JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Determination of Antioxidant Power of Red and White Wines by a New Electrochemical Method and Its Correlation with Polyphenolic Content

ÁNGELES M. ALONSO,* CRISTINA DOMÍNGUEZ, DOMINICO A. GUILLÉN, AND CARMELO G. BARROSO

Departamento de Química Analítica, Facultad de Ciencias, Universidad de Cádiz, C/República Saharaui, s/n. 11510 Puerto Real, Cádiz, Spain

A new method for measuring the antioxidant power of wine has been developed based on the accelerated electrochemical oxidation of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS). The calibration ($R = 0.9922$) and repeatability study (RSD = 7%) have provided good statistical parameters. The method is easy and quick to apply and gives reliable results, requiring only the monitoring of time and absorbance. It has been applied to various red and white wines of different origins. The results have been compared with those obtained by the total antioxidant status (TAS) method. Both methods reveal that the more antioxidant wines are those with higher polyphenolic content. From the HPLC study of the polyphenolic content of the same samples, it is confirmed that there is a positive correlation between the resveratrol content of a wine and its antioxidant power.

KEYWORDS: Antioxidant activity; wine; ABTS; polyphenols; resveratrol

INTRODUCTION

In recent years, antioxidants have been the subject of many epidemiological studies that have related their consumption with a reduction in the incidence of cardiovascular diseases and cancers (*1*). A wide variety of foods and drinks demonstrate a high degree of antioxidant effect and are consequently recommended for inclusion in the diet to provide a prolonged and balanced dose of antioxidants beneficial to human health (*2*). Among the recognized antioxidants (vitamins C and E, carotenoids, tocopherols, etc.) there is an extensive family of diverse components that are found in all foodstuffs of vegetable origin, known generally as polyphenolic compounds (*3*).

The moderate consumption of wine, especially red wine, has also recently been associated with the reduction in mortality from cardiovascular diseases, an effect that is commonly known as the "French Paradox" (*4*). The polyphenolic compounds present in wines, which are known to have a high antioxidant capacity, are the components recently attributed with providing the protective activity against these diseases. The polyphenols are considered to slow the natural processes of thrombosis by inhibiting the agglomeration of platelets, the peroxidation of lipids, the oxidation of low-density lipoproteins, etc. In addition, these compounds have also been found to be active, through their oxidative capacity, against other kinds of diseases such as cancers, cell mutations associated with aging, etc. (*5*−*7*). As a result, the determination of the antioxidant activity of polyphenolic compounds in wines, grapes, and related products has generated much interest, and could be useful for interpretation in many epidemiological studies.

In recent years, many different methods have been proposed for measuring antioxidant power, many of which have been applied to wines, including the DPPH method (*8*) and the FRAP method (*9*); peroxy radical scavenging capacity (*10*) has been studied; and electron spin resonance (ESR) spectroscopy (*11*) has been used. Semi-automated methods, such as the oxygen radical absorbance capacity (ORAC) assay (*12*) and flow injection analysis (*13*), have been developed.

Most of the methods described in the bibliography for determining antioxidant activity are based on the study of a reaction in which a free radical is generated and how this reaction is inhibited by the addition of the compound or sample that is the object of the measurement of antioxidant power. One of the most frequently used methods is that of Miller with metmyoglobin/2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS), based on the generation of the highly stable chromophoric cation-radical of ABTS, and the ability of the presumed antioxidant either to delay its appearance or to capture it and diminish its absorbance (*14*). This and similar methods require complex stages over different prolonged periods of time. For this reason, these methods are not suitable for application when a large number of samples need to be tested in a short time, and they are even less suitable when the samples are of multiple types. Furthermore, each of these methods produces different or even contradictory results for the same compounds, making comparison between them impossible (*15*). For all these reasons, a direct, rapid, simple, and reliable method is needed to permit the determination of antioxidant power or activity.

* To whom correspondence should be addressed. Tel: 34 956 016 363. Fax. 34 956 016 460. E-mail: angeles.alonso@uca.es.

