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Phenolic Compounds and Antioxidant Properties of Grape Berries and Wines in Loess Plateau Region (China)

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Four grape samples (Cabernet Sauvignon1, Cabernet Sauvignon2, Cabernet Gernischet and Chardonnay) and corresponding wines, produced from Loess Plateau region of China, were examined in present studies. The antioxidant activity of grape berries and wines was measured by different analytical methods (DPPH, CUPRAC, SRSA and FTC) and the contents of total phenols, total flavonoids, total flavanols and total anthocyanins were determined. As expected, the red grape berries and corresponding wines had much higher phenolic content and antioxidant capacity than the white grape berries and corresponding wine, respectively. Furthermore, the results also indicated that ecological factor of vineyard strongly influenced the contents of the phenolic compounds and antioxidant capacity in grape berries and wines. Finally, the correlation between the phenolic contents and the antioxidant capacity as well as 9 individual phenolic compounds contents and antioxidant capacity of grape berries and wines has been investigated. The analyses show that the former correlation is close and the latter correlation exists discrepant. The fact is concluded that the grape berry and wine's antioxidant properties are influenced differently by each polyphenolic molecule.

Key Words: Grape berries and wines, Loess Plateau region, Phenolic compounds, Antioxidant property, Different analytical methods, HPLC.

INTRODUCTION

Polyphenolics are an important group of secondary metabolites present in a variety of plant species, *i.e.*, vegetables, peanuts, tea and grape. Moreover, grapes are probably one of the most important source of natural phenolics for humans, since the compound is also found in one of the end products of grapes, *i.e.*, wine[1]. Grapes (*Vitis* species) are members of the family Vitaceae and one of the most popular fruits and the most widely cultivated around the world. Grape berries are consumed as table fruit, wine, juice and raisins. Grapevines and their products, particularly wine, have been important elements in human life, foods and religions[2].

Epidemiological evidence indicates an inverse relationship between the intake of polyphenol-rich foods (*i.e.* flavonoids) and the risk of certain chronic diseases and coronary heart disease mortality[3-5]. In recent years, because of possible health benefits of phenolics[6-9], consumption of foods containing high phenolics has been increasing steadily[10]. Reactive oxygen species (ROS) include free radicals and non-free-radical species, naturally formed during normal metabolism. However, excess ROS can result in oxidative stress, damaging biological structures such as proteins, lipids or DNA. The innate antioxidant defensive system in the human body may not be adequate 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•. Many papers have been published on grape berries and wines and the antioxidant properties which were correlated with their polyphenol contents[11-16]. According to many authors, antioxidant activity of grape berries and wines results mainly from phenolics, whereas the phenolic content and composition depend on the grape variety, vineyard location, cultivation system, climate, soil types, vine cultivation practices, harvesting time, production process and ageing[17].

The most commonly used methods of determining the antioxidant capacity include determination of the total phenols, 2,2-diphenyl-1-picrylhydrazyl (DPPH• assay), superoxide radical-scavenger activity, lipid peroxidation inhibition, *etc*. These methods differ in terms of their assay principles and experimental conditions. Most of them 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 which is the object of measurement of antioxidant capacity[18].

Multiple reaction characteristics and mechanisms are usually involved, no single assay will accurately reflect all antioxidants in a mixed or complex system, therefore different antioxidant capacity assays may be needed.

With the development of Chinese wine industry, areas of grapevines have been increasing, the new producing regions of wine were constantly discovered in recent years, including Loess Plateau region of China. Rongzi Chateau of Xiangning County located in Loess Plateau region, is situated approximately between 35º-37º N, average altitude of 1100 m. Climatic characteristics of this region are dryer climate, stronger sunshine and a wide swing in diurnal temperature differences distinguished by lower night-time temperature.

But to date, there has been no published work on the chemical quality and antioxidant activity of grape berries and wines produced in Loess Plateau region of China and for this study, we aimed to fill that gap. The antioxidant properties of the grape berries and corresponding wines were tested for their total antioxidant capacity with four different methods. Furthermore, the correlation analysis was done between these parameters for all samples.

