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The antioxidant activity of wines determined by the ABTS$^{\bullet+}$ method: influence of sample dilution and time

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Abstract

The free radical scavenging activity of 42 Spanish commercial wines was determined using the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS$^{\bullet+}$). The ABTS$^{\bullet+}$ radical was generated enzymatically using a horseradish peroxidase and hydrogen peroxide. The presence of wine phenolic compounds caused the absorbance of the radical to decay at 414 nm. The measurement conditions were optimised. The total phenolic content of wines ranged from 1262 to 2389 mg l^{-1} for red wines and 70 to 407 mg l^{-1} for white wines, expressed as gallic acid equivalents. The phenolic content of Sherry wines was similar to that of white wines. Optimum dilutions for white and Sherry wines were set up as a function of their total phenolic content (for total phenol index, TPI $<$ 300 mg gallic acid per liter, dilution 2.5:10 to 5:10; for TPI $>$ 300 mg gallic acid per liter, dilution 1:10 to 3:10). Red wines absorb at the wavelength of measurement and dilutions between 0.35:10 and 0.1:10 are advisable. Reaction kinetics were also monitored and the antioxidant activity, expressed as Trolox Equivalent Antioxidant Capacity (TEAC), was determined at 2 and 15 min of reaction. The mean values for $TEAC_{2\,min}$ were 5.01 ± 1.40 mM for red wines, 0.46 ± 0.32 mM for white wines and 0.26 ± 0.19 mM for Sherry wines. At 15 min, mean values were 6.93 ± 2.41 mM for red wines, 0.67 ± 0.47 mM for white wines and 0.26 ± 0.19 mM for Sherry wines. The correlation coefficients were better at 2 min ($r = 0.9012$) than at 15 min ($r = 0.8462$) when compared with TPI. Hence, $TEAC_{2\,min}$ seems to be a more appropriate measure.

Keywords: Wine; Polyphenols; Antioxidant activity; ABTS$^{\bullet+}$; TEAC

1. Introduction

Wine polyphenols are widely known to have a protective action on the organism against cardiovascular and degenerative diseases [1,2]. Some of these beneficial effects are directly related to their ability for scavenging free radicals. This ability depends on their chemical structure, because polyphenols can donate an hydrogen atom from their hydroxyl groups and stabilize the phenoxy radical formed by delocalisation of the unpaired electron within the aromatic structure. This antioxidant activity has been thoroughly studied and a wide variety of methods have been developed to evaluate it [3]. The most frequently used are those that employ a chromogen of a radical nature. The presence of antioxidants leads to the disappearance of the radical, and is followed by absorbance measurement. Two strategies can be used to evaluate the antioxidant activity of a given sample: monitoring the inhibition in the generation of the radical (inhibition assay) or evaluating the scavenging properties against an already generated radical (post-addition assay).

One of the most commonly used methods is the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS$^{\bullet+}$) assay. It has been applied in beverages and foods [4–7] as well as in biological fluids [8,9]. The radical cation of the 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) has a characteristic absorption spectrum, with maxima at 414, 645, 734 and 815 nm [10,11]. The radical can be generated from ABTS in various systems: enzymatically using myoglobin [12] or horseradish peroxidase [13]; chemically with MnO_2 [4], potassium persulfate [14] or peroxide radicals [7]; or even electrochemically [15].

The myoglobin/ABTS assay was firstly designed as an inhibition assay in which antioxidants are added to the medium before the radical is formed [11]. This strategy has the drawback that antioxidants in the sample may interact with reagents, as occurs with the polyphenol quercetin, the

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activity of which is overestimated [16]. For this reason, Cano et al. [17] developed a post-addition assay in which the $ABTS^{\bullet+}$ radical is generated first; then, the sample is added, which decreases the radical concentration. Some advantages of this method are that high temperatures are not required to generate $ABTS^{\bullet+}$ radicals, the commercial peroxidase used does not need previous purifications, and the reaction is recorded at the optimal wavelength of 414 nm, which in turns yields better detection limits [13].

The $ABTS^{\bullet+}$ radical has been extensively used to evaluate the antioxidant activity of wines [18–23] but studies concerning the optimum conditions in which it should be estimated are scarce. Different authors have used different dilutions and times for measuring absorbance, which makes it difficult to interpret and compare data. Several studies have been made of how the pure polyphenol concentration affects the antioxidant activity evaluated by the $ABTS^{\bullet+}$ assay [21,24]. In this respect, Yu and Ong [25] found a linear relationship between antioxidant activity and concentration; correlation values increased if high concentrations of phenols were not considered. Van den Berg et al. [7] also reported that quercetin showed a concentration-dependent antioxidant activity. López-Vélez et al. [18] studied the behaviour of four wine polyphenols with significant biological activities and found a good correlation between the total antioxidant activity (TAA) and the concentration of each compound, both expressed as mmol/l. As far as we know, no studies have been made of complex mixtures such as foods and beverages.

Antioxidant activity also depends on the time at which the decrease in absorbance is measured. Some authors use a fixed time point [5,23,26] and others monitor the reaction kinetics [20,27–29]. In both cases there is a lack of consensus about the optimal time. Several authors have obtained different figures (expressed as Trolox equivalent antioxidant capacity (TEAC)) for antioxidant activity depending on the time of absorbance measurement (3 or 6 min, for example) [14]. Moreover, information about the reaction kinetics of wine samples with the $ABTS^{\bullet+}$ radical is scarce.