10.1021/jf0116101 CCC: $22.00
Published on Web 04/30/2002

This study describes a method devised to determine the antioxidant power of samples of grapes and wines, employing the $ABTS^{\bullet+}$ radical by means of an accelerated electrochemical oxidation. This enables determinations to be performed easily and quickly, allowing a considerable number of samples to be processed together, and it provides very reliable results. The electrochemical generation of the radical avoids the need to use other reagents and facilitates the control of the reaction; it does not require working at a controlled temperature, and thus provides great speed, reliability, and facility of application. The method is based on measurement of the amount of current necessary to generate the $ABTS^{\bullet+}$ cation-radical in the presence and absence of the compound or sample with presumed antioxidant activity. Then the difference between these two measurements is related to the antioxidant potential of the substance or sample in question.

MATERIALS AND METHODS

Reagents and Samples. The electrolytic system comprises a cathodic cell containing a saturated solution of $Zn(CH_3COO)_2$ (Panreac, Barcelona, Spain), a saline bridge containing a saturated solution of KCl (Panreac), and an anodic cell with a solution of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS; Sigma-Aldrich, Madrid, Spain), in a phosphate buffer medium, pH 6 ($I = 0.05$), prepared from solutions of KH_2PO_4 and Na_2HPO_4 (Fluka, Buchs, Switzerland) in ultrapure water obtained using a Milli-Q water purification system (Millipore, Bedford, MA). The sample is added to the anodic cell.

The substance used as the standard and from which the calibration curve is constructed (0−100 mM) is 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) supplied by Sigma-Aldrich and dissolved in MeOH of HPLC grade (Scharlau, Barcelona, Spain).

The results in terms of antioxidant power obtained using this proposed method were compared with those obtained by means of the total antioxidant status (TAS) kit for the measurement of antioxidant power from Randox (Crumlin, Co. Antrim, U.K.) (*16*).

The Folin reagent (Sigma-Aldrich) and sodium carbonate (Panreac) were employed for the measurement of the Folin−Ciocalteau total polyphenolic index (*17*).

The solvents employed for HPLC analysis were prepared with methanol and acetic acid of HPLC quality (Scharlau, Barcelona, Spain) and water purified in a Milli-Q system (Millipore). The solutions were filtered through cellulose acetate membranes (solvent A) and Teflon membranes (solvent B) of 0.45-μm pore size and 47-mm diameter (Micron Separation, Westboro, MA) and were degasified in an ultrasound bath.

Calibration curves were constructed for the following polyphenols: resveratrol and *p*-hydroxy-phenethyl alcohol (Sigma-Aldrich), gallic acid and *trans*-*p*-coumaric acid (Merck, Darmstadt, Germany), protocatechuic acid, 5-hydroxymethylfurfural, and caffeic acid (Fluka, Buchs, Switzerland), and syringic acid (Eastman Kodak, Rochester, NY). These were dissolved in a wine-like model solution, prepared with tartaric acid (Merck) (3 gr/L), ethanol (Panreac; 15% v/v), and purified water.

Regarding the origin of the samples, some of the wines were bought and others were supplied by Bodegas Osborne, produced in Oporto (Portugal) and Jerez and Rioja (Spain).

Electrochemical Antioxidant Test. The device constructed for the measurement of the antioxidant power consists of two cells, cathodic and anodic, each with a platinum electrode. In the cathode a flat electrode (60 × 50 mm) is used, and in the anode a cylindrical platinum mesh ($h = 50$ mm; $d = 40$ mm) is used. To facilitate contact between the electrodes and the solutions, they are kept in continuous agitation by a magnetic agitator fitted to each cell. The feed source used (FAC-307C from Promax) allows the working conditions to be set in constant intensity mode. An UV−Vis transmission probe coupled to a PC2000 miniaturized spectrophotometer from Ocean Optics, Inc. (Eerbeek, The Netherlands) with a DH-2000 halogen−deuterium light source from Top Sensor Systems (Eerbeek, The Netherlands) was used to monitor the reaction.

The test consists of the oxidation, by means of the electrolytic system described, of a solution of ABTS 50 μM, to which the sample to be tested is added. The procedure is as follows. First, the platinum electrodes are washed with nitric acid 60% and flame-calcined. Then they are placed, one in the cathode immersed in 0.5 L of a saturated solution of $Zn(CH_3COO)_2$, and the other in the anode with 150 mL of a solution of ABTS 50 μM (pH 6, $I = 0.05$). The quantity of sample added to the anodic cell is 150 μL, and both are continuously agitated. A constant current of 10 mA intensity is applied, and the absorbance at 414 and 734 nm (the two wavelengths at which $ABTS^{\bullet+}$ presents its principal maximum values) is continuously recorded. The final point of the assay is taken to be the moment when the ABTS begins to oxidize, considering that this marks the termination of the oxidation of the sample. At this moment, the spectrum of the cation-radical begins to appear, with the absorbance at 414 and 734 nm increasing, and the quotient Abs 414/Abs 734 becoming constant. When the variation between two consecutive measurements of this quotient falls below 10%, it is considered that the oxidation of the ABTS has begun, and therefore that the oxidation of the antioxidant or sample added has been concluded. Depending on the degree of antioxidant power of the sample added, the formation of the cation-radical is delayed, resulting in the consumption of a greater quantity of coulombs. This quantity of coulombs is then compared with a calibration curve previously obtained from solutions of known concentrations of Trolox.

