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Polyphenolic compounds and antioxidant properties of selected China wines

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ABSTRACT

Thirty-seven China wines, produced from different geographical origins, were examined in this study. The antioxidant activity of wines was measured by different analytical methods: oxygen radical absorbance capacity (ORAC), reducing power, 2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH·), hydroxyl radical-scavenger activity, superoxide radical-scavenger activity, lipid peroxidation and chelating capacity. Furthermore,total phenols, total flavonoids, total flavanols and total anthocyanins of wines were determined. As expected, the red wines had much higher phenolic content and antioxidant capacity than rosé wines or white wines. Among the red wines, Cabernet Sauvignon and Muscat Hamburg, respectively, represented the wines with the highest and lowest phenolic contents and antioxidant capacity. Among the white wines, Italian Riesling had the lowest phenolic content and antioxidant capacity. Taken together, a close relationship between phenolic contents and antioxidant capacity, for all wines, was observed.

1. Introduction

Oxidative stress has been implicated in over one hundred human disease conditions, such as cancer, cardiovascular disease, aging and neurodegenerative diseases (Bagchi et al., 2000). However, the innate defense in the human body may not be enough for severe oxidative stress. Hence, certain amounts of exogenous antioxidants are constantly required to maintain an adequate level of antioxidants in order to balance the ROS.

Epidemiological evidence indicates that the moderate consumption of wines reduces the incidence of coronary heart disease (CHD), atherosclerosis and platelet aggregation (Tedesco et al., 2000). This greater protection may be due to the phenolic components of wines, which are particularly abundant in the red wine, since they behave as reactive oxygen species-scavengers and metal-chelators. Polyphenolic substances in wines are usually subdivided into two groups: flavonoids and nonflavonoids. The most common flavonoids in wine are flavonols (quercetin, kaempferol, and myricetin), flavan-3-ols (catechin, epicatechin, and tannins), and anthocyanins (cyanin). Nonflavonoids comprise stilbenes, hydroxycinnamic acids and benzoic acids. Numerous papers have been published on red and white wines and the antioxidant properties of wines have been correlated with their polyphenol contents (Arnous, Makris, & Kefalas, 2002; Cimino, Sulfaro, Trombetta, Saija, & Tomaino, 2007; Fernández-Pachón, Villaño, Troncoso, & García-Parrilla, 2006; Minussi et al., 2003).

With increasing interest in the function and diversity of antioxidants in foods, several *in vitro* methods for measuring antioxidant activity of food, beverages and biological samples have been developed (Prior, Wu, & Schaich, 2005; Roginsky & Lissi, 2005). The most commonly used antioxidant capacity assays include oxygen radical absorbance capacity (ORAC), reducing power, determination of total phenols, 2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid) (ABTS assay), 2,2-diphenyl-1-picrylhydrazyl (DPPH· assay), hydroxyl radical-scavenger activity, superoxide radical-scavenger activity and lipid peroxidation inhibition. These methods differ in terms of their assay principles and experimental conditions. Because multiple reaction characteristics and mechanisms are usually involved, no single assay will accurately reflect all antioxidants in a mixed or complex system. Thus, to fully elucidate a full profile of antioxidant capacity, different antioxidant capacity assays may be needed.

The wine industry is growing and the wine market has a wider space to develop in China, it will be even more prosperous in future. To date, there has been no published research on the chemical quality and antioxidant activity of wines produced in China; by establishing an "antioxidant profile", this paper will help to better understand the quality of current wines and stimulate the development of enological techniques for their enrichment. For this study, we selected the most important and representative commercial wines available in China and investigated the polyphenolic composition and antioxidant capacity of selected red, rosé and white wines. Total phenols, total flavonoids, total flavanols and total anthocyanins in the whole wines were determined. The methods used to measure antioxidant capacity were ORAC, ABTS, DPPH·,

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CUPRAC, hydroxyl radical-scavenger activity, superoxide radical-scavenger activity, lipid peroxidation inhibition and chelating capacity. Furthermore, a correlation analysis was done between these parameters for all wines.

2. Materials and methods

2.1. Chemicals

Folin-Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH·), 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), 2,9-dimethyl-1,10-phenanthroline (neocuproine), 2-deoxyribose, nicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), nitroblue tetrazolium (NBT), ferrozine, egg lecithin, gallic acid, ascorbic acid, catechin, *p*-dimethylaminocinnamaldehyde (DMACA) and sodium fluorescein were obtained from Sigma–Aldrich (St. Louis, MO). 2,2′-Azobis(2-amidinopropane)dihydrochloride (AAPH) was obtained from Fluka (Buchs, Switzerland). Trichloroacetic acid (TCA), hydrogen peroxide, ethylenediaminetetraacetic acid (EDTA) tretrasodium salt, ferric chloride and sodium metabisulphite were obtained from Sinopharm (Shanghai, China). Thiobarbituric acid (TBA) and potassium persulfate were obtained from Merck (Darmstadt, Germany). All other reagents used were of analytical grade.

2.2. Instruments

Spectrophotometric measurements were performed on a UV–vis double beam Shimadzu UV-1600 spectrometer (Shimadzu, Kyoto, Japan). A Tecan Genios Multi-Detection Microplate Reader with injectors (Tecan, Raleigh, NC) was used in the ORAC assay with a 485, and 20 nm bandpass, excitation filter and a 528 nm, 20 nm bandpass, emission filter. The plate reader was controlled by Magellan version 5.03 (Tecan, Raleigh, NC).

