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Redox Chemistry of Red Wine. Quantification by an Oscillating Reaction of the Overall Antioxidant Power as a Function of the Temperature

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The redox and acid–base reactivity of red wines was studied from both the analytical and kinetic standpoint. Four homemade wines, made from Italian red grape varieties of two different vintages, were tested to study the effect of temperature (25 and 37 °C) on the overall antioxidant power, through the Briggs–Rauscher oscillating reaction. The reaction was monitored by potentiometry (platinum electrode) and by direct chronometric detection. A reference scale based on the response of gallic acid was also employed, so as to achieve a quantitative evaluation: the novel Briggs–Rauscher antioxidant index (BRAI) was developed to express the overall antioxidant power quantitatively versus the chosen standard molecule. Overall antioxidant power was found to be related to total polyphenol content measured using the Folin–Ciocalteu method: the older wines had a lower antioxidant ability. Total acidity was also estimated indirectly by means of coupled pH-metric/photometric titrations and visible spectrophotometric measurements; it revealed an overlap between acid–base and redox chemistry of red wine.

KEYWORDS: Antioxidant power measurement; redox chemistry; red wine; polyphenols; gallic acid; color-acidity relation; free radicals

INTRODUCTION

Free radicals are naturally occurring species in living organisms and can affect important biological and biochemical functions such as immunity, inflammation, growth, and repair. They can have negative effects in biological systems when they damage proteins, lipids, and nucleic acids but are normally held in balance by antioxidant defense mechanisms. However, environmental injuries, infection, chemical toxins, smoking, radiation, and sunlight can promote further free-radical formation. Nutrition is recognized as fundamental for protection against free radicals, because it can affect the redox status of plasma, thus inhibiting those chemical processes leading to degenerative diseases (*1*) related to the oxidation of basic molecules. Oxidation of lipids and other target molecules can be reduced by the action of various molecules present in protective foodstuffs, such as tea, fruit juices, fresh vegetables, and wines. Phenolic compounds are the commonest phytochemicals in both fruit and vegetables; they contribute greatly to both chemical and sensorial characteristics and can act as exogenous antioxidant agents, protecting human health. The present study investigated the overall antioxidant power of red wines, which is known mainly to be related to their polyphenol content (*2–7*). No other beverage has attracted the attention of modern medicine like red wine: its preventive role against coronary diseases has recently been demonstrated (*8–10*).

Various methods have been used to measure antioxidant activity; the differences among these lie in (a) the nature of the radical in the synthetic environment of the chemical test, (b) the detection technique, (c) the standard molecule versus which the result is expressed on a comparative scale. Commonly used testing methods for antioxidant ability are the TEAC (trolox equivalent antioxidant capacity) method (*11*) and the ORAC (oxygen radical absorbance capacity) method (*12*). A method has also been optimized to study clinical aspects related to low-density lipoprotein oxidation in blood (*13*). Other methods are based on β-carotene/linoleic acid (*14*) and on linoleic acid (*15*). For wine and other beverages such as beer, EPR investigations (*16*) and luminiscence assay (*17*) have also been employed. Furthermore, a method based on the oscillating reaction of Briggs–Rauscher (*18*) has recently been proposed for measuring the antioxidant ability of various molecules (*19*) and food (*20–22*).

Information related to the polyphenol equipment of red wines was obtained in this study through two types of chemical test. First, the Briggs–Rauscher oscillating reaction was used to measure the antioxidant ability of four homemade wines made from Italian grape varieties of two vintages, 1999 and 2000. This method allows the antioxidant ability of a fluid to be estimated in a free-radical-rich chemical environment created using the hydroperoxyl radical $HOO^{\bullet}$, mimicking a condition of oxidative cellular stress (hydrogen peroxide is a common and abundant precursor of free radicals). Each diluted red wine was placed in such an environment to estimate its scavenger

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capability. When antioxidant free-radical scavengers are added to an oscillating Briggs−Rauscher mixture, there is a break of the oscillating regime; the corresponding so-called "inhibition time" varies linearly with the concentration of the antioxidant added.

Because measurement of antioxidant capability is linked to a kinetic response, we chose to monitor the reaction both potentiometrically (platinum electrode) and by direct chronometric detection. The second method has been shown to be fast, reproducible, and accurate, and we believe it would be suitable for rapid screening. A reference scale based on the response of gallic acid, a molecule naturally present in red wines [as verified by means of HPLC analysis (*23*)], was also produced, applying the same experimental conditions as those employed for the wines, so as to obtain a quantitative response based on a comparison between the kinetic behavior of the standard molecule and that of the wine under investigation.

The temperature coefficient of the estimator of interest is negative (*19*); hence, the effect of temperature was studied to achieve an accurate investigation at 25 and 37 °C (physiological value).

The influence of aging of a wine on its overall antioxidant power was also studied, because wines were available that were neither artificially stabilized nor fortified with antioxidant compounds.

Total polyphenols were determined using the Folin−Ciocalteu method, and the correlation with overall antioxidant capability was examined. Other redox active substances, namely, sulfur dioxide and ascorbic acid, were quantified to extend the study.

