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Resistance to Oxidation of White Wines Assessed by Voltammetric Means

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This work concerns the development of a methodology suited to measure the resistance to oxidation of white wines by cyclic voltammetry. The voltammetric responses of several white wines of different origin and age were analyzed in the oxidation potential range (0.2−1.2 V vs SCE). Currents measured at fixed potentials were correlated to the concentration of ascorbic acid, SO_2, and total phenolics. A forced degradation study was monitored by cyclic voltammetry; from plots of current versus time, the consumption rates of oxidizable species in wine were estimated.

KEYWORDS: Cyclic voltammetry; resistance to oxidation; white wines

INTRODUCTION

Oxygen plays a paramount role in white wine sensory quality; in fact, the redox phenomenon is active during various stages of wine production, namely, prefermentation processing, fermentation, and wine aging (*1*). The determination of the antioxidant capacity, related to the rate of oxygen uptake, is also a major concern in the prevention of the precious and random oxidation phenomena. The incidence of this rapid onset of degradation could be related with the presence of phenylacetaldehyde, 3-(methylthio)propionaldehyde (methional), and 3-hydroxy-4,5-dimethyl-2(*H*)-furanone (sotolon). It was also demonstrated that the synergistic effects of increasing temperature and O_2 at lower pH increase greatly their rate of formation (*2*).

Because aromatic degradation occurs before chromatic degradation, also designated by browning, the winemaking industry seeks early indications to estimate the oxidation resistance of wine (*3*).

Wine antioxidant capacity can be related to the presence of both endogenous and exogenous substances, namely, some phenolic compounds for the first group and sulfur dioxide (SO_2) and ascorbic acid (AA) for the second. The balance between wine composition and oxygen exposure determines the so-called "resistance to oxidation", which dictates the wine shelf life, according to consumer expectations on a sensory basis. On the other hand, oxygen management requires greater information on the amount of dissolved oxygen, on the permeability of the container, and on the kinetics of its reduction in alcoholic solutions.

Molecular oxygen is not solely responsible for the flavor and aromatic deterioration of the wine. Radical species, such as the superoxide radical, which further gives rise to other radicals and oxygen species (produced in the chain reaction, where Fe^{2+} and Cu^{+} act as electron pumps transferring electrons to the oxygen molecule), contribute much more to wine degradation than molecular oxygen (*4*). The reactivity of radical species produced during oxygen reduction increases dramatically; thus, the end product of this cascade hydroxyl radical, produced by Fenton mechanism, is most effective in flavor deterioration of the wine, because it reacts with organic substances in a nonselective manner (*4*).

The quantification of specific antioxidants such as phenolic compounds and ascorbic acid has been carried out by different methods, including spectrophotometry (*5, 6*), chromatography (HPLC) (*5–7*), and electrochemistry (*5, 6, 8–10*). However, as the role of each antioxidant is not well established, this information does not allow estimation of the wine resistance to oxidation. Alternatively, quantification of the total oxidizable compounds present in wine has been proposed by coulometry determination (*11*). A potentiometric titration was proposed to measure the "first line of defense" of white wine against aroma spoilage (*12*). In these two last methods, oxidizable materials are quantified altogether independently of their antioxidant power.

In this work, voltammograms of wines acquired between 0.2 and 1.2 V versus SCE are analyzed to estimate their oxidation resistance. The current measured at two potentials is analyzed to obtain both quantitative and qualitative information concerning antioxidants present in the medium. Currents are examined

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Table 1. Reference Codes of the Wines Analyzed by Voltammetry and Some Characterization Parameters

wine code	age (years)	$[SO_2]_{free}$ (mg/L)	$[SO_2]_{total}$ (mg/L)	Folin−Ciocalteu index	A_{420nm}	[AA] (mg/L)
PMU	2	29	163	6.8	0.084	8.9
BOR	2	32	165	7.2	0.086	8.2
RSV	4	14	95	6.4	0.180	6.7
RVD	7	18	97	7.5	0.361	9.6
FPI	7	13	29	6.4	0.360	4.6
VRG	9	21	114	8.3	0.307	11.0
QCR	9	8	102	5.4	0.137	6.0
ENC	10	6	68	5.0	0.151	4.6
PLA	14	8	85	6.4	0.354	8.2

considering wines of different ages and during a forced degradation study.

