Prediction of Oxidative Browning in White Wines as a Function of Their Chemical Composition

P. Fernández-Zurbano,* V. Ferreira, C. Peña, A. Escudero, F. Serrano, and J. Cacho

Department of Analytical Chemistry, Faculty of Sciences, University of Zaragoza, 50009 Zaragoza, Spain

The use of principal component regression has helped to generate a mathematical model to relate wine browning during oxidation at 55 °C with phenolic composition (flavanols and hydroxycinnamic acids) and other wine parameters prior to oxidation such as pH, SO_2, and Fe. A linear regression has been found between the browning capacity of wines oxidized at room temperature (20 °C) and that of wines oxidized at 55 °C, pointing out that the difference between the oxidations is the amount of time in which wine browning took place.

Keywords: *Prediction; oxidation; browning; phenolic compounds; white wine*

INTRODUCTION

One of the most serious problems in wine stability is oxidative browning. Among the compounds present in wine, polyphenols are strongly involved in oxidative browning reactions (Amerine et al., 1982; Singleton, 1987). The nature of these reactions in must may be enzymatic (presence of polyphenol oxidase) or chemical (absence of polyphenol oxidase activity), which takes place during wine handling or aging. The main difference between these types of oxidation is the different rate of formation of quinones, which are activated species able to generate chain reactions yielding polymerized polyphenols. The result from this reaction is the formation of brown pigments of high molecular weight which absorb light in the area complementary to brown, although molar absorbance will depend on the type of product (Singleton, 1987; Cilliers and Singleton, 1990; Rigaud et al., 1990; Vaimakis and Roussis, 1993).

We can define wine stability with respect to oxidation as wine resistance to generate brown pigments or to browning. This stability will depend on (1) the tendency of the wine to produce the active species, (2) the type of substrates present in the wine that can react with those species, and (3) the absorption coefficients of the generated products. This situation is complex enough so that the prediction of wine stability from wine chemical composition is not straightforward. It has been described that species such as hydroxycinamic acids and esters, GRP, flavanols, Fe, Cu, and sulfur dioxide can take part in the formation of the active species (Simpson, 1982; Singleton, 1987; Cheynier et al., 1989; Cilliers et al., 1990). Many more species may take part during the following phases of oxidation (Singleton, 1987; Simpon, 1982).

Therefore, wines retain their organoleptic characteristics for a certain length of time until the oxidation reactions start, and then wines begin to lose those characteristics that define their quality. Oxidation can be accelerated by several factors such as increase in pH, oxygen availability, and high temperatures. To determine the browning capacity of wines over a short period of time, Singleton and Kramling (1976) and Simpson (1982) developed an accelerated browning test by keeping wines at high temperatures.

* Author to whom correspondence should be addressed [fax (76) 567920; e-mail pfernandez@msf.unizar.es].

The different stabilities of wine (some become oxidized earlier than others) are known to depend on wine composition; therefore, the purpose of this work was to predict wine oxidative stability by a semiempiric model, that is to say, by a model built with chemical components of known or suspected importance and with the simplicity of linearity. The interest of such a model is predictive as well as explanatory, since it can help to interpret the roles played by different variables or at least determine if they provide the information needed to predict the evolution of a wine.

MATERIALS AND METHODS

Samples. Thirty-two samples of white wines of Macabeo and Chardonnay grape varieties from the 1993 harvest and from the Spanish Denominations of Origin Borja, Cariñena, and Somontano in Aragon were studied (Table 1).

Samples were taken straight from the deposits after fermentation. Clarification was done by adding 1 g/L bentonites (Singleton and Kramling, 1976). Two hours later wines were frozen for 2 days, after which time they were decanted and filtered under pressure with CO_2 through 0.65 and 0.45 μm pore size filters.

Sample Analysis. Wine analysis consisted of the following.