2.3. Wines

Table 1 summarizes 24 red wines, 11 white and 2 rosé wines investigated from six provinces in China. Wines covered dominating viticultural areas of China, which were available in the China market, and widely consumed. All samples were kindly supplied by eleven wineries.

Table 1
Origin and varietal composition of the wines tested

Wine samples	Vintage	Winery	Cultivar(s)	Location
Red wines				
R1	2004	A	Cabernet Sauvignon	Henan[c]
R2	2004	A	Cabernet Gernischet	Henan[c]
R3	2005	A	Blend	Henan[c]
R4	2004	A	Cabernet Sauvignon	Henan[c]
R5	2006	B	Cabernet Sauvignon	Hebei[a]
R6	2003	C	Cabernet Sauvignon	Ningxia[b]
R7	2005	C	Cabernet Sauvignon	Ningxia[b]
R8	2003	D	Cabernet Sauvignon	Ningxia[b]
R9	2006	D	Cabernet Sauvignon	Beijing[c]
R10	2006	E	Cabernet Gernischet	Gansu[b]
R11	2006	E	Merlot	Gansu[b]
R12	2005	F	Rose Honey	Yunnan[d]
R13	2003	F	Blend	Yunnan[d]
R14	2006	G	Blend	Hebei[a]
R15	2006	G	Cabernet Sauvignon	Hebei[a]
R16	2003	H	Cabernet Sauvignon	Xinjiang[b]
R17	2005	H	Blend	Xinjiang[b]
R18	2006	I	Cabernet Sauvignon	Shandong[a]
R19	2006	J	Muscat Hamburg	Ningxia[b]
R20	2006	J	Cabernet Sauvignon	Ningxia[b]
R21	2006	J	Cabernet Gernischet	Ningxia[b]
R22	2006	J	Merlot	Ningxia[b]
R23	2005	K	Blend	Ningxia[b]
R24	2006	K	Blend	Ningxia[b]
White wines				
W1	2004	A	Italian Riesling	Henan[c]
W2	2006	B	Chardonnay	Hebei[a]
W3	2005	C	Longyan	Ningxia[b]
W4	2004	D	Italian Riesling	Beijing[c]
W5	2006	E	Semillon	Gansu[b]
W6	2003	F	Blend	Yunnan[d]
W7	2006	H	Chenin Blanc	Xinjiang[b]
W8	2006	I	Blend	Shandong[a]
W9	2006	J	Chardonnay	Ningxia[b]
W10	2006	J	Italian Riesling	Ningxia[b]
W11	2006	J	Riesling	Ningxia[b]
Rosé wines				
Rosé1	2006	B	Muscat Hamburg	Hebei[a]
Rosé2	2006	G	Muscat Hamburg	Hebei[a]

Note: a, b, c, and d assign east, west, central and south China, respectively.

2.4. Determination of total phenols (TP), total flavonoids (TFO), total flavanols (TFA) and total anthocyanins (TA) of the wine samples

The amount of total phenols in the whole wines was determined according to the Folin-Ciocalteu colorimetric method (Rapisarda et al., 1999). Absorbances were measured at 765 nm. Total phenols were expressed as gallic acid equivalents (mg l^{-1} of GAE). Gallic acid standard solutions were prepared at a concentration ranging from 0 to 500 mg l^{-1}. The amount of total flavonoids was determined according to a previously described protocol (Kim, Chun, Kim, Moon, & Lee, 2003). Absorbances were measured at 510 nm. Results were expressed as catechin equivalents (mg l^{-1} of CTE). Catechin standard solutions were prepared at a concentration ranging from 6.25 to 300 mg l^{-1}. The amount of total flavanol was estimated using the slightly modified *p*-dimethylaminocinnamaldehyde (DMACA) method (Li, Tanner, & Larkin, 1996). Absorbances were measured at 640 nm. Results were expressed as catechin equivalents (mg l^{-1} of CTE). Catechin standard solutions were prepared at a concentration ranging from 6.25 to 200 mg l^{-1}. The amount of total anthocyanins was determined by using the bisulfite bleaching method (Arnous et al., 2002). Absorbances were measured at 520 nm. Each determination was performed in triplicate and repeated three times. Results are expressed as means ± S.D.

2.5. Free radical-scavenging activity on DPPH·

The ability of wines to scavenge DPPH· free radicals was determined. Scavenging activity was based on the slightly modified method of Brandwilliams, Cuvelier, and Berset (1995). Briefly, 0.1 ml of red wine (diluted at 1:20) or white wine (diluted at 1:2) was added to 3.9 ml of a 6×10^{-5} M solution of DPPH· in methanol. A control sample, containing the same volume of solvent in place of extract, was used to measure the maximum DPPH· absorbance. After the reaction was allowed to take place in the dark for 30 min, the absorbance at 515 nm was recorded to determine the concentration of remaining DPPH·. Results were expressed as trolox equivalent antioxidant capacity. Trolox standard solutions were prepared at a concentration ranging from 100 to 1200 M.

2.6. Free radical-scavenging activity on ABTS

ABTS assay was based on the slightly modified method of Re et al. (1999). ABTS radical cation ($ABTS^{+}$) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate aqueous

solution and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The $ABTS^+$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 732 nm. After addition of 100 l of red wine (diluted at 1:20) or white wine (diluted at 1:2) to 3.9 ml of diluted $ABTS^+$ solution, absorbance was measured at exactly 6 min. Results were expressed as trolox equivalent antioxidant capacity. Trolox standard solutions were prepared at a concentration ranging from 100 to 1400 M.