MATERIALS AND METHODS

Chemicals. All of the chemicals employed were of analytical reagent grade and were used as received: sodium metabisulfite (Merck), ethanol (Merck), L-tartaric acid (Merck), NaOH (Merck), iodine (Merck), dichorophenolindophenol (Merck), oxalic acid (Fluka), H_2SO_4 (Fluka), and L-ascorbic acid (Merck).

Wine Material and Antioxidant Solutions. A 2-year-old white wine, from the Douro region in Portugal, was used for the forced degradation. Nine white wines from several Portuguese wine regions and from different vintages (between 2 and 14 years old) were used in voltammetric characterization. Winemaking procedures depended on the producers. **Table 1** presents some characterization parameters of these wines as well as their reference codes.

To simulate a wine matrix, a model wine solution consisting of 12% ethanol (v/v), 0.033 M tartaric acid, and pH 3.2 (adjusted with NaOH solution) was prepared (*8*). In the experiments conducted to identify the position of the antioxidants (SO_2 and AA), in the voltammogram of wine, the antioxidants were directly added to a solution of the model wine and the wine sample to prevent dilution effect.

Preparation of Wine Samples for the Forced Degradation Study. A volume of 2000 mL of white wine (2-year-old vintage, pH 2.92) was first divided into two portions of 1000 mL each. One portion was supplemented with oxygen by air bubbling (20:80; O_2/N_2) (Gasin) close to the saturation level, that is, 7.2 mg/L. The second portion, not treated with oxygen (control sample), contained 0.9 mg/L O_2. Subsequently, the saturated portion was divided into two 500 mL Shott flasks (Durant, Germany) sealed with screw-caps made of PBT, complete with a PTFE-protected seal, to withstand up to 180 °C hot-air sterilization, GL45 (Durant, Germany); one was stored at 15 °C and the other at 45 °C. The control sample was divided into 10 portions of 100 mL into headspace flasks (headspace < 5 mL) sealed with 20 mm crimp caps with septa of silicone/PTFE (Varian). Half of the control samples were stored at 15 °C and the leftover at 45 °C. The wine samples and control samples were analyzed by cyclic voltammetry throughout the forced degradation experiment. At each sampling time, 50 mL of wine was withdrawn from the 500 mL Shott flasks for analysis while the remaining volume was resaturated with oxygen. At each sampling time the wine sample headspace suffered an increment corresponding to the volume withdrawn. For the control samples, the content of a flask was used at each sampling time.

Sample Characterization. The oxygen concentration was evaluated by means of the YSI 5000 220 V oxygen meter (Yellow Springs Instruments), using the YSI 5010 Bod probe with a dissolved oxygen accuracy of ±0.1 mg/L or ±2% of reading. pH measurements were performed using a Cyberscan 510 pH-meter, with a glass electrode (Hanna, HI 1131). The free sulfur dioxide was determined according to the Ripper method (*13*), and AA was quantified through a potentiometric titration with dichlorophenolindophenol (*14*). The total polyphenolics was evaluated by the Folin−Ciocalteu index used in the Folin−Ciocalteu procedure (*13*).

Voltammetry. Cyclic voltammetry experiments were performed using a potentiostat (Autolab type PGSTAT30, Ecochemie) controlled by GPES 4.9 software provided by Ecochemie. Voltammograms were obtained in the oxidation range of potentials (between ca. 0 and 1.3 V) at a scan rate of 100 mV/s using a 3 mm glassy carbon disk electrode (BAS M-2012) working electrode. The electrode surface was cleaned between runs by polishing; otherwise, a slight current decrease was observed between sequential scans. This electrode fouling effect was similar for young and aged wines. The polishing was performed with 3 μm alumina powder (PK-4 polishing kit) for 2 min between scans. The polishing time was established as the minimum time for which the current data did not differ by >5%. The saturated calomel electrode (SCE) was used as reference electrode in conjunction with a platinum counter electrode. Current data were obtained from the average of three to five determinations, and the presented uncertainty corresponds to the standard deviation. Wine and model wine samples were analyzed directly without the addition of supporting electrolyte.

