Analysis of Phenolic Compounds and Antioxidant Properties of Wines from Three Wine Grape-Growing Regions in China

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***Abstract*—In order to optimize two methods for the determination of antioxidant activity and compare the phenolic compounds (total phenols, total flavonoids, total flavanols and total anthocyanins) and antioxidant properties of the main wine grape varieties cultivated in three regions in China. The antioxidant activities of wines were measured by two different analytical assays: 2, 2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH+), the optimal reaction conditions of each method were also determined. Results showed that the dilution multiple of 0.15:10~0.2:10 and the reaction time of 200~240min were the optimal reaction condition for ABTS method. For DPPH method, the reaction time of 90~110 min and the dilution multiple of 0.4:10~0.8:10 were selected. Among the varieties tested, the contents of phenolic compounds and antioxidant activities in Cabernet Sauvignon wine from Ningxia and Xinjiang region were significantly higher than others, followed by the Merlot from Ningxia region. These parameters were the lowest in Cabernet Gernischet and Pinot Noir wines from Yantai and Xinjiang region, respectively. The phenolic contents and antioxidant capacity of four single varietal wines in the Ningxia region were in a higher value than in Yantai and Xinjiang region. In addition, a close relationship between phenolic contents and antioxidant capacity was observed. Analysis showed that the contents of phenolic compounds and the levels of antioxidant activity in the wine samples greatly varied with cultivar and environmental factors among regions of wine growth.**

***Index Terms*—China wine; antioxidant activity; phenolic compounds; correlation**

I. Introduction

We're told that, in general, alcohol is not good for us. But then we learn about the amazing health benefits that can come from having a glass or two of red wine every day. What's the real story here? Well, red wine contains powerful antioxidants, substances that protect your cells against damage caused by unstable molecules called free radicals, and is good for a man's health in a number of ways, according to many studies. Phenolic compounds, which are abundant in grape berries and wines, play one of the most important roles in the quality of grape berries and wines. They strongly contribute to the color, mouth feel and palatability of red wines [1]. Epidemiological evidence indicates that, polyphenols exert many favorable effects on human health, the moderate consumption of wines reduces the incidence of coronary heart disease (CHD), atherosclerosis and platelet aggregation [2]. This greater protection 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 [3].

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). The concentration of flavonoids in wine are strongly affected by the winemaking practices such as pressing and maceration that affect the degree of extraction from skins and especially from seeds which are rich in flavan-3-ol units [4]. 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 [5], [6]. Many epidemiological studies have found that regular intake of red wine or red wine polyphenols has positive effects on human health. Therefore, determination of the chemical composition, polyphenols content and antioxidant

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activity of red wine could be very useful for the interpretation of epidemiological studies [7].

Wine brewing areas are distributed all over the world, wine styles vary significantly between regions due to climate differences. Chile's grapes are mainly produced in the Mediterranean climate region, where wine enjoy a great reputation in the world. Summer here is full of light and large in diurnal temperature variation, which is conducive to the accumulation of organic matter and makes the grape have excellent quality. Bordeaux, France, one of the best well-known wine regions, has a relatively high annual rainfall of 850 mm. But the rainfall here is balanced and slightly less in the summer months. Napa Valley, located in northern California, USA. It is a vast hilly area famous for its wine production and is known as the "Wine Valley" of the United States. It has an average annual precipitation of 1045 mm, which is much higher than that in North China. But summer months here are arid, belonging to the typical Mediterranean climate. Compared with Europe and the United States, the climate conditions in China's wine grape producing areas are quite different. China is a famous monsoon climate region in the world with the characteristics of simultaneous heat and moisture, which grant China's wines a unique flavor.

"From cups of jade that glow with wine of grapes at night; drinking to pipa songs, we are summoned to fight." Today, as it has always been in the history of civilization of thousands of years, wine is loved by Chinese people with its special fragrance affords a lingering after-taste and has permeated almost every field in people's lives. 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.

