Comparison of the Phenolic Content and Total Antioxidant Activity in Wines as Determined by Spectrophotometric Methods

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Abstract

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Folin-Ciocalteu reagent (FCM) and Price and Butler method (PBM) were used for spectrophotometric determination of the total content of phenolic compounds in 29 wines (8 white, 21 red). The average contents of phenolic compounds determined by FCM and PBM were 108 (90–119) and 105 (90–129) for white wines, and 1545 (874–2262) and 547 (306–816) mg/l of gallic acid equivalents (GAE) for red wines, respectively. The reason for the lower PBM values in red wines is the higher reactivity in PBM of phenolic compounds, especially of gallic acid generally used as a standard in the above methods. The higher reactivity of the standard means that the measured absorbance of the sample responds to a lower concentration. The average total antioxidant activities determined by TEAC (Trolox Equivalent Antioxidant Capacity), FRAP (Ferric Reducing Antioxidant Power), and DPPH (using diphenyl-*p*-picrylhydrazyl radical) were 5.14 (4.30–6.14), 1.43 (0.86–2.14), and 0.71 (0.61–0.81) of Trolox equivalents (TE) and 26.44 (13. 9–34.4), 9.43 (4.92–13.9), and 5.52 (2.91–8.62) mmol/l TE for white and red wines, respectively. Almost the same molar absorptivities with TEAC and DPPH methods were found while with FRAP method it was somewhat higher (about 1.56-times). The ratio of the values determined by FRAP and DPPH methods for white and red wines were 2.0 and 1.7, respectively. The TEAC values were 2.8- and 4.8-fold higher than those determined by FRAP and DPPH methods, respectively. The radical $ABTS^{\bullet+}$ used in TEAC method is therefore the most reactive and responds to the highest number of hydroxyl groups of the phenolic compounds of wines.

Keywords: wine; phenolic compounds; total antioxidant activity; FCM; PBM; TEAC; DPPH; FRAP

Epidemiological studies show that a moderate wine consumption has beneficial effects on health (Renaud & De Lorgeril 1992; Klatsky & Armstrong 1993; Trichopoulou & Lagiou 1997; Hoffmeister *et al*. 1999; Renaud *et al*. 1999; Rimm *et al*. 1999). Wine consumption reduces the susceptibility of LDL to oxidation which is important for the prevention of arteriosclerosis development. A moderate wine consumption also increases serum antioxidant capacity (Cooper *et al*. 2004). A favourable influence on the reduction of cancer incidence and on chronic inflammatory diseases, the development of both being associated with oxygen free radical, is probable as well (Scalbert *et al*. 2005). Moreover, the presence of native antioxidants in wines at sufficient levels can significantly reduce the need for exogenous additives (ascorbic acid, SO_2 etc.) that can be

linked to allergic effects occurring during wine consumption in more than 15% consumers.

The more remarkable health promoting effect of wine in comparison to alcohol alone (in addition to other factors) is due to the biologically active compounds, present especially in red wine (so called French paradox). Among alcoholic beverages, red wine has been reported to be more protective against coronary heart disease than other alcoholic beverages (Gronbaek *et al*. 1995). Different wines have different quantities and spectra of native antioxidants and therefore different health benefits. Wine composition, including the contents of phenolic compounds, varies markedly depending on the grape cultivar, soil, nutrition, climatic conditions, weather, winemaking procedure, and conditions of maturation and storage.

Over 500 different compounds, of which 160 are esters, have been identified in different wine types. These include water (74–87%, w/w), ethanol (10–14%), saccharides (0.05–10%), organic acids (0.05–0.7%), phenols (0.01–0.2%), and glycerol (Soleas *et al*. 1997). Phenolic compounds have long been considered to be basic components of wines and over 200 compounds have been identified. The concentration of total phenolic compounds in commercially available red wines is rarely above 2.5 g/l (Singleton 1982). Two primary classes of phenolics that occur in grapes and wine are flavonoids and nonflavonoids.

Flavonoids commonly constitute > 85% of the phenolics content (≥ 1 g/l) in red wines. In white wines, flavonoids typically comprise < 20% of the total phenolics content (≤ 50 mg/l). Their dietary intake has been shown to be inversely related to coronary heart disease mortality (Hertog *et al*. 1993, 1995; Knekt *et al*. 1996).

The most common flavonoids in white and red wines are flavonols, catechins (flavan-3-ols), and anthocyanidins, the latter being found only in red wine. Small amounts of free leucoanthocyanins (flavan-3,4-diols) also occur. Flavonoids exist free or bound to other flavonoids, sugars, nonflavonoids, or combinations of these compounds. Flavonols and anthocyanidins originate predominately from the skin, whereas catechins and leucoanthocyanins originate mainly from the seeds and stems. Nonflavonoids partly originate also from yeast and the wood of oak barrels (Soleas *et al*. 1997).

The phenolic composition and the extractability of grapes largely depend on the grape variety and the winemaking process conditions (Soleas *et al*. 1997). The amount of flavonoids extracted during vinification is influenced by many factors, including temperature, mixing, the parameters of the fermentation vessel, the duration of skin maceration, ethanol concentration, SO_2, yeast strain, pH, and pectolytic enzymes. The concentration of phenolic compounds in wine increases during skin fermentation and subsequently begins to decrease as phenols bind with proteins and yeast hulls (cell remnants), and precipitate. During fining and maturation, the concentration of phenolic compounds continues to decrease. Their concentration is further substantially decreased at aging.

Aging in oak-wood barrels (barrique wines) can also increase the contents of particular phenolic compounds (Matějíček *et al*. 2005). Some phenols in wine arise by the activity of micro-organisms as secondary aromatic compounds in the course of the degradation of phenolic acids or lignin as well. They arise as by-products of lactic and alcoholic fermentations. Such a compound is e.g. ferulylalcohol (or 4-vinylguajacol) and other similar alcohols.

The taste and other sensory characteristics are primarily due to a few compounds that occur individually at concentrations above 100 mg/l. Lower phenolic acids account for flat flavour while larger polyphenols contribute to bitterness and astringency. Tannins present in red wine are rarely found in white wines in significant amounts.

Literature data about the contents of phenolic compounds and total antioxidant activity are insufficient and partly contradictory. This is due not only to different contents and proportions of particular phenolic compounds in different sorts of wines, but above all to different methods and various methodological approaches used. The comparison of the literature data is thus very complicated and even impossible in some cases.