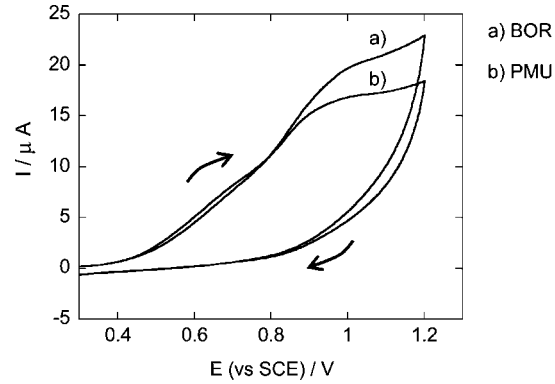

Figure 1. Cyclic voltammograms of two 2-year-old wines acquired using a 3 mm glassy carbon disk electrode.

Oxygen in wine samples did not interfere with the voltammetric signals, as similar currents were obtained (13.6 ± 0.6 μA at 0.7 V or 20.1 ± 0.7 μA at 1.0 V) in wine samples containing different oxygen concentrations (0.25–6.65 mg/L). Therefore, deoxygenation was not carried out in wine samples or model wine solutions prior to voltammogram recording.

RESULTS AND DISCUSSION

Voltammetric Characterization of the Wine Samples. The voltammetric characterization of white wines in the range of the oxidation potentials was carried out in wines of different origin and age. In **Figure 1**, voltammograms from two 2-year-old wine samples are presented. These voltammograms display, in the direct scan of potential, two broad peaks or bands resulting from the electrochemical oxidation of the wine components that occur at potentials lower than that of the media (water and ethanol) oxidation, which starts at approximately 1.2 V. The two wines present similar current responses at the lower potentials, defining a band close to 0.7 V, indicating that the amount of charge used to oxidize the most easily oxidizable compounds in both wines is comparable. This means that both wines contain similar concentrations of these readily oxidizable components. For the range of higher potentials both wines display a band close to 1.0 V, with different magnitudes, showing that the concentrations of the substances oxidized at these potentials are different in the two wines.

Ascorbic acid (AA) and SO_2 are the two most important antioxidants commonly used in wine. The antioxidant power of these substances is assigned to their prompt reaction with oxygen, preventing the oxidation of the compounds responsible for the flavor and aroma of wine. To identify the contribution of these two antioxidants in a white wine voltammogram, additions of sodium metabisulfite and AA were made. **Figure 2A** displays the voltammograms obtained from a model wine solution (solid line) and the same model wine solution supplied

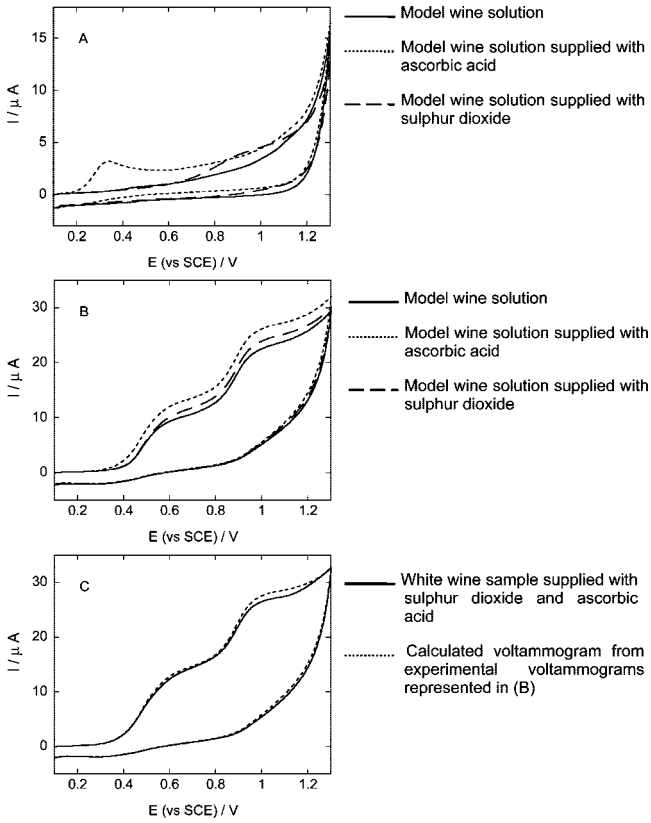

Figure 2. (**A**) Cyclic voltammograms of a model wine solution at pH 3.2 and a model wine solution supplied with 20 mg/L of free sulfur dioxide or with 25 mg/L of ascorbic acid. (**B**) Cyclic voltammograms of a white wine sample and a white wine sample supplied with 20 mg/L of free sulfur dioxide or with 25 mg/L of ascorbic acid. (**C**) Comparison of the experimental cyclic voltammogram of a white wine sample, supplied with 20 mg/L of free sulfur dioxide and 25 mg/L of ascorbic acid, with a calculated voltammogram from the experimental voltammograms represented in (**B**): dashed line + dotted line − solid line.