Total Antioxidant Status Test of Randox. The TAS test is performed in accordance with the supplier's instructions. Measurement of the antioxidant activity is made by pipetting into 3 cuvettes (of 1-cm light step) 20 μL of blank (bi-distilled water), standard (Trolox), and sample, respectively, together with 1 mL of chromogene (metmyoglobin and ABTS). The initial absorbance is read (A_1), taking care that the temperature is kept constant at 37 °C. Then a volume of 200 μL of substrate (H_2O_2) is added to each cuvette, and the absorbance (A_2) is read again *precisely* 3 min later. The antioxidant activity is calculated as follows:

$$Factor = \frac{conc.\ standard}{(A_2 - A_1)_{blank} - (A_2 - A_1)_{standard}} \quad (1)$$

$$mmol/L = Factor \times [(A_2 - A_1)_{blank} - (A_2 - A_1)_{sample}] \quad (2)$$

Measurement of the Total Polyphenolic Index: Folin-Ciocalteu Method. Observing the sequence specified here, the following are introduced into a calibrated 100-mL flask: 1 mL of wine, 50 mL of distilled water, 5 mL of Folin−Ciocalteu reagent, 20 mL of a solution of sodium carbonate at 20%, and distilled water to make up the total volume of 100 mL. The solution is agitated to homogenize it and left to stand for 30 min for the reaction to take place and stabilize. The absorbance at 750 nm is determined in a cuvette of 1 cm, with relation to a control prepared with distilled water. If the absorbance read is not close to 0.3, it is necessary to dilute the wine until this value is reached. (In principle, white wines can be tested undiluted and the red wines diluted by 1/5.) The index of Folin−Ciocalteu is calculated by multiplying the value of absorbance obtained by 20 and, if applicable, by the factor of dilution employed (*18*).

Analysis by High-Performance Liquid Chromatography (HPLC). The analysis was performed using a Waters HPLC system (Waters/Millipore, Milford, MA) consisting of a model 616 pump, a model 600S gradient controller, a model 717 automatic sampler, and a model 996 photodiode detector.

The separation, identification, and quantification of the polyphenols was performed using the method optimized by Domínguez et al. (*19*): the separation was conducted in a Luna Phenyl-Hexyl column (150 × 2 mm i.d., 3 μm particle size) (Phenomenex, Inc., Torrance, CA). The chromatographic conditions were the following: 0.15 mL/min flow rate; 25 μL injection volume; eluents A (0.1% methanol, 2% acetic acid, pH 2.5) and B (84% methanol, 2% acetic acid). The detection by UV absorption was conducted by scanning between 240 and 390 nm, with a resolution of 1.2 nm, and the quantification was conducted at 320 nm for the derivatives of cinnamic acid and at 280 nm for the rest of the polyphenols. The data acquisition and treatment were conducted using the Millenium 2010, version 2.21, software.

RESULTS AND DISCUSSION

The electrochemical method for the determination of antioxidant power was calibrated using Trolox as the reference

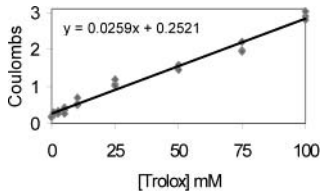

Figure 1. Calibration curve representing the concentration in mM of Trolox against the coulombs consumed in its oxidation.