2.7. Determination of reducing power (CUPRAC)

The cupric reducing antioxidant capacity of wines was determined according to the method of Apak, Guclu, Ozyurek, and Karademir (2004). To a test tube, 1 ml each of 10 mM Cu(II), 7.5 mM neocuproine, NH_4Ac buffer (1 M, pH 7.0) solutions and 0.6 ml of water were added. About 0.5 ml of red wine (diluted at 1:100) or white wine (diluted at 1:10) was added to the initial mixture so as to make the final volume 4.1 ml. The tubes were stoppered and, after 30 min, the absorbance at 450 nm was recorded against a reagent blank. Results were expressed as trolox equivalent antioxidant capacity. Trolox standard solutions were prepared at a concentration ranging from 50 to 500 M.

2.8. Oxygen radical absorbance capacity (ORAC)

The ORAC assay was performed essentially as described by Huang, Ou, Hampsch-Woodill, Flanagan, and Prior (2002). Briefly, AAPH was dissolved in 10 ml of 75 mM phosphate buffer (pH 7.4) to a final concentration of 153 mM and made fresh daily. A fluorescein stock solution (4×10^{-3} mM) was made in 75 mM phosphate buffer and stored. The stock solution was diluted 1:1000 with phosphate buffer. To all experimental wells, 150 µl of working sodium fluorescein solution were added. In addition, blank wells received 25 µl of 75 mM phosphate buffer, while standards received 25 µl of trolox dilution and samples received 25 µl of wine (diluted at 1:500 or diluted at 1:100). Reactions were initiated by the addition of 25 µl of AAPH solution using the microplate reader's injector. Results were expressed as trolox equivalent antioxidant capacity. Trolox standard solutions were prepared at a concentration ranging from 6.25 to 100 M.

2.9. Hydroxyl radical-scavenging activity (HRSA)

For the determination of the hydroxyl free radical-scavenging capacity of wines, the deoxyribose method was used, as described by Ghiselli, Nardini, Baldi, and Scaccini (1998), slightly modified. About 0.05 ml of wine (diluted at 1:100 or diluted at 1:50) was mixed with 0.345 ml of 10 mM phosphate buffer, pH 7.4, containing 2.5 mM 2-deoxyribose. An aliquot of 0.05 ml of 2 mM iron ammonium sulfate premixed with 2.08 mM EDTA was added, the mixture was vortexed, and the reaction was initiated by adding 0.05 ml of 1 mM ascorbic acid and 0.02 ml of 1.5 mM hydrogen peroxide. Samples were maintained at 37 °C for 30 min, and then treated with 0.5 ml of 30% TCA and 0.5 ml of 1% TBA. Samples were heated at 90 °C for 20 min, cooled, and the absorbance at 532 nm measured. Control sample was prepared by adding distilled water instead of wine. The result was expressed as inhibition in relation to a control test.

2.10. Superoxide radical-scavenging activity (SRSA)

Superoxide anion scavenging activity was measured, based on the described method by Robak and Gryglewski (1988). In this experiment, the superoxide radical was generated in 3 ml of sodium phosphate buffer (100 mM, pH 7.4) containing 1 ml of NBT (150 M) solution, 1 ml of NADH (468 M) solution and 1 ml of wine (diluted at 1:50 or diluted at 1:20). The reaction was started by adding 1 ml of PMS solution (60 M) to the mixture. The reaction mixture was incubated at 25 °C for 5 min, and the absorbance was measured against the corresponding blank solution. The result was expressed as inhibition in relation to a control test.

2.11. Lipid peroxidation inhibition

Liposomes were prepared according to the method of Tsuda et al. (1994). Egg lecithin (5 g) was dispersed in a sodium phosphate buffer (500 ml, 20 mM, and pH 7.4) and sonicated in a sonicator for 30 min under N_2 atmosphere in an ice-cold water bath. About 0.5 ml of wine (diluted at 1:50 or diluted at 1:20) was mixed with liposomes (2 ml), 25 mM $FeCl_3$ (0.1 ml), 25 mM H_2O_2 (0.1 ml), 25 mM ascorbic acid (0.1 ml) and 0.2 M phosphate buffer (1.2 ml, pH 7.4). The reaction mixture was incubated at 37 °C for 4 h. At the end of the incubation, 1 ml of BHA (20 mg/ml in methanol) was added to stop the oxidation reaction. The extent of oxidation of liposomes was subsequently determined by measuring the thiobarbituric acid-reactive substances (TBARS). The absorbance of the supernatant was measured spectrophotometrically at 532 nm. The result was expressed as inhibition in relation to a control test.

2.12. Metal chelation (MC)

The ferrous ion-chelating activity was determined by the method of Dinis, Madeira, and Almeida (1994). Wine was mixed with 2 mM $FeCl_2 \cdot 4H_2O$ and 5 mM ferrozine [4,4′-(3-(2-pyridinyl)-1,2,4-triazine-5,6-diyl)bisbenzenesulfonic acid, disodium salt] and the mixture was shaken. After 10 min, the Fe^{2+} was monitored by measuring the formation of ferrous ion-ferrozine complex at 562 nm.

2.13. Statistical analysis

Experimental results were means ± SD of three parallel measurements. Analysis of variance was performed by ANOVA procedures and correlation was calculated by linear regression (DPS 7.55 for Windows).

