorbance data obtained for the wines during the oxidative storage: intense [>0.5 absorbance unit (AU) in 3 months], moderate (between 0.2 and 0.5 AU), and slight (<0.2 UA) (Table 1).

The results of the repeated-measures ANOVA are show in Tables 2 and 3. The columns headed "time", "browning" and "time × browning" give the probability that the content and the observed composition changes are not significant with the different browning tendencies of the wines. The average concentrations of phenolic compounds for each category during the accelerated oxidation and oxidative storage are shown in the last four columns in Tables 2 and 3, respectively. For example, the data corresponding to the catechin in Table 2 relate to the average content of the three wine categories for the eight wines over the whole oxidation period, 8 days at 55 °C. The initial figure, for those wines with intense browning, was 11.39 ppm, falling to 6.84 ppm after 3 days, to 1.38 ppm after 5 days, and to 0.78 ppm after 8 days, and the same tendency was seen for the moderate and slight categories. These reductions can be attributed to the oxidation process; the "time" column gives 0.0001, and the possibility that it could be due to another cause is therefore <0.01%. The level of significance in the "browning" column for this compound is 0.0060, which means that the catechin content of the three wine classifications is related to their browning with a risk of error of <0.60%. The wines with a high catechin content showed the most intense browning; those with a low browning, classified as "slight", had the lowest catechin levels, whereas those with moderate browning had catechin levels intermediate between the two. With regard to the significance of the "time × browning" column, we can say with an error of 0.01% that the reduction in the catechin concentration during oxidation was significantly different for the different wines, being greater for the intensely browned samples, low for the slightly browned ones, and intermediate for the moderately affected ones.