Table 1. Antioxidant Activity of Various Wines Measured by the Electrochemical Method and by the TAS Test, Together with Their Total Polyphenolic Index (TPI) According to the Method of Folin–Ciocalteu

wine[a]	TPI	$[Trolox]_{eq}$ (mM)	TAS (mM)
DMR2	70.00	25.22	27.99
DMR1	53.84	28.51	24.58
R9	34.25	8.83	14.42
R1	33.40	10.42	12.48
R3	31.75	9.48	13.96
R7	30.35	8.09	13.49
R5	30.30	7.13	12.67
R11	26.10	6.52	10.24
R6	24.85	6.41	10.07
R4	24.28	4.70	10.11
R10	24.20	5.96	9.62
W1	17.43	4.24	5.51
W2	12.86	1.70	4.17
W3	10.66	1.50	3.08
W4	7.03	1.68	3.04

[a]DMR, double-macerated red; R, red; W, white.

substance. Solutions of Trolox ranging from 0 to 100 mM (0, 2.5, 5, 10, 25, 50, 75, and 100 mM) were processed, each in triplicate. Once the number of coulombs consumed had been calculated, the data were introduced into the ALAMIN program (*20*) to obtain, from the corresponding calibration curve (**Figure 1**), the characteristic properties of the method and its analytical parameters. For the slope of the curve ($b = 0.0259$) a standard deviation SD_b and relative standard deviation RSD_b of 0.0007 and 2.51%, respectively, were obtained. For the intercept ($a = 0.2521$) the SD_a and RSD_a obtained were 0.0299 and 11.86%, respectively. A very good coefficient of correlation ($R = 0.9922$) and linearity (LOL = 97.49%) can be observed, and the limit of detection (LOD = 8.652 mM) obtained is acceptable for an electrochemical method. The repeatability of the method was studied with a real sample of red wine, with 11 aliquots of the wine being processed, giving a RSD of 7%. Also, it was checked that the sulfite content of the wines did not interfere in the results of the method, given that sulfite is a compound that is readily sublimated and can be eliminated from the samples by agitation.

With a view to checking the validity of the electrochemical method for determining the antioxidant power, in comparison with another widely accepted method, the measurements of antioxidant potential obtained were compared with those obtained using the commercial TAS test, based on the method of Miller (*14*). First, the repeatability of the TAS method, applied manually, was measured, with 11 aliquots of the same sample. An RSD of 11% was obtained; this is somewhat higher than that of the electrochemical method. Then a selection of white and red wines of different origins were collected and their antioxidant power was measured by both methods. At the same time, the total polyphenolic index (TPI) was measured in accordance with the method of Folin−Ciocalteau, to enable a comparison between the measured values of antioxidant activity with the content of the samples in polyphenols. **Table 1** gives the results.

The comparison between the two methods of measuring antioxidant power was conducted using two approaches: first, a comparison was made by means of the Student's *t* test applied to pairs of values, producing the result that there were no significant differences at a 95% confidence level. Second, a regression curve was constructed between the values obtained by each method; in this case, the values obtained are shown in **Figure 2**. As can be seen by the coefficient of regression obtained ($R = 0.9516$) the two methods give similar results. In addition, from the slope of the curve (0.8537), it can be concluded that the sensitivity of the two methods is similar, although rather better for the electrochemical method.

Regarding comparison of the antioxidant power with the polyphenolic content of the wine, it can be seen that a high degree of correlation exists between the TPI and the results of the antioxidant activity measured by both methods (**Table 1**), which confirms the role played by the family of phenolic compounds as antioxidants.

Table 2. Quantities (in mg/L) of Certain Polyphenols in the Studied Wines[a]

polyphenol	DMR1	DMR2	R1	R3	R4	R5	R6	R7
resveratrol	18.56	18.92	3.55	1.94	2.36	3.85	3.39	
gallic acid	40.64	65.40	47.21	18.92	20.13	34.06	31.85	36.23
protocatechuic acid	7.15	5.88	5.86	4.76	8.37	11.81	10.45	6.17
p-OH-phenethyl alcohol	11.18	6.81		6.07	5.34	10.25	6.97	7.17
syringic acid	13.96	12.58	12.21	13.32	11.97	14.57	12.43	10.79
5-OH-CH_3-furfural	0.22	0.58	0.41	0.33	0.54	0.56	0.56	0.54
caftaric acid	12.22	18.15	5.08	3.55	5.60	5.70	6.84	7.12
cis p-coutaric acid	0.92	0.95	0.33	0.15	0.28	0.30	0.38	0.50
trans p-coutaric acid	3.04	2.96	2.01	1.38	2.74	1.97	2.62	2.41
caffeic acid	1.39	1.51	1.38	2.13	1.27	1.42	1.47	2.89