1. Esters, hydroxycinnamic acids, and 2-*S*-glutathionylcaffeoyltartaric acid (GRP) were analyzed by HPLC through direct injection of must or wine, after being filtered through a 0.45 μm pore size filter (Baranowski and Nagel, 1981; Singleton et al., 1984). The chromatographic conditions used were as follows: solution A, H_2O/acetic acid (pH 2.6); solution B; CH_3CN. Flow rate was 1.2 mL/min. The gradient used was 3–10% of B for the first 10 min, 10–24% for 14 min, and 24–40% for 20 min. The reversed phase column, C_{18}, used was Nucleosil C-120 (25 × 4.6 cm) and 5 μm particle size. Detection was done at 320 nm (Fernández, 1994).

Calibration of chromatographic response for esters, hydroxycinnamic acids, and GRP was done from known solutions of caffeic, coumaric, and ferulic acids.

2. Neutral phenolic compounds were extracted following Nagel and Wulf (1979) and Salagoïty-Auguste and Bertrand (1984) procedures. The extracts were analyzed by HPLC. The chromatographic conditions used were as follows: solution A, H_2O/acetic acid (pH 4); solution B, CH_3CN (80%). Flow rate was 0.9 mL/min. The gradient used was 3–10% of B for the first 10 min, 10–17% for 10 min, and 17–25% for 35 min. The same type of column was used but of 30 cm length. Detection was done at 280 nm.

Determination of catechin and epicatechin was done from their respective standards. The rest of the peaks in the chromatogram were named F-1, F-2, F-3, ... since standards were not available for those neutral phenolic compounds.

0021-8561/95/1443-2813$09.00/0

Table 1. Origin and Variety of White Wines

white wine	origin of wine	label	variety
Coop. Borja 1	Borja	B2	Macabeo
Coop. Borja 2	Borja	B3	Macabeo
Coop. Borja 3	Borja	B4	Macabeo
Ainzón 1	Borja	B5	Macabeo
Coop. Ainzón	Borja	B7	Macabeo
Coop. Fuendejalón	Borja	B8	Macabeo
Coop. Pozuelo	Borja	B9	Macabeo
Coop. Tabuenca	Borja	B10	Macabeo
COVINCA1	Cariñena	C1	Macabeo
COVINCA 3	Cariñena	C3	Macabeo
San Valero 1	Cariñena	C4	Macabeo
San Valero 2	Cariñena	C5	Macabeo
San Valero 3	Cariñena	C6	Macabeo
Paniza 1	Cariñena	C7	Macabeo
Paniza 2	Cariñena	C8	Macabeo
Paniza 3	Cariñena	C9	Macabeo
COVISA 1	Somontano	S1	Chardonnay
COVISA 2	Somontano	S2	Macabeo, Chardonnay, Riesling, Chenin Blanc
COVISA 3	Somontano	S3	Macabeo, Chardonnay
Pirineos1	Somontano	S4	Macabeo
Pirineos 2	Somontano	S5	Macabeo
Pirineos 3	Somontano	S6	Macabeo
Enate1	Somontano	S7	Macabeo
Enate 2	Somontano	S8	Chardonnay
COVISA 4	Somontano	S9	Chardonnay
COVISA 5	Somontano	S10	Chardonnay
COVISA 6	Somontano	S11	Chardonnay
COVISA 7	Somontano	S12	Chardonnay
COVISA 8	Somontano	S13	Chardonnay
COVISA 9	Somontano	S14	Chardonnay
COVISA 10	Somontano	S15	Chardonnay
COVISA 11	Somontano	S16	Chardonnay

These peaks were identified according to their retention times and with respect to their UV−vis absorption.

3. Other analyses were made for pH, free and total SO_2, acetaldehyde, color (420 and 520 nm), Fe, and Folin−Ciocalteu index.

4. Wine oxidation was done by the accelerated oxidation test or browning test as proposed by Singleton and Kramling (1976). Wines were filtered through 0.45 μm membrane filters, and 10 mL was poured in test tubes. The tubes were sealed and maintained at 55 °C. Absorbance at 420 nm was measured before wines were subjected to the oxidation test and after being in the oven for 5 and 7 days.