with sodium metabisulfite corresponding to 20 mg/L of free sulfur dioxide (dashed line) or with 25 mg/L of AA (dotted line). **Figure 2B** displays the voltammogram obtained from a sample of wine (solid line) and the same sample of wine supplied with sodium metabisulfite (dashed line) or AA (dotted line) in the same concentrations as in **Figure 2A**. The voltammetric oxidation of SO_2 in the model wine solution displays an irreversible process wave shaped at about 0.9 V versus SCE (dashed line in **Figure 2A**). The addition of the same concentration of SO_2 to a wine sample originated a current enhancement both at 0.7 V and at 1.0 V (**Figure 2B**). The AA oxidation potential in the model wine solution is about 0.35 V versus SCE (dotted line in **Figure 2A**), and the addition of the same concentration of AA leads to the current increase of both bands, as shown in **Figure 2B**. The variation of AA oxidation potential can be mainly due to a matrix composition effect, namely, the pH. Whereas the model wine solution was adjusted to pH 3.2, the wine sample pH was not adjusted and displayed pH 2.7 after the addition of AA.

Figure 2C presents the voltammogram obtained from the sample of wine supplied with 20 mg/L of free sulfur dioxide and 25 mg/L of ascorbic acid to a sample of wine (solid line) and the calculated voltammogram (dotted line) that simulates the contribution of both antioxidants from their individual voltammetric response in the same sample of wine. The calculated voltammogram was obtained as described in the legend of **Figure 2**. As the two curves in **Figure 2C** are similar, within experimental error, the additive nature of the voltammetric currents of SO_2 and AA oxidation is confirmed.

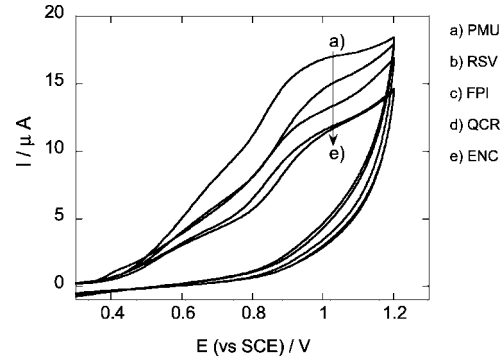

Figure 3. Cyclic voltammograms of white wines of different ages: (a) 2 years; (b) 4 years; (c) 7 years; (d) 9 years; (e) 10 years.

Analysis of Wines of Different Ages. The voltammetric responses from white wines of different ages are presented in **Figure 3**. The voltammograms display a similar pattern concerning the presence of two bands that are similar in terms of position, although the current magnitudes are different at certain potentials. As the shape of the whole voltammogram is the same, it can be pointed out that the nature of the oxidizable compounds is similar in terms of their oxidability. It can also be also remarked that current tends to be lower for the older wine voltammograms, indicating that the concentrations of the wine oxidizable components are lower. This observation is compatible with the aging effect, associated with the oxygen action, which leads to a decrease of the concentration of oxidizable compounds.