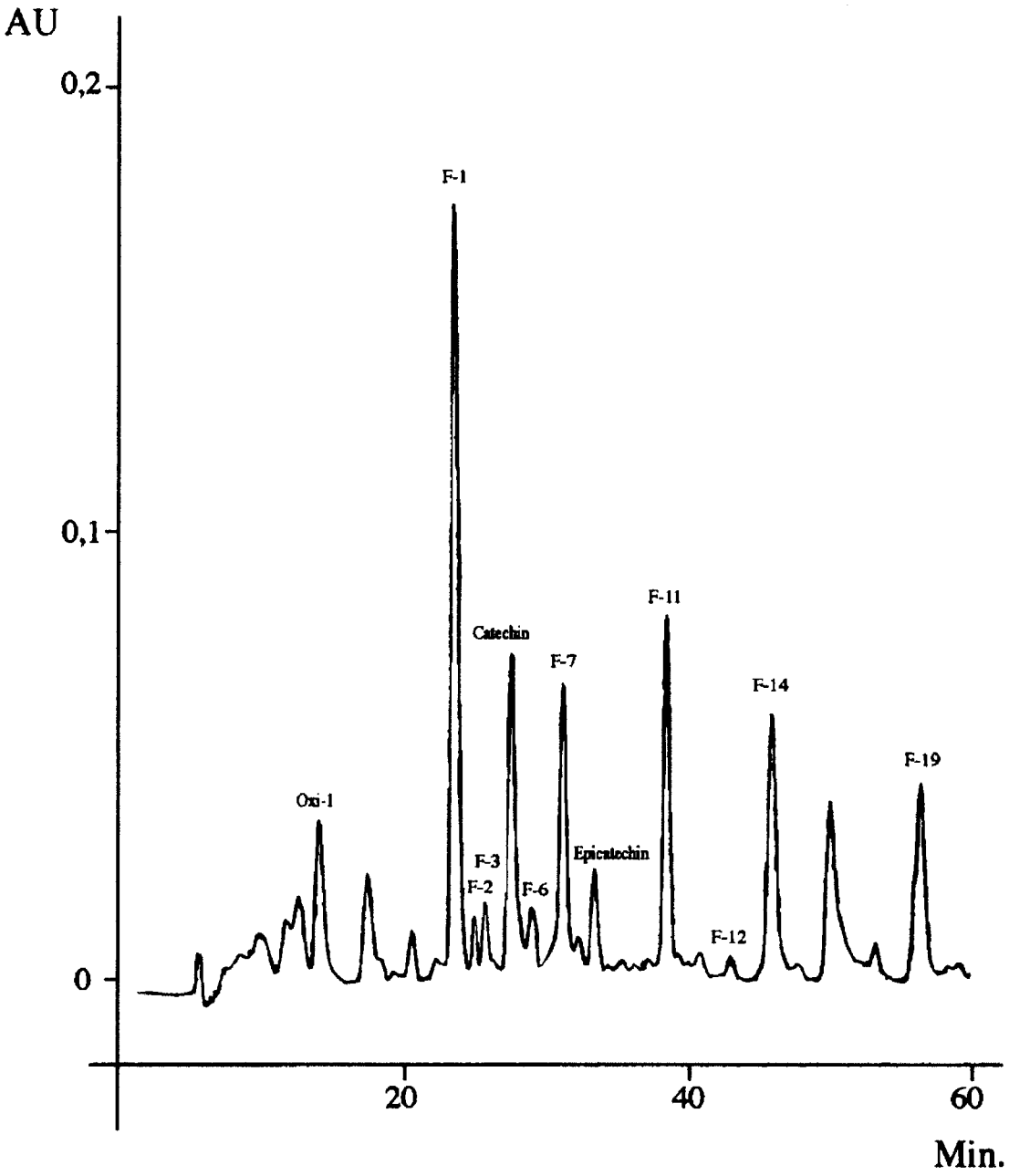

Figure 1. Chromatogram of a flavanols extract from a white wine.

When the probability is >0.05 (<95%), it is described as not significant (ns).

Changes in Hydroxycinnamic Acid Content. *Accelerated Oxidation.* Table 2 gives the results of the repeated-measures ANOVA for these compounds during oxidation of the wines at 55 °C. As can be seen, the contents of these compounds changed significantly during the process, except in the cases of *cis*-caftaric, *trans*-coutaric, and *cis*-fertaric; also, all of the contents decreased, though the extent by which they did so was seemingly not correlated with the rate of browning exhibited by the wines.

Oxidative Storage. Table 3 shows the results of the repeated-measures ANOVA of these compounds during oxidation of the wines at 20 °C. As can be seen, the contents decreased significantly during the process except in the cases of *cis*-caftaric and *trans*-coutaric, even though the extent to which they did was seemingly not related to the rate of browning of the wines.

During the process, the concentrations of *cis*-caftaric and *trans*-coutaric acid underwent no change, whereas those of all others decreased significantly.

As can be seen from the results, the behavior of the compounds studied was virtually identical at the two temperatures used (20 and 55 °C). All of the concentrations except those of *cis*-caftaric and *trans*-coutaric acid decreased during the process. The final contents in the wines oxidized at 55 °C were lower than in those browned at 20 °C, because the oxidation in the wines kept at 55 °C was higher than in those kept at 20 °C.

The decrease in the contents of these compounds during the oxidation of musts and wines has been demonstrated by several authors. Most studies in this respect were carried out on must (with intact enzyme systems) or model solutions containing the enzyme polyphenol oxidase. Cheynier et al. (Cheynier et al., 1988, 1989, 1991; Cheynier and Van Hulst, 1988;

Table 2. Repeated-Measures ANOVA and Average Concentration of Phenolic Compounds during Oxidation at 55 °C[a]