3. Results and discussion

3.1. Polyphenolic composition

The total phenols, the total flavonoids, the total flavanols and the total anthocyanins were measured for all the samples. The results are given in Table 2. There was a wide range of phenol concentrations in selected wines. As expected, the red wines had significantly higher amounts of total phenols, flavonoids, flavanols and anthocyanins than had white wines or rosé wines. The content of phenolics decreased in the order: red > rosé > white wine. These results are in agreement with those available in the literature (Katalinić, Milos, Modun, Musić, & Boban, 2004; Paixão, Perestrelo, Marques, & Câmara, 2007; Woraratphoka, Intarapichet, & Indrapichate, 2007). This is due to a greater grape skin and seed contact time and temperature for the fermentation process for red wines.

The content of total phenols varied from 1402 to 3130 mg l^{-1}, averaging 2068 mg l^{-1}, for the red wines and from 189 to 495 mg l^{-1}, averaging 302 mg l^{-1}, for the white wines. For the rosé wines, we obtained 741 and 1086 mg l^{-1} (Table 2). The content of total flavonoids varied from 396 to 1596 mg l^{-1}, averaging 873 mg l^{-1}, for the red wines and from 31 to 242 mg l^{-1}, averaging 87 mg l^{-1}, for the white wines. For the rosé wines, we obtained 283 and 634 mg l^{-1}. The content of total flavanols varied from 196 to 680 mg l^{-1}, averaging 386 mg l^{-1}, for the red wines and from 0.3

Table 2
Total amount of polyphenolic substances in the analyzed wines

Wine samples	TP[a] (mg l^{-1} GAE)	TFO[b] (mg l^{-1} CTE)	TFA[c] (mg l^{-1} CTE)	TA[d] (mg l^{-1})
R1	2268 ± 118	905 ± 59	379 ± 29	59.0 ± 3.4
R2	1596 ± 88	513 ± 32	196 ± 15	67.6 ± 4.5
R3	1410 ± 152	461 ± 42	163 ± 18	68.5 ± 8.2
R4	2027 ± 132	763 ± 45	400 ± 22	70.1 ± 6.7
R5	1649 ± 105	510 ± 28	273 ± 16	107 ± 9.2
R6	2360 ± 184	1028 ± 112	540 ± 70	71.3 ± 5.8
R7	2182 ± 147	760 ± 51	428 ± 35	133 ± 10.4
R8	2488 ± 93	1041 ± 69	430 ± 33	69.3 ± 6.8
R9	1880 ± 120	805 ± 93	399 ± 29	147 ± 15.1
R10	1655 ± 79	576 ± 48	308 ± 15	91.8 ± 5.2
R11	1977 ± 201	1086 ± 55	509 ± 18	71.4 ± 3.9
R12	1877 ± 126	650 ± 35	272 ± 14	86.8 ± 7.7
R13	2260 ± 93	620 ± 64	269 ± 37	287 ± 15.9
R14	2271 ± 132	808 ± 71	353 ± 24	134 ± 10.3
R15	1635 ± 108	768 ± 53	307 ± 19	129 ± 11.4
R16	2396 ± 302	1323 ± 89	472 ± 28	95.1 ± 5.8
R17	3130 ± 228	1396 ± 125	602 ± 51	96.7 ± 10.1
R18	1580 ± 101	716 ± 61	245 ± 16	170 ± 10.8
R19	1402 ± 74	396 ± 24	196 ± 12	61.0 ± 3.5
R20	2927 ± 159	1596 ± 33	680 ± 57	175 ± 13.6
R21	2046 ± 155	933 ± 79	423 ± 29	237 ± 20.6
R22	2246 ± 230	1110 ± 145	505 ± 46	154 ± 12.4
R23	1963 ± 147	876 ± 59	380 ± 25	192 ± 15.5
R24	2418 ± 156	1315 ± 87	527 ± 40	93.5 ± 7.6
AV	2068	873	386	119
W1	325 ± 38	89.7 ± 10.2	5.84 ± 0.47	ND
W2	218 ± 33	31.0 ± 4.5	0.27 ± 0.04	ND
W3	189 ± 12	58.5 ± 3.5	4.33 ± 0.12	ND
W4	303 ± 19	42.5 ± 2.8	4.76 ± 0.28	ND
W5	387 ± 31	119 ± 7.4	34.0 ± 1.75	ND
W6	495 ± 25	242 ± 13.6	61.4 ± 3.56	ND
W7	261 ± 14	56.4 ± 4.8	7.36 ± 0.42	ND
W8	325 ± 16	77.9 ± 3.8	11.9 ± 0.61	ND
W9	298 ± 22	94.1 ± 5.9	24.1 ± 2.19	ND
W10	219 ± 16	52.9 ± 4.4	7.55 ± 0.50	ND
W11	300 ± 17	95.4 ± 6.9	17.5 ± 2.05	ND
AV	302	87.3	16.23	ND
Rosé1	741 ± 58	283 ± 19	146 ± 11	4.33 ± 0.16
Rosé2	1086 ± 134	634 ± 59	280 ± 18	6.84 ± 0.43
AV	914	458	213	5.58

Note: Values represent means of triplicate determination ± S.D. a: Total phenols expressed as gallic acid equivalents; b: total flavonoids; c: total flavanols expressed as catechin equivalents; and d: total anthocyanins.

to 61 mg l^{-1}, averaging 16 mg l^{-1}, for the white wines. For the rosé wines, we obtained 146 and 280 mg l^{-1}. The grape pigments or anthocyanins are present in red grapes only. The content of total anthocyanins varied from 59 to 286 mg l^{-1}, averaging 119 mg l^{-1}, for the red wines and from 4.3 to 6.8 mg l^{-1}, averaging 5.6 mg l^{-1}, for the rosé wines.