Finally, many safety and health aspects of food are related to their measurable acid−base and redox properties. In a previous paper, we investigated the acid−base status of red wines (*23*), while their antioxidant ability is the subject of the present study, which also examined the link between acid−base and redox fields of reactivity. Because redox properties are mainly related to the pigments, the total acidity parameter of each wine was estimated using a coupled pH-metric and visible spectrophotometric measurement, plotting A_λ versus $V_{titrant}$.

MATERIALS AND METHODS

Chemicals. Folin−Ciocalteu reagent, ethanol, hydrogen peroxide (30%, w/w), malonic acid (>99%), and gallic acid (>98%) were from Fluka. Salts, KCl and KIO_3, were from Merck, and $MnSO_4$ was from Carlo Erba. The analytical kit for the determination of ascorbic acid was from R-Biopharm. Starch was from AnalaR (BDH). Standard NaOH, KOH, and HCl solutions were prepared by diluting Fluka concentrated products and standardized against potassium hydrogenphthalate (Fluka, puriss.) or sodium carbonate (Fluka, puriss.), respectively. Iodide volumetric solution was prepared by diluting AnalaR (BDH) concentrated products. All solutions were prepared using grade A glassware and deionized plus twice-distilled water.

Sample Storage. Four red wines produced in Piedmont (North-West Italy) known as Grignolino and Barbera were studied. Two vintages were considered for each wine, i.e., Grignolino 1999 and 2000 (henceforth, G99 and G00) and Barbera 1999 and 2000 (henceforth, B99 and B00). The wines were stored at room temperature in the dark and were subdivided into small glass bottles (250 mL capacity) to avoid air contact and other contamination.

The Briggs−Rauscher Reaction. The Briggs−Rauscher (henceforth, BR) oscillating reaction (*19*) was employed to measure the overall antioxidant ability of each red wine. The ioduration of the malonic acid was conducted in the presence of hydrogen peroxide, as indicated by Briggs and Rauscher (*18*). Hydrogen peroxide must always be maintained in excess, so that the inhibition time measured may be fully ascribed to the wine added to the oscillating mixture. Starch paste is added as a visual indicator for direct chronometric detection, because oscillations appear as the sequence colorless → yellow → blue. Starting with a freshly prepared BR mixture, at the second oscillation to blue, the diluted wine or gallic acid solution is added. The inhibition time corresponds to the interval between the second and third blue oscillation (chronometer measurement) or to the absence of oscillating regime of the potential (recorded potentiometrically with a Pt electrode).

Table 1. Experimental Details of the Briggs–Rauscher (BR) Oscillating Reaction[a]

reagent	stock solution	V (mL) for BR mixture	BR mixture initial composition
starch	3% w/v	1.00	0.1%
deionized and bidistilled water		2.00	
H_2O_2	30% w/w	10.0	5.87 N
malonic acid	0.3 M	5.0	0.050 M
IO_3^-/H_2SO_4	0.2 M/0.077 M	10.0	0.0667 M/0.0266 M
Mn^{2+}	0.04 M	2.00	0.002 67 M
gallic acid/wine dilution		0.30	*b*

[a] Reagents are listed according to the order of addition. [b] See text for details in the *Overall Antioxidant Power at 25 and 37 °C: The Novel Briggs–Rauscher Antioxidant Index (BRAI)* paragraph.

Experimental details are provided in **Table 1**.

Finally, the pH of the Briggs−Rauscher test environment is very acidic, which is compatible with the natural conditions of acidity of the product under examination.

Kinetic Measurements. The kinetic response was monitored potentiometrically (with a platinum electrode, see "Potentiometric Apparatus") as well as by direct chronometric detection. A chronometer (Oregon Scientific, model SL888T) was used to record the kinetic trend, with the agreement between potentiometric and chronometric measurements of inhibition time being found to be very close. Temperature control, at 25 or 37 °C, was achieved by means of circulation of water around the reaction vessel, from a thermo cryostat (model DI-G Haake). Each reactant was maintained at a controlled temperature before and during the experiment. Each measurement of inhibition time (both wine and reference standard molecule) was repeated at least 3 times using potentiometric and chronometric devices.

Potentiometric Apparatus. The pH-metric measurements were carried out at $T = 25 \pm 0.1$ °C with a Metrohm 713 potentiometer equipped with a combined glass electrode (Metrohm model 6.0204.100). The titrant was dispensed with a 765 Dosimat buret from Metrohm (minimum volume deliverable of ± 0.001 cm^3). The combined glass electrode was calibrated in $-\log[H^+]$ units (pH) employing alkalimetric titrations of hydrochloric acid with standard, carbonate-free potassium hydroxide at ionic strength $I = 0.05$ M (KCl) with C_2H_5OH at 12% level (see explanations in ref *23*). The alkalimetric titrations were carried out in a stream of purified nitrogen gently bubbled in the titration cell to avoid O_2 and CO_2 contamination. Temperature control was achieved as described in the previous section. Each titration was repeated at least twice.

