Research Article Phenolic Profile and Antioxidant Capacity of Ten Dry Red Wines from Two Major Wine-producing Regions in China

¹Feng-mei Zhu, ²Bin Du, ¹Peng-bao Shi and ¹Feng-ying Li ¹College of Food Science and Technology, ²Analysis and Testing Center, Hebei Normal University of Science and Technology, Qinhuangdao 066600, Hebei, P.R. China

Abstract: Ten dry red wines, produced two major wine-producing regions from China-Changli in Hebei Province and Yantai in Shandong Province, were examined in this study. The antioxidant activities of wines were measured by different analytical methods: 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging activity, 2, 2-azino-di-(3-ethylbenzothialozine-sulphonic acid) (ABTS) radical scavenging activity and Ferric Reducing Antioxidant Power (FRAP). Furthermore, total phenols, total flavonoids and seven individual phenolic compounds (Gallic acid; Catechin; Chlorogenic acid; Caffeic acid; Ferulic acid; Rutin and Morin) of wines were also determined. The results showed that sample 9 (College dry red wine, Cabernet Sauvignon, 2011) contained the highest total phenol content, up to 2131 mg/L; sample 7 (Great Wall dry red wine, Cabernet Sauvignon, Yantai) contained the highest total flavonoids, up to 1282 mg/L. Seven individual phenolics in wine samples were detected by Reverse Phase High Performance Liquid Chromatography (RP-HPLC). Different types of dry red wine polyphenols on DPPH scavenging rate from 78.88 to 98.55%, the highest FRAP scavenging activity was up to 222.66 µmol/L, the lowest was 189.51 µmol/L, different types of wine polyphenols have high ABTS scavenging activity. The current findings will provide useful information for evaluating the wine quality and for educating consumers to choose specific dry wine for specific health-promoting effects.

Keywords: DPPH, dry red wines, FRAP, HPLC, major wine-producing regions from china, phenolic compounds, TPC

INTRODUCTION

The wine industry is growing and the wine market has a wider space to develop in China (Li et al., 2009). The constituents of wine are water, ethanol, saccharides, amino acids, phenolic compounds, pigments and trace metals (Roig and Thomas, 2003; Katalinic et al., 2004; Nilsson et al., 2004; Monaci et al., 2003). The compositions and properties of grape wine are related to the wine origin and age. 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 speciesscavengers and metal-chelators. Positive correlations between total phenolics and antioxidant capacity have been reported (Gómez-Plaza et al., 2006; Orak, 2007). The most commonly used antioxidant capacity assays include 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 per oxidation inhibition. 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 (Li *et al.*, 2009).

The goal of this study is to investigate and analyze the phenolic compositions and antioxidant activity of 10 different dry red wines made in China and to compare the phenolic characteristics of these wines. This study will help to better understand the quality of current wines and stimulate the development of enological techniques for their enrichment.

MATERIALS AND METHODS

Materials: The investigation included 10 red wines from China's major wine-producing regions (Changli in

Corresponding Author: Feng-mei Zhu, College of Food Science and Technology, Hebei Normal University of Science and Technology, Qinhuangdao 066600 Hebei, P.R. China, Tel.:+86-335-2039074; Fax: +86-335-2039074 This work is licensed under a Creative Commons Attribution 4.0 International License (URL: http://creativecommons.org/licenses/by/4.0/).

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Sample No.	Region	Grape variety	Comments	TPC (mg/L)	TFC (mg/L)
1	Changli	Cabernet	Selected	1815±62.77 ^b	802±6.65 ^e
2	Yantai	Cabernet	-	2055±73.56 ^a	1074±12.50°
3	Changli	Cabernet sauvignon	Selected	1845±160.25 ^b	1204±30.53bc
4	Changli	Cabernet franc	-	1796±84.55 ^{bc}	1101±11.13 ^{bc}
5	Changli	Cabernet sauvignon	Cellaring 3 years	1680 ± 79.00^{bc}	1127±5.85 ^{bc}
5	Changli	Cabernet	-	1721±53.50 ^{bc}	951±7.50 ^d
7	Yantai	Cabernet sauvignon	-	1642±66.16°	1281±10.53 ^a
3	Changli	Cabernet sauvignon	2010	1469 ± 28.67^{d}	1137±16.00 ^{bc}
)	Changli	Cabernet sauvignon	2011	2149±108.21ª	1180±19.50 ^b
10	Changli	Zuoyouhong	2011	$1612 \pm 70.21^{\circ}$	$1092 \pm 17.00^{\circ}$