The results confirm a variation in phenolic content among wine samples tested. As is well known, the amounts of phenolic materials vary considerably in different types of wines, depending on the grape variety, environmental factors in the vineyard, the wine processing techniques and wood maturation (Lachman, Šulc, & Schilla, 2007; Villaño, Fernández-Pachón, Troncoso, & García-Parrilla, 2006). The high phenolic content in red wine contributes to its increased antioxidant potential in comparison to white wine.

In order to compare the phenolic contents of different grape varieties, we chose several wines in winery J (Table 2). These wines had the same situations, including same age, vintage and wine-making techniques. The phenolic contents of four red wines (Cabernet Sauvignon, Cabernet Gernischet, Merlot and Muscat Hamburg) and three white wines (Chardonnay, Italian Riesling and Riesling) were analyzed and compared. For the red wines, the total phenols, the total flavonoids and the total flavanols decreased in the order: Cabernet Sauvignon > Merlot > Cabernet Gernischet > Muscat Hamburg, while the total anthocyanins decreased in the order: Cabernet Gernischet > Cabernet Sauvignon > Merlot > Muscat Hamburg. Interestingly, Cabernet Gernischet wines contained significantly less total phenols and flavanols than did Cabernet Sauvignon and Merlot wines, but significantly more anthocyanins than the other red cultivar wines. Among the white wines, Chardonnay and Italian Riesling, respectively, represented the wines with the highest and lowest phenolic contents.

3.2. Antioxidant activity

3.2.1. General

The antioxidant activities found by different assays in the white and red wine varieties differed significantly (Table 3). As can be observed, red wines' values were higher than those of white and rosé wines in every antioxidant test used. The magnitude of the difference depends on the method employed. This result is well in accordance with recent reports in the literature (Fernández-Pachón, Villaño, García-Parrilla, & Troncoso, 2004; Katalinić et al., 2004; Paixão et al., 2007), suggesting a high polyphenol content in red wine varieties.

3.2.2. ORAC

The antioxidant capacity of selected wines was determined by the ORAC-FL method and the results are presented in Table 3. The ORAC assay depends on the free radical damage to a fluores-