EXPERIMENTAL

Folin-Ciocalteu's phenol reagent, gallic acid, catechin, *p*-dimethylaminocinnamaldehyde (DMACA), linoleic acid free acid, nicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), nitroblue tetrazolium (NBT), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) and neocuproine free base were purchased from Sigma-Aldrich (St. Louis, MO). Tris (base) was purchased from Sanland Chemical Co., Ltd (Los Angeles, CA, USA). All other chemicals and solvents were analytical reagent grade and purchased in China.

Grape samples and extraction procedures of phenolic compounds: Four grape samples of three cultivars (*Vitis vinifera* L.) were harvested at optimum technological maturity, as judged by indices of sugar and acid content in 2009. All vineyards are located in the Rongzi Chateau of Xiangning County in Loess Plateau region. Four grape samples include Cabernet Sauvignon1 (CS1), Cabernet Sauvignon2 (CS2), Cabernet Gernischet (CG) and Chardonnay (CY). The CS1 and CS2 were separately from different vineyard, average altitude of CS1 vineyard is higher almost 300 m than CS2 vineyard and up to 1200 m. In the collection all the vineyards have similar characteristics (age and cultivation management), which are representative of this region. All the vines were cultivated in 2007 spring and seedling root system with multiple main vine fan-training and 2.5 × 1.0 m (row × vine) spacing. Each sample consisted of 10 bunches picked randomly from 10 different plants and a total of 300 grape berries per sample was collected and transferred quickly to the laboratory and stored at -40 ºC until extraction.

Grape berries from 15 randomly chosen grapes were manually removed their seeds, flash frozen in liquid nitrogen, ground into a fine powder using mortar and pestle, 3.0 g of the freeze-dried powder was rapidly weighted into 50 mL centrifuge tube with 30 mL volumes of acidified methanolic solution (MeOH:HCl = 100:0.1, v/v), then centrifuge tubes were placed in a sonicator bath for 20 min and left for 24 h under stirring at room temperature. The mixture was then centrifuged at 10000 rpm for 10 min at 4 ºC. After pouring out the supernatant, the precipitate was re-extracted with 15 mL of the same solvent two more times. Finally supernatant was collected and stored at -40 ºC in dark until further analysis[19]. All procedures of extraction should be done in the dark and extractions were performed in three replicates.

Vinification: *Vitis vinifera* (CS, CG and CY varieties) grapes were harvested manually during the 2009 season in a supervised experimental vineyard were used to produce the wine studied in this work. Pre-fermentation treatments and winemaking were done as described by Lihua[20]. In brief, grapes were crushed on an experimental destemmer-crusher and then transferred to stainless-steel containers. 30 L of each treatment wine were produced in three replications. 50 mg/L of SO_2 and 30 mg/L of pectinase (Lallzyme Ex) were added to the musts, respectively and 200 mg/L of dried active yeast (Saccharomyces cerevisiae strain, Lallemand, Danstar Ferment AG, Switzerland) was added, according to commercial specifications. After fermentation, the wine samples were stored at 5 ºC prior to analysis. All the samples were 6 months old at the time of analysis and were analyzed at Northwest A&F University.

HPLC analysis: Phenolic compounds were extracted from grape berries and wines and analyzed by HPLC system and were separated at 35 ºC on C_{18} Hibar RT Lichrospher column (250 mm × 4.0 mm, 5 µm) with two gradient elution (flow rate 0.8 mL/min). The mobile phases consisted of A, water: acetic acid (98:2, v/v); B, acetonitrile. The step gradient started with 16 % mobile phase B for 0-10 min, 20-40 % mobile phase B for 10-25 min and 40 %-0 mobile phase B for 25-30 min. Grape and wine samples and mobile phases were filtered with 0.45 µm membrane filter. 100 mL of samples were injected directly into the column, using detection at 280 nm on UV spectra. The quantification of compounds was carried out by the external standard method[21].

Determination of total phenols (TP), total flavonoids (TFO), total flavanols (TFA) and total anthocyanins (TA): The total phenols content was determined by the Folin-Ciocalteu colorimetric method with slight modification[22]. An aliquot of 0.1 mL of sample solution (with appropriate dilution if necessary) was mixed with 0.5 mL of Folin-Ciocalteu reagent and allowed to react at 30 ºC for 5 min in the dark. Then 1.5 mL of saturated Na_2CO_3 solution was added and the mixture was allowed to stand for 2 h before the absorbance of the reaction mixture was read at 765 nm. The total phenols concentration was calculated from a calibration curve, using gallic acid as standard (0-500 mg/L).