In the present paper we applied FCM and PBM methods for the determination of the total contents of phenolic compounds, and TEAC, FRAP, and DPPH methods for the determination of total antioxidant activity in 29 wines. Another aim of the paper was to describe the basic principles applicable for the comparison of the values determined with those published in literature, and to compare the sale prices with the quality of wines expressed by the content of phenolic compounds and antioxidant activity.

MATERIAL AND METHODS

Chemicals. 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$, ≈ 90.0%) and 2,2´-azinobis(3- ethylbenzothiazolin-6-sulfonate) diammonium salts (ABTS, ≈ 98.0%) were purchased from Sigma-Aldrich (St. Louis, USA); Folin-Ciocalteu reagent (FC reagent), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ, puriss, ≥ 99.0%), gallic acid monohydrate (≥ 98.0%), and Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, a hydrophilic derivative of tocopherol, purum, ≥ 99%, for HPLC), were from Fluka (Buchs, Switzerland). Methanol and acetonitrile of gradient grade were purchased from Merck (Darmstadt, Germany). Other chemicals of p.a. purity were from Pliva-Lachema (Brno, Czech Republic). All reagents and standard solutions were prepared using deionised reverse osmosis water (AquaDem-2, Aqua Osmotic, Tišnov, Czech Republic) further purified by Milli Q-RG (Millipore, Bedford, USA) apparatus.

Sample preparation. Samples of 29 species of wines (8 white, and 21 red) produced in the Czech Republic or imported in the year 2006 were obtained from private cellars or purchased from local stores (Table 1). The wines were used directly from bottles or barrels. The samples of wines were stored at 5–8°C in dark until analysed. Each wine was analysed three times.

Determination of phenolic compounds in wines. *Folin-Ciocalteu method.* FCM based on the reduction of a phosphotungstate-phosphomolybdate complex by phenolic compounds to blue reaction products was used (Wildenradt & Singleton 1974; Vinson *et al*. 1998; Singleton *et al*. 1999). The total volume of the reaction mixture was minimised to 1 ml. Each sample (white wines 100 µl, red wines 50–100 µl diluted 10-fold) was read at 760 nm after 30 min of standing against blank (100 µl water instead of sample). Five-point calibration using 2 mmol/l gallic acid as the standard was linear (R^2 > 0.997) up to the concentration of 0.2 mmol/l in the reaction mixture and the absorbance range up to 3.000 AU. The determined values were expressed as gallic acid equivalents (GAE). Highly repeatable results for standards and samples were obtained.

Method according Price and Butler (1977). In this method, phenolate anion is oxidised to phenolate radical and at the same time hexacyanoferrite (ferricyanide) ion is reduced to hexacyanoferrate (ferrocyanide) forming Prussian blue $K_x(Fe_n^{(III)})\,[Fe^{(II)}(CN)_6]_3$)(Huang *et al*. 2005) according to the equation:

$$K_3\,[Fe^{(III)}(CN)_6] + \text{Ph-OH} \rightarrow K_x(Fe_n^{(III)}\,[Fe^{(II)}(CN)_6]_3) + \text{Ph-O}^{\bullet} + H^+$$

The Waterman and Mole's procedure (Waterman & Mole 1994) was modified to a semi micro-scale (reaction volume 1 ml). The samples of wines (25 µl) of 2- and 10-fold diluted white and red wines, respectively, were mixed with 750 µl of deionised water and 100 µl 0.1 mol/l $FeCl_3$ in 0.1 mol/l HCl. After 3 min standing, 100 µl 8 mmol/l potassium ferricyanide was added and the volume was adjusted to 1000 µl with deionised water and mixed. The reaction reached the maximum between 20 and 30 minutes. Colour (green to blue) was measured against blank (water instead of sample) at 720 nm after 30 minutes. Gallic acid (0.5 mmol/l) was applied as the standard and the values were expressed as gallic acid equivalents (GAE).

Determination of antioxidant activity/capacity of wines. Three methods, TEAC, FRAP, and DPPH$^{\bullet}$, based on the reaction with electron donating or hydrogen radicals (H$^{\bullet}$) producing compounds/antioxidants according to the following reaction were used:

$$R^{\bullet} + \text{Aox-H} \rightarrow RH + \text{Aox}^{\bullet}$$

where:

R$^{\bullet}$ = DPPH$^{\bullet}$, ABTS$^{\bullet+}$ or other reactive radical
Aox-H = Ph-OH, Trolox, ascorbic acid, etc.
Ph = polyphenolic compound

Despite the similar mechanisms of the methods, the reagents and products are different. Trolox was used as a common standard for the calibration of the methods, which makes the comparison of the measured values easier. The values of all three methods were expressed as a Trolox equivalent (TE).

TEAC Method (Trolox Equivalent Antioxidant Capacity or Total Antioxidant Activity – TAA) method (Miller *et al*. 1993; Rice-Evans *et al*. 1996; Lien *et al*. 1999; Plumb *et al*. 1999). The total volume used in the original procedure (Re *et al*. 1999; Long & Halliwell 2001) was reduced to 1 ml. The stock solution, a 1:1 (v/v) mixture of ABTS (7 mmol/l) and potassium persulfate (4.95 mmol/l), was left to stand for 12 h at laboratory temperature in dark to form radical-cation