Table 3
Antioxidant properties of the wines tested

Wine	ORAC[a] (TE)	DPPH[a] (TE)	ABTS[a] (TE)	CUPRAC[a] (TE)	MC[b] (%)	SRSA[b] (%)	HRSA[b] (%)	TBARS[b] (%)
R1	16838 ± 984	9816 ± 562	13232 ± 792	24015 ± 1308	50.9 ± 3.5	69.2 ± 5.2	79.3 ± 4.5	68.9 ± 7.1
R2	9620 ± 631	5010 ± 311	9155 ± 568	18498 ± 1145	68.3 ± 6.8	53.6 ± 3.4	79.1 ± 6.1	58.1 ± 3.6
R3	14520 ± 1084	4893 ± 206	10818 ± 755	16165 ± 998	69.5 ± 2.2	51.5 ± 2.9	55.3 ± 3.3	35.5 ± 2.4
R4	24371 ± 1521	8952 ± 477	13549 ± 1008	21681 ± 1546	66.2 ± 7.0	63.7 ± 6.1	70.3 ± 3.8	53.3 ± 3.3
R5	16766 ± 982	6124 ± 398	11820 ± 1112	17598 ± 1032	72.6 ± 5.3	72.2 ± 6.7	68.1 ± 2.7	49.6 ± 2.9
R6	14999 ± 1005	12439 ± 985	15542 ± 994	27281 ± 1752	70.4 ± 5.9	74.5 ± 4.6	50.6 ± 3.2	52.9 ± 6.5
R7	15840 ± 1428	11208 ± 1005	14380 ± 861	24998 ± 965	76.1 ± 4.1	68.9 ± 3.8	64.4 ± 4.1	51.7 ± 4.7
R8	15527 ± 899	10344 ± 726	13531 ± 582	24548 ± 2013	66.2 ± 2.7	70.8 ± 60	69.8 ± 2.6	52.4 ± 4.5
R9	8890 ± 760	7193 ± 367	12601 ± 495	19848 ± 1754	74.0 ± 26.4	71.2 ± 5.2	68.3 ± 5.3	40.8 ± 4.0
R10	15212 ± 1178	5421 ± 453	9441 ± 763	17181 ± 1305	33.6 ± 1.5	69.8 ± 5.5	74.6 ± 7.6	50.8 ± 3.8
R11	20956 ± 1562	10827 ± 765	13225 ± 825	22998 ± 1578	62.8 ± 3.6	73.1 ± 2.4	54.6 ± 2.9	63.1 ± 6.1
R12	20039 ± 1698	10798 ± 820	12000 ± 1105	20565 ± 1045	64.8 ± 3.3	68.5 ± 5.3	79.9 ± 6.1	43.9 ± 2.8
R13	22766 ± 1805	13259 ± 884	14187 ± 998	24715 ± 1136	67.1 ± 6.8	74.9 ± 3.1	82.3 ± 5.2	40.6 ± 1.9
R14	18860 ± 635	10725 ± 1027	12984 ± 794	24015 ± 1329	59.9 ± 4.9	77.3 ± 2.7	75.9 ± 3.6	50.3 ± 3.5
R15	13350 ± 1216	8512 ± 693	9102 ± 836	19698 ± 965	66.0 ± 7.2	76.9 ± 2.9	64.3 ± 2.9	48.0 ± 3.2
R16	20036 ± 1564	15018 ± 976	16063 ± 886	26298 ± 2134	63.9 ± 2.9	75.3 ± 4.6	56.1 ± 2.9	54.0 ± 3.8
R17	19942 ± 588	21362 ± 1024	30509 ± 1982	31931 ± 2219	63.6 ± 4.6	73.1 ± 3.9	57.2 ± 3.5	44.3 ± 3.9
R18	15753 ± 1041	6373 ± 631	12262 ± 630	16115 ± 881	65.5 ± 3.5	62.9 ± 2.5	71.0 ± 4.8	33.1 ± 2.2
R19	15208 ± 1257	4190 ± 184	9562 ± 584	18598 ± 1321	73.6 ± 6.8	45.4 ± 1.8	48.1 ± 3.2	31.0 ± 2.0
R20	22860 ± 1812	17172 ± 1028	25782 ± 2108	27431 ± 1985	61.6 ± 4.6	75.2 ± 4.2	65.3 ± 3.7	60.9 ± 4.7
R21	18016 ± 989	10080 ± 842	14650 ± 1325	19581 ± 1568	71.5 ± 7.8	69.7 ± 1.9	67.4 ± 4.5	45.9 ± 3.7
R22	19014 ± 963	11399 ± 653	15956 ± 792	20981 ± 1347	68.7 ± 6.6	70.0 ± 5.8	70.2 ± 3.8	50.9 ± 3.4
R23	17795 ± 895	8651 ± 469	13237 ± 899	16748 ± 1036	79.3 ± 5.4	63.7 ± 3.6	75.0 ± 6.2	41.1 ± 3.6
R24	21271 ± 1800	12541 ± 1135	14913 ± 652	22365 ± 1528	64.0 ± 3.2	66.3 ± 5.4	59.6 ± 3.7	46.8 ± 2.7
AV	17435	10096	14104	21827	65.8	68.2	66.9	48.5
W1	3276 ± 208	602 ± 31	1221 ± 100	3178 ± 167	55.9 ± 3.1	38.8 ± 1.9	40.3 ± 2.1	38.0 ± 2.1
W2	2113 ± 135	82 ± 8	947 ± 32	2106 ± 132	55.9 ± 2.8	40.6 ± 2.4	35.1 ± 1.8	41.9 ± 3.5
W3	2982 ± 156	171 ± 13	1252 ± 54	2098 ± 89	44.6 ± 2.1	38.6 ± 1.8	43.4 ± 3.5	44.5 ± 1.7
W4	3523 ± 98	253 ± 18	1510 ± 98	2883 ± 195	53.4 ± 5.6	36.7 ± 1.2	48.0 ± 2.5	36.4 ± 1.5
W5	4723 ± 307	593 ± 39	2321 ± 158	3394 ± 245	68.6 ± 5.6	50.2 ± 3.4	37.3 ± 1.5	38.1 ± 2.2
W6	6307 ± 299	1122 ± 52	2671 ± 161	14557 ± 681	58.7 ± 5.3	55.7 ± 3.9	39.1 ± 2.3	46.7 ± 5.2
W7	1664 ± 82	458 ± 28	1517 ± 120	2358 ± 134	56.8 ± 2.9	34.9 ± 2.1	41.9 ± 1.8	40.6 ± 3.7
W8	3223 ± 175	402 ± 17	1707 ± 87	2729 ± 215	53.8 ± 4.0	36.3 ± 5.2	35.7 ± 1.9	38.3 ± 2.8
W9	1792 ± 172	836 ± 73	1507 ± 92	2884 ± 193	50.7 ± 5.2	54.2 ± 4.1	38.5 ± 2.0	46.0 ± 4.2
W10	1989 ± 137	260 ± 15	905 ± 32	2069 ± 156	52.7 ± 4.4	41.4 ± 3.0	34.3 ± 1.6	38.7 ± 1.5
W11	2894 ± 141	451 ± 22	1562 ± 97	3151 ± 228	66.9 ± 5.0	46.3 ± 2.8	38.2 ± 2.2	42.1 ± 3.4
AV	3135	475	1556	3764	56.2	43.1	39.2	41.0
Rosé1	8780 ± 523	1402 ± 102	14309 ± 984	7169 ± 399	64.2 ± 4.8	48.8 ± 3.4	53.1 ± 4.5	51.1 ± 4.6
Rosé2	13805 ± 852	3410 ± 186	8841 ± 458	19815 ± 875	63.5 ± 3.7	56.6 ± 2.9	49.7 ± 2.3	54.2 ± 2.9
AV	11293	2406	11575	13492	63.9	52.7	51.4	52.6

Note: Values represent means of triplicate determination ± SD. a: ORAC, DPPH, ABTS and CUPRAC expressed as M trolox equivalents (TE). b: MC, SRSA, HRSA and TBARS expressed as an inhibition in relation to a control test.

cent probe through the change in its fluorescence intensity. The inhibition of free radical damage by an antioxidant is reflected in the protection against the fluorescence change in the ORAC assay (Huang et al., 2002). The ORAC value of the wines tested was found to vary from 8890 to 24,371 M, averaging 17,435 M for the red wines, 1664 to 6307 M, averaging 3135 M for the white wines, and 8780 to 13,805 M, averaging 11,293 M (TE) for the rosé wines.