polyphenol	R9	R10	R11	W1	W2	W3	W4
resveratrol	5.84	3.29	4.03	n.d.	n.d.	n.d.	n.d.
gallic acid	34.82	13.89	16.67	62.37	7.52	5.58	5.37
protocatechuic acid	4.37	3.83	0.00	0.00	0.00	0.00	1.27
p-OH-phenethyl alcohol		4.69	8.07		0.00		8.70
syringic acid	14.41	11.92	17.66	16.71	5.55	4.66	
5-OH-CH_3-furfural	0.46	0.22	18.72	81.33	188.58	137.09	0.90
caftaric acid	0.86	1.49	11.00	4.14	1.09	5.84	5.22
cis p-coutaric acid	0.17	0.12	0.65	0.35	0.30	0.64	0.66
trans p-coutaric acid		0.23	2.16	0.88	0.15	1.32	0.65
caffeic acid	5.49	3.86	1.28	0.62	0.28	1.28	0.48

[a] DMR, double-macerated red; R, red; W, white; n.d., not detected.

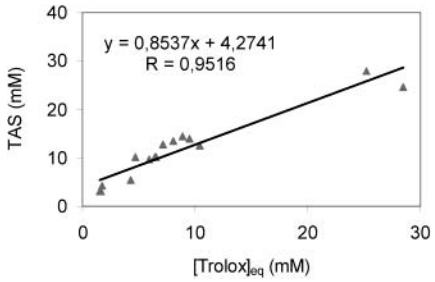

Figure 2. Regression curve for the values of antioxidant power obtained by the electrochemical method and the TAS method.

Table 3. Correlation Matrix between the Quantified mg/L of Various Polyphenolic Compounds and the Indexes of Total Polyphenols (TPI) and Antioxidant Power ($[Trolox]_{Eq}$ and TAS Methods)

	TPI–Folin	$[Trolox]_{eq}$ (mM)	TAS (mM)
resveratrol	0.9419	0.9672	0.9591
gallic acid	0.6763	0.6009	0.6235
protocatechuic acid	0.4498	0.3613	0.4914
p-OH-phenethyl alcohol	0.3646	0.4614	0.4316
syringic acid	0.3465	0.2913	0.3569
5-OH–CH_3-furfural	–0.4823	–0.4132	–0.5320
caftaric acid	0.6553	0.7895	0.6875
cis p-coutaric acid	0.4378	0.5887	0.4429
trans p-coutaric acid	0.7367	0.6976	0.7598
caffeic acid	0.0772	–0.0023	0.0838

To study this effect in more depth, some of the more common phenolic compounds were quantified (**Table 2**) by the chromatograms obtained by HPLC. As can be seen, each wine presents a distinct polyphenolic composition. To determine whether a correlation exists between the polyphenols individually quantified and the TPI and values of antioxidant power, the correlation matrix was prepared for these data (**Table 3**). It is observed that there is a high degree of correlation (more than 0.9) between the content in resveratrol and the antioxidant power of the wines tested. Other polyphenols also showing a good correlation (more than 0.7) are caftaric acid and *trans p*-coutaric acid; and for their particularly low correlation (practically zero and even negative), 5-OH-methyl-furfural and caffeic acid are notable.

It can be concluded that the results obtained by the two methods of measuring antioxidant power, the electrochemical and the TAS, are in general agreement. However, the electrochemical method presents several advantages: it is more sensitive; it has higher repeatability; it provides reliable results with the monitoring of only the time and absorbance, without the need for complicated instrumentation; and it is extremely fast, taking less than two minutes, thus enabling a large number of samples to be measured in a very short time. In general terms, both methods find that those wines with a higher overall content of polyphenols show higher antioxidant activity.

Among the many polyphenolic compounds present, resveratrol, which is already widely recognized as a powerful antioxidant beneficial to human health, is notable for the high correlation found between its content in wines and their antioxidant power.

ACKNOWLEDGMENT

We are indebted to Bodegas Osborne & Cía. for supplying samples.

LITERATURE CITED

(1) Renaud, S. C.; Guénguen, R.; Schenker, J.; d'Houtand, A. Alcohol and mortality in middle-aged men from Eastern France. *Epidemiology* **1998**, *9*, 184−188.

(2) Rice-Evans, C.; Packer, L., Eds. *Flavonoids in Health and Disease*; Marcel-Dekker: New York, 1998.