The total flavonoids content was measured according to a colorimetric assay[23]. In this method, 1 mL aliquot of appropriately diluted sample (with appropriate dilution if necessary) was added to a 10 mL volumetric flask containing 4 mL of distilled H_2O. At zero time, 0.3 mL of 5 % $NaNO_2$ was added to the flask. After 5 min, 0.3 mL of 10 % $AlCl_3$ was added. At 6 min, 2 mL of 1 M NaOH was added to the mixture. Immediately, the contents of the reaction flask were diluted to volume with the addition of 2.4 mL of distilled H_2O and thoroughly mixed. Absorbance of the mixture was determined at 510 nm *versus* a prepared water blank. The total flavonoids concen-

tration was calculated from a calibration curve, using catechin as standard (0-500 mg/L).

The total flavanols content was estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method[24,25]. This method has a great advantage over the widely used vanillin assay, since there is no interference by anthocyanins. Further, it provides higher sensitivity and specificity[24]. An aliquot of 0.2 mL of sample solution (with appropriate dilution if necessary) was introduced into a 1.5 mL Eppendorf tube and added 1 mL DMACA solution (0.1 % in 1 M HCl in MeOH). The mixture was vortexed and allowed to react at room temperature for 10 min. Following this, the absorbance at 640 nm was read against blank prepared similarly without DMACA. The total flavanols concentration was calculated from a calibration curve, using catechin as standard (0-16 mg/L).

The total anthocyanins content was determined by the pH-differential method[26] using two buffer systems-potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M). In brief, 1.0 mL of sample solution was mixed with 9.0 mL of corresponding buffers and read against a blank at 520 and 700 nm. Absorbance (A) was calculated as:

$$A = (A_\lambda 520\text{-}A_\lambda 700)_{pH = 1.0} - (A_\lambda 520\text{-}A_\lambda 700)_{pH = 4.5}$$

The total anthocyanins (TA) pigment was calculated (TA) as malvidin-3-O-glu

$$TA = A \times MW \times DF \times 1000/(\xi \times 1)$$

where A: absorbance; MW: molecular weight (493.5); dilution factor (DF); ξ: molar absorptivity (28,000). The total anthocyanins content was calculated as milligram of malvidin-3-O-glu per 1 kg grape or per 1 L wine.

Antioxidant activity

Free radical-scavenging activity on DPPH•: The ability to scavenge DPPH• free radicals was determined. Scavenging activity was based on the slightly modified method of Brandwilliams, Cuvelier and Berset[27]. 0.1 mL of sample solution (with appropriate dilution if necessary) was added to 3.9 mL of a 60 μM 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 0.5 h, the absorbance at 515 nm was recorded to determine the concentration of remaining DPPH•. Results were expressed as trolox equivalent antioxidant capacity.

Cupric reducing antioxidant capacity (CUPRAC): The cupric reducing antioxidant capacity was determined according to the method of Apak, Guclu, Ozyurek and Karademir[28]. To a test tube, 1 mL each of 5 mM Cu(II), 3.75 mM neocuproine, NH_4OAc buffer (1 M, pH 7.0) solutions and distilled water were added. About 0.1 mL of sample solution (with appropriate dilution if necessary) was added to the initial mixture so as to make the final volume 4.1 mL. The tubes were stoppered and, after 0.5 h, the absorbance at 450 nm was recorded against a reagent blank. Results were expressed as Trolox equivalent antioxidant capacity (TEAC).

Superoxide radical-scavenging activity (SRSA): The method used by Robak and Gryglewski[29] for determination of superoxide anion scavenging activity was followed after modification. The superoxide radical was generated in 3 mL of *Tris*-HCl buffer (20 mM, pH 8.3) containing 1 mL of nitroblue tetrazolium (NBT) (150 μM) solution, 1 mL of nicotinamide adenine dinucleotide (NADH) (468 μM) solution and 1 mL of sample solution (with appropriate dilution if necessary). The reaction was started by adding 1 mL of phenazine methosulfate (PMS) solution (60 μM) to the mixture. The reaction mixture was incubated at 25 ºC for 5 min and the absorbance was measured at 560 nm against the corresponding blank solution. The result was expressed as inhibition in relation to a control test.