A combined platinum electrode [Metrohm model 6.0402.100 (LE)] was used to monitor potentiometrically the kinetic trend of the oscillating reaction, together with a Metrohm automatic computer-assisted potentiometric apparatus (Basic Titrino 794).

Spectrophotometric Apparatus. The visible spectrophotometric determinations were carried out with a Jasco V-550 UV−vis double-beam spectrophotometer (optical path length of 1.000 cm). As for the coupled measurements with the alkalimetric titration of wines, the examined solution [wine diluted in a 0.05 M (KCl) water/ethanol solution] was transferred from the potentiometric to the optical cell using a peristaltic pump to record visible spectra as a function of the pH value of the solution, greatly reducing equilibrium inconveniences.

Sulfur Dioxide Determination. Free (mainly as HSO_3^-) and combined (bisulfate adducts, Bertagnini reaction) SO_2 had been measured previously (*23*) by titration with standard I_2 solution on the distillate obtained for the volatile acidity determination.

Ascorbic Acid Determination. L-Ascorbic acid was determined by means of an enzymatic (enzyme: ascorbic oxidase) reaction (37 °C,

Table 2. Total Polyphenols Concentration and BRAI for Each Red Wine under Investigation

wine	total polyphenols[a]	BRAI (25 °C)[b]	BRAI (37 °C)[b]
G99	185.3	974[c]	9942[d]
G00	213.4	1121	9494
B99	141.2	834	5885
B00	191.7	921	8928

[a] Concentration expressed as GAE per 100 mL of wine. [b] Overall antioxidant power (BRAI has been defined per 100 mL of wine). [c] Uncertainty on BRAI at 25 °C ranges between 10.7 and 12.6%. [d] Uncertainty on BRAI at 37 °C ranges between 4.9 and 7.8%.

pH 3.5) using a double-beam spectrophotometer (578 nm). A differential colorimetric measurement was made between a cuvette in which only the ascorbic acid is specifically oxidized by the ascorbic oxidase (the sample blank) and another cuvette in which all of the substances of the sample able to be reduced are reduced thanks to MTT [(3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide)] and PMS (5-methylphenazinium methosulfate). A commercial kit (see Chemicals) was employed.

Data Analysis and Calculations. Standard software was used to perform the linear regression analysis of the experimental data and to locate the end point of the alkalimetric titrations. The nonlinear, least-squares computer program ESAB2M (*24*) was used to evaluate the purity of the reagents (starting from the acid−base titration data) and to refine all parameters related to the calibration of the electrode system.

RESULTS

Sulfur Dioxide. The results obtained for free and combined sulfur dioxide are as follow. Free SO_2 (mg/L): G99, 4.4; G00, 15.1; B99, 2.8; and B00, 5.4. Combined SO_2 (mg/L): G99, 15.9; G00, 7.7; B99, 6.1; and B00, 6.7.

Ascorbic Acid. The results obtained for ascorbic acid are (mg/L): G99, 5.0; G00, 23.6; B99, 2.2; and B00, 13.7.

Spectrophotometric Determinations of Total Polyphenols. The total polyphenol content was determined by means of the Folin−Ciocalteu method (*25*). Because dry wines containing very small amounts of SO_2 were tested, no correction factors were used (*25*). The quantitative result is expressed using gallic acid (gallic acid equivalent units, GAE) as the standard molecule for the calibration. The working curve was constructed at 752 nm, according to the position of the experimental maximum of absorbance recorded. The results are shown in **Table 2**.

Overall Antioxidant Power at 25 and 37 °C and the Novel Briggs−Rauscher Antioxidant Index (BRAI). The kinetic trend (Pt electrode) of the BR reaction with inhibition because of the different dilutions of the wine is shown in **Figure 1**. The inhibition times recorded from both potentiometric and chronometric sources were found to be very close (generally discrepancies were within ±1 s). The uncertainty rate on replicates (at least three) of the inhibition time was about 3% as RSD%. If necessary, analysis time can thus be reduced by eliminating instrumental recording, using a simple chronometer, which was found to provide accurate results. However, instrumental detection can easily be used, if preferred, in particular with a computer-assisted potentiometer that ensures automatic data acquisition.

The inhibition time was first measured on a standard molecule to construct a calibration curve. In this connection, gallic acid was chosen as a standard phenolic substance (benzoic series) to obtain a reference scale and moreover to simplify a comparison with the total polyphenol content, expressed as units of GAE. The overall antioxidant power was then expressed by means of the novel BRAI per 100 mL of each wine at each temperature. A wide range of gallic acid concentrations in the BR mixture was examined: from 0.15 to 0.75 mg at 25 °C and from 0.25 to 4.50 mg at 37 °C. A volume of 0.30 mL of gallic acid aqueous solution was added to the freshly prepared BR mixture, starting with a stock solution of 20.0 mM and related dilutions in calibrated flasks. The diagram $t_{inhib.}$ versus $mass_{gallic\ acid}$ (milligrams of gallic acid added to the mixture) is shown in **Figure 2** at 25 and 37 °C. Two segments are easily located and can be modeled with a linear function; **Table 3** gives details of the linear functions of gallic acid.