In addition, statistically significant differences were also found between individual red wine varieties and between white varieties under the same conditions. Fig. 1 depicts the kinetic behavior of the fluorescein/AAPH system in the absence and in the presence of different wines. The differences in the ORAC kinetic behavior observed for wines were attributed to the different natures of the antioxidant substances present in different samples. Muscat Hamburg wine's ORAC values were the lowest (15,208 M) and Cabernet Sauvignon wine's ORAC values were the highest (22,860 M) in the four red varieties examined in winery J. Chardonnay wine's average ORAC values were the lowest (1792 M) and Riesling wine's ORAC values were the highest (2894 M) in the three white varieties examined in winery J.

3.2.3. DPPH and ABTS

The free radical-scavenging activity of different wines was determined by the DPPH· and ABTS methods and the results are shown in Table 3. The $ABTS^{+}$ and the DPPH· radicals are the two most widely used and stable chromogen compounds to measure the antioxidant activity of biological material. High TEAC value indicates that the mechanism of antioxidant action of extracts was as a hydrogen donor and it could terminate the oxidation process by converting free radicals to the stable forms. The free radical-scavenging activities found by ABTS and DPPH assays in the white and red wine varieties differed significantly, which was well in accordance with recent reports in the literature (Cimino et al., 2007; Paixão et al., 2007; Staško, Polovká, Brezova, Biskupič, & Malík, 2006). For DPPH·, the values varied from 4190 to 21,362 M for the red wines, 82 to 1122 M for the white wines and 1402 to 3410 M for the rosé wines. For ABTS, the values varied from 9102 to 30,509 M for the red wines, 905 to 2671 M for the white wines and 8841 to 14,309 M (TE) for the rosé wines.

In the case of individual wine varieties under the same conditions, the DPPH· and ABTS of the red wines decrease in the order: Cabernet Sauvignon > Merlot > Cabernet Gernischet > Muscat Hamburg. Italian Riesling wine's average values were the lowest in the three white varieties examined in winery J. The results of investigation show that the higher the concentration of antioxidant, the lower is the amount of remaining DPPH· and the higher is the free radical-scavenging activity. The same is observed for the $ABTS^{+}$ radical cation.

3.2.4. CUPRAC

The reducing power property indicates that the antioxidant compounds are electron donors and can reduce the oxidized inter-

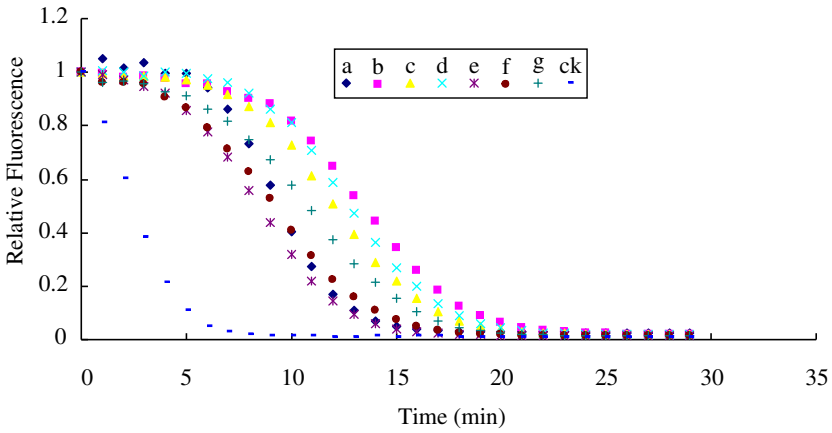

Fig. 1. Time course of the reaction of fluorescein with AAPH in the absence (CK) and in the presence of four red wines and three white wines. (a) Muscat Hamburg; (b) Cabernet Sauvignon; (c) Cabernet Gernischet; (d) Merlot; (e) Chardonnay; (f) Italian Riesling; and (g) Riesling.

mediates of the lipid peroxidation process. FRAP assay is the most widely used method for determining the reducing power of antioxidants. However, FRAP has two major flaws: (1) FRAP assay is conducted at acidic pH (3.6) to maintain iron solubility; (2) FRAP assay does not measure thiol antioxidants, such as glutathione (Prior et al., 2005). Thus, FRAP may not give comparable relative values in physiological conditions. In the present study, we use the CUPRAC assay which is based on reduction of Cu(II) to Cu(I) by antioxidants. All analyzed wines demonstrated significant antioxidant capacity with the CUPRAC test (Table 3). Red wines had stronger reducing power (16,115–31,931 M) than had white wines (2069–14,557 M) or rosé wines (7169–19,815 M). The mean CUPRAC of red wines was 21,827 M, mean CUPRAC of white wines was 3764 M and mean CUPRAC of rosé wines was 13,492 M (TE).

In the case of individual wine varieties in winery J, the CUPRAC of the red wines decreased in the order: Cabernet Sauvignon > Merlot > Cabernet Gernischet > Muscat Hamburg. For the white wines, the CUPRAC decreased in the order: Riesling > Chardonnay > Italian Riesling.