Ferric thiocyanate (FTC): The ferric thiocyanate method was used to evaluate the effects of samples antioxidants on preventing peroxidation of linoleic acid described previously[30] with slightly modified. A mixture of sample extract (1.0 mL) in absolute methanol, an emulsion of 2.51 % (v/v) linoleic acid in absolute ethanol (1.0 mL), 0.05 M sodium phosphate buffer, pH 7.0 (2 mL) and distilled water (1.0 mL) were placed in a screw capped tube, incubated in an oven at 40 ºC in the dark. The same reaction mixture without sample extract was used as the control. To 0.1 mL of this solution was added 9.7 mL of 75 % ethanol and 0.1 mL of 30 % ammonium thiocyanate. Precisely 3 min after addition of 0.1 mL of 0.02 M ferrous chloride in 3.5 % hydrochloric acid to the reaction mixture, the absorbance was measured against a reagent blank at 500 nm, each 24 h until 1 day after absorbance of the control reached a maximum (120 h). Antioxidant activity was calculated as per cent inhibition of linoleic acid peroxidation *versus* control.

Statistical analysis: The analysis on the same sample was made in three replications and the results were expressed as mean value ± standard deviation. Correlation was calculated by linear regression (SPSS 16.0 for Windows).

RESULTS AND DISCUSSION

Phenolic composition: Total phenols (TP), total flavonoids (TFO), total flavanols (TFA) and total anthocyanins (TA) were measured for all the grape berries and corresponding wines. The results are shown in Table-2. As expected, red grape varieties and corresponding wines had significantly higher amounts of TP, TFO, TFA and TA than had the white grape variety and wine. These results are in agreement with those available in the literature[31-33]. This is due to a greater grape skin and seed contact time and temperature for the fermentation process for red wines.

For the red grape berries and corresponding wines, the content of TP separately varied from 2790 to 3460 mg GAE/kg FW and 1152 to 1881 mg GAE/L. For the white grape and corresponding wine, the content of TP was separately was 1760 mg GAE/kg FW and 192 mg GAE/L, the content of TP decreased in the order: CS2 > CS1 > CG > CY grape and CS2 > CG > CS1 > CY wine (Abbreviations were explained in Table-1). For the red grape berries and corresponding wines, the content of TFO separately varied from 2054 to 2324 mg CTE/kg FW and 858 to 1594 mg CTE/L. For the white grape and corresponding wine, the content of TFO was separately 967 mg CTE/kg FW and 164 mg CTE/L, the content of TFO decreased in the order: CS1 > CS2 > CG > CY grape and CS2 > CG > CS1 > CY wine. For the red grape berries and corresponding wine, the content of TFA separately varied from 209 to 348 mg CTE/kg FW and 96 to 333 mg CTE/L. For the white grape and corresponding wine, the content of TFA was separately

TABLE-1
PHYSICO-CHEMICAL INDEX OF GRAPE BERRIES AND CORRESPONDING WINES

Cultivar	Altitude (m)	North latitude	East longitude	Grape berries				Corresponding wines				
				Sugar (g/L)	Acidity[a] (g/L)	pH	Tannins[b] (mg/L)	Residual sugar (g/L)	Acidity[a] (g/L)	pH	Alcohol vol. (%)	Tannins[b] (mg/L)
CS1	1280	36º01'05"	110º49'11"	194.0	10.4	3.09	4060	1.68	8.93	3.0	11.8	933
CS2	909	35º57'09"	110º47'47"	195.3	9.6	3.29	4300	1.78	8.36	3.1	12.8	1630
CG	1296	36º01'20"	110º49'07"	196.5	10.4	3.06	3490	1.98	8.93	3.1	12.2	1038
CY	1200	35º59'34"	110º47'36"	185.3	8.7	3.70	1850	1.33	5.98	3.4	12.7	191

CS1 = Cabernet Sauvignon1; CS2 = Cabernet Sauvignon2; CG = Cabernet Gernischet; CY = Chardonnay; [a]Acidity expressed as grams of tartaric acid equivalents per liter; [b]Tannins of grape and wine expressed separately as milligrams of tannin acid equivalents per kilogram fresh weight basis (mg/kg FW) and per liter wine basis (mg/L).