The correlations between the voltammetric current, at the potential of the two band maxima, and some quality indicators related to the oxidative resistance of wine were evaluated considering white wines. In **Figure 4A** it is shown that the current measured at 0.7 V followed a linear relationship with the concentration of AA (measured by potentiometric titration) with a correlation coefficient of 0.93. The unexplained variance can be related with noise on the quantification of AA as the oxidizing agent employed may also react with other wine constituents with lower oxidation potentials. Similar correlation ($R = 0.97$) is obtained for the representation of current at 1.0 V as a function of sulfur dioxide concentration (assessed by the Ripper method), as presented in **Figure 4B**. The Folin−Ciocalteu method is commonly employed as a reference method to compare resistance to oxidation in different matrices. This technique is affected by several interfering substances, such as sugars, aromatic amines, organic acids like ascorbic acid, or sulfur dioxide in wines (*15, 16*); thus, an overestimation on the Folin−Ciocalteu index is expected to be obtained in white wines. The correlation between current data and Folin−Ciocalteu index was calculated. The representation of current measured at both wave maxima versus the Folin−Ciocalteu index for the same set of wines is shown in **Figure 4C**. Although current from both bands displays linear correlations, a better fit is obtained for current acquired at 0.7 V, where the most powerful reducing agents of the wine emerge including AA, SO_2 to some extent, and polyphenolic compounds with a triphenol group on the flavonoid B-ring [e.g., flavonol myricetin, anthocyanin delphinidin ($E = 0.3$ V), and to some extent cathecol-containing polyphenolic compounds (*8*)].

Forced Aging Study. To check the capability of voltammetry to reveal the change in composition associated with the oxidative

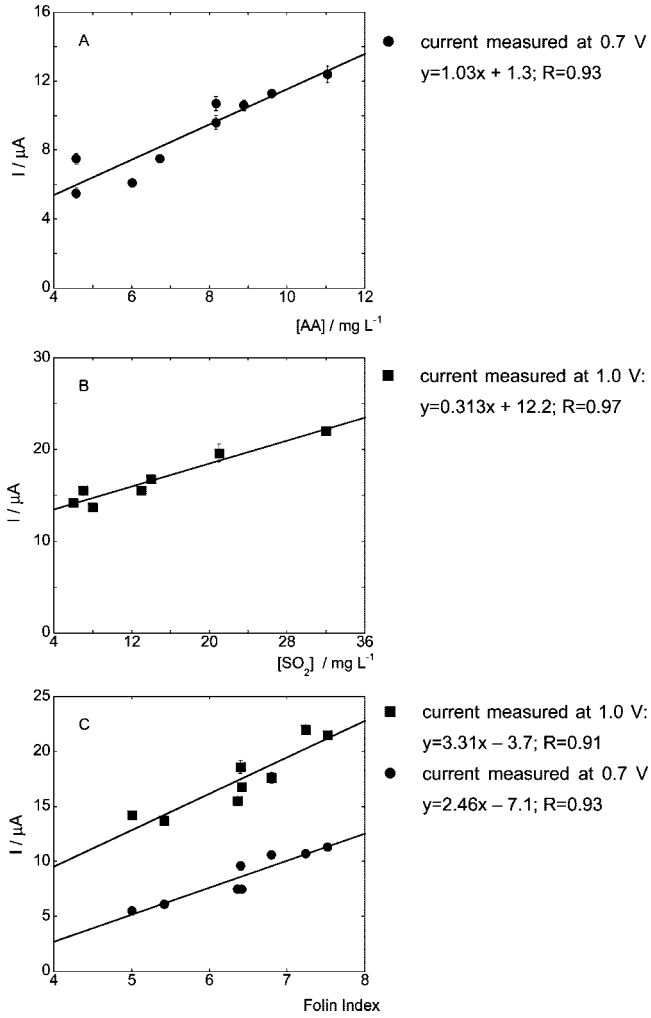

Figure 4. Representation of current versus ascorbic acid concentration (**A**) ($y = 1.03x + 1.3$; $R = 0.93$) or versus SO_2 concentration (**B**) ($y = 0.313x + 12.2$; $R = 0.97$) or versus the Folin index (**C**).