Table 1. Content of phenolic compounds and total antioxidant activity in wines

No.[1]	pH[2]	EtOH[2] (%)	FCM[3]		PBM[3]		TEAC[4]	FRAP[4]	DPPH[4]	A_{520} (AU)[6]
			(mmol/l)[5]	(mg/l)	(mmol/l)[5]	(mg/l)		(mmol/l)[5]		
White wines										
1	3.55	12.32	0.53 ± 0.01	90	0.53 ± 0.03	90	4.30 ± 0.19	1.79 ± 0.11	0.72 ± 0.02	0
2	3.42	11.16	0.61 ± 0.06	104	0.53 ± 0.04	91	4.78 ± 0.28	1.00 ± 0.02	0.63 ± 0.02	0
3	3.58	12.62	0.60 ± 0.01	103	0.53 ± 0.05	90	5.13 ± 0.04	0.88 ± 0.03	0.61 ± 0.08	0
4	3.51	11.96	0.63 ± 0.01	107	0.56 ± 0.00	95	5.08 ± 0.02	0.86 ± 0.02	0.66 ± 0.06	0
5	3.53	11.98	0.73 ± 0.01	125	0.76 ± 0.10	129	6.14 ± 0.32	2.14 ± 0.01	0.81 ± 0.01	0
6	3.54	11.53	0.65 ± 0.01	110	0.70 ± 0.01	119	5.08 ± 0.05	1.93 ± 0.03	0.73 ± 0.01	0
7	3.42	12.41	0.70 ± 0.02	119	0.72 ± 0.05	122	5.57 ± 0.01	1.00 ± 0.04	0.79 ± 0.01	0
8	3.57	11.54	0.97 ± 0.01	166	1.21 ± 0.01	206	8.44 ± 0.91	1.47 ± 0.01	1.78 ± 0.03	0
Red wines										
9	3.49	10.50	5.66 ± 0.01	963	2.22 ± 0.01	377	17.84 ± 0.01	6.34 ± 0.01	3.70 ± 0.01	1.06
10	3.56	12.06	10.17 ± 0.01	1730	3.80 ± 0.01	647	30.85 ± 0.01	5.65 ± 0.01	3.38 ± 0.01	5.21
11	3.21	11.07	11.22 ± 0.01	1908	3.50 ± 0.01	595	32.30 ± 0.01	11.38 ± 0.01	7.33 ± 0.01	3.57
12	3.59	13.61	5.14 ± 0.01	874	1.80 ± 0.01	306	13.89 ± 0.01	4.92 ± 0.01	2.91 ± 0.01	2.16
13	3.48	12.44	7.17 ± 0.01	1219	2.60 ± 0.01	442	20.15 ± 0.01	7.41 ± 0.01	4.20 ± 0.01	2.16
14	3.40	12.31	8.95 ± 0.01	1523	3.48 ± 0.01	592	25.62 ± 0.01	8.60 ± 0.01	4.77 ± 0.01	3.18
15	3.45	11.70	8.41 ± 0.01	1431	3.46 ± 0.01	588	24.44 ± 0.01	9.98 ± 0.01	4.64 ± 0.01	2.68
16	3.63	11.64	9.11 ± 0.01	1550	3.26 ± 0.01	554	26.36 ± 0.01	9.99 ± 0.01	5.88 ± 0.01	5.02
17	3.75	12.83	11.60 ± 0.01	1973	3.46 ± 0.01	589	34.69 ± 0.01	12.41 ± 0.01	7.78 ± 0.01	4.11
18	3.62	12.57	9.80 ± 0.01	1666	3.14 ± 0.01	534	28.38 ± 0.01	10.37 ± 0.01	6.21 ± 0.01	3.13
19	3.54	12.67	6.19 ± 0.01	1054	2.20 ± 0.01	375	16.74 ± 0.01	6.84 ± 0.01	3.73 ± 0.01	3.53
20	3.57	11.49	6.87 ± 0.01	1169	2.60 ± 0.01	443	20.50 ± 0.01	7.34 ± 0.01	4.05 ± 0.01	3.16
21	3.63	11.78	7.72 ± 0.01	1314	2.65 ± 0.01	451	23.72 ± 0.01	8.62 ± 0.01	5.47 ± 0.01	2.72
22	3.53	12.56	8.47 ± 0.01	1440	3.28 ± 0.01	558	25.41 ± 0.01	9.97 ± 0.01	4.48 ± 0.01	3.41
23	3.69	11.37	10.78 ± 0.01	1833	3.56 ± 0.01	606	30.53 ± 0.01	11.35 ± 0.01	7.05 ± 0.01	3.17
24	3.68	12.68	8.91 ± 0.01	1515	2.91 ± 0.01	496	25.26 ± 0.01	9.52 ± 0.01	5.86 ± 0.01	4.06
25	3.44	11.21	10.73 ± 0.01	1825	3.93 ± 0.01	669	32.37 ± 0.01	11.56 ± 0.01	6.60 ± 0.01	8.68
26	3.85	11.84	8.86 ± 0.01	1508	3.04 ± 0.01	516	25.07 ± 0.01	8.94 ± 0.01	5.51 ± 0.01	4.25
27	3.45	11.32	10.66 ± 0.01	1813	3.70 ± 0.01	629	32.50 ± 0.01	11.65 ± 0.01	6.65 ± 0.01	8.13
28	3.67	12.07	11.00 ± 0.01	1871	4.13 ± 0.01	703	34.38 ± 0.01	11.34 ± 0.01	7.12 ± 0.01	4.91
29	3.48	–	13.30 ± 0.01	2262	4.80 ± 0.01	816	34.27 ± 0.01	13.94 ± 0.01	8.62 ± 0.01	4.01

[1]1. Ryzlink vlašský[a], 2. Ryzlink vlašský[b], 3. Müller-Thurgau[b], 4. Veltlín zelený[b], 5. Veltlín zelený[a], 6. Muscadel Moravia (MOPR)[a], 7. Chardonnay, late gathering[a], 8. Tramín rosé, late gathering[a], 9. Frankovka blue-dry, Hungary, La Fiesta, area Duna Mellék, year 2004, Supermarket Billa, 10. Frankovka, Macedonia[c], 11. Frankovka[a], 12. Modrý Portugal[b], 13. Modrý Portugal, shop, 14. Modrý Portugal[b], 15. Rulandské modré, cellar Stráchotin[c], 16. Cabernet Sauvignon[b], 17. Cabernet Sauvignon, Argentina[c], 18. Cabernet Moravia[a], 19. Svatovavřinecké[b] (Saintlaurence), 20. Svatovavřinecké[b], 21. Svatovavřinecké, private cellar Krumvíř, 22. Svatovavřinecké klasik, Rakvice, area Small Carpathian Mountains, SO_2 added, hypermarket, 23. Svatovavřinecké[a], 24. Zweigeltrebe[a], 25. Zweigeltrebe[d], 26. André, private cellar[a], 27. André + Portugal[d], 28. Syrah, Argentina[c], 29. Italian red wine[c]

[a]private cellar from south Moravia, [b]salesroom of wines from south Moravia, [c]salesroom of domestic and imported wines, [d]private salesroom of wines from south Moravia

[2]average values from three repetition, [3]gallic acid equivalents (GAE), [4]Trolox equivalent (TE), [5]mean ± SD, n = 3, [6]AU = absorbance units (absorbance × dilution)

$ABTS^{\bullet+}$. The final solution was stable for at least one week at 4°C in dark.

The stock solution was diluted with phosphate buffer solution to give the absorbance values between 1.0 and 1.5 AU at 734 nm (the same absorbance value must be used for the standard and samples). The standard or sample (20 µl) of 4-fold and 20- or 40-fold diluted white and red wines (according to the reaction intensity), respectively, were mixed with the working solution (975 µl) and adjusted to 1000 µl with deionised water. The decrease of the absorbance at 734 nm was measured after 30 min (after reaching plateau). Aqueous phosphate buffer solution (1 ml, without $ABTS^{\bullet+}$) and Trolox (0.5 mmol/l) were used as a control and a calibrating standard, respectively.