An excellent linear trend was found for each diluted wine under investigation at both 25 and 37 °C (see **Figure 3**); **Table 3** gives the straight-line parameters for each wine. The relationship was linear over the following dilution ranges (v/v): from 5:100 to 9:100 at 25 °C and from 1:10 to 3.5:10 at 37 °C. A

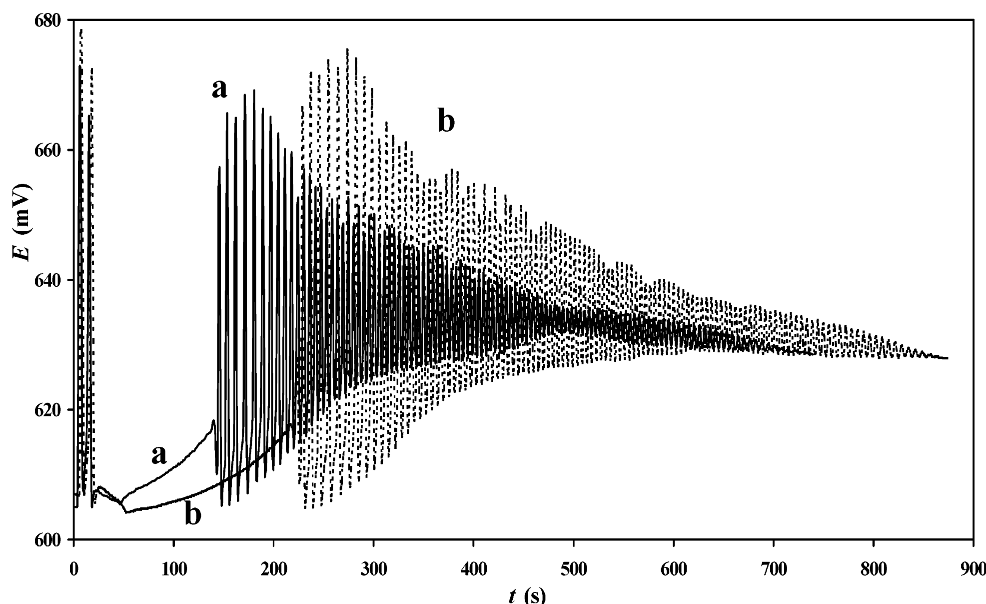

Figure 1. Kinetic trend by potentiometric measurement (Pt electrode) of the BR reaction with two different dilutions of G99 (T = 25 °C): (a) 10:100 (v/v) and (b) 14:100 (v/v). In the mixture, 0.30 mL of each dilution of wine was added. The inhibition time is clearly evidenced by the stop of the oscillating regime of E (mV).

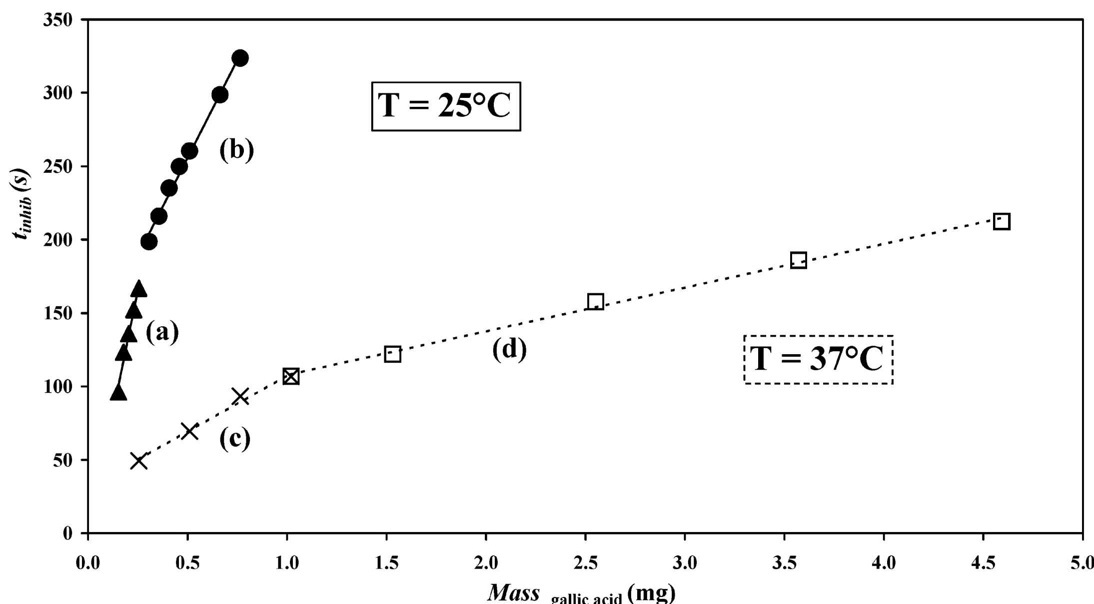

Figure 2. Calibration curve (BR reaction) with standard gallic acid: diagram $t_{inhib.}$ (s) versus $mass_{gallic\ acid}$ (mg) with linear fittings, at 25 °C [▲ and ●, calibration curves (a) and (b), see **Table 3**] and 37 °C [× and □, calibration curves (c) and (d), see **Table 3**]. A volume of 0.30 mL of gallic acid aqueous solution was added to the BR mixture freshly prepared, starting by a stock solution of 20.0 mM and related dilutions in calibrated flasks.