5. Eight of the previous wines were oxidized under less strict temperature conditions (20 °C). Absorbance at 420 nm was measured for 3 months.

Equipment. The chromatography equipment used had a Waters automatic gradient control, two Waters 510 bombs, a Waters 712 Wips automatic injector, and a Waters 991 detector. Signals were received and processed by an NEC computer with Waters PDA-991 software version 3.3.

Absorbance at 420 nm was done with a diode spectrophotometer (Hewlett-Packard 8452A) connected to an HP Vectra C5 computer.

Statistical Analysis. The statistical program used was Statview TM SE and Graphics 1.03. Chemical composition data of 30 compounds were first treated by principal component analysis (PCA) to generate orthogonal variables. The new variables containing a significant percentage of the variance were treated by stepwise linear regression. This regression was calculated with an acceptance F value of 0.1 and a rejecting F value of 0.05.

The validation of the prediction model obtained consisted of dividing the number of samples in two sets: one of them was used to build again the prediction model and the other to evaluate the prediction model.

RESULTS AND DISCUSSION

Validation of the Slope Data. Data from browning evolution both with accelerated oxidation and with oxidation at 20 °C were adjusted by linear regression to obtain a slope value (browning slope) to compare wine rates of browning (Singleton et al., 1979).

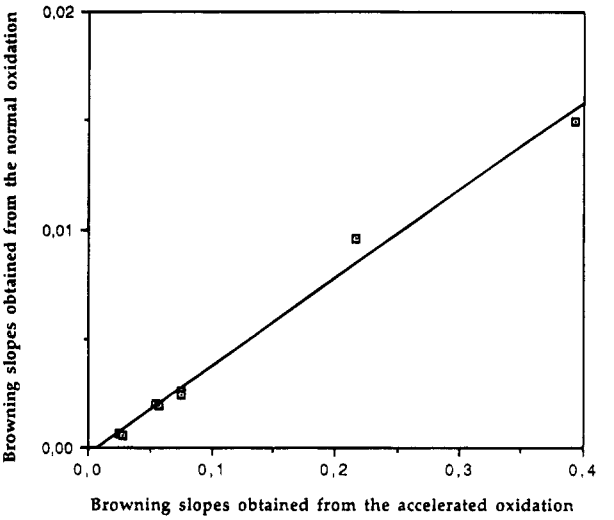

Figure 1. Relation among browning slopes.

A linear relation of correlation coefficient of 0.988 (n = 8), (Figure 1) was found for the browning slopes obtained from the accelerated oxidation experiment (55 °C) and from the oxidation at normal temperature (20 °C). This result shows, although the number of samples was only eight, the main difference between the two kinds of oxidation is the kinetics of the processes.

Study of Wine Oxidation as a Function of Composition by PCA. Figure 2 shows the results from the PCA of 32 white wines. The variables used were the parameters analyzed before oxidation together with the browning slope obtained from the oxidation process suffered by the wines. The first component accounts for 19.6% of the variance and separates varietal Macabeo wines (central and left part of the figure) from varietal Chardonnay wines (right side of the figure) independently of origin. Macabeo wines from Somontano (S4, S5, S6, and S7) were grouped with the same variety of wines from Borja and Cariñena. Variables responsible for this separation were pH, color, and some unidentified flavanols (F-7, F-12, F-14, F-11, and F-19).

The second component accounts for 16.4% of the variance and seems to be related to the browning value of wines; that is, negative values of this component correspond to wines with more browning, while wines with lower browning slopes had positive values of this component. However, since the variance accumulated by the two components is 35.6%, the results obtained by PCA are merely indicative.