All the climate and soil characteristics of China's wine grape-growing regions can influence the wine grapes' quality to a different extent, which leads to the wine produced also have their own characteristics. In this study, phenolic compounds and antioxidant properties of the main wine grape varieties cultivated in three regions were compared. The northern foot of Tianshan Mountain in Xinjiang region has been a high-quality grape-growing area in China since ancient times. It is located in the middle part of the northern foot of Tianshan Mountains and the southern margin of Zhungeer Basin. The terrain is flat and the average altitude is 450.8 meters above sea level. It belongs to temperate continental arid climate, with the characteristics of long and severe winter, short and hot summer, dry climate and long sunshine time. The east foothills of Helan Mountain in Ningxia region in China is the most promising high-quality wine production area. It belongs to the typical Continental climate in temperate and cold zone, with the characteristics of four distinct seasons, late spring and short summer, early autumn and long winter, large temperature difference between day and night, few rain and snow, strong evaporation, dry climate and much wind and sand. As the cradle of China's wine industry, Yantai area in Shandong province is a traditional quality wine producing area in China with a history of wine grape cultivation for hundreds of years. It belongs to the warm temperate humid monsoon climate. However, due to the influence of the ocean, compared with the same latitude inland, it has the characteristics of ample sunshine, abundant rainfall and mild climate, no cold here in winter, and summer without heat.

Wines, as one of the products with antioxidant effect, has attracted the attention of the majority of consumers. At present, several studies for measuring antioxidant activity of wine have been reported. However, for the antioxidant compositions of samples are fairly complex, usually involving multiple reaction characteristics and mechanisms, the best conditions for determination using Enzyme - labelled meter were not explicitly stated by the investigators. The determination of antioxidant activity is mostly based on the methods in the literature. Researchers also used different sample dilution schemes and reaction times. Thus, to fully elucidate a full profile of antioxidant capacity, the best factors for analysis to compare a smorgasbord of wines antioxidant capacity assays may be needed. The objectives of this study were to: (1) establish a rapid, accurate, high throughput method for the determination of total antioxidant activity of red wine, the reaction conditions and analytical parameters of DPPH and ABTS assays were optimized by the Enzymatic marker and (2) compare the phenolic content and antioxidant activity of five varieties of wine from three major grape producing areas in China.

By establishing an "antioxidant profile", this paper will help to better measuring the quality of wines with multi - species and providing consumers with a more basis for choice. Further studies on antioxidant activity of wine will be summarized in our next study.

II. Materials and Methods

A. Chemicals

The phenolic compounds were selected for their usual occurrence in wines and their availability as commercial standards. Gallic acid, as the standard of phenolic compounds and Folin-Ciocalteu's reagent (FCR) were all purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were obtained from Xiya Reagent (Shandong, China). 6-hydroxy-2,5,7,8-tetramethylchro-man-2-carboxylic acid (Trolox), as the standards of antioxidant activity, and p-dimethylaminocinnamaldehyde (DMACA) were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). (+)-Catechin was obtained from Nanjing Oddfoni biological Science Technologies Co. (Nanjing, China). All reagents were of analytical grade.

B. Instruments

Absorbance measurements of the contents of total phenols, total flavonoids, total flavanols and total anthocyanins were performed on a UV/VIS Spectrometer, manufactured by PerkinElmer Singapore. While, the absorbance measurements of the whole wine samples and Trolox were recorded on a 96-well plate of the Lab

microplate reader/ Multiskan FC, Thermo Fisher in Shanghai, China. ABTS and DPPH assays were measured with an absorbance of 734 nm and 517 nm respectively. The plate reader was controlled by Skanlt Software 4.1. Operating conditions were set at room temperature.

C. Wines Sample

The study summarizes 5 species, a total of 63 red wines investigated from three provinces in China. All wine samples were produced in 2018 that have not been aged. Various species wines covered three dominating viticultural areas of China, which were available in the China market. All samples were kindly supplied by Technology Center Changyu Group Co., Ltd.