compound	time	browning	time × browning	category	wine (SD)	3 weeks (SD)	8 weeks (SD)	13 weeks (SD)
				Hydroxycinnamic Acids				
cis-caftaric	ns	ns	ns	intense	3.92 (1.17)	4.44 (1.06)	4.17 (0.53)	3.66 (0.38)
				moderate	2.80 (0.94)	3.00 (0.88)	2.95 (0.67)	2.87 (0.43)
				slight	2.54 (0.55)	2.75 (1.22)	2.57 (0.77)	2.23 (0.42)
trans-caftaric	<0.0001	ns	ns	intense	24.69 (5.38)	21.75 (3.09)	21.11 (4.99)	17.58 (3.11)
				moderate	25.06 (7.26)	20.50 (4.78)	19.53 (6.62)	16.62 (7,26)
				slight	15.89 (7.72)	12.45 (7.31)	11.84 (7.36)	10.46 (7.25)
GRP	<0.0001	ns	ns	intense	28.28 (7.26)	22.81 (6.79)	18.33 (0.41)	12.41 (0.05)
				moderate	26.83 (2.37)	19.36 (2.70)	14.09 (5.30)	10.70 (4.74)
				slight	24.10 (10.23)	20.09 (5.92)	14.12 (5.18)	10.21 (4.57)
cis-coutaric	<0.0001	ns	ns	intense	9.69 (1.44)	8.66 (2.39)	8.57 (1.20)	7.78 (1.42)
				moderate	7.95 (1.85)	6.27 (0.50)	6.22 (1.33)	5.83 (1.75)
				slight	5.74 (1.51)	5.20 (1.56)	4.72 (1.38)	4.11 (1.19)
trans-coutaric	ns	ns	ns	intense	11.13 (6.93)	11.13 (5.68)	12.01 (6.30)	10.63 (4.63)
				moderate	7.93 (3.55)	8.57 (3.92)	8.15 (2.76)	7.81 (2.79)
				slight	7.80 (3.91)	6.48 (4.36)	6.89 (4.75)	7.15 (4.58)
cis-fertaric	ns	ns	ns	intense	1.55 (0.26)	1.57 (0.13)	1.53 (0.78)	1.26 (0.08)
				moderate	1.04 (0.34)	1.15 (0.46)	1.08 (0.23)	1.07 (0.33)
				slight	1.10 (0.18)	0.80 (015)	0.92 (0.15)	0.92 (0.18)
trans-fertaric	0.0332	ns	ns	intense	4.21 (1.25)	3.94 (1.49)	4.42 (0.87)	4.17 (1.68)
				moderate	3.77 (0.44)	3.07 (0.19)	3.17 (0.16)	3.19 (0.56)
				slight	2.99 (0.74)	2.56 (0.49)	2.56 (0.54)	2.51 (0.58)
caffeic acid	0.0012	ns	ns	intense	3.03 (1.13)	2.42 (1.13)	2.07 (0.47)	1.84 (0.19)
				moderate	2.38 (0.80)	1.66 (0.26)	1.82 (0.54)	1.42 (0.36)
				slight	2.49 (0.57)	1.72 (0.48)	1.89 (0.72)	1.62 (0.49)
				Flavanols				
oxi-1[b]	<0.0001	ns	ns	intense	1.36 (0.90)	5.96 (2.44)	9.34 (2.74)	18.20 (11.17)
				moderate	0.66 (0.33)	3.45 (0.43)	8.18 (2.55)	12.69 (4.10)
				slight	0.36 (0.06)	3.25 (0.43)	7.10 (0.90)	9.97 (2.83)
F-1[b]	0.0074	ns	ns	intense	28.98 (15.99)	33.63 (15.49)	31.09 (14.72)	34.51 (19.82)
				moderate	20.72 (9.98)	21.76 (9.44)	22.43 (9.41)	23.81 (9.80)
				slight	21.07 (14.35)	21.74 (14.23)	23.47 (17.00)	23.20 (16.99)
F-2[b]	0.0008	ns	<0.0001	intense	6.17 (2.48)	1.84 (0.09)	0.83 (0.33)	0.72 (0.21)
				moderate	1.14 (0.10)	1.04 (0.32)	0.78 (0.27)	0.88 (0.18)
				slight	1.02 (0.65)	1.45 (1.72)	1.24 (1.64)	0.98 (1.37)
F-3[b]	ns	0.0033	<0.0001	intense	1.23 (0.42)	0.57 (0.27)	0.19 (0.14)	0.19 (0.20)
				moderate	0.12 (0.04)	0.16 (0.09)	0.26 (0.12)	0.24 (0.12)
				slight	0.01 (0.02)	0.10 (0.03)	0.11 (0.03)	0.06 (0.05)
catechin	<0.0001	0.0060	<0.0001	intense	11.39 (3.00)	6.84 (0.07)	1.38 (1.53)	0.78 (1.08)
				moderate	3.71 (0.51)	2.46 (0.70)	0.80 (0.35)	0.64 (0.34)
				slight	1.76 (1.09)	1.12 (0.79)	0.29 (0.13)	0.25 (0.19)
F-6[b]	<0.0001	ns	0.0056	intense	1.76 (1.30)	5.50 (3.08)	6.80 (3.10)	9.87 (5.57)
				moderate	0.76 (0.56)	2.11 (1.12)	3.51 (1.42)	4.18 (1.67)
				slight	0.64 (0.18)	1.33 (0.56)	1.88 (0.39)	2.48 (0.30)
F-7[b]	ns	ns	ns	intense	2.34 (0.81)	1.67 (0.99)	0.94 (0.79)	2.56 (1.61)
				moderate	1.57 (1.36)	1.33 (0.56)	1.58 (0.91)	1.68 (0.87)
				slight	1.01 (0.41)	1.30 (0.75)	1.44 (0.42)	1.28 (0.76)
epicatechin	<0.0001	<0.0001	0.0008	intense	3.38 (0.30)	1.49 (0.59)	0.46 (0.52)	0.47 (0.55)
				moderate	1.36 (0.17)	0.34 (0.09)	0.14 (0.05)	0.10 (0.08)
				slight	0.80 (0.26)	0.14 (0.05)	0.10 (0.04)	0.09 (0.03)
F-11[b]	ns	ns	ns	intense	7.73 (7.50)	7.11 (3.85)	9.04 (6.94)	8.98 (6.60)
				moderate	3.22 (2.56)	2.28 (1.09)	3.01 (1.31)	2.45 (2.00)
				slight	1.60 (1.66)	3.17 (0.30)	2.76 (0.54)	3.41 (2.81)
F-12[b]	0.0023	0.0066	ns	intense	1.85 (0.70)	0.93 (0.05)	0.48 (0,09)	0.54 (0.24)
				moderate	0.67 (0.26)	0.34 (0.17)	0.16 (0.11)	0.32 (0.31)
				slight	0.39 (0.01)	0.25 (0.07)	0.12 (0.01)	0.13 (0.01)
F-14[b]	ns	ns	ns	intense	1.23 (0.58)	0.39 (0.01)	0.13 (0.04)	0.23 (0.04)
				moderate	0.94 (0.32)	0.68 (0.17)	0.68 (0.57)	0.71 (0.45)
				slight	0.48 (0.09)	0.64 (0.51)	0.60 (0.56)	0.35 (0.20)
F-19[b]	<0.0001	ns	ns	intense	11.56 (11.63)	6.78 (6.04)	2.23 (1.58)	0.66 (0.16)
				moderate	17.24 (3.86)	4.71 (1.83)	3.81 (3.46)	1.79 (2.02)
				slight	7.38 (3.02)	1.86 (2.71)	1.37 (1.89)	0.33 (0.14)