Principal Component Regression (PCR). Since the variance accounted for by the first two components is very low, a higher number of components needs to be considered. There is no fixed rule regarding the number of principal components that must be taken into account in multiple regression; usually it is an arbitrary decision (Vallejo-Córdoba and Nakai, 1994). In this case, the first 10 components that accounted for 86.96% of the variance have been considered (see Table 2) to obtain satisfactory results in the regression model built by stepwise linear regression analysis. Table 3 shows the model and the statistical parameters. The estimated standard error for the proposed prediction model (n = 32) was 0.008, 0.0059 being the average of the residual absolute values; the obtained error is equivalent to 11% of the average browning slope value.

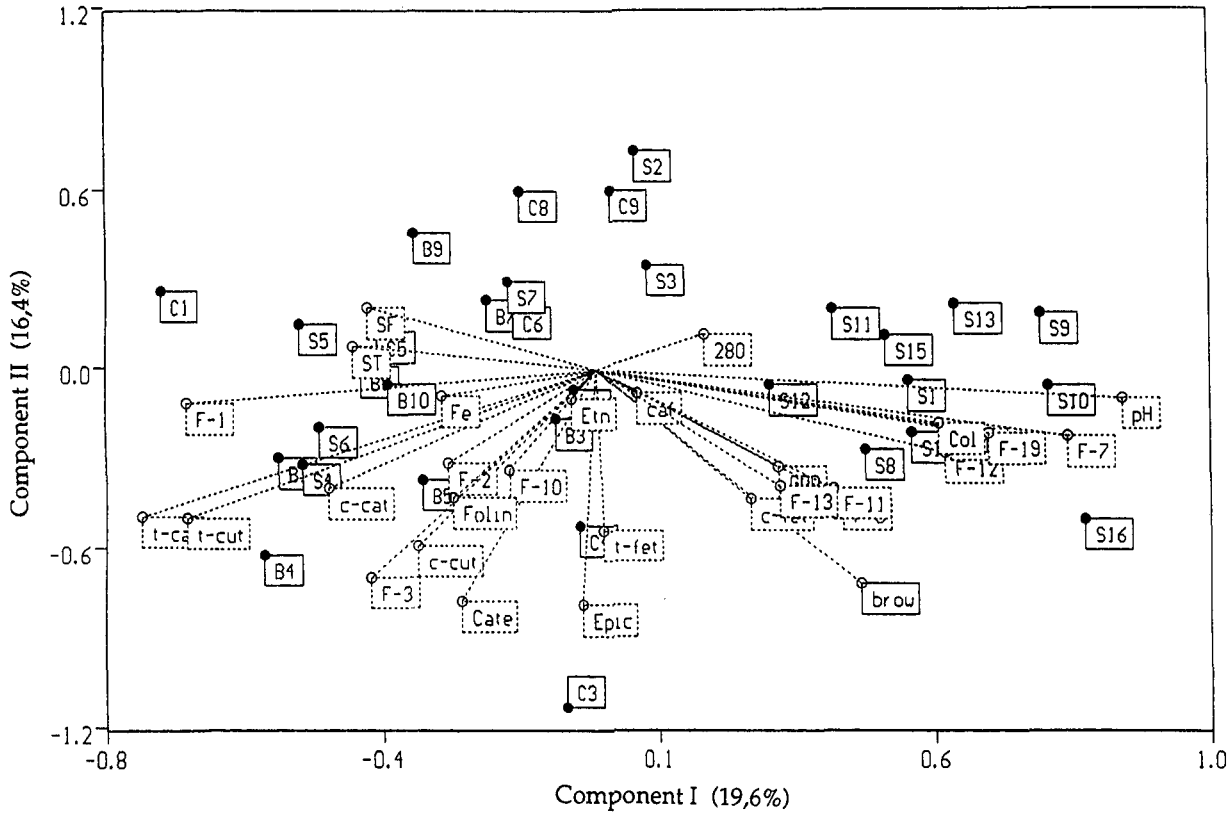

Figure 2. Principal component analysis of 32 white wines.