Table 3. Equation Parameters of the Straight Lines Optimized for Gallic Acid and Wines at 25 and 37 °C

		slope (*m*)	±*s*	intercept (*q*)	±*s*	*R*
T = 25 °C						
gallic acid	(a)	667.2	54.7	–1.3	11.3	0.9901
	(b)	265.9	10.1	122.8	5.2	0.9964
G99		6496.0	235.0	–62.5	5.3	0.9987
G00		7481.8	200.7	–61.7	4.6	0.9993
B99		5565.9	141.1	–43.7	3.2	0.9993
B00		6143.0	273.7	–53.6	6.23	0.9980
T = 37 °C						
gallic acid	(c)	76.9	5.7	30.7	4.0	0.9945
	(d)	29.9	1.0	77.7	3.0	0.9983
G99		2972.8	47.6	–48.2	3.0	0.9997
G00		2838.7	128.5	–48.4	8.9	0.9979
B99		1759.6	59.9	–36.3	5.0	0.9988
B00		2669.6	106.3	–42.6	7.4	0.9984

[a] Letters (a) and (b) are referred to Gallic acid at 25 °C, while letters (c) and (d) are referred to gallic acid at 37 °C (see **Figure 2**).

volume of 0.30 mL of each diluted wine was added to the BR mixture.

The BRAI is calculated as the ratio of the slopes of the kinetic straight-line equations obtained for each wine (slope = $t_{inhib.}/mL_{wine}$) and for the standard molecule (slope = $t_{inhib.}/mg_{gallic\ acid}$). To ensure compatibility of inhibition time amplitude between gallic acid and each of the wines, we used the straight-line equations for gallic acid at 25 °C (a) and 37 °C (d) (see **Figure 2**). The ratio of slopes thus obtained, $mg_{gallic\ acid}/mL_{wine}$, was finally referred to 100 mL of wine, which is the BRAI sought: "milligrams of gallic acid equivalent per 100 mL of wine". The results are in **Table 2**. Moreover, on the basis of the standard deviation (±*s*) of the gallic acid and wine slopes, we estimated the following mean uncertainty values for BRAI: 11.5% at 25 °C and 6.7% at 37 °C.

Color−Acidity Relation and Photometric Estimation of the Total Acidity. Polyphenols account for both the chromatic and the redox properties of red wine. The color of red wine and that of other beverages such as tea depends on its pH value. The relation between color and pH was investigated through coupled pH-metric and photometric measurements. Each wine was diluted 1:20 (v/v) to ensure the absence of precipitation during alkalimetric titration and to provide reasonable absorbance values. Prior to titration, the visible absorption spectra of each diluted wine sample was recorded to identify the best wavelength to build the photometric titration diagram. Parts a and b of **Figure 4** show visible absorption spectra with varying pH values for diluted Barbera and Grignolino. Two absorption maxima are present, at about 500 and 630 nm, in the visible spectrum of each red wine, and thus, a choice is necessary. The photometric/volumetric diagram obtained at 630 nm provides less accurate results, probably because of the low absorbance recorded in the first segment (acidic−neutral field). Hence, the fixed-wavelength working diagram was constructed using the absorbance at 500 nm (A_{500}). The natural color tends to be red-purple for Barbera and to be red-brick for Grignolino. Chromogens of the investigated red wines are strongly modified during alkalimetric titration: a blue-green color is obtained around the end point. Two colors were found to vary as a function of pH, showing significant changes in absorption features around the inflection point (pH ∼ 7.5) of the alkalimetric titration.

DISCUSSION

Meaning of BRAI as a Function of the Temperature. The inhibition time depends on the temperature: it is shorter at higher temperatures, in agreement with what was found by Cervellati et al. (*19*, *26*) for substances. The temperature here was found to be of the utmost importance in regard to the overall antioxidant power of red wine. To interpret the meaning of BRAI, we initially considered that, if the temperature is constant, the antioxidant power increases with increasing the index value. In fact, the inhibition time increases with increasing amounts of gallic acid, as is clear in **Figure 2**. Temperature variations were next considered: in terms of inhibition time, we recorded a clear decrease in antioxidant activity with increasing temperature, which is obviously due to the kinetic activity of the radicals formed in the test environment, which is higher at 37 °C. As a consequence, we found that the antioxidant activity