TABLE I. WINE SAMPLES

Cultivars	Location	Num	Winery
Cabernet Sauvignon	Xinjiang	C1	A
		C 2	A
		C 3	B
		C 4	B
		C 5	B
		C 6	B
		C 7	B
		C 8	B
		C 9	C
		C 10	C
		C 11	C
	Ningxia	C 12	D
		C 13	D
		C 14	D
		C 15	D
		C 16	D
		C 17	D
		C 18	D
		C 19	D
		C 20	D
		C 21	D
	Yantai	C 22	E
		C 23	E
		C 24	E
		C 25	E
		C 26	E
		C 27	E
		C 28	E
		C 29	E
		C 30	E
		C 31	E

Cultivars	Location	Num	Winery
Merlot	Xinjiang	M1	C
		M2	C
		M3	C
		M4	C
		M5	C
	Ningxia	M6	D
		M7	D
		M8	D
Cabernet Gernischet	Ningxia	G1	D
		G2	D
		G3	D
		G4	D
	Yantai	G5	E
		G6	E
		G7	E
		G8	E
		G9	E
		G10	E
		G11	E
		G12	E
		G13	E
		G14	E
Syrah	Xinjiang	S1	B
		S 2	B
		S 3	B
	Ningxia	S 4	D
		S 5	D
		S 6	D
Pinot noir	Xinjiang	P1	B
		P2	B
		P3	B
		P4	B

D. Determination of Total Phenols (TP)

The amount of total phenols was determined according to the Folin-Ciocalteu colorimetric method, with gallic acid as a standard [8], [9]. Gallic acid standard solutions were prepared at a concentration of 0, 50, 100, 250, and 500 mg/L. Wine samples (0.2 mL) was added to a 100 mL volumetric flask containing 60 mL H2O. 15 mL 20% Na_2CO_3 was added 30 seconds later and 8 minute ago. The volume was completed with H_2O and after 2 hours the absorbance was read at 765 nm. Each sample was analyzed in triplicate. Total phenols were expressed as milligrams of gallic acid equivalents (mgL^{-1} of GAE). Data are expressed as mean ± SD (n = 3).

E. Measurement of Total Anthocyanins (TA)

The amount of total anthocyanins was measured using the pH-differential method [10]. An aliquot of 2mL wine sample was added to a 25 mL tube containing 18 mL buffer with a pH of 1 and 4.5 respectively. Absorbance of the samples in each buffer was determined at 520 nm and 700nm against prepared water blank. The differential absorbance of wine samples was determined as follows:

$$A_{diff} = (A_{520} - A_{700})_{pH1:0} - (A_{520} - A_{700})_{pH4:5}$$

The concentration of total anthocyanin content $T_{\text{anthocyanin}}$, expressed as cyanidin 3-glycoside (C3G), was calculated with the following formula:

$$T_{\text{anthocyanin}} = \frac{A_{diff} \times MW \times DF \times 1000}{\varepsilon \times 1}$$

where MW is the molecular weight (449 for cyanidin 3-glycoside), DF is the dilution factor and Ɛ (26,900) is the molar absorptivity coefficient of C3G. Total anthocyanin content was expressed as milligrams of C3G equivalents per 1L of wines for the triplicate sample.

F. Quantification of Total Flavonoids (TFO)

Flavonoids in wine were investigated by spectrometric method with rutin as standard material. The total amount of flavonoids was estimated using the slightly modified a

previously described method [11]. Rutin standard solutions were prepared at a concentration ranging from 10 to 80 mg/L. Briefly, 1 mL of diluted wine was mixed with 5 mL of distilled water and subsequently with 2 mL of 5 % sodium nitrite solution and was allowed to react for 6 min. Then a 2 mL of 10% aluminum nitrate was added and allowed to further react for 6 min before 10 mL of 1 M sodium hydroxide was added. Distilled water was added to bring the final volume of the mixture to 50 mL. The absorbance of the mixture was immediately measured at a 510 nm wavelength against a prepared blank. The flavonoid content was determined by a rutin standard curve and expressed as the mean (milligrams of rutin equivalents per 1 L of wine sample) ± SD for the triplicate.