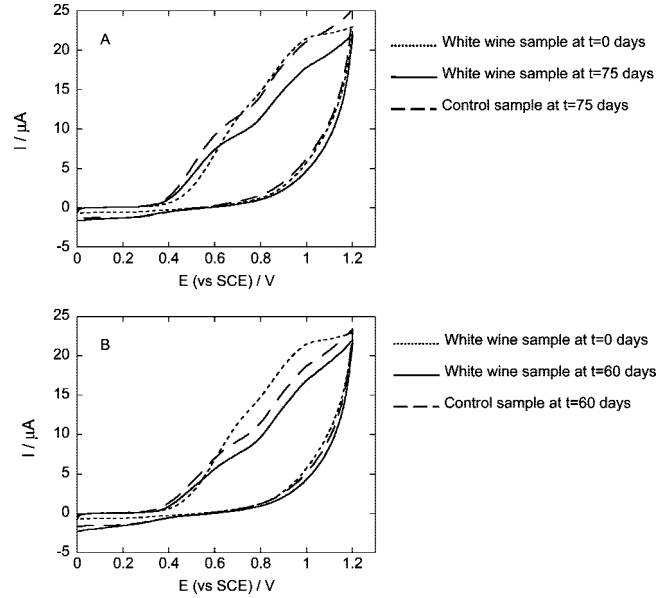

Figure 5. Cyclic voltammograms of wine samples submitted to accelerated spoilage: (**A**) $T = 15$ °C; (**B**) $T = 45$ °C.

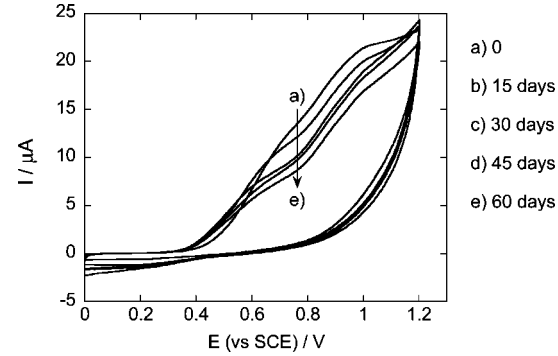

Figure 6. Cyclic voltammograms of wine samples submitted to accelerated spoilage at $T = 45$ °C acquired along time.

degradation of wine, a study was performed in a wine submitted to an accelerated spoilage process. Two samples of wine saturated with oxygen were stored at 15 and 45 °C and analyzed over several weeks. The corresponding control experiments were performed on individual samples stored at the same temperatures, as described under Materials and Methods. In **Figure 5** two sets of voltammograms illustrate the effect of the accelerated spoilage. The dotted voltammograms were obtained from the wine sample at $t = 0$, that is, immediately after the wine bottles were opened. The dashed voltammograms were acquired from the control samples after 75 days, for storage at $T = 15$ °C (**Figure 5A**), or after 60 days, for storage at $T = 45$ °C (**Figure 5B**). The solid line voltammograms correspond to O_2-saturated samples at the same storage temperature and sampling times. The accelerated spoilage, conducted at both temperatures, promoted a modification of the wine composition that could be sensed by voltammetry. The composition alteration was followed by a current decrease for potentials larger than 0.6 V (compare the dotted line with the solid line voltammograms). The current decrease is more important for the degradation conducted at the higher temperature. Simultaneously, a slight current increase is displayed at potentials lower than 0.6 V. This current increase can be due to the presence of readily oxidizable dimers or other larger structures formed by oxidative polymerization of polyphenolic compounds (*17–19*). The voltammogram recorded from the control sample at 45 °C also reveals a composition change. Although O_2 was not supplied for this control sample, the presence of oxygen both dissolved and in the headspace, at this higher temperature, could promote a noticeable consumption of antioxidants in samples. The voltammogram recorded from the control sample at the lower storage temperature displays a current increase at potentials lower than 0.6 V. As stated above, this current increase can be due to the oxidation of larger phenolic structures formed from the recombination of oxidized polyphenols.

The set of voltammograms obtained over time in the forced degradation experiment conducted at 45 °C is displayed in **Figure 6**. A continuous current variation is observed in the whole voltammogram. The most noticeable trend is a gradual current decrease at both band potentials, indicating a concentration decrease of the species oxidized at these potential ranges. Simultaneously, a slight current increase is displayed at potentials lower than 0.6 V for the voltammogram recorded at $t = 15$ days, associated with the oxidation of easily oxidizable phenolic compounds formed during the forced aging process (*17–19*). Current in these regions tends to decrease at subsequent sampling times. The analysis of the current decrease performed at the two potentials, 0.7 and 1.0 V, corresponding to the two wave maxima is presented in **Figure 7**. In each plot, current data from the wine samples (solid symbols) are compared to control samples (open symbols) at both potentials. Current data measured at 0.7 and 1.0 V are represented by circles