Table 1: The TPC and TFC in 10 dry red wines

a^{-e}: Bar with no letters in common are significantly different (p<0.05) in the same column; TPC: Total phenolic content; TFC: Total flavonoid cotent

Hebei Province and Yantai in Shandong Province). Sample 1, 2, 3, 4, 5, 6 and 7 were purchased at local supermarkets and sample 8, 9 and 10 were acquired from the Experimental Winery at the College of Food Science and Technology, Hebei Normal University of Science and Technology. All information regarding the analyzed samples was summarized in Table 1. Folin-Ciocalteu reagent was purchased from Beijing Aoboxing Biotechnology Co., Ltd. (Beijing, China). Gallic acid, Catechin, Chlorogenic acid, Caffeic acid, Ferulic acid, Rutin, Morin, 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH), 2, 4, 6-tri (2-pyridyl) -striazine (TPTZ) and 2, 2-azino-di-(3ethylbenzothialozine-sulphonic acid) (ABTS) were purchased from National Standard Samples Center (Beijing, China). All other reagents were of analytical grade.

Determination of Total Phenolic Content (TPC): The TPC of samples was determined using the Folin-Ciocalteu reagent-based colorimetric assay as described by Singleton et al. (1999). Phenolic content was calculated as Gallic Acid Equivalents (GAE) and reported as mg/L wine sample. Briefly, 0.5 mL appropriately diluted sample (or gallic acid standard at 0, 50, 100, 150 or 200 ppm, respectively) was mixed with 0.5 mL of 2 N Folin-Ciocalteu reagent and 7.5 mL deionized water and allowed to stand for 10 min at room temperature; then 3 mL of 20% (w/v) Na₂CO₃ was added to the reaction mixture and it was placed in a 40°C water bath for 20 min. After the 20 min reaction period, the samples were cooled to room temperature and the absorbance measured at 760 nm (Dong et al., 2013).

Determination of Total Flavonoid Content (TFC): The TFC of samples was determined using a modified colorimetric method (Jia *et al.*, 1999). 0.25 mL of 1:10 diluted wine sample was mixed with 1.25 mL of distilled water and subsequently with 0.075 mL of 5% sodium nitrite solution and was allowed to react for 5 min. Then, a 0.15 mL of 10% aluminium chloride was added and allowed to further react for 6 min before 0.5 mL of 1 M sodium hydroxide was added. Distilled water was added to bring the final volume of the mixture to 3 mL. The absorbance of the mixture was immediately measured at 510 nm wavelength against a prepared blank using a SHIMADZU UV-2201 spectrophotometer. The flavonoid content was determined by a rutin standard curve and expressed as the mean (milligrams of rutin equivalents per L of wine sample) ±Standard Deviation (S.D.) for three replications.

Extraction of phenolics: Phenolics in wine samples was extracted according to a method by Li *et al.* (2011). The samples were extracted with 100 mL ethyl acetate thrice and then filtered through 0.22 μ m organic membranes.

Determination of individual phenolics content: For determination of the individual phenolic compounds we used an HPLC Agilent-1200 series instrument equipped with a UV-Vis photodiode array (DAD) detector. After injecting 5 µL of sample, separation was performed in an Agilent-Eclipse XDB C-18 (4.6×150 mm) column. The column temperature was at 30°C. Two solvents were used for the gradient elution: A- (H₂O+1% CH₃COOH) and B- (Methanol). The elution program used was as follows: from 0 to 3 min, 85% B, flow rate was 1.2 mL/min; from 3 to 7 min, 70% B, flow rate was 1.2 mL/min; from 7 to 8 min, 55% B, flow rate was 0.6 mL/min; from 8 to 14 min, 40% B, flow rate was 1.2 mL/min; from 14 to 14.1 min, 85% B, flow rate was 1.2 mL/min. The gradience with five concentrations of mixture standard (Gallic acid, Catechin, Chlorogenic acid, Caffeic acid, Ferulic acid, Rutin and Morin) were set with three replications, seven groups of standard curves of the concentration were made based on the average area of individual compounds and seven polyphenol compounds were qualified by the external standard method.