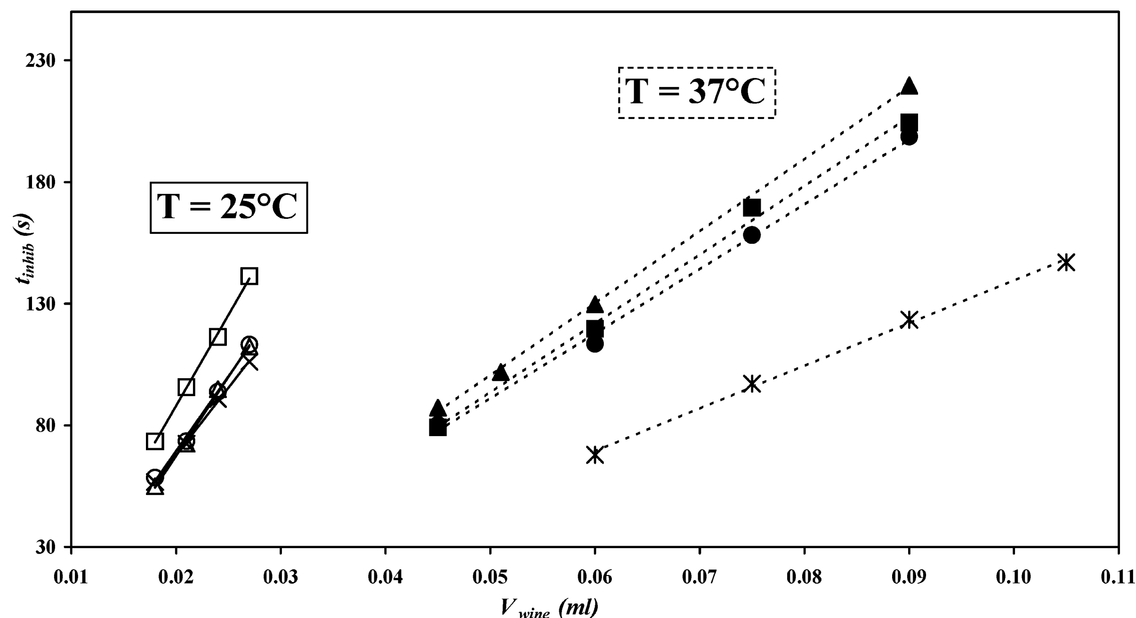

Figure 3. Kinetic test (BR reaction) of each wine [abscissa units of measurements indicate the real volume (mL) of each wine added]: diagram $t_{inhib.}$ (s) versus V_{wine} (mL) with linear fittings, at 25 °C (△, G99; □, G00; ×, B99; ○, B00) and 37 °C (▲, G99; ■, G00; *, B99; ●, B00).

decreased, because of the enhanced radical reactivity induced by the higher temperature. With the lower antioxidant activity at 37 °C, more gallic acid than is needed at 25 °C must be used to stop the radical reactivity, under equal inhibition time conditions. Thus, $BRAI_{37} > BRAI_{25}$ in all cases, correctly indicating (overall antioxidant power)$_{37}$ < (overall antioxidant power)$_{25}$. The direct experimental evidence is clear in **Figures 2** and **3**, which give the straight lines of gallic acid and wines, respectively. Thanks to the use of a standard molecule, the novel index is independent of inhibition time, which is not the real target of the experiment but only the quantity that is directly measurable.

Polyphenols and Overall Antioxidant Power. The availability of genuine homemade wines, even if there was only a few types and vintages, was fundamental for this study, because we could be certain that the products had neither been artificially stabilized (with physical or chemical methods) to prevent precipitation of tannins or inorganic salts nor supplemented with antioxidant compounds (natural or synthetic). Thus, only the effects of natural redox-active substances have to be considered. This enabled us to investigate the correlation between total polyphenol concentration, overall antioxidant power, and aging of the wine, without interference from other species or treatments (inhibitors of precipitation, etc.).

As shown in **Table 2**, the higher the polyphenol content, the higher the antioxidant activity and vice versa. Nevertheless, comparing G99 with B00, we found an inversion: higher activity did not correspond to higher phenolic content. Even if the gap was very small and not too different from the overall experimental uncertainty, it would appear to be better to avoid comparing activities of fluids produced from plants of different genetic origins, because these are natural products with complex mixtures of phenolic molecules whose individual effects are mostly unknown. Furthermore, the possibility of some contribution from other redox-active substances, even if present in very low concentrations, must be taken into account. On the other hand, to strengthen previous comments, significantly different behaviors were found by Cervellati et al. (*19*) in their study of various antioxidant substances, in which they found wide variability. Moreover, antagonistic or synergic effects on real multicomponent products cannot be excluded, leading us to a very complex situation.

Aging and Overall Antioxidant Power. An attempt was made to establish the role of aging on overall antioxidant power of genuine red wines: the older wines exhibited lower antioxidant ability. Natural precipitation and/or oxidation of polyphenol substances tends to impoverish wine of its antioxidants. In light of our findings, it appears clear that the antioxidant properties of genuine red wines do not improve with aging. In this connection, young red wines are probably much more efficient, because the polyphenol concentration tends naturally to decrease with aging, particularly in genuine wines not artificially stabilized such as those considered in this study.