Ferric Reducing Antioxidant Power (FRAP) method (Benzie & Strain 1996; Pulido *et al*. 2000; Imeh & Khokhar 2002) was modified to a semi microscale using the total volume of 1 ml. A portion of an aqueous 10 mmol/l solution of TPTZ reagent in 40 mmol/l HCl was mixed with the same volume of 20 mmol/l $FeCl_3 \cdot 6\ H_2O$ and ten times higher volume of acetate buffer of pH 3.6 (3.1 g sodium acetate and 16 ml acetic acid per litre). The mixture was incubated at 37°C for five minutes. A portion (900 µl) of the Fe^{3+}-TPTZ mixture and the sample of wine (25 µl; red wines diluted 5 to 10-fold) or the standard or water (for blank) were adjusted to 1000 µl with deionised water, incubated for 30 min (after reaching plateau), and the absorbance at 593 nm was read. Trolox (0.5 mmol/l) was used for calibration (Gardner *et al*. 2000).

Diphenyl-p-picrylhydrazyl (DPPH) method. The original procedure (Sánchez-Moreno *et al*. 1998) was modified (to 1 ml total volume). The working solution was prepared by dilution of methanolic $DPPH^{\bullet}$ solution (98 mg/l, absorbance ≈ 1.9) to the absorbance of ≈ 1.5 AU (the same for the sample and standard) to provide a sufficient reaction capacity for higher contents of antioxidants in the extracts. A portion (950 µl) of the working solution and the sample of wines (50 µl; red wines diluted 5 to 10-fold) or standard were adjusted to 1000 µl with aqueous methanol (1:1, v/v) and incubated 30 minutes to reach plateau (Kim *et al*. 2002). The absorbance was read at 515 nm against blank (aqueous methanol). Fresh 0.5 mmol/l working solutions of Trolox were used for calibration.

Determination of ethanol. A gas chromatograph HP-4890D with a flame-ionisation detector was used for the assessment of ethanol using an HP-5MS (5% Phenyl Methyl Siloxane, 30 m × 0.25 mm × 0.25 µm film) analytical column (all from Hewlett Packard, Waldbronn, Germany). The flow rate of helium was 1 ml/min, split ratio 20:1, spray temperature 240°C, detector temperature 250°C, and thermal programme: T_1 = 30°C, t_1 = 5 min, 40°C/min on T_2 = 70°C, t_2 = 1 min, ca 7 min. The applied volume of the samples was 1 µl.

Determination of color intensity. Colour intensity of the red wines was determined spectrophotometrically at 520 nm. Optimal wavelength for measuring was selected by scanning full spectrum from 200 to 700 nm. All samples of wines were diluted 5-fold before measuring. The measured values shown in Table 1 and Figures 2 are expressed in absorbance units (AU).

RESULTS AND DISCUSSION

The determined contents of phenolic compounds and antioxidant activity values are presented in Figures 1 and 2. Both methods for the determination of phenolic compounds were standardised using the same standard; however, the reactivity with the standard differed with the individual methods. The average values of phenolic compounds content in white wines determined by FCM were in the interval of 103–125 mg/l GAE except of one Ryzlink vlašský (90 mg/l). The values of phenolic compounds content in red wines were approximately 10- to 15-times higher, namely from 874 to 1973 mg/l, and in one Italian red wine reached as much as 2261 mg/l. The determined content of phenolic compounds (166 mg/ml) was notably higher in Tramin than in other white wines. Our values for white wines were about 30 to 50% lower in comparison with the results published by three other authors and very close to the values published by Simonetti *et al*. (1997) (Table 2).

The differences in the values are probably not of a methodological origin because the most commonly used FCM for the assessment of phenolics is relatively robust and trouble-free.

The differences between our values and the published results could be primarily affected by the nature of the analysed wines, i.e. by their actual contents of phenolic compounds. The great differences in the contents of phenolic compounds in white and red wines indicate that anthocyanins form the most important part of the phenolic compounds in red wines. Beside other factors, the grape variety, intensity of solar irradiation at the

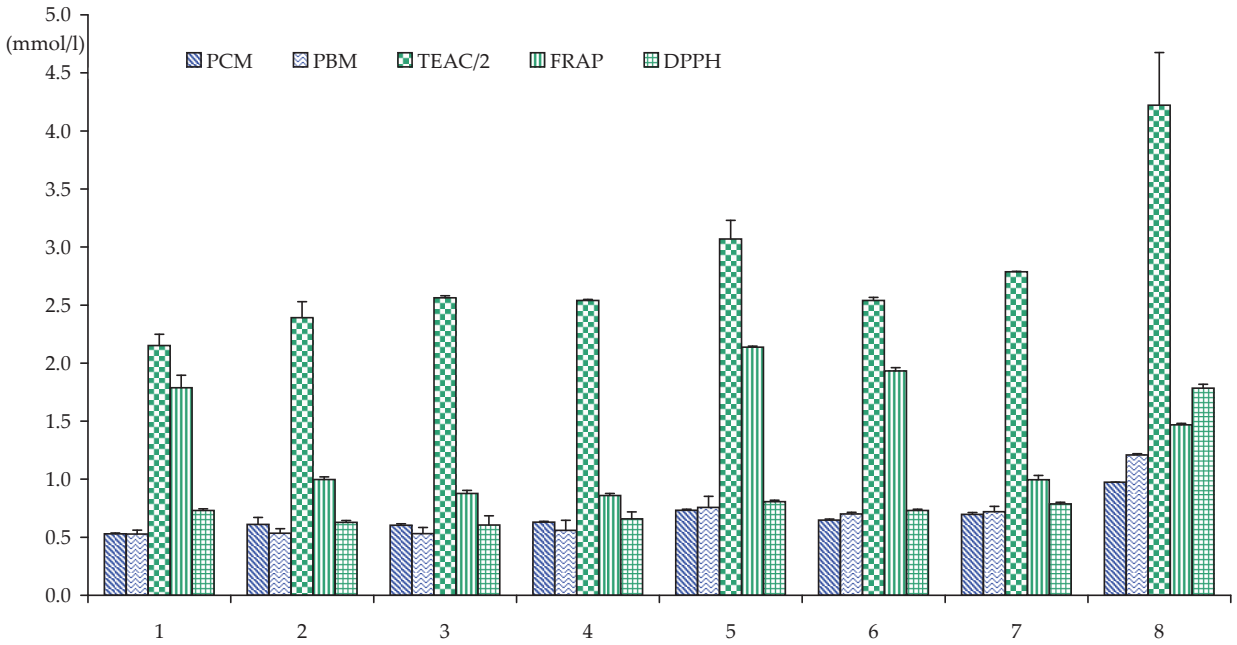

Figure 1. Contents of phenolic compounds of white wines determined by the FC and PB ethods (GE μmol/l), and total antioxidant activity determined by the TEAC, FRAP and DPPH methods (TE mmol/l)

ripening time of grapes, and winemaking procedure mostly influence the phenolic compounds content. Therefore, much higher contents of anthocyanins and total contents of phenolic compounds are present especially in red wines from sunny regions (Italy, Spain, California etc.).