DPPH assay: DPPH radical scavenging capacity of samples was evaluated according to the method of Xu and Chang (2007) with slightly modifications. DPPH radicals have an absorption maximum at 515 nm, which disappears with reduction by an antioxidant compound. The DPPH solution in methanol (6×10^{-5} M) was prepared daily and 3 mL of this solution was mixed with 100 µL sample solution. The mixture was incubated for 20 min at 37°C in a water bath and then

the decrease in absorbance at 515 nm was measured (A_s) . A blank sample containing 100 µL of methanol in the DPPH solution was prepared daily and its absorbance was measured (A_B) . The experiment was carried out in triplicate. Radical scavenging activity was calculated using the following formula:

% inhibition =
$$\left[\left(A_s - A_B \right) / A_B \right] \times 100$$

where,

 A_B = Absorbance of the blank sample A_s = Absorbance of the wine sample

Ferric Reducing Antioxidant Power (FRAP) assay: This method is based on the reduction, at low pH, of a colorless ferric complex (Fe³⁺-tripyridyltriazine) to a blue-colored ferrous complex (Fe²⁺-tripyridyltriazine) by the action of electron-donating antioxidants. The reduction is monitored by measuring the change of absorbance at 593 nm. The working FRAP reagent was prepared daily by mixing 10 volumes of 300 mM acetate buffer, pH 3.6, with 1 volume of 10 mM TPTZ in 40 nm hydrochloric acid and with 1 volume of 20 mM ferric chloride. A standard curve was prepared using various concentrations of FeSO₄·7H₂O. All solutions were used on the day of preparation. One hundred micro L of sample solutions and 300 µL of deionized water were added to 3 mL of freshly prepared FRAP reagent. The reaction mixture was incubated for 30 min at 37°C in a water bath. Then, the absorbance of the samples was measured at 593 nm. A sample blank reading using acetate buffer was also taken. The difference between sample absorbance and blank absorbance was calculated and used to calculate the FARP value. In this study, the reducing capacity of the sample tested was calculated with reference to the reaction signal given by a Fe^{2+} solution. FRAP values were expressed as mmol Fe^{2+}/g of sample. All measurements were done in triplicate (Xu and Chang, 2007).

ABTS assay: ABTS was dissolved in deionized water to a 7 mM concentration. ABTS radical cation (ABTS⁺⁻) was produced by reaction ABTS solution potassium persulfate with 2.45 mМ (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. For the study, the ABTS⁺⁻ solution was diluted in deionized water or ethanol to an absorbance of $0.7 (\pm 0.02)$ at 734 nm. An appropriate solvent blank reading was taken (A_B). After the addition of 100 μ L of aqueous or ethanolic (according to solubility) sample solutions to 3 mL of ABTS⁺⁻ solution, the absorbance reading was taken at 30°C 10 min after initial mixing (As). All solution was used on the day of preparation and all determinations were carried out in triplicate (Dudonne et al., 2009). The percentage of inhibition of $ABTS^+$ was calculated using the following formula:

% inhibition =
$$[(A_s - A_B) / A_B] \times 100$$

where,

 A_B = Absorbance of the blank sample A_s = Absorbance of the wine sample

Statistical analysis: All results in this study were expressed as mean±S.D. of three replicates. Data in triplicate were analyzed by one-way analysis of variance using SPSS 11.5 software package for Windows (SPSS Inc, USA).

RESULTS AND DISCUSSION

Total phenolic content: TPC of 10 dry red wines were measured (Table 1). Sample 9 presented the highest TPC (2149±108.21 mg/L), followed by sample 2, 3, 1, 4, 5, 6, 7, 10 and 8, respectively. The TPC of sample 9, 1, 7 and 8, respectively was significantly different from each other (p < 0.05). However, significant differences in TPC were not found among sample 4, 5, 6, 7 and 10, respectively (p>0.05). The results indicated that sample 9 and 2 contained high concentrations of total phenolics. It was well known that the composition of phenolics in wines varied with grape variety, species, seasons, environment and management factors. Both genetic and agronomic or environmental factor play important roles in phenolic composition and concentration.

Total flavonoid content: TFC of 10 dry red wines are presented in Table 1. Among all the wines analyzed, sample 7 had the highest TFC (1281 ± 10.53 mg/L), followed by sample 3, 9, 8, 5, 4, 10, 2, 6 and 1, respectively. There was significant difference (p<0.05) in TFC between sample 7, 9, 10, 6 and 1, respectively. However, significant differences in TFC were not observed among sample 3, 4 and 5, among 2, 8 and 10, respectively (p>0.05). In this study, there was 1.6-fold difference in TFC between the highest and lowest ranked wines, sample 7 and 1.