G. Determination of Total Flavanols (TFA)

The amount of total flavanol was estimated based on the p-dimethylaminocinnamaldehyde (DMACA) method [12]. Catechin standard solutions were prepared at a concentration ranging from 10 to 100 mg/L. Diluted wine (0.1mL) was added to a 10 mL volumetric tube containing 3.0 mL of a 0.1% p-DMACA solution. The mixture was left at room temperature for 10 min and the absorbance was measured at 640 nm. The results were calculated from a calibration curve using catechin as a standard as the mean ± SD.

H. Free radical-scavenging Activity on ABTS

1) Reaction solution configuration and sample dilution

A total amount of 25.03 mg of Trolox antioxidant powder was dissolved in 100 mL methanol and prepare Trolox standard reserve solution with 1mmol/L concentration. ABTS assay was based on the slightly modified method of Ozgen M, (2006) and Li et al. (2017) [13, 14]. Briefly, the Trolox standard reserve solution of 1mmol/L was diluted to different concentration gradient (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 mmol/L) with methanol, which contained 10%~90% free radical scavenging rate. 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+ solutions were diluted with Acetic acid buffer to an absorbance of 0.74 ± 0.03 at 734 nm.

Based on the results of the pre-experiment, 5 species of wines were diluted to different concentration gradients (the ratio of the volume of the wine sample to the total volume after dilution). Cabernet Sauvignon, Cabernet Gernischet and Merlot were diluted to 0.05:10~0.4:10, at the same time, Pinot noir and Syrah were diluted to 0.05:10~0.6:10. The decrease in absorbance was determined at 734 nm within 280 min every 20 min. Results were expressed as Trolox equivalent antioxidant capacity, unit mmol/L.

2) Determination of optimal reaction conditions

Analyses of reaction kinetic curves were as a purpose to determining the best reaction time. The wine samples with different dilution ratios were reacted with ABTS working fluid at the determined reaction time. If there is a good dose-effect relationship between the wine dilution and the ABTS free radical scavenging rate within a certain interval, then determined it as the best substrate concentration region of this species of wine.

I. Free Radical-scavenging Activity on DPPH

1) Reaction solution configuration and sample dilution

The Trolox standard reserve solution of 1mmol/L was diluted to different concentration gradient (0.1, 0.2, 0.3, 0.35, 0.45, 0.5, 0.6, 0.8, 1.0mmol/L) with methanol, which contained 10%~90% free radical scavenging rate. In addition, 5 species of wines were all diluted to different concentration gradients (the ratio of the volume of the wine sample to the total volume after dilution): 0.2:10, 0.3:10, 0.4:10, 0.6:10, 0.8:10, 1.0:10, 1.5:10.

The ability of wines to scavenge DPPH+ free radicals was determined. Scavenging activity was based on the slightly modified method of Li (2017). Briefly, on the 96-well plate of the Enzyme - labelled meter, 10uL of Trolox solution and wine (diluted to different concentration gradients) was added to 200uL of DPPH+ in methanol. A control sample containing the same volume of methanol in place of wine sample was used to measure the DPPH+ absorbance. After the reaction was allowed to take place, the absorbance at 517 nm was recorded to determine the free radical-scavenging activity on DPPH+. Results were expressed as Trolox equivalent antioxidant capacity, unit mmol/L.

2) Determination of optimal reaction conditions

The analytical method was in common with ABTS. The time at which the reaction reaches an equilibrium position is determined to be the optimal time interval for the reaction by analyzing the reaction kinetics curve. In equilibrium, regarding it as the optimal substrate concentration if the reducing power showed an excellent dose-effect relationship with the mass concentration.

J. Statistical Analysis

One-way ANOVA was used to evaluate the differences among the three regions for each grape cultivar. Significance analysis was performed using SPSS 16.0 program. Correlations between various parameters were also investigated. Significance was determined at $p < 0.05$. All data were reported as the mean ± SD of three replications.