TABLE-2
TOTAL AMOUNT OF PHENOLIC SUBSTANCES AND ANTIOXIDANT PROPERTIES OF GRAPE BERRIES AND CORRESPONDING WINES

Sample	TP[a] (GAE)	TFO[b] (CTE)	TFA[b] (CTE)	TC[c]	DPPH[d]	CUPRAC[d]	SRSA[e] (%)	FTC[f] (%)
Grape berries								
CS1	3430±28.3	2324±166.5	330±7.0	1182±17.23	9715±178.2	26033±1054	71.6±1.2	55.65±0.77
CS2	3460±71.5	2316±90.3	348±18.9	929±2.02	9824±95.2	37109±2144	74.0±0.15	55.55±0.74
CG	2790±74.1	2054±90.3	209±30.9	1226±8.91	9355±55.0	32707±697	69.5±1.14	46.67±4.49
CY	1760±147.2	967±71.5	135±4.4	ND	4990±170.7	8284±437	46.7±3.13	45.85±1.16
Corresponding wines								
CS1	1152±26.3	858±34.8	101±6.7	231±2.19	4598±126.4	9207±321	63.4±1.23	54.96±1.48
CS2	1881±41.0	1594±29.8	333±17.1	230±0.75	7878±198.8	15921±306	70.7±0.60	55.85±2.00
CG	1193±73.7	943±13.9	96±4.2	259±6.00	4526±231.4	9428±179	56.9±0.74	50.47±2.06
CY	192±0.6	164±11.0	7.1±0.06	ND	514±21.6	1046±29.10	35.9±0.38	45.88±3.14

Note: Four grape samples (CS1, CS2, CG and CY) and their corresponding wine samples were examined; Results are the means ± SD (n ≥ 3); ND: not detected; [a]Total phenolics (TP) of grape berry and wine expressed separately as milligrams of gallic acid equivalents per kilogram fresh weight basis (mg GAE/kg FW) and per liter wine basis (mg GAE/L); [b]Total flavonoids (TFO) of grape berry and wine expressed separately as milligrams of catechin equivalents per kilogram fresh weight basis (mg CTE/kg FW) and per liter wine basis (mg CTE/L), expression method of TFO and total flavanols (TFA) were identical; [c]Total anthocyanins (TA) of grape berry and wine expressed separately as milligrams of malvidin-3-O-glucoside equivalents per kilogram fresh weight basis (mg/kg FW) and per liter wine basis (mg/L); [d]DPPH and CUPRAC of grape berry and wine expressed separately as μM of Trolox equivalents per kilogram fresh weight basis (μM TE/kg FW) and per liter wine basis (μM TE/L); [e]SRSA of grape berry and wine expressed as % inhibition of superoxide anion free radical of grape and wine (red wine: 20 × diltuted; white wine: 5 × diluted) in relation to a control test; [f]FTC of grape berry and wine expressed as % inhibition of lipid peroxidation of grape berry and wine in relation to a control test.

was 135 mg CTE/kg FW and 7.1 mg CTE/L, the content of TFA decreased in the order: CS2 > CS1 > CG > CY grape and CS2 > CS1 > CG > CY wine. The grape pigments or anthocyanins are present in red grapes only, for the red grape berries and corresponding wines, the content of TA separately varied from 929 to 1226 mg/kg FW and 231 to 259 mg/L, the content of TA decreased in the order: CG > CS1 > CS2 grape and CG > CS1 > CS2 wine.

The results confirm a variation in phenols content among grape varieties and corresponding wines tested. As is all 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[34]. This research shows that the environmental factors in the vineyard does influence the phenols content of grape and wine,CS1 and CS2 of the same variety come from vineyards of different subregions, the content of TP, TFO and TFA exhibited different degree of discrepancies and the discrepancies among CS1and CS2 wines were in particularly conspicuous, the content of TP, TFO and TFA of CS2 wine was nearly 1.6, 1.9 and 3.3 times higher in CS2 wine than that of CS1 wine, respectively, whereas the content of TA was nearly equal. Since total phenol content is an index of potent antioxidant capability[35], the high phenolic content in wine contributes to its increased antioxidant potential.

In order to identify the phenols content of different grape varieties and corresponding wines, we chosen representative grape varieties from several different subregion. These grapes and corresponding wines had the same situations, including the same age, vintage, cultivation management and winemaking techniques. Interestingly, for four grape samples, CS variety contained significantly more the content of TP, TFO and TFA than did CG and CY varieties, but significantly less anthocyanins than CG variety, especially for CS2 variety. For four corresponding wines, CS2 contained significantly more the content of TP, TFO and TFA than did CS1 and CS2 as well as CY, but significantly less anthocyanins than the CG.

