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Development of a Potentiometric Method To Measure the Resistance to Oxidation of White Wines and the Antioxidant Power of Their Constituents

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This work describes a new potentiometric method to evaluate the resistance to oxidation of white wines. Reduction and oxidation titrations were made, and coefficient of variation obtained were 10.87 and 2.65%, respectively. The antioxidant powers of ascorbic acid (Aas) and sulfur dioxide (SO_2) were evaluated by this method, SO_2 proving to be much less active in this respect than ascorbic acid. The two agents did not demonstrate any antioxidant synergy. A relationship between oxygen present and ascorbic acid was found by the proposed method (1 mmol of $O_2 \leftrightarrow$ 0.84 mmol of Aas). This method enables the distinction of different wines on the basis of their resistance to oxidation.

KEYWORDS: Resistance to oxidation; white wines; potentiometric titrations

INTRODUCTION

In the wine-making process redox phenomena are responsible for profound modifications leading to alterations principally in chemical wine color and aroma. These mechanisms are active during prefermentation processing, fermentation itself, and various configurations of aging that a wine might suffer.

The aromatic degradation associated with oxidative spoilage is largely caused by these redox mechanisms (*1*). A wine's resistance to oxidation is a function of three main parameters: redox potential, the total concentration of native or added antioxidants, and the amount of dissolved oxygen. Different methods are available in the literature to measure the resistance to oxidation in different matrices. They can be divided into two groups: those based on free radical formation (*2*−*7*) and on electrochemical methods (*8*−*11*).

It is important to note that the measured antioxidant power of specific molecules, reported in the literature, presents differences in their relative strengths, depending on the method used. Furthermore, it has been demonstrated, using data available in the literature, that even when the same methodology is used, the ranking of molecules by their relative antioxidant power is substantially different (*10*). The small repeatability described by some authors, the complexity of the matrix that is proposed to work, the type of response for each method, and the great cost associated with the described tests justify the development of a new method.

In the present study, a potentiometric titration is used to measure the resistance to oxidation of white wines. The antioxidant power of molecules present in wines is also estimated using the same methodology.

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The concentration of dissolved oxygen has a direct influence on the instantaneous potential (*8*), and this molecule is the principal agent of wine oxidation. Besides native wine antioxidants, additional antioxidants are used in wine-making, namely, ascorbic acid (Aas) and sulfur dioxide (SO_2). The latter is ubiquitous in wine-making, whereas the former is much less widespread. The antioxidant power of these two compounds is currently a source of some controversy. The antioxidant power of sulfur dioxide is frequently contested, and the utilization of ascorbic acid has some associated risks, being an oxidation promoter at high concentration (*12*). Moreover, at present, there is a concern in the food industry to reduce the maximum limits of SO_2. Thus, several wine-making suppliers have proposed products that combine these two compounds, to take advantage of a hypothetical "synergistic"effect.

MATERIALS AND METHODS

Method Approach. Potentiometric titrations, based on oxidation−reduction reactions, have many analytical applications in foodstuff. Oxidizing agents such as $KMnO_4$, K_2CrO_7, I_2, and dichlorophenolindophenol (PIP) are used as titrants to obtain redox titration curves with a single potentiometric end-point. Of these, PIP is employed to provide a standard oxidation challenge in a selective potentiometric measurement of ascorbic acid, when a specific sample treatment is applied (*13*).

It has been observed, during our experiments with potentiometric titrations in white wines, that it was possible to obtain also a titration curve with a single potentiometric end-point. That result led to the assumption on which this work based: that all redox species present in wine could be approximate to a single redox behavior.

The instantaneous potential is the equilibrium potential between both the oxidized and reduced fractions of a wine composition. In the determination of the oxidized and reduced fractions, titrant additions, in the method proposed, begin with a volume of reductant followed by a volume of oxidant. Thus, after the first titration, all species present

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in wine are likely to be found in the reduced form, with a final potential at a constant value close to −400 mV. On the other hand, in the second titration, assuming that all reactions in the first titration are reversible, these species will revert to their oxidized form, with a final potential at a constant value close to +400 mV.