Table 2. Eigenvalues, Proportion of Variance Explained, and Cumulative Percent of Total Variance Explained in PCA

principal component	eigenvalue	proportion of variance explained	cumulative % of total variance
CP1	5.88	19.60	19.60
CP2	4.91	16.37	35.96
CP3	2.88	9.62	45.59
CP4	2.70	8.99	54.58
CP5	2.50	8.33	62.91
CP6	1.94	6.46	69.38
CP7	1.53	5.09	74.47
CP8	1.47	4.90	79.37
CP9	1.23	4.10	83.47
CP10	1.04	3.48	86.96

Table 3. Regression Model and Statistical Parameters for the Browning Prediction

$$y\ (\text{browning slope}) = 0.054 - 0.011CP_1 - 0.016CP_2 + 0.036CP_3 - 0.007CP_4 + 0.014CP_5 + 0.006CP_6 - 0.016CP_7 - 0.027CP_8 + 0.06CP_{10}$$

Statistical Parameters			
R	R^2	R^2 adj	test F
0.925	0.855	0.796	14.46

The validation of the model is the final step to build the model. The sample set was divided into two subsets. The first, called the model-building data set, was used to develop the model, and the second set, called the validation of prediction data set, was used to evaluate the predictive ability of the selected model. The obtained residuals for the group of wines included in the validation of prediction data set were never higher than 0.014, having values around 0.009, showing that the prediction capacity of the model was consistent.

Table 4 shows the browning slope values predicted by the model, the experimental values, and the residual values (difference between the estimated and experimental values). A normalized distribution of residual values (data not shown) has been done to make sure that the assumed normality and linearity were correct. Figure 3 shows residual values plotted against estimated values; it can be seen that residual values of wines are distributed at both sides of the zero value at

Table 4. Browning Slope Values Predicted by the Model, the Experimental Values, and the Residual Values (Data Expressed as UA/Day)

white wine	label	predicted value	exptl value	residual value
Coop. Borja 1	B2	0.058	0.063	0.005
Coop. Borja 2	B3	0.061	0.063	0.002
Coop. Borja 3	B4	0.064	0.071	0.007
Ainzón 1	B5	0.061	0.054	−0.007
Coop. Ainzón	B7	0.052	0.040	−0.012
Coop. Fuendejalón	B8	0.030	0.035	0.005
Coop. Pozuelo	B9	0.023	0.021	−0.002
Coop. Tabuenca	B10	0.024	0.034	0.010
COVINCA1	C1	0.024	0.025	0.001
COVINCA 3	C3	0.082	0.092	0.010
San Valero 1	C4	0.074	0.075	0.001
San Valero 2	C5	0.030	0.035	0.005
San Valero 3	C6	0.044	0.040	−0.004
Paniza 1	C7	0.071	0.067	−0.004
Paniza 2	C8	0.035	0.036	0.001
Paniza 3	C9	0.036	0.028	−0.008
COVISA 1	S1	0.076	0.076	0
COVISA 2	S2	0.043	0.050	0.007
COVISA 3	S3	0.059	0.057	−0.002
Pirineos1	S4	0.057	0.045	−0.012
Pirineos 2	S5	0.050	0.040	−0.010
Pirineos 3	S6	0.080	0.065	−0.015
Enate1	S7	0.042	0.034	−0.008
Enate 2	S8	0.067	0.063	−0.004
COVISA 4	S9	0.051	0.046	−0.005
COVISA 5	S10	0.061	0.072	0.011
COVISA 6	S11	0.052	0.051	−0.001
COVISA 7	S12	0.061	0.056	−0.005
COVISA 8	S13	0.063	0.059	−0.004
COVISA 9	S14	0.077	0.081	0.004
COVISA 10	S15	0.067	0.060	−0.007
COVISA 11	S16	0.056	0.068	0.012