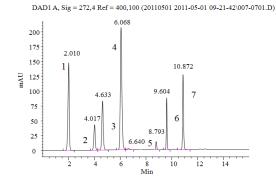


Fig. 1: High Performance Liquid Chromatography-Diode Array Detection (HPLC-DAD) chromatogram of the individual phenolic compounds mixture standard. Peak identification: (1) gallic acid, (2) catechin, (3) chlorogenic acid, (4) caffeic acid, (5) ferulic acid, (6) rutin and (7) morin

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Table 2: Statistical analysis for linearity of the calibration curves of phenolic compounds

Phenolic compounds	Retention time (min)	Linear range (mg/L)	Regression equation	Correlation coefficient
Gallic acid	2.010	9.5~380	y = 11.2466907x -11.173928	0.99997
Catechin	4.015	10.6~424	y = 3.16083587x - 2.241366	0.99997
Chlorogenic acid	4.630	10.2~408	y = 6.70393222x - 22.538271	0.99991
Caffeic acid	6.061	10.0~400	y = 18.3117056x-32.742016	0.99997
Ferulic acid	8.783	10.0~400	y = 0.76399556x-1.8972963	0.99995
Rutin	9.592	9.8~392	y = 3.82695765x-4.4373345	0.99997
Morin	10.872	4.9~392	y = 7.2603x - 15.383	0.99995

Table 3: The recovery results of phenolic compounds

	Phenolic compounds content	Added content		Recovery
Phenolic compounds	in sample (mg/L)	(mg/L)	The determined content (mg/L)	(%)
Gallic acid	62.665	95	143.5195	85.110
Catechin	120.867	106	217.3270	91.000
Chlorogenic acid	47.216	102	151.1540	101.900
Caffeic acid	42.641	100	133.4210	90.780
Ferulic acid	86.777	100	197.1370	110.360
Rutin	10.670	98	101.6434	92.830
Morin	7.022	98	97.8778	92.710

Table 4: The content of gallic acid, catechin, chlorogenic acid, caffeic acid, ferulic acid, rutin and morin in 10 dry red wines

	Gallic acid	Catechin	Chlorogenic	Caffeic acid	Ferulic acid	Rutin	Morin
Sample No.	(mg/L)	(mg/L)	acid (mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
1	62.670	120.87	47.22	42.64	86.78	10.67	7.02
2	468.970	582.43	168.27	216.20	501.31	70.41	34.24
3	465.890	684.06	256.11	245.24	868.46	128.91	61.18
4	57.040	94.33	32.27	38.20	89.53	20.10	14.24
5	164.260	258.29	124.84	94.76	350.00	69.99	53.36
6	128.540	211.76	99.45	68.41	272.64	61.44	24.10
7	342.880	466.82	241.98	159.19	620.55	105.81	45.54
8	309.080	590.30	198.08	100.83	614.53	85.82	33.58
9	128.700	319.83	131.99	49.47	477.62	59.30	18.61
10	96.541	217.44	72.34	27.46	167.27	18.95	7.15

Analysis of individual phenolics in dry red wines: The High Performance Liquid Chromatography-Diode Array Detection (HPLC-DAD) chromatogram of the individual phenolics mixture standard were shown in Fig. 1. The mixed standard substances were separated at various scanning wavelengths and the wavelength selected can simultaneously determine a variety of phenolics. After analysis and comparison of the spectrograms at each wavelength, it has been found there was no wavelength at which 7 kinds of phenols can be simultaneously determined. This problem can be overcome by using a multiple wavelength scanning program which is capable of monitoring several wavelengths simultaneously, in which 7 phenolics were able to achieve maximum absorption and the baseline was stable, separation and repeatability was good.

The statistical analysis for linearity of the calibration curves of phenolic compounds were indicated in Table 2. The correlation coefficient of each phenolic compound was good. As shown in Table 3, the calculated recovery of each phenolic compound ranged from 85.11 to 110.36%, respectively. These results allowed us to conclude that good accuracy was reached in the dry red wine phenolic compounds determination.

The content of Gallic acid, Catechin, Chlorogenic acid, Caffeic acid, Ferulic acid, Rutin and Morin in 10

dry red wines were shown in Table 4. The content of phenolic compounds ranked the samples in descending order, as follows: Ferulic acid>Catechin>Gallic acid>Chlorogenic acid>Caffeic acid>Rutin>Morin. Sample 2, 3, 5, 6, 7, 8 and 9, respectively had the highest Ferulic acid content. Ferulic acid is an abundant phenolic phytochemical found in plant and food. Kikuzaki *et al.* (2002) suggested that ferulic acid was most effective radical-scavenging activity among the tested phenolic acids. Sample 1, 4 and 10 presented the highest Catechin content: 120.87, 94.33 and 217.44 mg/L, respectively.