Link between Redox and Acid−Base Reactivity. Three properties of red wine, namely, (i) the possibility of conserving them over time (long-lasting wines), (ii) their organoleptic characteristics (mainly taste and color), and (iii) their antioxidant ability, are known to be linked with the total acidity of the product. The tests performed here enabled us to ascertain the mutual influence between acid−base and redox reactivity in red wines. They show the importance of equilibrium chemistry principles in the description of both acid−base (*23*) and the redox properties of red wines.

We found that the photometric trend with varying pH, recorded during the alkalimetric titration of diluted red wines, can be used to estimate total acidity (C_H) by means of a diagram like that in **Figure 5**. A comparison with the data obtained starting from pH-metric or conductometric titration (*23*) is displayed in **Table 4**. The agreement is fairly satisfactory, because the largest discrepancy observed in B99 was 3%, assuming as a reference the pH-metric equivalent volume (*27*). Grignolino produced the most accurate result. A reasonable comparison requires one also to consider that (a) the chemical equilibrium tends to be slow around and after the end point (pH ∼ 7.5), (b) oxidation of polyphenols in the basic field may cause inaccuracy in the measurements (i.e., oxidized molecules provide new color but overlapped acid−base and redox processes hinder rapid achievement of equilibrium during titration), and (c) the circulation from potentiometric to optical cell might introduce a further small amount of noise, in particular on slow

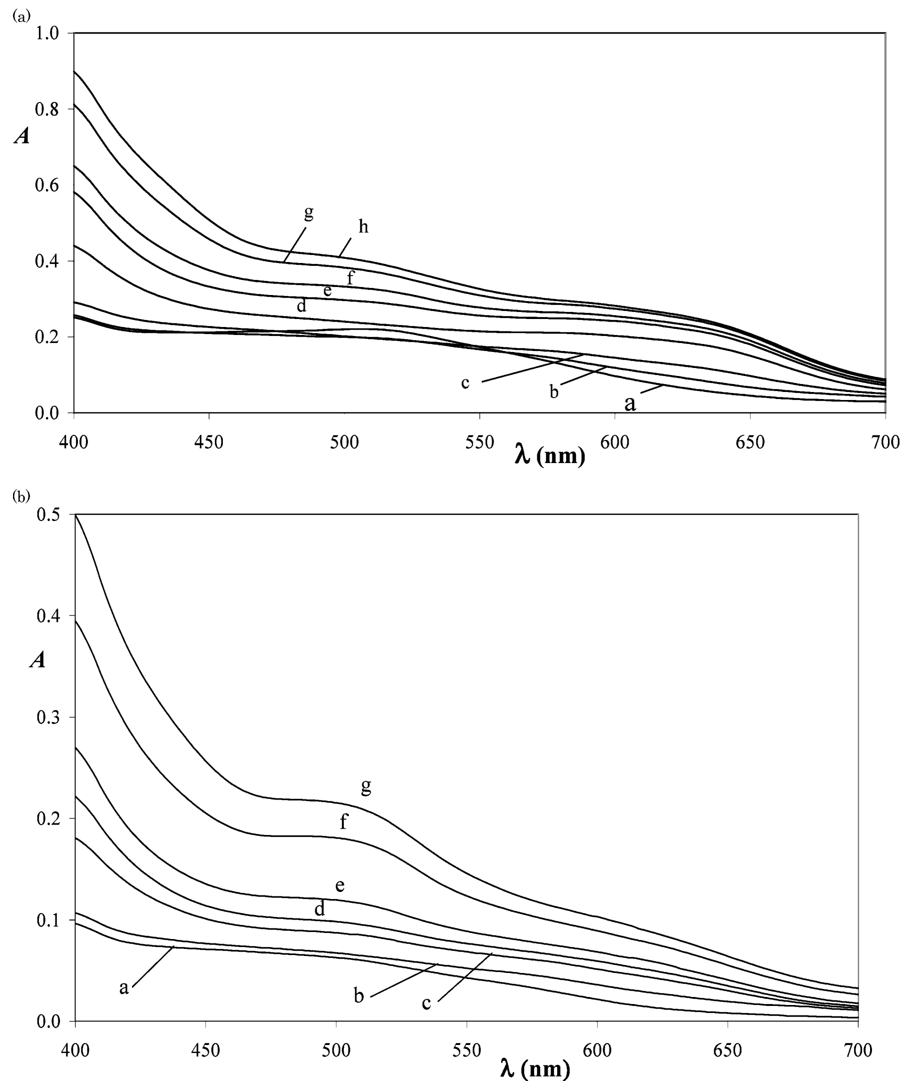

Figure 4. Visible absorption spectra of two diluted [1:20 (v/v)] red wines. (a) Barbera 2000. a, pH 3.69; b, pH 5.14; c, pH 6.67; d, pH 8.04; e, pH 8.56; f, pH 8.68; g, pH 9.13; h, pH 9.36. (b) Grignolino 2000. a, pH 3.83; b, pH 5.91; c, pH 7.67; d, pH 8.03; e, pH 8.32; f, pH 8.73; g, pH 9.08.