The content of phenolics determined by FCM and PBM was approximately the same in white wines. The values determined in FCM in red wines were 2- to 3-times higher than those determined by PBM for identical samples. This difference could be at least partly explained by a different reactivity of anthocyanins with the reagent in the respective methods. This presumption is supported by the fact that millimolar absorption coefficient of gallic acid for PBM is approximately 5.4-times higher than that for FCM, and therefore the concentration values measured by PBM are several-fold lower in the measured samples for the identical absorption (Stratil *et al*. 2007).

The sequence of the 10 "best" red wines according to the total content of phenolic compounds determined by FCM were as follows (in decreasing order): Italian red wine (2261 mg/l) > Cabernet Sauvignon, Argentina > Frankovka[a] > Syrah, Argentina > Svatovavřinecké > Zweigeltrebe > mixture André + Modrý Portugal > Frankovka, Macedonia > Cabernet Moravia > Cabernet Sauvignon (1973 mg/l). The total content of phenolic compounds determined by FCM and PBM methods correlated very significantly with the colour intensity of the red wines (Table 1).

Table 2. Comparison of our and published values of phenolic compounds contents in wines (mg/l GAE)

	White wines		Red wines	
Our values (Czech Republic)	103–125	(n = 7)	963–2262	(n = 20)
Sánchez-Moreno *et al*. (1999) (Spain)	178–293	(n = 5)	1019–2446	(n = 7)
Heinonen *et al*. (1998) (Finland)	265	(n = 1)	1390–1600	(n = 3)
Jewell and Ebeler (2001) (California)	163	(n = 1)	2220, 2390	(n = 2)
Stevanato *et al*. (2004) (Italy)*	170–260	(n = 16)	1921–3659	(n = 21)
Schoonen and Sales (2002) (Portugal)	–		938–1820	(n = 5)
Simonetti *et al*. (1997) (Italy)	96–146	(n = 3)	1365–3326	(n = 10)

*catechin equivalents converted to GAE

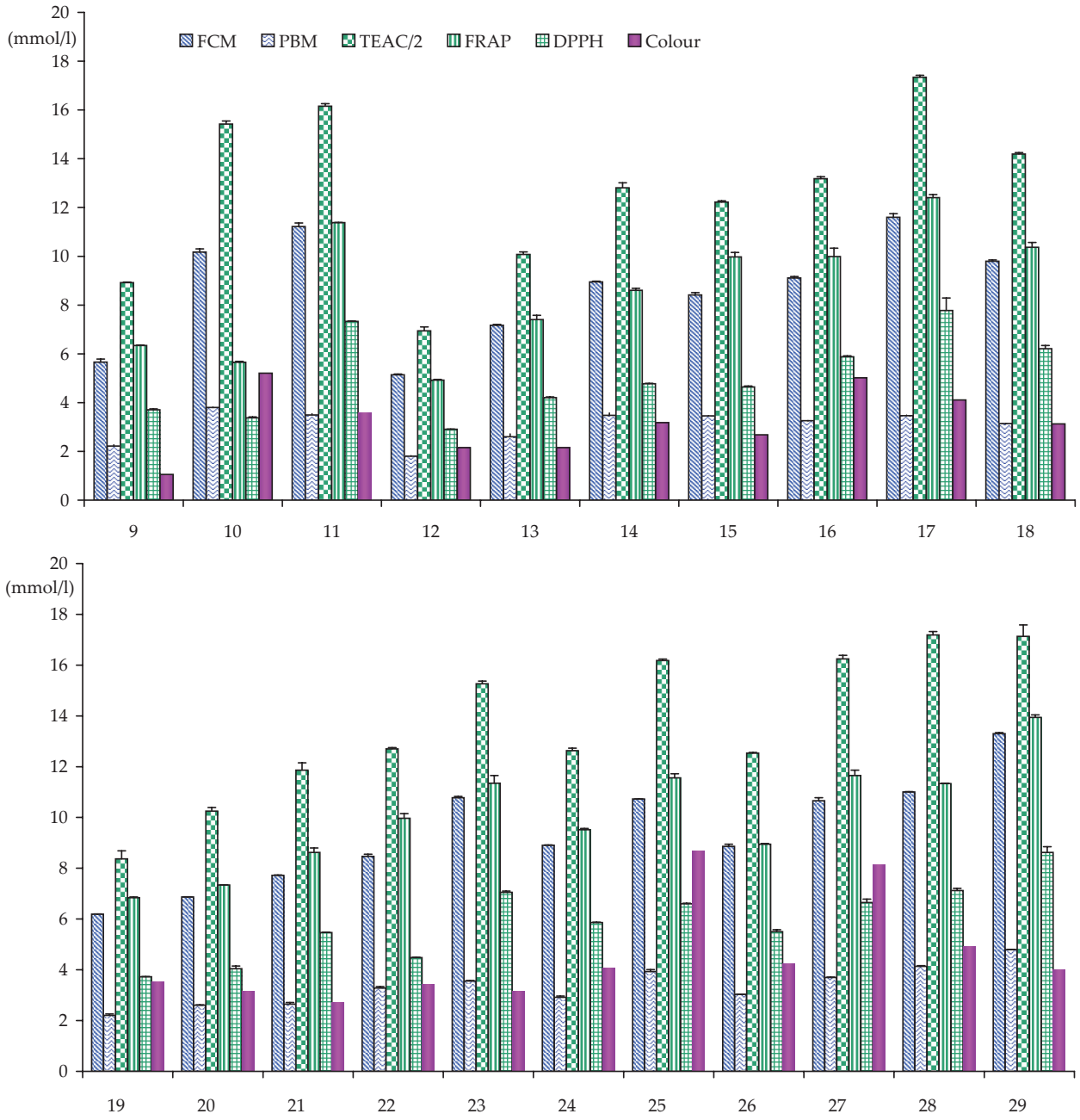

Figure 2. Contents of phenolic compounds of red wines determined by the FC and PB methods (GE μmol/l), and total antioxidant activity determined by the TEAC, FRAP and DPPH methods (TE mmol/l). Colour is expressed in AU (absorbance × dilution)

Trolox was used as a common standard for the calibration of all the methods used for the assessment of the total antioxidant activity. Millimolar absorption coefficients of Trolox for TEAC and DPPH methods are almost the same, and they are approximately about 56% higher than for FRAP method (Stratil *et al*. 2007). TEAC method is the most reactive one in the reaction with phenolic compounds, yielding approximately 2.8- and 4.8-times higher values than FRAP and DPPH methods, respectively. The higher reactivity of ABTS reagent with phenolic compounds is the most important factor.