Antioxidant activity

The antioxidant activities found by different assays in the grape berries and wines differed significantly (Table-3). As can be observed, the values of red grapes and corresponding wines were higher than those of the white grape and wine 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[31,36], suggesting a high polyphenol content in the red grape varieties and wine.

DPPH: The free radical-scavenging activity of grape berries and respective wines was determined by the DPPH$^{\bullet}$ methods and the results are shown in Table-2. The free radical-

TABLE-3
PEARSON'S CORRELATION COEFFICIENTS OF ANTIOXIDANT CAPACITY (DPPH, CUPRAC, SRSA AND FTC), TOTAL PHENOLS (TP), TOTAL FLAVONOIDS (TFO), TOTAL FLAVANOLS (TFA) AND TOTAL ANTHOCYANINS (TA) IN GRAPE BERRIES AND CORRESPONDING WINES

	TP	TFO	TFA	TA	DPPH	CUPRAC	SRSA	FTC
TP	1	0.980**	0.871**	0.889*	0.958**	0.930**	0.806*	0.444^{ns}
TFO		1	0.910**	0.869*	0.988**	0.946**	0.886**	0.512^{ns}
TFA			1	0.474^{ns}	0.915**	0.792*	0.855**	0.679^{ns}
TA				1	0.837*	0.868*	0.620^{ns}	-0.279^{ns}
DPPH					1	0.922**	0.916**	0.522^{ns}
CUPRAC						1	0.820*	0.374^{ns}
SRSA							1	0.745*
FTC								1

ns = non-significant; *Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

scavenging activities in the red and the white grape varieties differed significantly, which was well in accordance with recent reports[12,31]. For DPPH$^{\bullet}$, the values of grape berries and wines varied from 4990 to 9824 μM TE/kg FW and 514 to 7878 μM TE/L, respectively. For grape berries and corresponding wines, the values of DPPH· decrease in the same order: CS2 > CS1 > CG > CY. For red grape berries and corresponding wines, TEAC value was averagely 9631 μM TE/kg FW and 5667μM TE/L, respectively, the averaging values were separately 1.9 and 11 times higher than that of white grape and corresponding wine. Trolox equivalent antioxidant capacity value of CS2 wine was equally 1.7 times higher than that of CS1 and CG wines, the discrepancy was very notably. The results of investigation show that the higher the concentration of antioxidant, the lower is the amount of remaining DPPH$^{\bullet}$ and the higher is the free radical-scavenging activity.

CUPRAC: The reducing power property indicates that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid peroxidation process. In this research, we use the CUPRAC assay which is based on reduction of Cu(II) to Cu(I) by antioxidants. All analyzed grape samples and corresponding wines demonstrated significant antioxidant capacity with the CUPRAC test (Table-2). The TEAC values of grapes and corresponding wines decreased in the same order: CS2 > CG > CS1 > CY. Red grapes and corresponding wines had stronger reducing power (26,033-37,109 μM TE/kg FW and 9207-15,921 μM TE/L) than had white grape (8284 μM TE/kg FW) and corresponding wine (1046 μM TE/L). The mean CUPRAC of red grapes and corresponding wines was separately 31949 μM TE/kg FW and 11518 μM TE/L, the averaging values were separately 3.9 and 11 times higher than that of white grape and corresponding wine. Moreover, TEAC value of CS2 wine was equally 1.7 times higher than that of CS1 and CG wines.

SRSA: The superoxide radical is one of the extremely reactive free radicals formed in biological systems and have been implicated as highly damaging specie in free radical pathology, capable of damaging almost every molecule found in living cells[37]. The superoxide radical-scavenging activity of four grape samples and corresponding wines are shown in Table-2. In this research, grape samples and corresponding wines respectively exhibited from 47 to 74 % and 36 to 71 % superoxide radical-scavenging activity. Whether grape samples or corresponding wines, the inhibition of superoxide radical decreased in the following order: CS2 > CS1 > CG > CY. For wine samples, the results were from different dilution folds, red wines and white wine were separately diluted at 1:20 and 1:5.