The titrants chosen were trichlorotitanium (TCT) as reductant and PIP as oxidant, their choice being based on the above-mentioned assumption and other published work (*8*, *14*). The titration sequence (reduction/oxidation) was established to ensure the end-point detection for the oxidation titration in all samples.

A nonselective electrode (Pt) measures the potential (*E*), and the equivalent volumes ($V_{red.}$ and V_{oxi}), estimated after titration, are a measure of the oxidized and reduced fractions, respectively. Therefore, the equivalent volume of PIP is a measure of the resistance to oxidation (ROX) of a wine.

Potentiometric Titration. Fifty milliliters of white wine was titrated with 7 mL of a reductant solution (1 mL of 15% $TiCl_3$ in 100 mL of 1 M HCl), followed by an oxidation titration with a 0.05% (w/v) PIP solution. The titrant concentrations employed were determined, by experimentation, the selection criteria being to obtain repeatable curves.

A combined platinum electrode with a reference system, Ag/AgCl in a KCl reference electrolyte (Mettler-Toledo), followed these two titrations in an N_2 atmosphere (100 mL/min). The titrations were made by an automatic system Titralab (Radiometer).

The titration function criteria are as follows: (i) (for the reduction titration) stop point −400 mV, maximum volume 7.00 mL, increments 0.15 mL, stability 0.80 mV/s; (ii) (for the oxidation titration) stop point 400 mV, maximum volume to determine, increments 0.15 mL, stability 0.80 mV/s.

The reduction titration is stopped after the addition of excess reductant (7 mL), and the oxidation titration is stopped after complete oxidation of the entire reduced fraction (total reduced fraction of wine plus the reductant excess).

Repeatability. The repeatability study was obtained by repetitive analysis of seven samples of the same wine. The coefficients of variation (CV) obtained for the reduction and oxidation are, respectively, 10.87 and 2.65%. The higher precision obtained for oxidation is explained by a better reading of the equivalent volume, related to titrant concentration.

Sample Preparation (Effect of Dissolved Oxygen and Antioxidant Additions). *Effect of Dissolved Oxygen.* A volume of white wine with 1.1 mg/L of dissolved oxygen was divided into five parts. Four parts were separately charged with oxygen to values of, respectively, 2.01, 2.95, 4.25, and 4.70 mg/L. The concentration of dissolved oxygen was determined using an oxygen meter, WTW 340. Wines were titrated, and the equivalent volumes of reduction and oxidation titration were determined.

Antioxidant Additions. To evaluate the effect of ascorbic acid on the titration curves, different additions to the same white wine were made giving, respectively, 25, 50, 75, 125, and 150 mg/L of Aas, in the final solution. Wines were titrated and equivalent volumes determined. For sulfur dioxide, sodium metabisulfite additions were made, to the same white wine, giving values of free SO_2 of 0, 5, 15, 20, and 30 mg/L, in the final solution. Samples were left overnight and then titrated. To evaluate a possible "synergistic" effect between these two antioxidants, the following experiment was performed: a white wine was divided into six parts, a blank and five reference samples with (i) 20 mg/L of free SO_2, (ii) 25 mg/L of Aas, (iii) 25 mg/L of Aas + 20 mg/L of free SO_2, (iv) 50 mg/L of Aas, and (v) 50 mg/L of Aas + 20 mg/L of free SO_2. The free sulfur dioxide was determined according to the Ripper method (*13*). All samples were titrated using the potentiometric method above.

White Wines. Seven samples of white wine of different ages (1, 2, 3, 4, 8, 9, and 10 years old) were evaluated by direct titration, without dilution.

Sulfate Determination. Sulfate were determined by an HPLC, ion exchange Dionex 4500i using a condutimetric detector *(15)*.

Potential determination (*E*) was performed by direct reading of the combined platinum electrode and the **ascorbic acid** concentration by potentiometric titration (*13*).

Amount of Total Phenolics. The amount of total phenolic was determined according to the Folin−Ciocalteu procedure (*16*).