According to the TEAC values determined in white wines the total antioxidant activity of wines can be qualified in this decreasing order (TE mmol/l): Veltlín zelený[b] (6.14) > Chardonnay (5.57) > Muscadel Moravia (5.08) > Veltlín zelený[a] (5.08) > Müller-Thurgau (5.13) > Ryzlink vlašský[b] (4.78) >

Ryzlink vlašský[a] (4.30). A remarkably higher antioxidant activity was observed in rosé Tramín (8.44). The highest antioxidant activity was found in Italian red wine and in two Argentinian wines (34.3 to 34.7 mmol/l, i.e. 5- to 6-times higher than that in white wines). The lowest value, less than half the highest value, was found in Modrý Portugal (13. 9). The average antioxidant activity values determined by TEAC method were 4.95 and 26.4 mmol/l in white and red wines, respectively. The values of antioxidant activity found in bottled Hungarian Frankovka were inconsistent with its colour. The values, which were slightly higher compared to the Modrý Portugal, did not correspond to the intensity of red colour since the colour intensity was lower than a half (possible influence of the contents of sulfites, ascorbic acids or saccharides).

It is difficult to confront our values of antioxidant activity with the literature data. The majority of authors used various methods such as the inhibition of lipid oxidation, DPPH method with the evaluation of EC_{50} (the sample concentration necessary to reduce the remaining DPPH by 50%), and ORAC method (Oxygen Radical Absorbance Capacity). It is possible to compare, in part, some of the values determined by the method TEAC using ABTS radical, e.g. Simonetti *et al*. (1997) mentioned values with intervals 0, 1.1 and 3.6 mmol/l for Italian white wine (n = 3) and values with intervals 7.8–14.1 (average 12.3) mmol/l (TE) for red wine (n = 10). These values are less than one half in comparison to our values. The differences could be caused, above all, by the different reaction times used in the experiments. The authors used a very short incubation time (only 3 min) that corresponded to about one third to one half of the reaction time since the reaction can proceed for up to much as 30 minutes (Stratil *et al*. 2006).

Fernández-Pachón *et al*. (2006) presented the values of TEAC in the range from 0.14 to 1.45 mmol/l TE for white wines (n = 13). These TEAC values were approximately 5 to 10-times lower then ours. This could be caused by methodological differences. The authors used the wavelength of 414 nm that gives about 13–16% lower values than the more commonly used and preferable wavelength of 734 nm (Arnao 2000) and the reaction time about 15 min at which the reaction reached approximately only two thirds of its maximal response. The determined TEAC ABTS values, 11.4–17.5 in Portugal red wines (n = 8) and 1.4–2.9 mmol/l TE in white wines (n = 10), using sequential injection analysis (SIA) method (Pinto *et al*. 2005), differed likewise; the short reaction time may have been the cause.

The evaluation of the red colour intensity is used for a fast subjective visual appreciation of red wines. Therefore the intensity of the red colour of the wines was measured at the maximum wavelength of wine colour (520 nm) determined by scanning the full VIS spectrum. The intensity of the red colour of wines correlated very significantly with the values of phenolics determined by both methods used, and with the values of antioxidant activity determined by TEAC method. The correlation with the values determined by FRAP and DPPH methods was significant and after the elimination of extreme values of two wines (No. 25 and 27, André + Modrý Portugal and Zweigeltrebe) was also highly significant. The highest red colour of these two wines could be influenced by the addition of some exogenous colouring agent that was not of anthocyanin origin.

Significant differences in antioxidant activity and colour existed not only between particular types and sorts of red wines but also inside one variety of wine due to different origins and winemaking procedures. Five different kinds of Svatovavřinecké wine (Czech Republic) of different origins (region, winemaking) were analysed and the values of antioxidant activity determined were in the interval from 16.7 to 30.5 mmol/l. Thus, they differed almost by 100%, however, the difference in the intensity of the red colour was approximately only 23%.

The determined contents of ethanol in white and red wines were rather steady, except two more extreme values (10.5% and 13.6%). Fourteen wines had the concentration of ethanol in the interval of 11–12% and twelve in that of 12–13% (Table 1). Also the acidity values of the wines were very similar and fell within the pH range of 3.40–3.85 (Table 1).

Sulfite influence on phenolic compounds determination

The addition of free SO_2 to wines to maximum concentration of 30 mg/l is preferable to the addition of ascorbic acid. Substantial interference of SO_2 with the FC reagent was reported in the literature (Ough & Amerine 1988). For the reaction of 1 mmol/l solution (126 mg Na_2SO_3/l) with FC reagent we determined an equation A = 0.560× concentration, which means approximately 30-

Table 3. Determined molar absorption coefficients of individual methods for the reaction with main saccharides in fruits (× 1000)

Method	Glucose	Fructose	Saccharose
FCM	0.6	2.2	0.5
PBM	0.7	2.3	0.6
TEAC	–0.2	–0.6	–1.1
FRAP	0.0	0.4	0.0
DPPH	0.0	0.0	0.0

times lower reactivity than in the case of gallic acid. Sulfite content in wines was usually in the intervals of 1 to 75 and 10 to 250 mg/l for free and total SO_2, respectively, but mostly 50–100 mg/l (Santos & Korn 2006). According to the calibration equation is it possible to calculate that sulfites at a concentration of 100 mg/l increased the absorbance by about 0.050 and 0.006 absorbance units for white and red wines, respectively. These values are quite insignificant for red wines and they correspond only to 1.7 mg GAE for white wines. From these facts, it is possible to conclude that 100 mg of sulfite per liter increased the real content of phenolic compounds approximately by 1 to 2%.