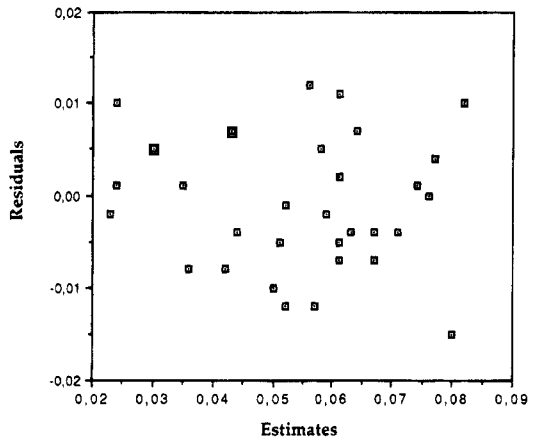

Figure 3. Relationship between residual and estimated values for the browning prediction regression model.

random for all of the estimated browning values. This indicates that errors are independent. The graph of the normal function also showed that the assumption was correct.

The role played by certain groups of phenolic compounds, as well as pH, temperature, available oxygen, etc. in wine oxidation is sufficiently recognized in the literature (Singleton, 1987; Cheynier et al., 1989; Cilliers and Singleton, 1990). However, only after a wine has gone through the accelerated oxidation test (Singleton and Kramling, 1976; Simpson, 1982) is it possible to know its capacity for oxidation. Without having the wines go through an oxidation test to determine browning, the browning of white wines can be predicted by the mathematical model previously described, which makes it possible to determine the oxidative capacity of wine once the described analyses have been done.

The high correlation coefficient value, 0.925, of the mathematical function obtained shows that the compounds analyzed can explain a high percentage of the tendency of white wines from different varieties and origins to oxidize.

With this mathematical model we can predict white wine browning, and this is a first step in the prediction of life span for this type of wine.

Main Compounds in Browning Process. Principal components have been applied to avoid collinearity of variables. However, it is difficult to interpret the predictive and explanatory role of the variables in such complex functions. We can assess that the system has the needed information; therefore, it is convenient to study the variables individually. Simple regressions between browning and the rest of the compounds have been done. Table 5 shows the correlation values obtained.

Folin index and OD measurements at 280 nm are used to determine the total phenol content and often to indicate the browning potential of white wines. However, low correlation between browning and such indexes has been obtained; therefore, these indexes are not good indicators of the oxidation susceptibility of white wines. These results agree with those obtained by Simpson (1982). A better correlation between iron content and browning was expected. Several authors attributed to ferrous ions a catalytic role in oxidation reactions; however, a relation between iron content and browning has not been found (Table 5).

While free sulfur dioxide content presents a poor correlation with browning, total sulfur dioxide is inversely related to browning (negative regression slope); therefore, it is bound sulfur dioxide that delays the onset of oxidation. As could be expected, pH is another factor contributing to oxidation; high pH values accelerate wine oxidation.

Table 5. Simple Regressions between Browning and the Parameters Analyzed

parameter analyzed	correlation regression ($n = 32$)	significance (p)
optical density at 280 nm	0.028	0.8802
Folin index	0.122	0.5532
color	0.419	0.0333
pH	0.503	0.0034
$SO_{2\ total}$	0.409	0.0392
$SO_{2\ free}$	0.266	0.1884
Fe	0.007	0.9727
catechin	0.610	0.0002
epicatechin	0.567	0.0007
F-1	0.255	0.1587
F-2	0.010	0.9587
F-3	0.377	0.0575
F-6	0.343	0.0548
F-10	0.276	0.1719
F-11	0.303	0.1324
F-13	0.066	0.7526
F-19	0.489	0.0045
cis-caftaric	0.197	0.2807
trans-caftaric	0.028	0.8800
trans-coutaric	0.012	0.9470
trans-fertaric	0.203	0.2649
caffeic acid	0.060	0.7439
GRP	0.219	0.2292

All derivatives of hydroxycinamic acids, GRP included, show low correlation to browning for the 32 wines studied. This result indicates that these compounds do not play a main role in chemical oxidation of wines. They do not initiate the oxidation process of wines even though these compounds may intervene during oxidative polymerization with other phenolic compounds.