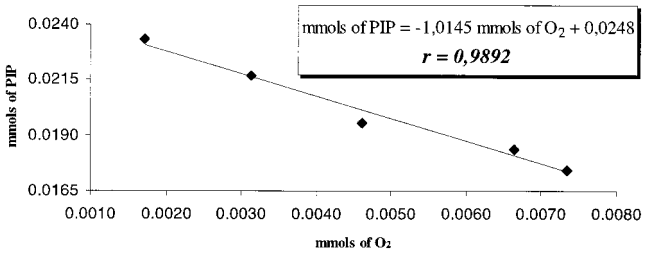

Figure 1. Scatter diagram of molar relationship between dissolved oxygen with equivalents of PIP.

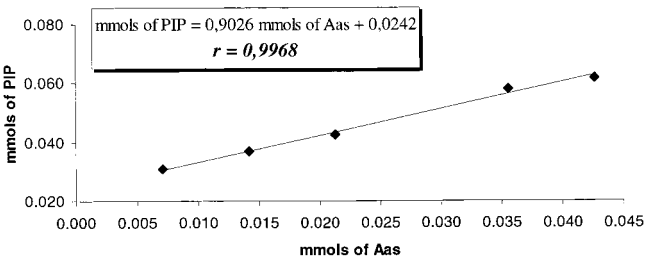

Figure 2. Scatter diagram of molar relationship between ascorbic acid with equivalents of PIP.

RESULTS AND DISCUSSION

Effect of Dissolved Oxygen and Ascorbic Acid Additions. **Figure 1** represents the scatter diagram of the molar relationship between dissolved oxygen and equivalents of PIP. The *X*-axis represents the millimoles of dissolved oxygen, and the *Y*-axis represents the consumed millimoles of PIP caused by dissolved oxygen increments. **Figure 2** represents the scatter diagram of the molar relationship between ascorbic acid and equivalents of PIP. The *X*-axis represents the millimoles of ascorbic acid, and the *Y*-axis represents the consumed millimoles of PIP caused by Aas additions. The results over the range of dissolved oxygen and ascorbic acid values described under Sample Preparation were evaluated by the consumption of PIP in the potentiometric titration.

There is a direct correlation between the dissolved oxygen in a wine and the consumed volume of PIP ($r = 0.9892$) with a slope of −1.015 (**Figure 1**). Thus, for white wine, 1 mmol of PIP is required to react with 1 mmol of dissolved oxygen (competition).

To know the molar relationship between consumed PIP and the oxygen consumed by a wine stored at 15 °C for 60 days, reduction and oxidation titration curves were evaluated at $t =$ 0 and 60 days, and the dissolved oxygen was measured at these times. Results obtained have demonstrated that a volume of 1.65 mL (2.59E−3 mmol) of PIP is necessary to titrate a wine sample that had consumed 1.69 mg/L (2.64E−3 mmol) of oxygen. These results indicate that PIP and oxygen are oxidants of similar strengths. Further work is needed to better explore to what extent PIP could be used as a measure of the quantitative oxygen that a wine could consume before aromatic degradation occurred.

Ascorbic acid has a direct influence on the consumption of PIP ($r = 0.9968$), and this variation is well adjusted with a linear model. The oxidant power of PIP and the antioxidant power of ascorbic acid are similar with a slope of 0.903 (**Figure 2**). The molar relationship calculated in this experiment for both molecules is (mmol of PIP/mmol of Aas) = 0.903. Relating this value with that obtained for the oxidation with oxygen experiment, to oxidize 1 mmol of ascorbic acid ∼1 mmol of oxygen will be necessary (1 mmol of $O_2 \Leftrightarrow$ 0.84 mmol of Aas).