[a] Data are given in milligrams per liter except where indicated otherwise. [b] Data are absolute areas × 100.

Cheynier and Moutounet, 1992) obtained decreased contents of these compounds in every case. Nonenzymatic oxidation of wines is less widely documented. Singleton et al. (1979) observed a decrease in the contents of nonflavonoid compounds in young white wines periodically exposed to the air. More recently, Cilliers and Singleton (1990) showed the decrease of caffeic acid in nonenzymatic autoxidation reactions in wines of the Garnacha variety.

The results obtained at both 20 and 50 °C (Tables 2 and 3) reveal that neither the contents in the compounds studied nor their changes were related to the extent of browning observed (intense, moderate, or slight).

Hydroxycinnamic acids possess the lowest redox potential among the phenol compounds naturally occurring in grapes (Cheynier et al., 1988). Therefore, the *o*-quinones produced by oxidation of these compounds are the most efficient conductors for the coupled reac-

Table 3. Repeated-Measures ANOVA and Average Concentration of Phenolic Compounds during Oxidative Storage[a]

compound	time	browning	time × browning	category	wine (SD)	3 weeks (SD)	8 weeks (SD)	13 weeks (SD)
				Hydroxycinnamic Acids				
cis-caftaric	ns	ns	ns	intense	3.92 (1.17)	4.57 (0.15)	3.67 (0.41)	4.23 (0.66)
				moderate	2.80 (0.94)	3.46 (0.69)	2.47 (0.44)	2.96 (0.80)
				slight	2.54 (0.55)	2.52 (0.67)	3.17 (0.67)	2.89 (1.47)
trans-caftaric	0.0305	ns	ns	intense	24.69 (5.38)	24.54 (2.79)	22.22 (6.23)	17.49 (8.33)
				moderate	25.06 (7.26)	25.02 (6.49)	23.06 (6.03)	19.17 (7.40)
				slight	15.89 (7.72)	11.08 (2.81)	14.20 (7.71)	13.33 (7.56)
GRP	0.0024	ns	ns	intense	28.28 (7.26)	26.69 (0.13)	17.66 (6.81)	15.88 (0.42)
				moderate	26.83 (2.37)	21.92 (2.63)	15.68 (4.85)	18.75 (1.64)
				slight	24.10 (10.23)	17.74 (3.53)	16.12 (4.52)	15.44 (5.34)
cis-coutaric	0.0061	ns	ns	intense	9.69 (1.44)	9.28 (1.36)	8.94 (0.26)	6.59 (1.95)
				moderate	7.95 (1.85)	7.27 (1.56)	6.70 (2.06)	5.88 (2.79)
				slight	5.74 (1.51)	5.54 (1.34)	5.45 (1.63)	4.88 (1.28)
trans-coutaric	ns	ns	ns	intense	11.13 (6.93)	11.68 (4.36)	9.15 (7.93)	9.61 (5.69)
				moderate	7.93 (3.55)	9.80 (3.22)	7.09 (3.51)	6.94 (2.68)
				slight	7.80 (3.91)	4.70 (1.80)	7.51 (4.73)	5.93 (5.10)
cis-fertaric	0.0076	ns	ns	intense	1.55 (0.26)	1.59 (0.04)	1.06 (0.60)	1.05 (0.15)
				moderate	1.04 (0.34)	1.29 (0.27)	0.90 (0.46)	0.65 (0.12)
				slight	1.10 (0.18)	0.80 (0.09)	1.02 (0.31)	0.58 (0.04)
trans-fertaric	0.0363	ns	ns	intense	4.21 (1.25)	3.79 (0.72)	2.76 (0.16)	3.98 (0.99)
				moderate	3.77 (0.44)	2.79 (0.47)	2.55 (0.96)	3.10 (0.41)