DPPH radical scavenging activity: As a rapid and simple measure of antioxidant activity, the DPPH radical scavenging capacity has been widely used. DPPH is a stable free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers (Sun *et al.*, 2011). The DPPH radical scavenging activity of 10 dry red wines were shown in Table 5 and the values were significantly different. Sample 7 had the highest DPPH radical scavenging activity (98.55±0.52%). The scavenging effect of samples on the DPPH radical decreased in the order of sample 7>sample 1>sample 3>sample 8>sample 10>sample 4>sample 5>sample

Table 5: DPPH, FRAP and ABTS scavenging capacity of 10 dry red wines

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Sample No.	DPPH (%)	FRAP (µmol/L)	ABTS (%)
1	98.33±0.50 ^a	222.09±9.62 ^{ab}	87.64±3.48 ^{abc}
2	80.11±0.34 ^h	204.97±12.92 ^{cd}	91.66±1.31 ^{ab}
3	97.44±0.38 ^b	222.84±9.27 ^{ab}	75.57±7.32 ^d
4	91.70±0.46 ^e	202.84±8.58 ^{cd}	83.62±2.28 ^{bcd}
5	90.33±0.33 ^f	191.63±11.10 ^d	76.12±3.24 ^d
6	86.44±0.16 ^g	189.51±9.43 ^d	79.59±3.02 ^{cd}
7	98.55±0.52 ^a	213.45±5.19 ^{cd}	76.14±4.73 ^d
8	95.22±0.39°	211.63±13.24 ^{bc}	83.90±8.63 ^{bcd}
9	78.88±0.44 ⁱ	229.66±6.17 ^a	92.54±2.65 ^a
10	94.11±0.42 ^d	196.18±4.33 ^{cd}	83.90±2.16 ^{bcd}

 $\frac{a}{a}$: Bar with no letters in common are significantly different (p<0.05) in the same column

6>sample 2>sample 9. It has been reported that the antioxidant activity of many compounds of botanical origin is proportional to their phenolics contents, suggesting a causative relationship between TFC and DPPH radical scavenging activity. Interestingly, sample 7, which exhibited the highest TFC, registered the highest DPPH radical scavenging activity.

FRAP assay: The FRAP assay is often used to measure the antioxidant capacity of foods, beverages and nutritional supplements containing polyphenols. The antioxidant capacities of wine samples using FRAP assay were indicated in Table 5. The wine samples in general exhibited higher antioxidant capacities and the values ranged from 189.51±9.43 µmol/L (sample 6) to 229.66±6.17 µmol/L (sample 9). The results showed that sample 9, which exhibited the highest TPC, registered the highest FRAP activity. This is in agreement with the result obtained by Loots et al. (2006), who suggested that the FRAP of Kei-apple iuice correlated well with the polyphenol concentrations.

ABTS scavenging activity: This ABTS method determines the antioxidant activity of hydrogen donating antioxidants and of chain breaking antioxidants. The ABTS assay is applicable on both lipophillic and hydrophilic compounds (Sasidharan and Menon, 2011). The antioxidant capacities of wine samples using the ABTS assay were shown in Table 5. In present study, the wine samples showed notable A^{+} cation radical scavenging activity. Sample 9 had the $ABTS^{+}$ highest radical scavenging activity $(92.54\pm2.65\%)$. The results showed that wine samples with high TPC, also presented a high antioxidant capacity in ABTS and FRAP model. Bao et al. (2005) reported the similar results that the highest ABTS scavenging activity of bayberry was attributed to the presence of higher levels TPC, TFC and anthocyanins.

CONCLUSION

The present results describe TPC, TFC and 7 individual phenolic compounds (Gallic acid, Catechin, Chlorogenic acid, Caffeic acid, Ferulic acid, Rutin and

Morin) in 10 dry red wines made from China's two major wine-producing regions. Our results have found that significant differences in TPC, TFC and individual phenolic compounds can exist among the wines. These dry red wines also showed higher antioxidant activity when evaluation by FRAP, DPPH and ABTS radical scavenging ability assays. This study has shown that the phenolic present in wines has potent antioxidant and that the antioxidant activity in wines is positive correlated with TPC and TFC. These dry red wines from Changli and Yantai may serve as natural antioxidants for human nutrition and health.

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