Ferric thiocyanate: Lipid peroxidation may cause peroxidative tissue damage in inflammation, cancer, toxicity of xenobiotics and aging[38]. Some authors have reported inhibition of peroxidation of wine grapes and wines in different model systems[39-41]. We measured the potential of grape samples and corresponding wines to inhibit lipid peroxidation in the linoleic acid emulsion system. In this research, all samples showed inhibition of peroxidation but at different levels (Table-3). The values equally varied from 46 to 56% for the grape samples and corresponding wines, the inhibitory effect of lipid oxidation of samples decreased in the order: CS1 > CS2 > CG > grapes and CS2 > CS1 > CG > CY wines.

Individual phenolic composition: The phenolic composition is an important quality parameter of grape and wine. In this research, nine individual phenolic compounds were identified and quantified with HPLC (Table-4). Flavonoid was the principal phenolic compound and had the most proportion of total phenolics quantified. The content of individual flavonoid separately varied from 8.79 to 232.80 mg/kg FW and 1.57 to 77.44 mg/L in grapes and corresponding wines. In the flavonoid, catechin was the most abundant compound in the different grapes and wines except that CS1 grape was found to contain a slightly higher quercetin content than the catechin content and followed by quercetin and rutin. The highest catechin content was identically found in grapes and wines from CS2 variety and the lowest in grapes from CS1 and wines from CY. The grapes and wines with the highest contents of quercetin were separately CS2 and CG, the lowest in grapes and wines from CY variety.

For grapes and corresponding wines, the content of the individual hydroxybenzoic acid was found to separately from 1.28 to 91.28 mg/kg FW and 0.02 to 29.18 mg/L. The content of the individual hydroxycinnamic acid ranged from 1.81 to 67.90 mg/kg FW and 0.49 to 12.70 mg/L, respectively. For grape samples, the predominant hydroxybenzoic was benzoic acid, followed by gallic acid and syringic acid (except for CY grape). The predominant hydroxycinnamic acid was ferulic acid, followed by caffeic acid and *p*-coumaric acid. The highest benzoic acid, syringic acid, caffeic acid and ferulic acid contents were identically found in grapes from CS2, the lowest in grapes from CY. For wines, the predominant phenolic acid was gallic acid except that CY white wine contained much less phenolic acid than that of red wines.

TABLE-4
CONCENTRATIONS OF INDIVIDUAL PHENOL IN GRAPES BERRIES AND CORRESPONDING WINES

Polyphenolic compounds	Retention time (min)	Grape berries (mg/kg FW)				Corresponding wines (mg/L)			
		CS1	CS2	CG	CY	CS1	CS2	CG	CY
Non-flavonoid phenolics									
Hydroxybenzoic acids		79.42	148.31	75.36	48.41	38.29	39.00	32.16	2.16
Gallic acid	5.13	31.07	47.03	24.88	42.27	29.18	27.22	24.17	0.02
Benzoic acid	5.48	40.67	91.28	44.07	1.28	5.88	7.47	7.99	1.07
Syringic acid	8.38	7.68	10.00	6.41	4.86	3.23	4.31	*	1.07
Hydroxycinnamic acids		52.96	87.47	62.27	28.32	16.70	17.41	22.79	2.70
Caffeic acid	8.10	11.80	17.76	11.11	8.96	8.06	6.84	12.70	0.63
Ferulic acid	14.76	38.73	67.90	49.06	16.55	6.08	7.41	7.06	1.58
p-Coumaric acid	21.65	2.43	1.81	2.10	2.81	2.56	3.16	3.03	0.49
Flavonoid phenolics		211.96	402.40	206.91	215.86	110.51	127.75	105.27	20.05
Catechin	6.25	98.11	232.80	104.21	145.42	64.57	77.44	54.01	12.39
Quercetin	14.15	105.35	159.90	93.91	53.44	41.00	43.93	44.86	6.09
Rutin	23.54	8.50	9.70	8.79	17.00	4.94	6.38	6.40	1.57

Note: Four grape samples (CS1, CS2, CG, CY) and their corresponding wines were examined, abbreviations of all samples are as in Table-1; values represent means of triplicate determinations (n = 3); *Trace, the amount of phenols less than 0.04 mg/L.

It was worth mentioning here that the contents of catechin and benzoic acid in the CS2 grape were more than 2 times higher than that of the CS1grape. The results indicated that ecological environment of vine growth had an strong effect on phenolic composition and the effect could exhibit in flavonoid and nonflavonoid phenolics, apart fom genetic background, the finding was in agreement with previous studies[42-44].