				slight	2.99 (0.74)	2.29 (0.76)	2.65 (0.48)	2.78 (0.84)
caffeic acid	0.0004	ns	ns	intense	3.03 (1.13)	2.60 (0.58)	1.77 (0.11)	1.98 (0.58)
				moderate	2.38 (0.80)	2.01 (0.74)	1.44 (0.04)	2.04 (0.50)
				slight	2.49 (0.57)	1.96 (0.80)	1.75 (0.48)	2.11 (0.56)
				Flavanols				
oxi-1[b]	0.0001	ns	ns	intense	1.36 (0.90)	1.63 (1.25)	2.08 (1.39)	3.50 (0.62)
				moderate	0.66 (0.33)	1.06 (0.35)	1.60 (0.81)	1.76 (1.53)
				slight	0.36 (0.06)	0.77 (0.04)	1.86 (0.42)	2.70 (0.15)
F-1[b]	ns	ns	ns	intense	28.98 (15.99)	29.75 (17.04)	31.32 (17.63)	29.92 (10.91)
				moderate	20.72 (9.98)	23.74 (11.22)	21.75 (12.06)	22.80 (9.82)
				slight	21.07 (14.35)	19.67 (11.44)	22.62 (12.89)	20.93 (14.88)
F-2[b]	0.0335	ns	0,0106	intense	6.17 (2.48)	3.34 (2.28)	1.50 (1.90)	0.32 (0.31)
				moderate	1.14 (0.10)	0.51 (0.17)	0.07 (0.01)	0.08 (0.08)
				slight	1.02 (0.65)	1.59 (1.80)	1.39 (2.32)	1.62 (2,73)
F-3[b]	ns	0.0017	0.0075	intense	1.23 (0.42)	0.59 (0.60)	0.73 (0.26)	0.13 (0.12)
				moderate	0.12 (0.04)	0.14 (0.08)	0.14 (0.08)	0.17 (0.13)
				slight	0.01 (0.02)	0.13 (0.03)	0.04 (0026)	0.06 (0.02)
catechin	<0.0001	0.0002	0.0034	intense	11.39 (3.00)	9.77 (2.39)	5.18 (3.95)	0.63 (0.33)
				moderate	3.71 (0.51)	3.28 (0.78)	0.66 (0.44)	0.72 (0.34)
				slight	1.76 (1.09)	1.02 (0.90)	0.31 (0.35)	0.24 (0.18)
F-6[b]	0.0002	ns	0.037	intense	1.76 (1.30)	3.54 (2.31)	4.39 (3.62)	5.50 (2.39)
				moderate	0.76 (0.56)	1.42 (1.50)	2.33 (0.16)	3.17 (0.85)
				slight	0.64 (0.18)	0.89 (0.38)	1.26 (0.60)	1.36 (0.31)
F-7[b]	ns	ns	ns	intense	2.34 (0.81)	2.45 (1.43)	2.71 (1.43)	1.99 (0.84)
				moderate	1.57 (1.36)	1.54 (1.41)	0.49 (0.32)	1.57 (0.99)
				slight	1.01 (0.41)	1.38 (0.36)	1.23 (0.57)	1.04 (0.11)
epicatechin	<0.0001	0.0083	0.0043	intense	3.38 (0.30)	2.41 (0.72)	1.51 (1.71)	0.20 (0.01)
				moderate	1.36 (0.17)	0.68 (0.42)	0.15 (0.04)	0.14 (0.03)
				slight	0.80 (0.26)	0.37 (0.22)	0.13 (0.02)	0.11 (0.02)
F-11[b]	ns	0.0472	ns	intense	7.73 (7.50)	7.83 (6.73)	6.66 (6.82)	8.74 (5.94)
				moderate	3.22 (2.56)	3.86 (3.89)	0.94 (0.15)	4.15 (2.85)
				slight	1.60 (1.66)	2.67 (0.70)	1.78 (0.13)	2.78 (1.08)
F-12[b]	0.0371	ns	ns	intense	1.85 (0.70)	1.04 (0.62)	0.87 (0.07)	0.47 (0.48)
				moderate	0.67 (0.26)	0.39 (0.19)	0.41 (0.36)	0.45 (0.36)
				slight	0.39 (0.01)	0.29 (0.09)	0.47 (0.05)	0.22 (0.29)
F-14[b]	0.0351	ns	ns	intense	1.23 (0.58)	1.00 (0.13)	0.69 (0.54)	0.29 (0.22)
				moderate	0.94 (0.32)	1.33 (0.73)	0.49 (0.36)	0.84 (0.76)
				slight	0.48 (0.09)	0.70 (0.57)	0.40 (0.46)	0.50 (0.18)
F-19[b]	<0.0001	ns	ns	intense	11.56 (11.63)	7.67 (8.37)	3.23 (2.96)	1.40 (0.58)
				moderate	17.24 (3.86)	12.47 (4.37)	2.43 (2.37)	1.74 (1.20)
				slight	7.38 (3.02)	2.97 (2.62)	1.12 (0.95)	0.75 (0.73)