III. RESULTS AND DISCUSSION

A. Comparison on Phenolic Compounds

The main climate parameters in the studied regions are given in Table II. The total phenols, total flavonoids, total flavanols and total anthocyanins were measured for all the samples. The results were given in Table III. As expected, the Cabernet Sauvignon had significantly higher amounts of total phenols, flavonoids and flavanols than had in Cabernet Gernischet, Syrah or Pinot noir. The TA contents of two single varietal wines in the Ningxia and Xinjiang region were the highest whether the

phenolic content in the wines of Yantai was at a relatively low level. What causes this result? As a matter of fact, the amounts of phenolic materials vary considerably in different wine grape-growing regions, to some degree, depending on the grape variety, environmental factors of wine growth [15]. As a result of being landlocked, Ningxia and Xinjiang region have the characteristics of low temperature, large diurnal temperature variation, few and concentrated rainfall, long sunshine time, strong solar radiation, which allows sufficient accumulation of sugars and phenols. However, due to the short frost-free period, frequent frost and extremely low temperature, it is essential to bury soil to prevent frost. This is also a vital limiting factor for the development of wine grapes in less rainy areas in the north of China. By contrast, 210 frost-free days in Yantai region can ensure the safety of wine grape overwintering. However, compared with Ningxia and Xinjiang region, it has the characteristics of smaller Dryness index and heavy rainfall. From June to September, the precipitation in the mature stage of grapes reached 651.9 mm, accounting for 70% of the whole year. Therefore, it is not conducive to the accumulation of sugar, resulting in thin phenols in grape berries. In addition, moisture over makes grape susceptible to disease and affects the quality of wine.

TABLE II. MAIN CLIMATE PARAMETERS IN THE STUDIED REGIONS

Regions	Xinjiang	Ningxia	Yantai
Frost-free season (d)	170	183	210
Annual sunshine hours (h)	2721~2818	2800~3000	2698
Active accumulated temperature (℃)	4023~4118	3298~3351	3756~4174
Diurnal temperature variation (℃)	>20	10-15	<10
Rain (mm)	190	200	651.9
Dryness index	28.62	4.77	1.17
Extremely low temperature (℃)	-13~-32	-19~-22	-10~-14

TABLE III. TOTAL AMOUNT OF PHENOLIC SUBSTANCES OF THE MAIN WINE GRAPE VARIETIES FROM THREE DIFFERENT WINE GRAPE-GROWING REGIONS

Cultivars	Location	TP (mg/L)	TA (mg/L)	TFO (mg/L)	TFA (mg/L)
Cabernet Sauvignon	Xinjiang	2963.56±358.37^{a}	260.41±60.12^{a}	93.14±10.67^{a}	1800.54±337.42^{a}
	Ningxia	3158.46±333.09^{a}	237.89±35.7^{ab}	94.04±12.99^{a}	1958.85±290.09^{a}
	Yantai	2227.94±299.52^{b}	201.83±33.12^{b}	71.67±11.93^{b}	1843.81±302.42^{a}
Merlot	Xinjiang	2455.19±326.79^{b}	269.57±39.51^{a}	77.88±16.53^{b}	1529.26±121.01^{b}
	Ningxia	2717.25±188.72^{a}	272.39±33.58^{a}	83.8±3.78^{a}	1708.67±215.41^{a}
Cabernet Gernischet	Ningxia	2554.37±330.76^{a}	354.4±30.62^{a}	72.12±7.3^{a}	1274.12±179.32^{a}
	Yantai	1374.03±294.69^{b}	246.8±68.43^{b}	37.46±12.92^{b}	806.34±427.5^{b}
Syrah	Xinjiang	1677.93±9.53^{b}	127.09±10.75^{b}	60.94±5.84^{b}	1005.51±12.51^{b}
	Ningxia	2174.08±11.56^{a}	271.71±11.04^{a}	78.81±9.66^{a}	1659.16±10^{a}
Pinot noir	Xinjiang	1243.67±131.05	138.1±5.72	53.35±7.96	1027.53±510.24

Note: Values are means ±S.D. Different letters in each column in a same variety are significantly different at the 0.05 level according to ANOVA.