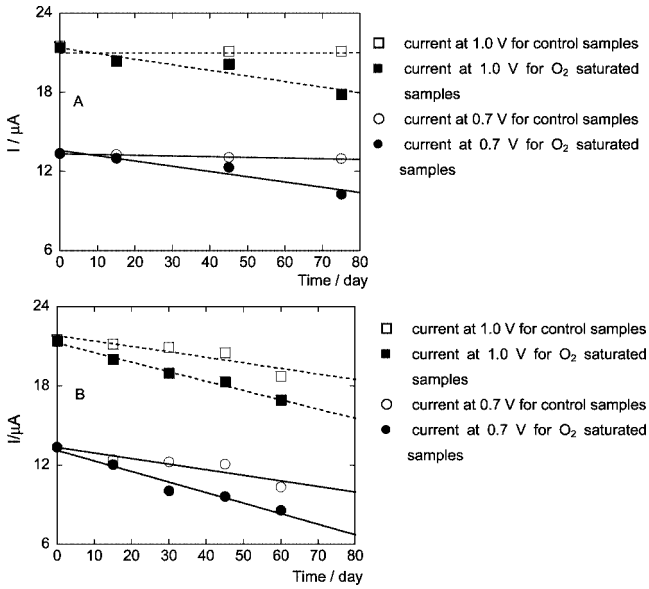

Figure 7. Variation of the voltammetric current in degradation spoilage experiments conducted at (**A**) 15 °C and (**B**) 45 °C.

Table 2. Parameters of the Straight Lines, Represented in Figure 7, Used To Describe the Current Variations in the Accelerated Degradation Experiment

T/°C	*E*/V	control samples	O_2-saturated samples
15	0.7	$y = 13.6 - 0.006x$, $R = 0.98$	$y = 13.6 - 0.040x$, $R = 0.96$
	1.0	$y = 21.0 + 0.0003x$, $R = 0.021$	$y = 21.4 - 0.043x$, $R = 0.95$
45	0.7	$y = 13.4 - 0.042x$, $R = 0.92$	$y = 13.1 - 0.080x$, $R = 0.98$
	1.0	$y = 21.8 - 0.042x$, $R = 0.90$	$y = 21.3 - 0.071x$, $R = 0.994$

and squares, respectively. The current follows a linear trend during the storage time, decreasing more rapidly for the O_2-saturated samples, regardless of the potential selected for the current measurement. Nevertheless, no oxygen was intentionally added to the control samples, and a continuous current decrease was observed for the control samples kept at 45 °C, whereas those kept at 15 °C did not show any appreciable current change. The current decrease in the control samples results from the partial oxidation of oxidizable species. This reaction could be promoted by the dissolved oxygen (0.9 mg/L) and present in the headspace (<5 mL; 20:80; O_2/N_2). Although adequate flasks and seals (described under Materials and Methods) were used, the possibility that there was some O_2 uptake through the seals cannot be discounted, as the permeability of the flask seals was not evaluated. As the evaluation of the O_2 uptake was not attempted, the correlation between the O_2 consumption and the voltammetric current decrease was not established.

The equations of the straight lines used to describe the current variations are presented in **Table 2**. The correlation coefficients obtained for the O_2-saturated and control samples were good, $R > 0.9$, except that obtained for the control samples kept at 15 °C, when current was measured at 1.0 V ($R = 0.021$). This low correlation coefficient indicates that the two variables are not correlated, which means that there was no significant variation of current throughout the degradation experiment for control samples kept at 15 °C. The slopes of the straight lines from the control samples are independent of the selected potential for the current measurement and are null for the control samples kept at 15 °C. For the O_2-saturated samples, the slopes are also independent of the potential selected for the current measurement. The slope of the current versus time corresponds to the rate of current decrease and is an estimate of the consumption rate of oxidizable species. These assessed rates, for the higher degradation temperature, were twice those of the lower temperature and were independent of the potential used for the current measurement (0.7 and 1.0 V).

Future works will concern the exploitation of all information contained in the whole voltammograms. This information in conjunction with the chemical characterization of a great number of wines could be utilized for the prediction of white wine shelf life.

LITERATURE CITED

(1) Oliveira, C. L.; Silva Ferreira, A. C.; Guedes de Pinho, P.; Hogg, T. Development of a potentiometric method in order to measure the resistance to oxidation of white wines and the antioxidant power of their constituents. *J. Agric. Food Chem.* **2002**, *50*, 2121–2124.