[a] Data are given in milligrams per liter except where indicated. [b] Data are absolute areas × 100.

tions involved in the oxidation (Nicolas, 1994) and hence appear to play a central role in the degradation of other phenols. Although these compounds obviously play some part in starting the process and their *o*-quinones in its development, their contents in wines seemingly bear no relationship to the brown pigments formed during the oxidation. We calculated the rate of browning by measuring the absorbance of the brown compounds formed in the oxidation process, during which the concentrations of hydroxycinnamic acids and esters—the obvious triggers for the formation of the oxidation products (Cheynier and Moutounet, 1992)—decreased; however, the change was not significantly related to the extent of browning.

The fall in the concentration of these compounds during oxidative storage of the white wines may be due to their suffering degradations, such as hydrolization of the esters to their corresponding acids, which then

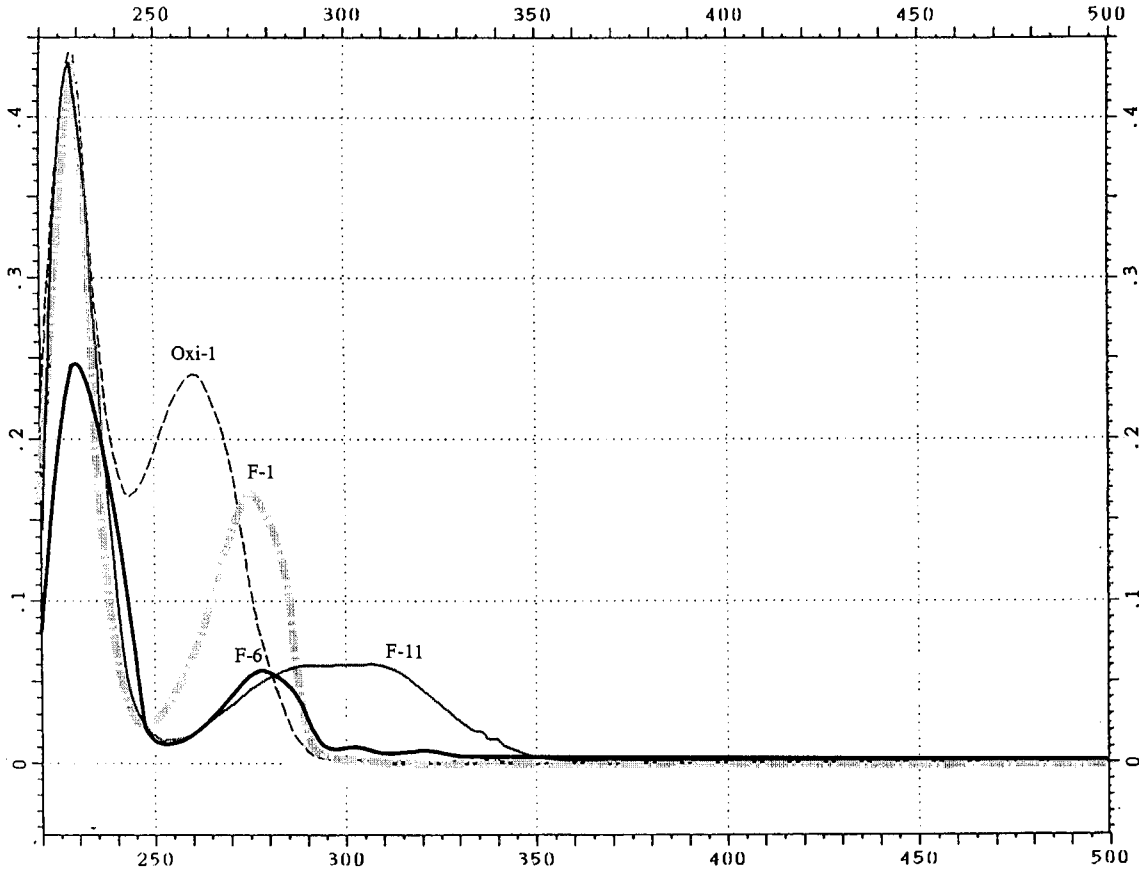

Figure 2. UV spectra for the unidentified compounds oxi-1, F-1, F-6, and F-11.

decarboxylize. This process could give rise to the formation of phenols, cresols, vinylphenols, eugenol, etc., all of which are considered off-flavors. The degradation of hydrocinnamic compounds to vinylphenols has been described for the storage of orange juice (Rouseff et al., 1992; Peleg et al., 1992; Fallico et al., 1996).