The objective of the present paper is to study how these variables affect the determination of the antioxidant activity of wines and that the optimal conditions depend on the characteristics of the wine selected (white, Sherry and red wines). Once the conditions have been optimized, it will be possible to compare the antioxidant activity of commercial wines and even changes in the antioxidant activity throughout wine production.

2. Experimental

2.1. Apparatus

Absorbance measurements were recorded on a Milton-Roy Spectronic® 3000 Array spectrophotometer (Dosapro Milton Roy Iberica, Madrid, Spain). Operating conditions were at 25 °C. An Orion® pH-meter (Boston, USA) was used for buffer preparation.

2.2. Chemicals and standards

2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) in the crystallized diammonium salt form, horseradish peroxidase type VI-A, hydrogen peroxide 30% (v/v), Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid, a water soluble tocopherol analogue) and gallic acid were obtained from Sigma–Aldrich Quimica (Alcobendas, Spain). Glycine, ethanol, hydrochloric acid (32%) and Folin–Ciocalteau's phenol reagent were provided by Merck (Mollet del Vallés, Spain). All reagents were of analytical grade. Double distilled water (Millipore Co.) was used throughout.

2.3. Samples

Forty-two wines from different origins and vintages were purchased from local supermarkets: white ($n = 17$), Sherry ($n = 9$) and red wines ($n = 16$). The characteristics of the wine samples are presented in Table 1. Samples were opened, protected from sunlight and stored at 4 °C. Analyses were performed within a few days.

2.4. Methods

2.4.1. Total phenol index (TPI)

The total phenol index (TPI) was determined using the Folin–Ciocalteau reagent and data were expressed as gallic acid equivalents (GAE, $mg\,l^{-1}$) [30]. A calibration gallic acid curve was prepared for concentrations ranging from 3 to 30 μM.

2.4.2. Determination of antioxidant activity

Antioxidant activity was determined using the $ABTS^{\bullet+}$ method described by Cano et al. [17] for fruit juices, with some modifications. Free radicals are generated by an enzymatic system consisting of the horseradish peroxidase enzyme, its oxidant substrate (hydrogen peroxide) and the ABTS chromophore. The radical is generated by a reaction between 1.5 mM ABTS, 15 μM hydrogen peroxide and 0.25 μM peroxidase in 50 mM glycine–HCl buffer (pH 4.5). The final volume is 60 ml, which yields 30 μM of the $ABTS^{\bullet+}$ radical cation (final concentration). These concentrations have to be checked by measuring their absorbances and using their molar extinction coefficients ($\varepsilon_{340nm} = 36000\,M^{-1}\,cm^{-1}$ for ABTS, $\varepsilon_{240nm} = 43.6\,M^{-1}\,cm^{-1}$ for hydrogen peroxide, $\varepsilon_{403nm} = 100\,mM^{-1}\,cm^{-1}$ for peroxidase and $\epsilon_{414nm} = 31100\,M^{-1}\,cm^{-1}$ for $ABTS^{\bullet+}$ radical cation). The blank reference cuvette contained glycine–HCl buffer. Absorbance was recorded to check the radical stability throughout the analysis. Moreover, five different concentrations of the radical (between 30 and 3 μM) were

Table 1
Commercial wines analyzed

Sample	Origin	Year
White wines		
W1	Condado de Huelva	2000
W2	Condado de Huelva	2001
W3	Valdepeñas	2001
W4	Valdepeñas	2000
W5	Cataluña	2001
W6	Rioja	2001
W7	Rioja	2001
W8	Rioja	2001
W9	Montilla-Moriles	2001
W10	Rueda	2001
W11	Condado de Huelva	2001
W12	Cádiz	2000
W13	Cádiz	2000
W14	Extremadura	2000
W15	Table wine	
W16	Table wine	
W17	Table wine	
Red wines		
R1	Valdepeñas	2000
R2	Ribera de Duero	2000
R3	Rioja	2001
R4	Jumilla	2000
R5	Navarra	2001
R6	Navarra	1997
R7	Cataluña	1998
R8	Campo de Borja	1996
R9	Cariñena	1997
R10	La Mancha	1997
R11	Extremadura	1999
R12	Table wine	
R13	Table wine	
R14	Table wine	
R15	Table wine	
R16	Table wine	
Sherry wines		
S1	Manzanilla	
S2	Manzanilla	
S3	Manzanilla	
S4	Manzanilla	
S5	Fino	
S6	Oloroso	
S7	Oloroso	
S8	Amontillado	
S9	Amontillado	

prepared every day and a linear relationship between radical concentration and absorbance at 414 nm was established.

Once the radical was formed, the pure antioxidant or sample was added and the decrease in absorbance was monitored. The assay was carried out at 25 °C. The reaction started by adding 100 μl of test sample to 2 ml of $ABTS^{\bullet+}$ radical cation and absorbance at 414 nm was measured at 2–6, 10, 15 min of reaction. For each wine sample, five to six different dilutions (from 0.1:10 to undiluted wine) were prepared in an aqueous ethanolic solution (ethanol content depending on the alcoholic degree) and analyzed. All measurements were performed in duplicate. Standard Trolox solutions (40–200 μM) were also evaluated against the radical.