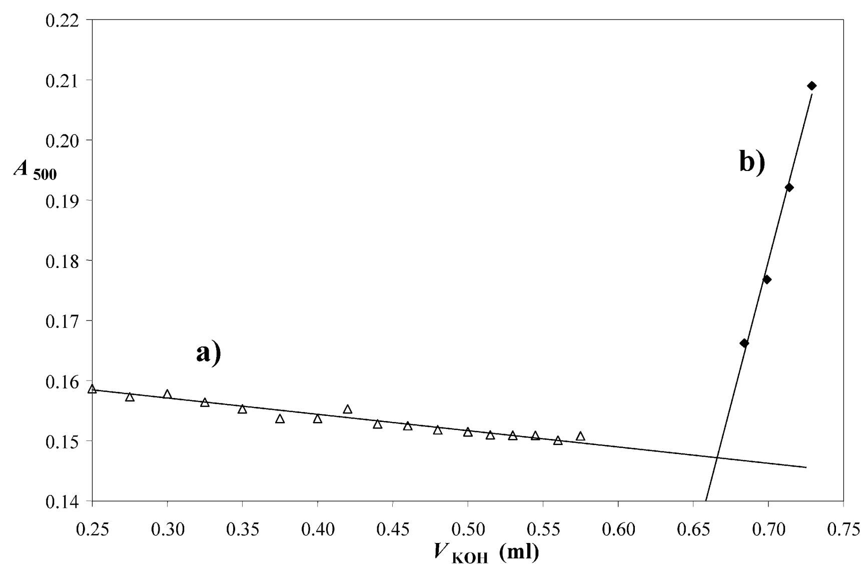

Figure 5. Estimation of the total acidity. Plot A_{500} versus $V_{titrant}$: photometric detection during the alkalimetric titration (titrant, 0.2 M KOH) of B00 [diluted 1:20 (v/v)]. Linear fittings: (a) $y = -0.0272x + 0.1653$ ($R = 0.9919$), and (b) $y = 0.958x - 0.4908$ ($R = 0.9950$).

equilibria. We believe further studies on single pigments are necessary to interpret our results, in combination with reports already presented in the literature (*28*).

What we believe significant is the quantitative relation found between wine color and pH and the estimation of total acidity via coupled pH-metric/photometric titrations. This shows that

Table 4. Total Acidity Evaluation in Red Wine by pH-metric (pH versus $V_{titrant}$), Conductometric (Conductivity versus $V_{titrant}$), or Photometric (A_{500} versus $V_{titrant}$) Detection[a]

			$V_{e.p.}$[b] 1 M KOH (mL)		
wine	percent volume	$pH_{exp.}$[c]	pH-metric detection[d]	conductometric detection[d]	photometric detection[e,f]
G99	12.2	3.38	1.07_5	1.10_0	1.06_4
G00	11.5	3.40	1.17_0	1.15_5	1.16_4
B99	12.0	3.07	1.75_9	1.76_0	1.81_2
B00	12.0	3.41	1.63_1	1.69_0	1.66_4

[a] Alcoholic grade and pH of each wine are also reported from ref. *23.* [b] e.p. = equivalent point. [c] exp. = experimental. [d] Reference *23.* [e] Referred to 1 M KOH [0.2 M KOH used for photometric determination on 1:20 (v/v) dilution]. [f] This paper.

(a) acid−base (mainly because of carboxylic fraction) and redox (mainly because of polyphenol fraction) reactivity are not independent, (b) there is an active and mutual influence of acidity on the polyphenol equipment and, as a consequence, on the overall antioxidant power. The protective role of acidity on the polyphenol molecules is thus confirmed.

On the other hand, we do not propose a photometric-based analytical method to quantify total acidity; rather, it was our intention to investigate the overlap between acid−base and redox chemistry of red wines. Nevertheless, the indirect and reliable estimation of total acidity based on the dependence of color on pH (**Figure 5**) provided a linkage between acid−base and redox-based properties of red wine.

Finally, color was employed as a discriminating factor among red wines from different grape varieties by means of a specific chemometric analysis (*29*).

Conclusive Remarks. As a significant although slightly discouraging conclusion, if compared to usual clinical practice, we observe that, during diseases with fever, the physiological ability to scavenge free radicals (abundantly produced by biochemical detoxification processes) is lowered, owing to the negative temperature coefficient (time/temperature curve). Hence, any specific therapy in the presence of fever must always be fortified with strong doses of nutritional supplements containing a blend of antioxidants (of both the hydrosoluble and liposoluble type) to supply the organism with important aid against molecular damage (the start of degenerative pathologies) and to speed up the recovery time.

The concentration (analytical aspect) as well as the activity (kinetic aspect) of specific substances of a food may be of great meaning in determining its protective capability against oxidative stress, also bearing in mind the important role of significant variables such as pH, titratable acidity, and temperature. New or additional findings concerning the composition−property relationship in food and dietary supplements should be of great interest to prevent diseases (cardio- and cerebrovascular diseases, senescence, cancer, etc.) by means of nutritional intervention alone. The close dependence of antioxidant power on temperature might determine a new trend in clinical chemistry evaluation of the redox status of plasma or other biological fluids.

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