In a study of the aromatic profile of these wines, the concentration of eugenol was seen to increase over the whole oxidation process; the concentrations of 4-ethylphenol and phenol increased significantly for the first weeks and then fell, whereas the 4-vinylguaiacol disappeared during the time of the oxidative storage (Ferreira et al., 1997). Although the transformation of these compounds in some of the aromatic phenols seems to be possible, it is not clear which type of compounds may be driving the degradation, because the compounds can degrade again and reduce their concentration as described above.

Changes in Flavanol Contents. *Accelerated Oxidation.* Table 2 gives the ANOVA results for flavanol content, which exhibited two different trends. Thus, some increased in concentration while the others behaved identically with hydroxycinnamic esters (i.e., they disappeared to a great extent from the wine during oxidation at 55 °C). On the other hand, the contents of some of these compounds in the wine were significantly related to the browning categories established, and so were the changes in some contents during the oxidation process.

The compounds that increased in concentration in the course of the accelerated oxidation experiments were those designated oxi-1, F-1, F-6, and F-11. The chemical structures of these compounds have not yet been determined, but their spectra are shown in Figure 2.

Table 4. Repeated-Measures ANOVA of CIELab Coordinates for Oxidized Wines

CIELab coordinate	time × browning oxidation	
	55 °C	20 °C
*a**	<0.0001	0.0004
*b**	0.0005	0.0059
*C**	0.0002	0.0049
*L**	0.0027	<0.0001
H°	ns	ns

The spectra for F-1 and F-6 are similar to those of the procyanidin dimers. The concentration increase in these compounds was particularly significant for oxi-1 and F-6.

All of the other compounds—catechin and epicatechin included—underwent a concentration decrease during the oxidation process, the effect being significant for all except F-3. The spectra for these compounds are shown in Figure 3. Those for compounds F-2 and F-3 are similar to the spectra for procyanidins.

As can be inferred from the ANOVA results shown in Table 2, the contents of some compounds (F-3, catechin, epicatechin, and F-12) varied significantly with the extent of browning. Therefore, differences in the contents of these compounds during the oxidation process seemingly determine whether the wine browns to a high, medium, or low degree.

As noted earlier, the statistical analysis of the results revealed that changes (increases or decreases) in the concentrations of some compounds during the oxidation process are related to browning. Thus, wines that exhibited high browning were found to lose compounds F-2, F-3, catechin, and epicatechin at a higher rate and to produce compound F-6 also at a higher rate than the other wines. On the other hand, wines that exhibited