The good correlation found between browning and some flavanols, mainly catechin and epicatechin (Table 5), shows that these compounds have an important role in browning and therefore in wine oxidation. Among the wines studied, those with a low polyphenolic content showed a lesser browning capacity than those with a higher one. From a browning point of view, it seems necessary to obtain wines with low content in the polyphenols previously mentioned, limiting in this manner the formation of quinones that can yield brown pigments. We believe the elimination of part of these compounds should be done before fermentation and not on the finished wine since this could alter its organoleptic characteristics.

In conclusion, it can be stated that these compounds together with pH and total sulfur dioxide play an important part in the prediction function obtained.

LITERATURE CITED

Amerine, M. A.; Berg, H. W.; Kunkee, R. E.; Ough, C. S.; Singleton, V. L.; Webb, A. D. *The Technology of Winemaker*, 4th ed.; AVI Publishing: Westport, CT, 1982; 533 pp.

Baranowski, J. D.; Nagel, C. W. Isolation and identification of the hydroxycinnamic acid derivatives in white Riesling wine. *Am. J. Enol. Vitic.* **1981**, *32*, 5–13.

Cheynier, V.; Rigaud, J.; Souquet, J. M.; Barillère, J. M.; Moutounet, M. Effect of pomace contant and hyperoxidation on the phenolic composition and quality of Grenache and Chardonnay wines. *Am. J. Enol. Vitic.* **1989**, *40*, 36–42.

Cilliers, J. L.; Singleton, V. L. Nonenzymic autoxidative reactions of caffeic in wine. *Am. J. Enol. Vitic.* **1990**, *41*, 84–86.

Fernández, P. Thesis, Universidad de Zaragoza, 1994.
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.
Rigaud, J.; Cheynier, V.; Souquet, J. M.; Moutounet, M. Mechanisms of oxydation of polyphenols in Mouts Blancs. *Technique* **1990**, No. *124*, 27–31.
Salagoïty-Auguste, M. H.; Bertrand, A. Wine phenolics—analysis of low molecualr weight components by high performance liquid chromatography. *J. Sci. Food Agric.* **1984**, *35*, 1241–1247.
Simpson, R. F. Factors affecting oxidative browning of white wine. *Vitis* **1982**, *21* (3), 233–239.
Singleton, V. L. Oxygen with phenols and related reactions in must, wines, and model system: observations and practical implications. *Am. J. Enol. Vitic.* **1987**, *38* (1), 69–77.
Singleton, V. L.; Kramling, T. E. Browning of white wine and an accelerated test for browning capacity. *Am. J. Enol. Vitic.* **1976**, *27*, 157–160.
Singleton, V. L.; Trousdale, E.; Zaya, J. Oxidation of wines. I. Young white wines periodically exposed to air. *Am. J. Enol. Vitic.* **1979**, *30* (1), 49–54.
Singleton, V. L.; Zaya, J.; Trousdale, E.; Salgues, M. Caftaric acid in grapes and conversion to a reaction product during processing. *Vitis* **1984**, *23*, 113–120.
Vaimakis, V.; Roussis, I. G. Must oxygenation and polyphenoloxidase inhibition and the oxidation of white wine. *Lebensm. Wiss. Technol.* **1993**, *26*, 133–137.
Vallejo-Córdoba, B.; Nakai, S. Keeping-quality assessment of pasteurized milk by mutivariate analysis of dinamic headspace gas chormatographic data. 1. Shelf-life prediction by principal component regression. *J. Agric. Food Chem.* **1994**, *42*, 988–993.

Received for review January 24, 1995. Revised manuscript received June 8, 1995. Accepted July 24, 1995.[⊗] This research was funded by the Spanish CICYT (Project ALI 92-0672).

JF950056Q

[⊗] Abstract published in *Advance ACS Abstracts,* October 1, 1995.