Ascorbic acid interference with methods used

Ascorbic acid is a powerful native antioxidant present in grapes (about 50 mg/l) whose content continuously decreases during fermentation in the wine production (Ribéreau-Gayon *et al*. 1998*).* Nevertheless, it can be found in wines because it is sometimes used as an additive during processing to prevent oxidation. In Europe, the addition of ascorbic acid is legally limited to maximum concentration of 150 mg/l. According to our measurements, ascorbic acid interferes with all the used methods. The literature values of ascorbic acid content were 25–100 mg/l (100 mg/l = 0.568 mmol/l) in some white wines and zero in red wines (Lopes *et al*. 2006). Ascorbic acid should markedly increase the absorbance of white wines at the concentration of 100 mg/l. According to our measurement, the increase was equal to 0.980, 0.340, 0.084, 0.560, and 0.730 absorbance units for FCM, PBM, TEAC, FRAP, and DPPH methods, respectively. The parameters of the calibration equations of the methods for ascorbic acid were published in our previous paper (Stratil *et al*. 2006).

Saccharides interference with the methods used

Glucose, fructose, and sucrose belong to the most important saccharides present in fruits. These sugars react with different intensity (interfere) in both methods used for the assessment of phenolic compounds and for the assessment of antioxidant activity, DPPH method being an exception (it requires donation of hydrogen radical). Molar absorption coefficients (Table 3) of the individual methods for the reaction with sugars express their reactivity (Stratil *et al*. 2007). As to FCM, it is possible to evaluate their contributions in the reaction with percentual subtraction from the total values in the samples with their higher content according to the known content. According to Singleton, it is possible to express (Table 4) the quantitative influence of saccharides depending on the quantities of phenolic compounds and saccharides (Singleton *et al*. 1999). Heinonen *et al*. (1998) found the difference about 20% between the values for the determination of total phenolics with saccharides and without saccharides. According to the absorption coefficient for FCM, it is possible to estimate that the influence of fructose is approximately 3.5-times higher than that of glucose and perhaps 4.5-times higher than that of sucrose. The approximate influence of saccharides on the values determined by other methods (PBM, TEAC, FRAP and DPPH) can be calculated from the average contents of residual

Table 4. Correction (%) of determined values by FCM on the contents of saccharides (Singleton *et al*. 1999)

Phenolic compounds/sugars (mg/l)	25 g/l	50 g/l	100 g/l
100	–5	–10	–20
200	–5	–8	–20
500	–4	–6	–10
1000–2000	–3	–6	–10

saccharides and the determined absorption coefficients presented in Table 3.

CONCLUSIONS

The tradition is to classify the quality of wine based on its colour, smell, and taste rather then on its content of compounds beneficial to health. The determinable laboratory characteristics so far used like alcohol, organic acids, saccharides, and sulfites concentration relate more to the taste quality of wine as well. The determinations of total phenolic compounds and the total antioxidant activity of wine tell more about the health effect of a particular wine and can be used as critera of quality and beneficial health effect.

Several different methods for the evaluation of phenolic compounds and antioxidant activity of wines are used and little is known about the possibility of the comparison of the values obtained by different methods. Through the analyses of the same sample by different methods we investigated the correlations of values determined by the used methods. The values found with the methods for total phenolics (FCM and PBM) and total antioxidant activity (TEAC, FRAP, and DPPH) determination correlated with one another and also with the colour of wines very significantly with the exception of FRAP method for white wines (so far for unexplained reasons). The insignificant correlation between the contents of total phenolics or antioxidant activity and the intensity of red wines colour might indicate artificial colouring of red wine or mixing white and red wine.

FCM for the determination of total phenolics and TEAC method for the determination of total antioxidant activity can be particularly easy to use and may be the best of the tested methods for rapid objective evaluation of a large number of wine samples. The assessment of phenolics content and total antioxidant activity in white and red wines and the introduction of these values on the bottle label would better inform the consumer about the quality and health benefit of the purchased wine.

References

Arnao M.B. (2000): Some methodological problems in the determination of antioxidant activity using chromogen radicals: a practical case. Trends in Food Science and Technology, **11**: 419–421.

Benzie I.F.F., Strain J.J. (1996): The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry, **239**: 70–76.

Cooper K.A., Chopra M., Thurnham D.I. (2004): Wine polyphenols and promotion of cardiac health. Nutrition Research Review, **17**: 111–129.

Fernández-Pachón M.S., Villaño D., Troncoso A.M., García-Parrilla M.C. (2006): Determination of phenolic composition of sherry and table white wines by liquid chromatography and their relation with antioxidant activity. Analytica Chimica Acta, **563**: 101–108.

Gardner P.T., White T.A.C., McPhail D.B., Duthie G.G. (2000): The relative contribution of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. Food Chemistry, **68**: 471–474.

Gronbaek M., Deis A., Sorensen T.I.A., Becker U., Schnohr P., Jensen G. (1995): Mortality associated with moderate intakes of wine, beer, or spirits. British Medical Journal, **310**: 1165–1169.

Heinonen M., Lehtonen P.J., Hopia A.I. (1998): Antioxidant activity of berry and fruit wines and liquors. Journal of Agriculture and Food Chemistry, **46**: 25–31.

Hertog M.G.L., Feskens E.J.M., Hollman P.C H., Katan M.B., Kromhout D. (1993): Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. Lancet, **342**: 1007–1011.

Hertog M.G.L., Kromhout D., Aravanis C., Blackburn H., Buzina R., Fidanza F., Giampaoli S., Jansen A., Menotti A., Nedeljkovic S., Pekkarinen M., Simic B.S., Toshima H., Feskens E.J.M., Hollman P.C.H., Katan M.B. (1995): Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Archives of Internal Medicine, **155**: 381–386.

Hoffmeister H., Schelp F.P., Mensink G.B.M., Dietz E., Bohning D. (1999): The relationship between alcohol consumption, health indicators and mortality in the German population. International Journal of Epidemiology, **28**: 1066–1072.

Huang D., Ou B.X., Prior, R.L. (2005): The chemistry behind antioxidant capacity assays. Journal of Agriculture and Food Chemistry, **53**: 1841.

Imeh U., Khokhar S. (2002): Distribution of conjugated and free phenols in fruits: antioxidant activity and cultivar variations. Journal of Agriculture and Food Chemistry, **50**: 6301–6306.

Jewell W.T., Ebeler S.E. (2001): Tyrosine biosensor for the measurement of wine polyphenolics. American Journal of Enology and Viticulture, **52**: 219–222.

Kim D.O., Lee K.W., Lee H.J., Lee C.Y. (2002): Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic

phytochemicals. Journal of Agriculture and Food Chemistry, **50**: 3713–3717.