Table 2
Summary of Trolox calibration curves[a]

Time (min)	Intercept (b)	Slope (m)	R^2
2	0.04460	2.3778	0.99905
3	0.04960	2.3527	0.99904
4	0.05690	2.3052	0.99942
5	0.06210	2.2751	0.99955
6	0.05950	2.2952	0.99940
10	0.06920	2.2526	0.99896
15	0.09470	2.1150	0.99955

[a] Decrease in absorbance (at time t) = $b + mx$ (Trolox concentration).

Results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC). This index is defined as the millimolar concentration of a Trolox solution whose antioxidant capacity is equivalent to a 1.0 mM solution of the substance under study [3]. Several calibration curves were obtained for Trolox. Their parameters are summarized in Table 2. The relationship ($R^2 > 0.999$) between the Trolox concentration and the decrease in absorbance at different time points is nearly perfectly linear.

The TEAC value of a wine expresses the concentration of a Trolox solution whose antioxidant activity is identical to that of the wine itself. It is obtained by interpolating the decrease in absorbance (corresponding to a diluted wine sample) on the calibration curve, thus obtaining a concentration of Trolox [7]. Appropriate corrections are made taking into account the dilution (Fig. 1).

2.5. Statistical analysis

All tests were carried out in duplicate. One-way ANOVA was performed to determine whether dilution factors differed significantly and to differentiate between types of wine on the basis of the TEAC values. All statistical analyses were carried out using the STATISTICA'99® version software package.

3. Results and discussion

3.1. Total phenol index

Phenolic compounds are responsible for the antioxidant activity of wines. The concentration of phenolics, estimated as the Total Phenol Index (TPI) is shown in Table 3. Red wines contain high concentrations of phenols, ranging from 1262 to 2389 GAE mg l^{-1}, and averaging 1866 mg l^{-1}. For white wines, TPI ranged from 70 to 407 GAE mg l^{-1}, with an average of 254 mg l^{-1}. The figures for Sherry wines were similar to those of white wines, ranging from 201 to 446 GAE mg l^{-1}, with an average of 289 mg l^{-1}.

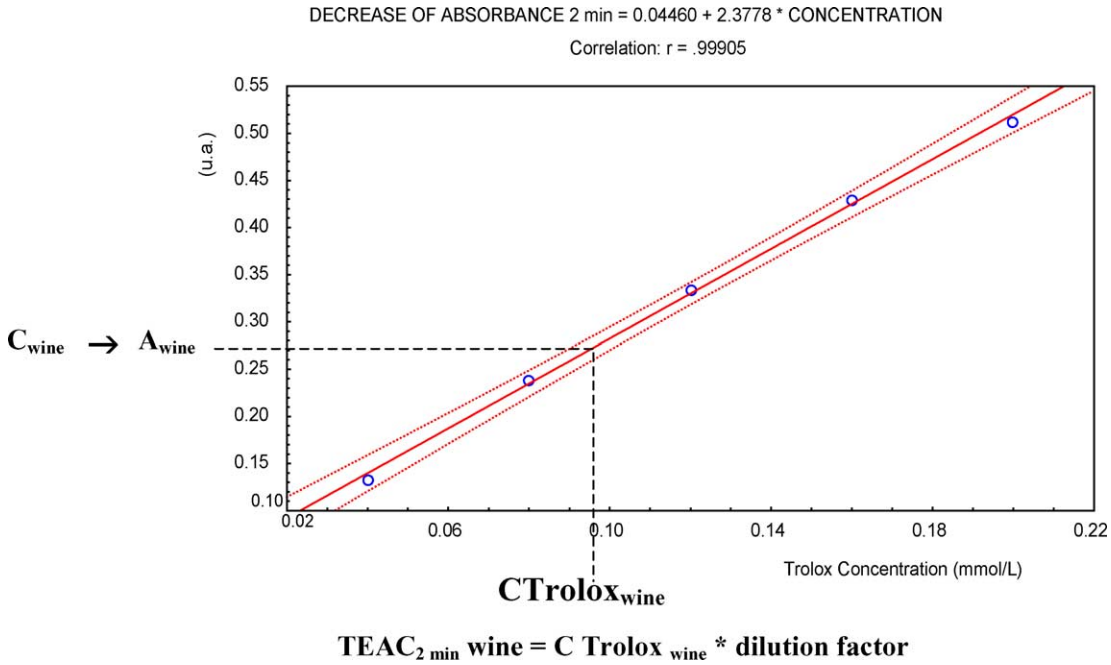

Fig. 1. Calculations for $TEAC_{2\,min}$ of wine samples.

3.2. Determination of antioxidant activity

Dilution of wines and time of measurement were optimised in the determination of the antioxidant activity by the $ABTS^{\bullet+}$ method.

3.2.1. Dilution of wine samples

Response is linear only if samples are properly diluted. Moreover, dilution must be such that the absorbance of wine compounds at the wavelength of measurement (414 nm) is avoided or at least minimized. For each sample, several dilutions, ranging from 0.1:10 to undiluted wine, were tested in duplicate. Antioxidant activity can be expressed as the percentage of inhibition of the radical absorbance versus the dilution applied, in accordance with the equations:

$$\%\,\text{inhibition at 2 min} = \left(\frac{1 - A_2}{A_0}\right) \times 100$$

$$\%\,\text{inhibition at 15 min} = \left(\frac{1 - A_{15}}{A_0}\right) \times 100$$

A_0, A_2, A_{15}: Absorbance of the radical measured at 0, 2 and 15 min of reaction, respectively.