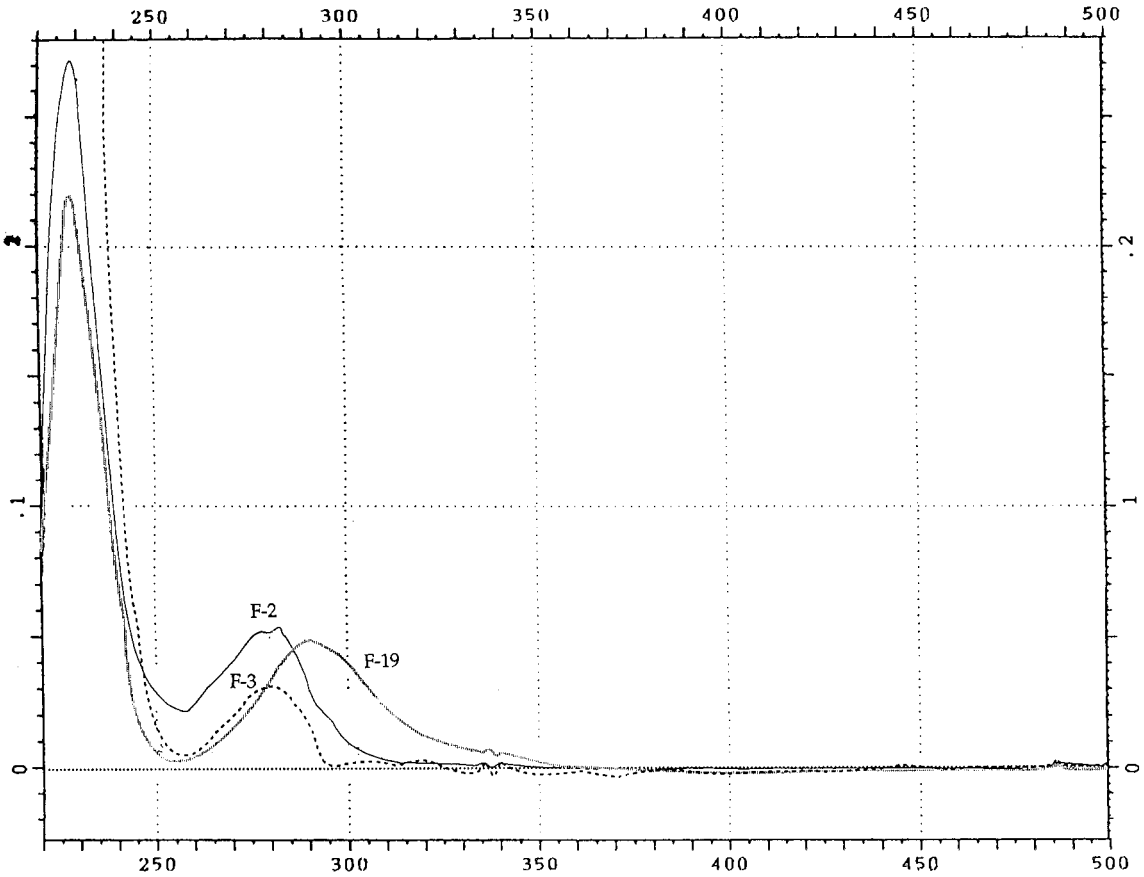

Figure 3. UV spectra for the unidentified compounds F-2, F-3, and F-19.

Table 5. CIELab Coordinates for Three Whites Wines Belonging to the Three Browning Categories Established: Intense, Moderate, and Slight

	wine with intense browning			wine with moderate browning			wine with slight browning		
CIELab coordinate	unoxidized	oxidized at 20 °C	oxidized at 55 °C	unoxidized	oxidized at 20 °C	oxidized at 55 °C	unoxidized	oxidized at 20 °C	oxidized at 55 °C
*a**	−0.677	14.403	16.632	−1.520	1.012	1.418	−0.911	−0.703	−0.589
*b**	8.425	67.848	65.352	6.743	22.182	31.271	3.363	10.899	14.505
*C**	8.450	69.360	67.430	6.910	22.200	31.300	3.480	10.920	14.520
*L**	101.300	79.220	65.800	100.160	94.030	86.340	97.110	99.620	90.370
H°	−85.400	78.000	75.700	−77.400	87.400	87.400	−74.800	−86.300	−87.700

little browning lost the above-mentioned compounds at a lower rate and produced compound F-6 at a higher rate than the rest.

Oxidative Storage. Table 3 shows the ANOVA results for flavanol compounds allowed to oxidize at 20 °C. As can be seen, the results were similar to those obtained in the oxidation at 55 °C.

The decrease in the contents of some flavanols (catechin, epicatechin, and some procyanidins) is consistent with reported results during the enzymatic oxidation. Thus, Cheynier et al. (1988, 1989), who used model solutions containing PFO, found (1) flavanols (catechin, epicatechin, ...) decreased during the oxidation and (2) the rate of degradation of all flavanols was much higher if the solution contained caftaric acid. This was the likely result of the mechanisms via which oxidative polymerization takes place in the wine. These reactions involve oxidation of caftaric acid, followed by a chemical reaction of a second compound (e.g., catechin or epicatechin) by the caftaric acid quinone with regeneration of caftaric acid that yields flavanol polymers principally. According to these authors, these compounds are formed more rapidly than those of caftaric acid oligomers. This explanation is consistent with the ANOVA results (Tables 2 and 3), according to which the contents of some flavanols (catechin and epicatechin included) in the wines were significantly correlated with their extent of browning. The decrease in such contents was also highly correlated with wine browning; in fact, highly browned wines exhibited large content changes; these were more moderate for the four wines included in the medium browning category and quite small in the two wines that exhibited low browning.

Changes in Color. Table 4 shows the relationship of the variation of CIELab coordinates during oxidation at 55 and 20 °C to the extent of browning of the wines.

Coordinates *a**, *b**, *C**, and *H*° increased during the oxidation process, the first three to a statistically significant degree. On the other hand, coordinate *L** decreased with increasing absorbance at 420 nm (i.e., with increasing browning) as a result of the oxidation of the wines. The two trends were exhibited by the wines subjected to both accelerated and ambient temperature oxidation.

The differences in the coordinates obtained for each wine at the end of the two oxidation processes are

probably the result of the browning in the wines kept at 55 °C being stronger than that in wines kept at 20 °C.

Table 5 shows the CIELab coordinates obtained for three wines that exhibited high, medium, and low browning after oxidation. As can be seen, the chromatic profiles of the three wines prior to oxidation were very similar. Also, the profile for each oxidized wine was related to the extent of browning undergone by each wine and therefore to the different phenolic composition of the white wines.

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