Klatsky A.L., Armstrong M.A. (1993): Alcoholic beverage choice and risk of coronary artery disease mortality: do red wine drinkers fare best? American Journal of Cardiology, **71**: 467–469.

Knekt P., Järvinen R., Reunanen A., Maatela J. (1996): Flavonoid intake and coronary mortality in Finland: a cohort study. British Medical Journal, **312**: 478–481.

Lien E.J., Ren S., Bui H.H., Wang R. (1999): Quantitative structure-activity relationship analysis of phenolic antioxidants. Free Radical Biology & Medicine, **26**: 285–294.

Long L.H., Halliwell B. (2001): Antioxidant and prooxidant abilities of food and beverages. Methods in Enzymology, **335**: 181–190.

Lopes P., Drinkine J., Saucier C., Glories Y. (2006): Determination of L-ascorbic acid in wines by direct injection liquid chromatography using a polymeric column. Analytica Chimica Acta, **555**: 242–245.

Matějíček D., Mikeš O., Klejdus B., Štěrbová D., Kubáň V. (2005): Changes in content of phenolic compounds during maturing of barrique red wine. Food Chemistry, **90**: 791–800.

Miller N.J., Rice-Evans C.A., Davies M.J., Gopinathan V., Milner A. (1993): A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clinical Science, **84**: 407–412.

Ough C.S., Amerine M.A. (1988): Methods for Analysis of Musts and Wines. 2nd Ed. Wiley, New York.

Pinto P.C.A.G., Saraiva M.L.M.F.S., Reis S., Lima J. L.F.C. (2005): Automatic sequential determination of the hydrogen peroxide scavenging activity and evaluation of the antioxidant potential by the 2,2´-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation assay in wines by sequential injection analysis. Analytica Chimica Acta, **531**: 25–32.

Plumb G.W., Price K.R., Williamson G. (1999): Antioxidant properties of flavonol glycosides from green beans. Redox Report, **4**: 123–127.

Price M.L., Butler L.G. (1977): Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grain. Journal of Agriculture and Food Chemistry, **25**: 1268–1273.

Pulido R., Bravo L., Saura-Calixto F. (2000): Antioxidant activity of dietary polyphenols as determined by a modified FRAP assay. Journal of Agriculture and Food Chemistry, **48**: 3396–3402.

Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. (1999): Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology & Medicine, **26**: 1231–1237.

Renaud S.C., Gueguen R., Siest G., Salomon R. (1999): Wine, beer, and mortality in middle-aged men from eastern France. Archives of Internal Medicine, **159**: 1865–1870.

Renaud S., De Lorgeril M. (1992): Wine, alcohol, platelets and the French paradox for coronary heart disease. Lancet, **339**: 1523–1526.

Ribéreau-Gayon P., Dubourdieu D., Donéche B., Lonvaud A. (1998): Traité d'Oenologie – Microbiologie du Vin – Vinifications. Dunod, Paris: 282.

Rice-Evans C.A., Miler N.J., Paganga G. (1996): Structure-antioxidant activity relationship of flavonoids and phenolic acids. Free Radical Biology & Medicine, **20**: 933–956.

Rimm E.B., Williams P., Fosher K., Criqui M., Stampfer M.J. (1999): Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. British Medical Journal, **319**: 1523–1528.

Sánchez-Moreno C., Larrauri J.A., Saura-Calixto F. (1998): A procedure to measure the antiradical efficiency of polyphenols. Journal of the Science of Food and Agriculture, **76**: 270–276.

Sánchez-Moreno C., Larrauri J.A, Saura-Calixto F. (1999): Free radical scavenging capacity of selected red rosé and white wines. Journal of the Science of Food and Agriculture, **79**: 1301–1304.

Santos J.C.C., Korn M. (2006): Exploiting sulphide generation and gas diffusion separation in a flow system for indirect sulphite determination in wines and fruit juices. Microchimica Acta, **153**: 87–94.

Scalbert A., Manach C., Morand C., Rémésy C. (2005): Dietary polyphenols and the prevention of diseases. Critical Reviews in Food Science and Nutrition, **45**: 1–20.

Schoonen J.W., Sales M.G.F. (2002): Determination of polyphenols in wines by reaction with 4-aminoantipyrine and photometric flow-injection analysis. Analytical and Bioanalytical Chemistry, **372**: 822–828.

Simonetti P., Pietta P., Testolin G. (1997): Polyphenol content and total antioxidant potential of selected Italian wines. Journal of Agriculture and Food Chemistry, **45**: 1152–1155.

Singleton V.L. (1982): Grape and wine phenolics; background and prospects. In: Grape and Wine Centennial Symposium, Proceedings of the University of California, Davis: 215–227.

Singleton V.L., Orthofer R., Lamuela-Raventós R.M. (1999): Analysis of total phenols and other oxidation substrates and antioxidants by means of Fo-

lin-Ciocalteu reagent. Methods in Enzymology, **299**: 152–178.

Soleas G.J., Tomlinson G., Diamandis E.P., Goldberg D.M. (1997): Relative contribution of polyphenolic constituents to the antioxidant status of wines: Development of a predictive model. Journal of Agriculture and Food Chemistry, **45**: 3995–4003.

Stevanato R., Fabris S., Momo F. (2004): New enzymatic method for the determination of total phenolic content in tea and wine. Journal of Agriculture and Food Chemistry, **52**: 6287–6293.

Stratil P., Klejdus B., Kubáň V. (2006): Determination of total content of phenolic compounds and their antioxidant activity in vegetables – evaluation of spectrofotometric methods. Journal of Agriculture and Food Chemistry, **54**: 607–616.

Stratil P., Klejdus B., Kubáň V. (2007): Determination of phenolic compounds and their antioxidant activity in fruits and cereals. Talanta, **71**: 1741–1751.

Trichopoulou A., Lagiou P. (1997): Healthy traditional Mediterranean diet: an expression of culture, history, and lifestyle. Nutrition Review, **55**: 383–389.

Vinson J.A., Hao Y., Su X., Zubík L. (1998): Phenol antioxidant quantity and quality in foods: vegetables. Journal of Agriculture and Food Chemistry, **46**: 3630–3634.

Waterman P.G., Mole S. (1994): Extraction and chemical quantification. In: Waterman P.G., Mole S. (eds): Analysis of Phenolic Plant Metabolites. Methods in Ecology. Blackwell Publishing, Oxford: 66–103.

Wildenradt H.L., Singleton V.L. (1974): The production of aldehydes as a result of oxidation of polyphenolic compounds and its relation to wine aging. American Journal of Enology and Viticulture, **25**: 119–124.

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