When % inhibition is plotted versus the dilutions applied, linearity can only be observed in a particular range of concentrations, above which the results are not valid. As a consequence, wines must be properly diluted when their activity towards the $ABTS^{\bullet+}$ radical is analyzed. An example can be seen in Fig. 2.

Table 3 shows the appropriate dilution range found for each wine under study. The upper limit corresponds to the maximum concentration of wine that gives a linear response and the lower limit is the minimum concentration that shows measurable activity against the $ABTS^{\bullet+}$ radical.

In order to predict the optimal dilution for a given wine and taking the total polyphenol index as a reference, we can observe that, for white wines, the maximum and minimum

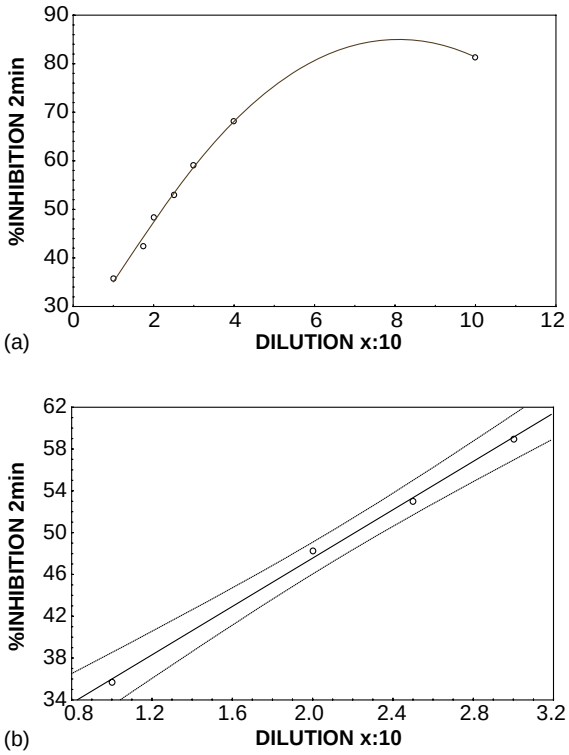

Fig. 2. Percentage of radical's inhibition measured after 2 min with different dilutions of the white wine W15: (a) all dilutions studied; and (b) dilutions giving a linear response (1:10 to 3:10).

Table 3
Total phenolic content (TPI), linear dilution range and antioxidant activity values of wine samples

Sample	TPI[a]	Dilution range	TEAC[b]$_{2\,min}$	TEAC[b]$_{15\,min}$
White wines				
W1	199	2.5:10 to 6:10	0.40 ± 0.10	0.52 ± 0.15
W2	305	1:10 to 4:10	0.59 ± 0.06	0.82 ± 0.07
W3	247	1:10 to 3.5:10	0.54 ± 0.05	0.78 ± 0.06
W4	239	4:10 to 8:10	0.29 ± 0.03	0.41 ± 0.04
W5	213	1:10 to 4:10	0.53 ± 0.07	0.69 ± 0.07
W6	278	3:10 to 6:10	0.37 ± 0.03	0.56 ± 0.05
W7	297	1:10 to 3:10	0.69 ± 0.06	0.93 ± 0.04
W8	222	3:10 to 6:10	0.30 ± 0.02	0.47 ± 0.03
W9	247	4:10 to 8:10	0.21 ± 0.02	0.31 ± 0.03
W10	239	3:10 to 7:10	0.28 ± 0.05	0.40 ± 0.08
W11	199	4:10 to 10:10	0.14 ± 0.01	0.21 ± 0.01
W12	70	4:10 to 10:10	0.08 ± 0.01	0.16 ± 0.01
W13	251	4:10 to 7:10	0.33 ± 0.03	0.45 ± 0.03
W14	140	3:10 to 10:10	0.27 ± 0.02	0.48 ± 0.05
W15	216	1:10 to 3:10	0.82 ± 0.22	1.22 ± 0.33
W16	407	0.5:10 to 1.75:10	1.45 ± 0.29	2.18 ± 0.54
W17	358	1:10 to 3.5:10	0.58 ± 0.06	0.80 ± 0.04
Red wines				
R1	1262	0.1:10 to 0.4:10	6.33 ± 1.13	11.15 ± 3.65
R2	2360	0.1:10 to 0.35:10	5.11 ± 0.50	6.32 ± 0.38
R3	2337	0.1:10 to 0.3:10	4.26 ± 0.15	5.36 ± 0.52
R4	2092	0.1:10 to 0.35:10	6.14 ± 0.66	7.94 ± 0.67
R5	1748	0.25:10 to 0.5:10	4.57 ± 0.34	6.06 ± 0.52
R6	1807	0.1:10 to 0.5:10	3.72 ± 0.32	4.69 ± 0.47
R7	2389	0.1:10 to 0.35:10	7.85 ± 1.61	11.06 ± 3.41
R8	2197	0.25:10 to 0.5:10	4.52 ± 0.70	5.95 ± 0.99
R9	2304	0.1:10 to 0.5:10	3.59 ± 0.22	4.56 ± 0.60
R10	1726	0.1:10 to 0.7:10	3.96 ± 1.08	5.28 ± 1.18
R11	1902	0.1:10 to 0.3:10	5.00 ± 0.26	6.53 ± 0.34
R12	1301	0.25:10 to 1:10	5.29 ± 0.62	8.15 ± 1.48
R13	1357	0.3:10 to 1:10	2.33 ± 0.23	3.06 ± 0.35
R14	1484	0.1:10 to 0.4:10	6.66 ± 0.80	10.37 ± 1.83
R15	1748	0.1:10 to 0.4:10	6.50 ± 1.09	8.75 ± 1.29
R16	1839	0.2:10 to 0.5:10	4.31 ± 0.68	5.72 ± 1.00
Sherry wines				
S1	318	3:10 to 10:10	0.21 ± 0.05	0.29 ± 0.04
S2	303	1:10 to 3:10	0.57 ± 0.04	0.70 ± 0.03
S3	201	2:10 to 7:10	0.26 ± 0.03	0.31 ± 0.03
S4	255	1:10 to 3:10	0.57 ± 0.05	0.74 ± 0.06
S5	206	3:10 to 8:10	0.19 ± 0.02	0.28 ± 0.03
S6	446	1:10 to 4:10	0.27 ± 0.03	0.38 ± 0.04
S7	297	4:10 to 10:10	0.08 ± 0.01	0.11 ± 0.02
S8	284	1:10 to 6:10	0.11 ± 0.01	0.16 ± 0.01
S9	290	3:10 to 7:10	0.11 ± 0.02	0.17 ± 0.01