Effect of SO_2 Addition. Small variations in PIP consumption were observed, after SO_2 additions to samples, as described

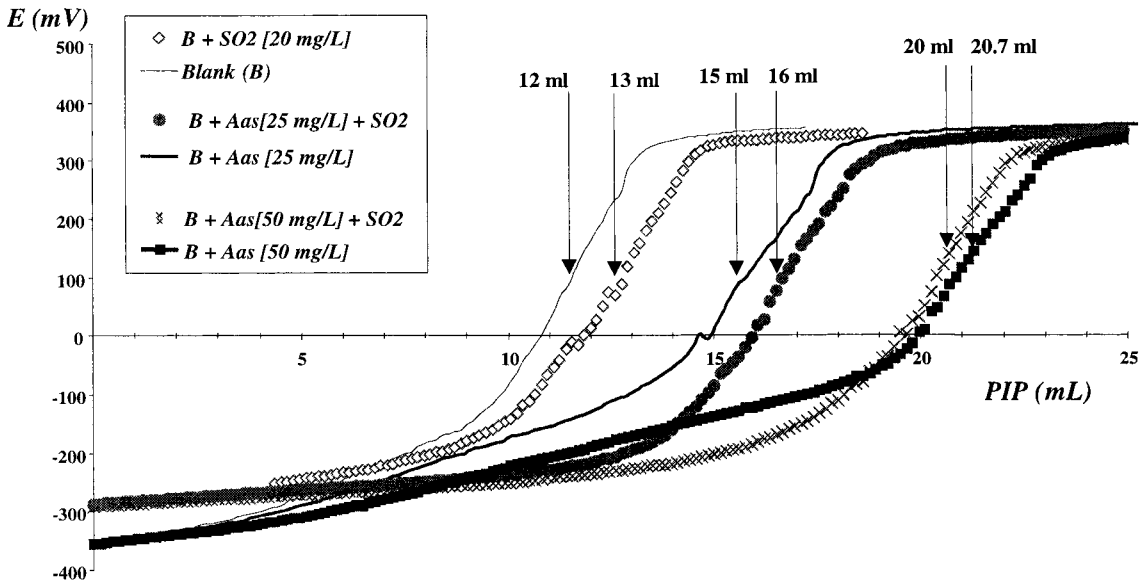

Figure 3. Synergistic effect of ascorbic acid + SO_2.

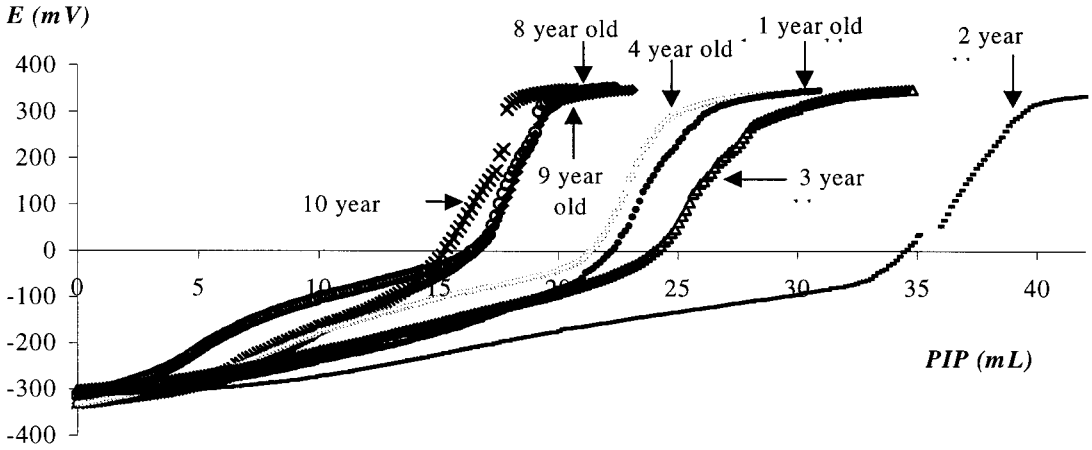

Figure 4. Oxidation curves for the evaluation of the resistance to oxidation of white wines.