3.2.5. ROS

The hydroxyl radical and superoxide radical are two extremely reactive free radicals formed in biological systems and have been implicated as two highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells (Tedesco et al., 2000). The hydroxyl radical- and superoxide radical-scavenging activities of all wines are shown in Table 3. In the present investigation, all samples exhibited between 48% and 82% hydroxyl radical-scavenging activity for red wines and between 34% and 48% hydroxyl radical-scavenging activity for white wines in the reaction mixture. For superoxide radical, all samples exhibited between 45% and 77% superoxide radical-scavenging activity for red wines and between 35% and 56% superoxide radical-scavenging activity for white wines.

In the case of individual wine varieties in winery J, the hydroxyl radical-scavenging activity of the red wines decreased in the order: Merlot > Cabernet Gernischet > Cabernet Sauvignon > Muscat Hamburg, while the superoxide radical-scavenging activity decreased in the order: Cabernet Sauvignon > Merlot > Cabernet Gernischet > Muscat Hamburg. For the white wines, the hydroxyl radical- and superoxide radical-scavenging activities decreased in the order: Chardonnay > Riesling > Italian Riesling.

3.2.6. TBARS

Lipid peroxidation may cause peroxidative tissue damage in inflammation, cancer, toxicity of xenobiotics and aging (Middleton, Kandaswami, & Theoharides, 2000). Some authors have reported inhibition of peroxidation of wines in different model systems (De Beer, Joubert, Gelderblom, & Manley, 2005; Faustino, Clark, Sobrattee, Czubryl, & Pierce, 2004; Sánchez-Moreno, Larrauri, & Saura-Calixto, 1999). We measured the potential of wine to inhibit lipid peroxidation in egg yolk phosphatidylcholine, induced by Fenton reaction. In the present study, all samples showed inhibition of peroxidation but at different levels (Table 3). The values varied from 31% to 69% for the red wines, 38% to 46% for the white wines and 51% to 54% for the rosé wines.

In the case of individual wine varieties in winery J, the inhibition of lipid oxidation of the red wines decreased in the order: Cabernet Sauvignon > Merlot > Cabernet Gernischet > Muscat Hamburg. For the white wines, the inhibition of lipid oxidation decreased in the order: Chardonnay > Riesling > Italian Riesling.

3.2.7. MC

Metal chelation by phenolic compounds, in theory, could prevent iron-dependent lipid peroxidation in organisms by rendering iron inactive. Phenolic compounds of wines may be able to play a protective role against oxidative damage by sequestering iron (II) ions that may otherwise catalyze Fenton-type reactions or participate in metal-catalyzed hydroperoxide decomposition reactions. The values varied from 51% to 74%, averaging 66%, for the red wines, 45% to 67%, averaging 56%, for the white wines and 64% for the rosé wines. In the case of individual wine varieties in winery J, the metal chelation of the red wines decreased in the order: Cabernet Gernischet > Merlot > Cabernet Sauvignon > Muscat Hamburg. For the white wines, the metal chelation decreased in the order: Riesling > Italian Riesling > Chardonnay.

3.3. Correlation

A correlation analysis was done between parameters for all wines (Table 4 and Table 5). The total phenol, flavonoids and flavanol contents of wines exhibited the strongest correlation with

Table 4
Lineal correlation coefficients (R) between polyphenolic composition and antioxidant capacity

	ORAC	ABTS	DPPH	CUPRAC	HRSA	SRSA	TBARS	MC
TP	0.93	0.93	0.96	0.96	0.77	0.89	0.50	0.44
TFO	0.87	0.91	0.94	0.91	0.62	0.85	0.55	0.39
TFA	0.88	0.92	0.93	0.92	0.64	0.86	0.57	0.41
TA	0.32	0.26	0.30	0.14	0.48	0.42	0.3	0.34

Table 5
Lineal correlation coefficients (R) among the different methods for quantifying the antioxidant capacities

	ORAC	ABTS	DPPH	CUPRAC	HRSA	SRSA	TBARS	MC
ORAC	1							
ABTS	0.86	1						
DPPH	0.87	0.93	1					
CUPRAC	0.92	0.88	0.92	1				
HRSA	0.79	0.64	0.65	0.72	1			
SRSA	0.85	0.78	0.85	0.89	0.73	1		
TBARS	0.46	0.44	0.46	0.53	0.41	0.55	1	
MC	0.45	0.43	0.38	0.45	0.31	0.39	0.05	1

antioxidant properties, while total anthocyanins exhibited weaker correlations. No significant correlation between phenolic content of tested wines and metal chelation was observed. Thus, the antioxidant efficiency of wines tested appears to be largely influenced by the total phenol, flavonoids and flavanol level, with anthocyanins playing a minor role. These results are in agreement with other reports in the literature (Arnous et al., 2002; Cimino et al., 2007; Fernández-Pachón et al., 2006; Minussi et al., 2003).

Regarding different methods, the significant correlation between methods was confirmed with six methods (ORAC, ABTS, DPPH, CUPRAC, SRSA and HRSA), while TBARS and metal chelation exhibited weaker correlations with other methods. Taken together, a relatively tight coupling of four parameters (ORAC, DPPH, ABTS and CUPRAC) indicates that every one of them can be considered as a relevant and reliable characteristic of the antioxidant capacity of wines.

4. Conclusions

It is verified that the red wines have higher phenolic content levels than white or rosé wines and the same result is obtained for antiradical activity and antioxidant capacity. The amounts of phenolic materials and antioxidant activity vary considerably in different types of wines, depending on the grape variety, environmental factors in the vineyard and the wine processing techniques. Because of a relatively tight coupling of the ORAC, DPPH, ABTS and CUPRAC methods, any of these methods can be used for the quick evaluation of antioxidant capacity of wines.

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