W: white; R: red; S: Sherry wines.
[a] Expressed as mg gallic acid per liter.
[b] Expressed as mmol Trolox per liter. Each value corresponds to the mean and standard deviation of the duplicate of five to six concentrations within the linear interval.

dilution factors can be different and, as a result, samples can be divided into three different groups (Fig. 3):

- *Group 1*: TPI $< 200\,mg\,l^{-1}$;
- *Group 2*: TPI between 200 and $300\,mg\,l^{-1}$ (most of the samples);
- *Group 3*: TPI $> 300\,mg\,l^{-1}$.

ANOVA did not show significant differences between groups 1 and 2, both of which were significantly different from group 3. Therefore, it can be concluded that, if TPI is lower than 300, optimum dilution ranges from 2.5:10 to 5:10 and, if TPI is higher than 300, dilution 1:10 to 3:10 is advisable.

Red wines absorb at the wavelength of measurement (414 nm) and, as a result, the decay in the $ABTS^{\bullet+}$ radical absorbance can be underestimated. To prevent this interference, authors generally use a wavelength at which only the radical absorbs (for example, 734 nm) [19,31]. However, the

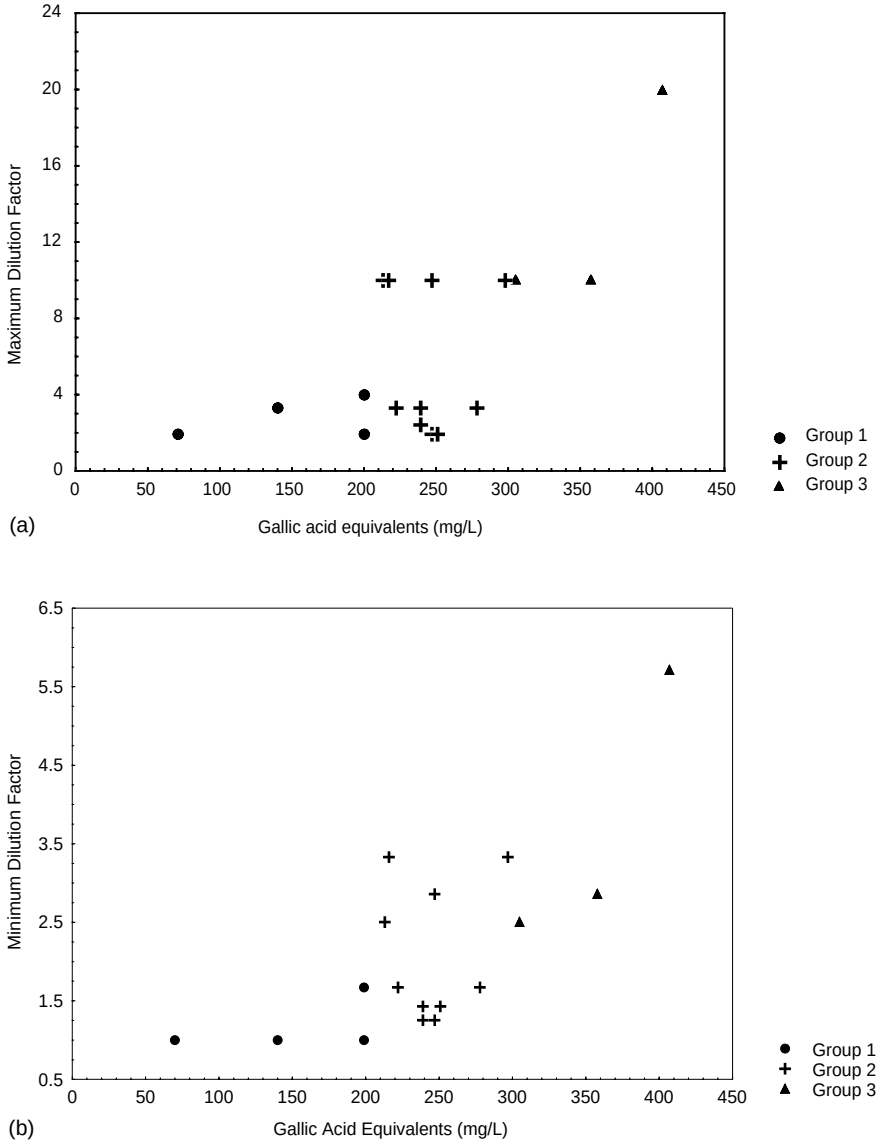

Fig. 3. Distribution of (a) maximum and (b) minimum dilution factors of white wines ($n = 17$) vs. total phenol content as gallic acid equivalents.