(2) Silva Ferreira, A. C.; Guedes de Pinho, P.; Rodrigues, P.; Hogg, T. Kinetics of oxidative degradation of white wines and how they are affected by selected technological parameters. *J. Agric. Food Chem.* **2002**, *50*, 5919–5924.

(3) Gilis, M.; Duriliat, H.; Comtat, M. Contribution of a zero current potential measurement to wine making. *Biotechnol. Tech.* **1999**, *13*, 195–199.

(4) Laurie, V. F.; Waterhouse, A. L. Oxidation of glycerol in the presence of hydrogen peroxide and iron in model solutions and wine. Potential effects on wine colour. *J. Agric. Food Chem.* **2005**, *54*, 4668–4673.

(5) Roginsky, V.; Lissi, E. A. Review of methods to determine chain-breaking antioxidant activity in food. *Food Chem.* **2005**, *92*, 235–254.

(6) Beer, D. D.; Harbertsone, J. F.; Kilmartin, P. A.; Roginsky, V.; Barsukova, T.; Adams, D. O.; Waterhouse, A. L. Phenolics: Aa comparison of diverse analytical methods. *Am. J. Enol. Vitic.* **2004**, *55*, 389–400.

(7) Vrhovsek, U.; Mattivi, F.; Waterhouse, A. L. Analysis of red wine phenolics: comparison of HPLC and spectrophotometric methods. *Vitis* **2001**, *40*, 87–91.

(8) Kilmartin, P. A.; Zou, H.; Waterhouse, A. L. A cyclic voltammetry method suitable for characterizing antioxidant properties of wine and wine phenolics. *J. Agric. Food Chem.* **2001**, *49*, 1957–1965.

(9) Huang, T.; Goa, P.; Hageman, M. J. Rapid screening of antioxidants in pharmaceutical formulation development using cyclic voltammetry − potential and limitations. *Curr. Drug Discovery Technol.* **2004**, *1*, 173–179.

(10) Bradshaw, M. P.; Prenzler, P. D.; Scollary, G. R. Square-wave voltammetric determination of hydrogen peroxide generated from the oxidation of ascorbic acid in a model wine based. *Electroanalysis* **2002**, *14*, 546–550.

(11) Alonso, A. M.; Dominguez, C.; Guillén, D. A.; Barroso, C. G. Determination of antioxidant power of red and white wines by a new electrochemical method and its correlation with poyphenolic content. *J. Agric. Food Chem.* **2002**, *50*, 3112–3115.

(12) Silva Ferreira, A. C.; Oliveira, C; Hogg, T.; Guedes de Pinho, P. Resistance to oxidation of white wines. Relationship between potentiometric measurements and substances responsible for aroma degradation. *J. Agric. Food Chem.* **2003**, *51*, 4668–4672.

(13) Ribereau-Gayon, J.; Peynaud, E.; Ribereau-Gayon, P.; Sudraud, P. *Traité d'Oenologie. Sciences et Techniques du Vin. Tome 1. Analyse et Contrôle des Vins*; Dunod: Paris, France, 1976.

(14) Iland, P.; Ewart, A.; Sitters, J. *Techniques for Chemical Analysis and Stability Tests of Grape Juice and Wine*; Patrick Iland Wind Promotions: South Australia, 1993.

(15) Box, J. D. Investigation of the Folin−Ciocalteu phenol reagent for the determination of polyphenolic substances in natural waters. *Water Res.* **1983**, *17*, 511–525.

(16) Peterson, G. L. Review of the Folin phenol protein quantitation method of Lowry, Rosebrough, Farr, and Randall. *Anal. Biochem.* **1979**, *18*, 201–220.

(17) Singleton, V. L. Oxygen with phenols and related reactions in musts, wines, and model systems: observations and practical implications. *Am. J. Enol. Vitic.* **1987**, *38*, 69–77.

(18) Tulyathan, V.; Boulton, R. B.; Singleton, V. L. Oxygen uptake by gallic acid as a model for similar reactions in wines. *J. Agric. Food Chem.* **1989**, *37*, 844–849.

(19) Cillierst, J. J. L.; Singleton, V. L. Characterization of the products of nonenzymic autoxidative phenolic reactions in a caffeic acid model system. *J. Agric. Food Chem.* **1991**, *39*, 1298–1303.

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