(3) Pratt, D. E. In *Phenolic Compounds in Food and Their Effects on Health*; Huang, M. T., Ho, C. T., Lee, C. Y., Eds.; American Chemical Society: Washington, DC, 1992; Vol. II.

(4) Renaud, S.; De Lorgeril, M. Wine, alcohol, platelets, and the Franch paradox for coronary heart disease. *The Lancet* **1992**, *339*, 1523−1526.

(5) Castelluccio, C.; Paganga, G.; Melikian, N.; Bolwell, G. P.; Pridham, J.; Sampson, J.; Rice-Evans, C. Antioxidant potential of intermediates in phenylpropanoid metabolism in higher plants. *FEBS Lett.* **1995**, *368*, 188−192.

(6) Soleas, G. J.; Diamandis, E. P.; Goldberg, D. M. Wine as a biological fluid: history, production, and role in disease prevention. *J. Clin. Lab. Anal.* **1997**, *11*, 287−313.

(7) Kinsella, J. E.; Frankel, E.; German, B.; Kanner, J. Possible mechanisms for the protective role of antioxidants in wine and plant foods. *J. Food Technol.* **1993**, *April*, 85−89.

(8) Larrauri, J. A.; Sánchez-Moreno, C.; Rupérez, P.; Saura-Calixto, F. Free radical scavenging capacity in the aging of selected red Spanish wines. *J. Agric. Food Chem.* **1999**, *47*, 1603−1606.

(9) Jamroz, A.; Beltowski, J. Antioxidant capacity of selected wines. *Med. Sci. Monit.* **2001**, *7* (6), 1198−1202.

(10) Rigo, A.; Vianello, F.; Clementi, G.; Rossetto, M.; Scarpa, M.; Vrhovsek, U.; Mattivi, F. Contribution of proanthocyanidins to the peroxy radical scavenging capacity of some Italian red wines. *J. Agric. Food Chem.* **2000**, *48*, 1996−2002.

(11) Gardner, P. T.; McPhail, D. B.; Crozier, A.; Duthie, G. G. Electron spin resonance (ESR) spectroscopic assessment of the contribution of quercetin and other flavonols to the antioxidant capacity of red wines. *J. Sci. Food Agric.* **1999**, *79*, 1011−1014.

(12) Caldwell, C. R. Oxygen radical absorbance capacity of the phenolic compounds in plant extracts fractionated by high-performance liquid chromatography. *Anal. Biochem.* **2001**, *293*, 232−238.

(13) Brenna, O. V.; Pagliarini, E. Multivariate analysis of antioxidant power and polyphenolic composition in red wines. *J. Agric. Food Chem.* **2001**, *49*, 4841−4844.

(14) Miller, N. J.; Rice-Evans, C. A. Spectrophotometric determination of antioxidant activity. *Redox Rep.* **1996**, *2* (3), 161−171.

(15) Alonso, A. M.; Guillén, D. A.; Barroso, C. G. Development of a new method for determining the antioxidant power of the phenolic compounds present in wines. *Bull. O. I. V.* **2000**, *73* (837−838), 794−808.

(16) Soleas, G. J.; Diamandis, E. P.; Goldberg, D. M. Wine as a biological fluid: history, production, and role in disease prevention. *J. Clin. Lab. Anal.* **1997**, *11*, 287−313.

(17) Singleton, V. L.; Rossi, J. A. J. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.

(18) Índice de Folin-Ciocalteau. In *Métodos de análisis comunitarios aplicables en el sector del vino*; A. Madrid Vicente: Madrid, Spain, 1991; pp 258−260.

(19) Domínguez, C.; Guillén, D. A.; Barroso, C. G. Method for determining polyphenols derived from cinnamic acid in Fino Sherry Wine: application to detection of contamination by yeasts of the *Bretanomyces* genus. *J. Chromatogr. A* **2002**, submitted for publication.

(20) García, A. M.; Cuadros, L.; Alés, F.; Román, M. ALAMIN: a chemometric program to check analytical method performance and to assess the trueness by standard addition methodology. *Trends Anal. Chem.* **1997**, *16* (7), 381−385.

Received for review December 7, 2001. Revised manuscript received February 28, 2002. Accepted March 4, 2002. This study was supported by the CICYT of the Spanish Ministry of Education and Science and FEDER of U.E. (Project 1FD97-0683-C05-01).

JF0116101