1) Comparison on total phenols

The TP contents of 5 wine varieties from 3 regions are presented in Table III. For the whole wine samples, the TP content decreased in the order: Cabernet Sauvignon > Merlot > Cabernet Gernischet > Syrah > Pinot noir, Ningxia > Xinjiang > Yantai. The TP content varied from 1243.67 ± 131.05 to 3158.46 ± 333.09 mg/L, averaging 1914.79 mg/L. Among all the wine varieties from three regions evaluated, Cabernet Sauvignon from Ningxia and Xinjiang had the highest content of TP, with 3158.46 ± 333.09 and 2963.56 ± 358.37 mg of GAE/L of wine, respectively, followed by Merlot from Ningxia and Xinjiang region, Cabernet Sauvignon from Yantai region, whereas the single varietal wine samples of Cabernet Gernischet from Yantai region and Pinot noir from Xinjiang region tested had the lowest values. Significant differences were found in total phenolic content in comparisons between Cabernet Sauvignon from Yantai and Ningxia, Xinjiang region ($p < 0.05$); however, the TP content in Cabernet Sauvignon from Ningxia region was non-significantly higher than that from Xinjiang region ($p > 0.05$). In this study, there was a 2.5-fold difference in TP content between the highest and lowest ranked varieties, Cabernet Sauvignon and Pinot noir ($p < 0.05$). It is well known that both genetic and agronomic or environmental factors play important roles in phenolic composition and thus nutritional quality of crops[16]. Results also showed that the phenolic content of the single varietal wine samples vary considerably in different wine grape-growing regions.

2) Comparison on total flavonoids

Flavonoids have been reported to play an important role in the antioxidant capacity of red wines. Besides, flavonoids have many biological activities such as the inhibition of plasma platelet aggregation and cyclooxygenase activity, the suppression of histamine release and SRS-A biosynthesis in vitro, potent nitric oxide radical scavenging activity and exhibiting anti-bacterial, antiviral, anti-inflammatory and antiallergenic effects [17]. As can be seen from Table III, the content of TFO varied from 71.67 ± 11.93 to 94.04 ± 12.99 mg/L, for the Cabernet Sauvignon wines and from 77.88 ± 16.53 to 83.80 ± 3.78 mg/L for the Merlot wines, while 37.46 ± 12.92 to 72.12 ± 7.3 mg/L for Cabernet Gernischet, 60.94 ± 5.84 to 78.81 ± 9.66 mg/L for Syrah and 53.35 ± 7.96 mg/L for Pinot nior wine from Xinjiang. The TFO content of Cabernet Sauvignon wines from Ningxia and Xinjiang region was significantly higher

than those of the other wine varieties. The content of TFO decreased in the order: Ningxia > Xinjiang > Yantai for Cabernet Sauvignon wines, Ningxia > Xinjiang for Merlot and Syrah wines, Ningxia > Yantai for Cabernet Gernischet wines, respectively. Flavonoids, as the most abundant phenolics, are important for their antioxidant and free radical scavenging activities; its content can reflect the antioxidant capacity of the fruit [18].

3) Comparison on total flavanols

As can be seen from Table III, for the whole wine samples, the TFA content decreased in the order: Cabernet Sauvignon > Merlot > Syrah > Cabernet Gernischet > Pinot noir. The Cabernet Sauvignon wines from three regions contained non-significantly differences content of TFA, and were significantly higher than those of the other wine varieties. The content of TFA varied from 806.34 ± 427.5 to 1274.12 ± 179.32 mg CTE/L, for Cabernet Gernischet wines and from 1005.51 ± 12.51 to 1659.16 ± 10 mg CTE/L for Syrah wines. In addition, total flavanols content in Merlot samples was more than 50% those in Pinot noir from Xinjiang region. The TFA content were more than double that between the highest and lowest ranked varieties.