molar extinction coefficient at 414 nm is considerably higher ($\varepsilon_{414} = 31100\,M^{-1}\,cm^{-1}$, $\varepsilon_{734} = 1.510^{-8}\,M^{-1}\,cm^{-1}$, in water) [31] and this improves sensitivity. Moreover, for purposes of comparisons, the antioxidant activities of white, Sherry and red wines must be determined in the same conditions of analysis. So, the contribution of the red wine absorbance at 414 nm to the determination of antioxidants has to be considered.

Two red wines with high TPI (R3, TPI = 2337 mg l^{-1}; R7, TPI = 2389 mg l^{-1}) were selected. To 0.1 ml of diluted wine (ranging from dilution 0.1:10 to undiluted wine), 2 ml of glycine–HCl buffer was added and the absorbances at 414 nm were recorded. These values were compared with their respective $A_{2\,min}$ of the ABTS$^{\bullet+}$–diluted sample mixture. As can be seen in Table 4, the contribution of wine depends on the dilution. For non diluted wines to those diluted at 0.35:10, the absorbance at 414 nm is a substantial percentage of the total absorbance (mean value 35%). On the contrary, for dilutions between 0.35:10 and 0.1:10, this contribution drops to 5.69–0.19%. These values are lower than the coefficient of variation of the method itself. So, we consider that this contribution is negligible. Besides, this dilution range complies with the requisite of linearity. Hence, dilutions between 0.35:10 and 0.1:10 (dilution factor between 29 and 100) are recommended to determine the antioxidant activity of red wines.

3.2.2. *Effect of time*

Wine is a complex mixture of phenolic compounds, with a diversity of chemical structures which give them particular properties of reaction and solubility. Some authors have described biphasic reaction kinetics between the ABTS$^{\bullet+}$ radical and some polyphenols in wine and foods [27,28] and we have also observed this when studying the scavenging

Table 4
Contribution of wine absorbance to measurements

Wine sample (red)	Dilution assayed	Sample absorbance	Absorbance after 2 min of reaction mixture radical-sample	Wine contribution (%)	Coefficient of variation of duplicates (%)
R3	Undiluted	0.148	0.146	101.37	5.00
	Dilution 1:10	0.014	0.098	14.29	6.12
	Dilution 0.5:10	0.005	0.208	2.40	3.85
	Dilution 0.3:10	0.004	0.318	1.26	1.57
	Dilution 0.25:10	0.001	0.369	0.27	2.17
	Dilution 0.2:10	0.002	0.402	0.50	1.74
	Dilution 0.1:10	0.001	0.519	0.19	1.93
R7	Undiluted	0.215	0.232	92.67	1.72
	Dilution 1:10	0.025	0.115	21.74	6.96
	Dilution 0.5:10	0.016	0.179	8.94	3.35
	Dilution 0.35:10	0.011	0.261	4.21	6.13
	Dilution 0.3:10	0.017	0.299	5.69	4.35
	Dilution 0.2:10	0.010	0.409	2.44	3.42
	Dilution 0.1:10	0.009	0.534	1.69	5.62

capacity of 19 wine polyphenols (data not shown). This reaction pattern consists of initial fast scavenging activity where the more active compounds react immediately with the radical. Reaction products are formed and, together with the less reactive polyphenols, give a second slow reaction.

Results obtained with the wines analyzed corroborate this type of kinetic behaviour, in all samples and dilutions assayed. An example can be seen in Fig. 4.

Therefore, there is a time dependency on the TEAC value. The first 2 min correspond to the "fast" scavenging activity, while the final point of 15 min is known as the "total" scavenging activity.

The magnitude also depends on the dilution assayed. In the case of sample R4, $TEAC_{15\,min}$ is 31% higher than $TEAC_{2\,min}$ when the dilution is 0.35:10. However, if the dilution is only 0.1:10, this percentage drops to 20%. The same behavior is also observed for white and Sherry wines.

When fixing the point in time at which measurement is to be performed, it should be taken into account that biological processes take place at very high rates and free radicals are species with short half-lives. Consequently, when searching for an explanation for the antioxidant activity of wine after ingestion it would be more representative to select short times as the end-point [7].

The contribution of reaction products to the total antioxidant activity as the reaction occurs should also be taken into account. Moreover, TEAC values at 2 min are better correlated with TPI ($r = 0.9012$) than at 15 min ($r = 0.8462$). One possible explanation is that the $TEAC_{15\,min}$ value is partly due to the scavenging activity of these intermediate products.