Table 1. Wine Sample Parameters

	age of wine						
	10 years	9 years	8 years	4 years	3 years	2 years	1 year
PIP consumption (mL)	15.30	16.80	17.25	21.30	24.45	34.65	22.65
dissolved O_2 (mg/L)	1.0	1.5	1.0	0.9	0.9	1.0	1.3
E (mV)	160	156	163	145	125	136	138
free SO_2 (mg/L)	6.4	6.4	6.4	9.6	16.0	28.8	6.4
combined SO_2 (mg/L)	35.2	38.4	48.0	38.4	48.0	60.8	64.0
sulfates (g/L)	0.20	0.16	0.21	0.20	0.19	0.18	0.18
Folin–Ciocalteu index	6.14	5.80	6.56	8.30	6.66	6.14	6.06
ascorbic acid (mg/L)	3.32	4.29	2.74	5.29	21.20	89.50	15.66

above. Statistical treatment of results showed that the consumption of PIP was 12.60 ± 0.35 mL, and the CV (2.79%) is near the value calculated in the repeatability (2.65%). These results seem to indicate that SO_2, for the quantities usually employed in wine-making, does not have a big impact on the resistance to oxidation of a wine. These results are in agreement with observations reported in the literature (*7*).

Synergistic Effect (SO_2 and Ascorbic Acid). Oxidation curves (PIP additions) for the six samples described under Sample Preparation are shown in **Figure 3**.

Increasing additions of ascorbic acid (25 and 50 mg/L) lead to a significant increase in PIP consumption, 3.0 and 8.7 mL, respectively.

Addition of SO_2 to 20 mg/L (free form) leads to an increase of 1 mL in PIP consumed. The difference between oxidant consumption in blank and 20 mg/L samples is 7.69%. This value is higher than the CV of the method (2.65%) (**Figure 3**).

The increase in PIP consumption by these two antioxidants together is 1 mL for the combination 25 mg/L Aas + 20 mg/L free SO_2 (6.25% greater than 25 mg/L Aas sample). For the combination 50 mg/L Aas + 20 mg/L free SO_2 no variations were found in relation with the 50 mg/L sample of Aas (**Figure 3**). These results show clearly the stronger antioxidant power of ascorbic acid over sulfur dioxide. Furthermore, the possible synergistic antioxidant effect between these two molecules was

not observed. Nevertheless, more experiments need to be made to verify this statement.

Resistance to Oxidation of White Wines. Oxidation curves for the evaluation of oxidation resistance of the seven white wines are represented in **Figure 4**, and **Table 1** lists the wine sample parameters.

PIP consumption for each wine is shown in **Table 1**. These samples can be grouped in three groups as shown in **Figure 4**. In the first group (from the left) are the oldest wines (10, 9, and 8 years old). The 10-year-old wine was the one that consumed less oxidant and was, therefore, the more easily oxidizable. These three wines also have the higher redox potential and the lower antioxidant concentrations (**Table 1**). The second group is made up of the 4-, 1-, and 3-year-old wines, in that order. Finally, the wine of 2 years of age, despite not being the youngest wine, is the one that consumed the highest quantities of PIP (34.65 mL). This result is in agreement with the data of **Table 1,** where this wine is shown to have the higher antioxidant concentrations (89.50 mg/L Aas; 28.8 mg/L free SO_2) and a low redox potential (E = 136 mV).

The application of this new method allowed the discrimination and ordering by resistance to oxidation of the seven wines.

SUMMARY

During this work a potentiometric titration method was developed to quantify the resistance to oxidation (ROX) of white wines. The coefficients of variation (CV) obtained for the reduction and oxidation titrations are, respectively, 10.87 and 2.65%.

This method enables the distinction of wines from different regions and ages on the basis of their ROX capacities.

There is a straight correlation between the amount of dissolved oxygen in wine and the consumed volume of PIP (r = 0.9892). One millimole of PIP is required to react with 1 mmol of dissolved oxygen.

The antioxidant power of sulfur dioxide is small, compared with that of ascorbic acid, and no antioxidant synergistic effect was observed between these two antioxidants for the quantities currently employed in wine-making.

The molar relationship between PIP and Aas is 1 mmol of PIP $\Leftrightarrow$ 0.90 mmol of Aas and, relating this relationship with that obtained for the oxygen experiment, 1 mmol of $O_2 \Leftrightarrow$ 0.84 mmol of Aas.

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