Correlation: Correlation analysis was used to explore the relationships amongst the different antioxidant variables measured for grapes and corresponding wines (Tables 3 and 5). The significant correlations ($p < 0.01$) among the TP, TFO and TFA strongly suggest that TFA is the major compound of TFO and TFO are the major compounds contributing to TP in grape and wine. The TA also exhibited a significant correlation ($p < 0.05$) with TP and TFO while it had no significant correlation with TFA. The TP, TFO, TFA and TA (except for CY sample) contents of grapes and wines exhibited a significant correlation ($p < 0.01$ or $p < 0.05$) with antioxidant properties with a decreasing order of TFO > TP > TFA > TA (Table-3). No significant correlation between phenolic content of tested grapes and wines and FTC was observed. Thus, the antioxidant efficiency of grapes and wines tested appear to be largely influenced by the TP, TFO and TFA, with TA playing a minor role. These results are in agreement with previous reports[11-14]. Amongst the methods used for quantifying antioxidant activities, the significant correlation ($p < 0.01$ or $p < 0.05$) between methods was confirmed with 3 methods (DPPH, CUPRAC and SRSA), while FTC exhibited weaker correlations with other methods. This result suggested that these three assays are almost comparable and interchangeable in characterizing the grapes and wines antioxidant activities. These results are in agreement with previous reports[45].

TABLE-5
PEARSON'S CORRELATION COEFFICIENTS OF ANTIOXIDANT CAPACITY (DPPH, CUPRAC, SRSA AND FTC) AND SINGLE PHENOLIC COMPOUNDS IN GRAPE BERRIES AND CORRESPONDING WINES

Polyphenolic compounds	DPPH	CUPRAC	SRSA	FTC
Gallic acid	0.657^{ns}	0.550^{ns}	0.567^{ns}	0.406^{ns}
Benzoic acid	0.744^{*}	0.919^{**}	0.652^{ns}	0.370^{ns}
Syringic acid	0.843^{**}	0.874^{**}	0.679^{ns}	0.397^{ns}
Hydroxybenzoic acids	0.812^{*}	0.907^{**}	0.705^{*}	0.428^{ns}
Caffeic acid	0.745^{*}	0.766^{*}	0.699^{ns}	0.421^{ns}
Ferulic acid	0.799^{*}	0.949^{**}	0.629^{ns}	0.212^{ns}
p-Coumaric acid	0.377^{ns}	0.053^{ns}	0.455^{ns}	0.381^{ns}
Hydroxycinnamic acids	0.829^{*}	0.953^{**}	0.677^{ns}	0.267^{ns}
Catechin	0.663^{ns}	0.720*	0.498^{ns}	0.262^{ns}
Quercetin	0.851^{**}	0.936^{**}	0.728^{*}	0.417^{ns}
Rutin	0.395^{ns}	0.278^{ns}	0.118^{ns}	-0.175^{**}

ns: non-significant; **Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed).

These results on correlation coefficients of antioxidant capacity (DPPH, CUPRAC, SRSA and FTC) and individual phenolic compounds in grapes and corresponding wines showed that benzoic acid, syringic acid, ferulic acid and quercetin correlate highly with antioxidant activities ($p < 0.05$). The caffeic acid rutin and catechin might have coefficients were relatively low ($p < 0.05$). The correlation of total hydroxybenzoic acid and the total hydroxycinnamic acid to the antioxidant capacities were significant ($p < 0.01$ or $p < 0.05$), which indicates that these kinds of phenolics have important effect on the antioxidant capacities of grape and wine.

Conclusion

In summary, it is verified that the red grapes and wines have higher phenolic content levels than the white grape and wine and the same result is obtained for antiradical activity and antioxidant capacity. A highly correlation between the total antioxidant potential of grapes and wines and the benzoic acid, syringic acid, ferulic acid and quercetin concentrations has been exhibited. The amounts of phenolic materials and antioxidant activity vary considerably in different types of wines, depending on the grape variety, environmental factors of vine growth. Because of a relatively tight coupling of the DPPH, CUPRAC and SRSA methods, any of three methods can be used for the quick evaluation of antioxidant capacity of grapes and wines.

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