Some authors conclude that, in the evaluation of the antioxidant activity of a compound, not only the chemical structure is important, but also the type of reaction products that are formed, as they may show scavenging activity against $ABTS^{\bullet+}$ [32]. This enhances the antioxidant value of the parental compound and makes it difficult to establish structure-activity relationships. Therefore, further

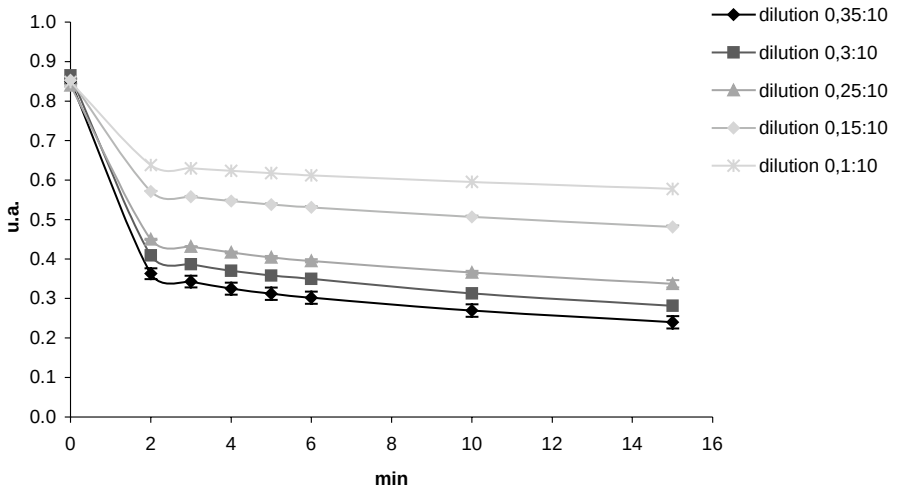

Fig. 4. Reaction kinetics of red wine R4 against the $ABST^{\bullet+}$ cation radical.

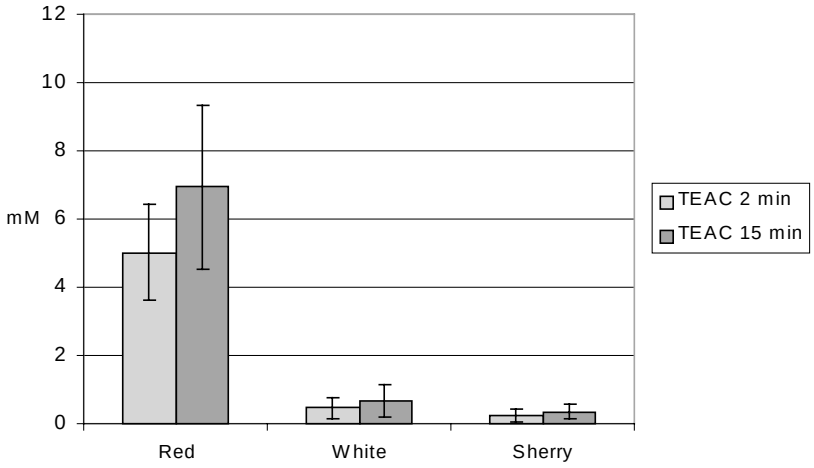

Fig. 5. TEAC values (mean and standard deviation) of red ($n = 16$), Sherry ($n = 9$) and white ($n = 17$) wines.

investigation is needed. Our findings for wine samples suggest that the $TEAC_{2\,min}$ value seems to be the best approach for determining the antioxidant activity of wines.

3.2.3. TEAC values of wines

The TEAC values reflect the relative ability of hydrogen- or electron-donating antioxidants of a sample to scavenge the $ABTS^{\bullet+}$ radical cation compared with that of Trolox. The results obtained for wines are summarized in Table 3 and Fig. 5. Red wines have a high antioxidant activity, with TEAC values 10 times higher than white and Sherry wines (evaluated at 2 and 15 min). This agrees with their greater phenol content and has been reported by other authors [33,34]. No significant differences were found between white and Sherry wines.

The correlation between total phenolic content and the antioxidant potential, in all the wines analyzed was good. The correlation values were always slightly better for $TEAC_{2\,min}$ ($r = 0.9012$). One explanation may be that reaction products are constantly forming and that their possible antioxidant activity may participate in the $TEAC_{15\,min}$ value ($r = 0.8462$).

A comparison of the $TEAC_{2\,min}$ values obtained with those obtained for other foods in the literature, using the $ABTS^{\bullet+}$ method, shows that one glass of red wine (125 ml) has the same antioxidant activity as 212 ml of grape juice [7], 190 ml of orange juice [35], 225 ml of black tea [35], 286 g of fresh spinach [26] or 926 g of tomatoes [26].

4. Conclusions

The $ABTS^{\bullet+}$ radical is an interesting tool for predicting the antioxidant activity of any food. It is advisable to dilute white wines before analysis, between 2.5:10 and 5:10 if their total phenolic content is lower than 300 mg l^{-1} or between 1:10 and 3:10 if it is higher. Because red wines absorb at the wavelength of measurement and have higher phenol contents, they need to be diluted further, between 0.35:10 and 0.1:10 (29 and 100 times).

TEAC values are time-dependent and we have demonstrated that in wines they can increase by 30–40% between $TEAC_{2\,min}$ and $TEAC_{15\,min}$. Correlations are best between TPI and $TEAC_{2\,min}$.

The TEAC values of red wines are 10 times higher than those of white wines, in accordance with their greater phenolic content, and there are no statistical differences between white and Sherry wines.

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References

[1] M.G.L. Hertog, E.J.M. Feskens, P.C.H. Hollman, M.B. Katan, D. Kromtout, Lancet 342 (1993) 1007.
[2] G.J. Soleas, E.P. Diamandis, D.M. Goldberg, J. Clin. Lab. Anal. 11 (1997) 287.
[3] M. Antolovich, P.D. Prenzler, E. Patsalides, S. McDonald, K. Robards, Analyst 127 (2002) 183.
[4] O. Benavente-García, J. Castillo, J. Lorente, A. Ortuño, Food Chem. 68 (2000) 457.
[5] N. Pellegrini, F. Visioli, S. Buratti, F. Brighenti, J. Agric. Food Chem. 49 (2001) 2532.
[6] L. Scalfi, V. Fogliano, A. Pentangelo, G. Graziani, I. Giordano, A. Ritieni, J. Agric. Food Chem. 48 (2000) 1363.
[7] R. van den Berg, G.R.M.M. Haenen, H. van den Berg, A. Bast, Food Chem. 66 (1999) 511.
[8] B. Kirschbaum, Clin. Chim. Acta 305 (2001) 167.
[9] G. Mazza, C.D. Kay, T. Cottrell, B.J. Holub, J. Agric. Food Chem. 50 (2002) 7731.

[10] M.B. Arnao, M. Acosta, J.A. Del Río, F. García-Cánovas, Biochim. Biophys. Acta 1038 (1990) 85.
[11] N.J. Miller, C.A. Rice-Evans, M.J. Davies, V. Gopinathan, A. Milner, Clin. Sci. 84 (1993) 407.
[12] C.A. Rice-Evans, N.J. Miller, Methods Enzymol. 234 (1994) 279.
[13] M.B. Arnao, A. Cano, J. Hernández-Ruiz, F. García-Cánovas, M. Acosta, Anal. Biochem. 236 (1996) 255.
[14] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Free Radic. Biol. Med. 26 (1999) 1231.
[15] A.M. Alonso, C. Domínguez, D.A. Guillén, C.G. Barroso, J. Agric. Food Chem. 50 (2002) 3112.
[16] M. Strube, G.R.M.M. Haenen, H. van den Berg, A. Bast, Free Radic. Res. 26 (1997) 515.
[17] A. Cano, J. Hernández-Ruiz, F. García-Cánovas, M. Acosta, M.B. Arnao, Phytochem. Anal. 9 (1998) 196.
[18] M. López-Vélez, F. Martínez-Martínez, C. Del Valle-Ribes, Crit. Rev. Food Sci. Nutr. 43 (2003) 233.
[19] D. de Beer, E. Joubert, W.C.A. Gelderblom, M. Manley, J. Agric. Food Chem. 51 (2003) 902.
[20] G.J. Soleas, G. Tomlinson, E.P. Diamandis, D.M. Goldberg, J. Agric. Food Chem. 45 (1997) 3995.
[21] J. Castillo, O. Benavente-García, J. Lorente, M. Alcaraz, A. Redondo, A. Ortuño, J.A. del Río, J. Agric. Food Chem. 48 (2000) 1738.
[22] A. Cano, J.R. Guerrero, M. Acosta, M.B. Arnao, Vit. Enol. Prof. 60 (1999) 45.
[23] M.I. Gil, F.A. Tomás-Barberán, B. Hess-Pierce, D.M. Holcroft, A.A. Kader, J. Agric. Food Chem. 48 (2000) 4581.
[24] N. Salah, N.J. Miller, G. Paganga, L. Tijburg, G.P. Bolwell, C. Rice-Evans, Arch. Biochem. Biophys. 322 (1995) 339.
[25] T.W. Yu, C.N. Ong, Anal. Biochem. 275 (1999) 217.
[26] S. Buratti, N. Pellegrini, O. Brenna, S. Mannino, J. Agric. Food Chem. 49 (2001) 5136.
[27] R. van den Berg, G.R.M.M. Haenen, H. van den Berg, W. van den Vijgh, A. Bast, Food Chem. 70 (2000) 391.
[28] M.B. Arnao, A. Cano, M. Acosta, Food Chem. 73 (2001) 239.
[29] J.F. Alcolea, A. Cano, M. Acosta, M.B. Arnao, Nahrung 46 (2002) 353.
[30] V.L. Singleton, J.A. Rossi, Am. J. Enol. Vitic. 16 (1965) 144.
[31] M.B. Arnao, Trends Food Sci. Tech. 11 (2000) 419.
[32] M.J.T.J. Arts, J.S. Dallinga, H-P. Voss, G.R.M.M. Haenen, A. Bast, Food Chem. 80 (2003) 409.
[33] N. Landrault, P. Poucheret, P. Ravel, F. Gasc, G. Cros, P.-L. Teissedre, J. Agric. Food Chem. 49 (2001) 3341.
[34] R.C. Minussi, M. Rossi, L. Bologna, L. Cordi, D. Rotilio, G.M. Pastore, N. Durán, Food Chem. 82 (2003) 409.
[35] N. Pellegrini, D. Del Río, B. Colombi, M. Bianchi, F. Brighenti, J. Agric. Food Chem. 51 (2003) 260.