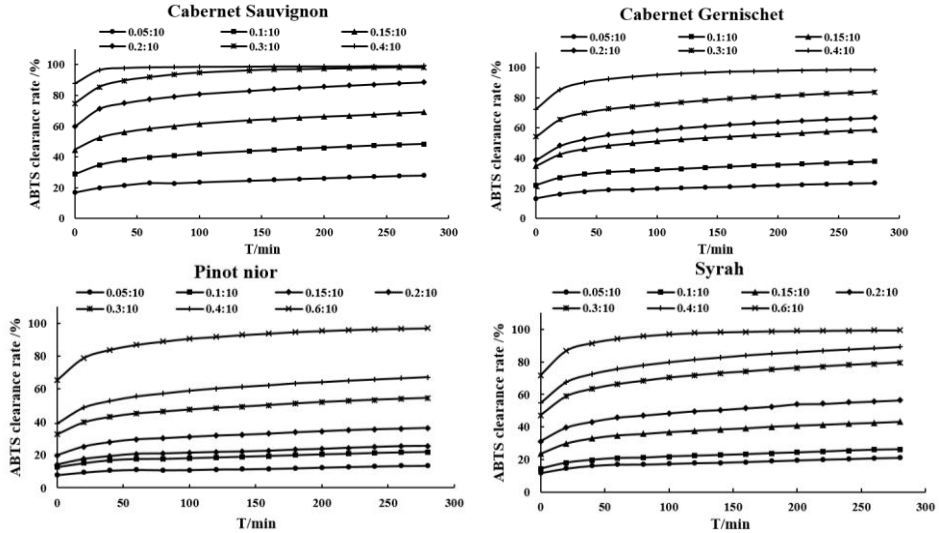
4) Comparison on total anthocyanins

Anthocyanins possess antioxidant activity, which is considered to be an important physiological function. Additively, anthocyanins are reported to have anti-inflammatory activity, anticancer activity, apoptotic induction effect, α -glucosidase inhibition activity, vision benefits and effects on collagen, blood platelet aggregation and capillary permeability and fragility [19]. The TA content of the wines was expressed as milligrams of C3G equivalents per 1 L of wine samples. The results were shown in Table III. As can be seen, different from other indicators, the TA content decreased in the order: Cabernet Gernischet > Merlot > Cabernet Sauvignon > Syrah > Pinot nior, Ningxia > Yantai > Xinjiang. The levels ranged from 127.71 ± 11.04 mg/L for Syrah from Xinjiang region to 354.40 ± 30.62 for Cabernet Gernischet from Ningxia region. The content of TA varied from 246.80 ± 68.43 to 354.40 ± 30.62 mg/L, for Cabernet Gernischet wines. No significant difference was found between the Merlot wines from Ningxia and Xinjiang ($p > 0.05$). On the whole, in the present study, either for Cabernet Gernischet or for Merlot, the wine samples from Ningxia region contained significantly more contents of TA than did the other regional wines. While on the contrary, its contents of Syrah and Pinot nior in Xinjiang region were the lowest. Significant differences were found in TA content in comparisons between Cabernet Sauvignon from Yantai and Xinjiang region ($p < 0.05$); however, significant differences in TA content were not found between Cabernet Sauvignon from Ningxia and Xinjiang region, Ningxia and Yantai region ($p > 0.05$). Intriguingly, Cabernet Gernischet wines from Ningxia region contained significantly fewer total phenols, flavanols and flavonoids than did Cabernet Sauvignon and Merlot wines, but significantly more anthocyanins than the other red cultivar wines. This result is well in accordance with those available in the literature [3].

As a characteristic associated with the variety, the level of anthocyanins in grapes may serve as an estimate of the red pigments and be useful for the classification of grape varieties and of relevant wines. Therefore, anthocyanins have been proposed as chemical markers to differentiate grape varieties and red wines [16]. As is well known, the amount of phenolic materials vary considerably in different wine grape-growing regions, depending on the grape variety, environmental factors of vine growth [20].

B. Determination of Optimal Reaction Conditions on ABTS and DPPH

In order to investigate the effects of reaction time and dilution ratio on antioxidant activity of red wine, several samples, including five grape varieties and a common antioxidant Trolox, were determined in this experiment. The free radical-scavenging activity of five varieties of wine and Trolox was determined by two methods and the results were shown in Fig. 1 to 3. As can be seen from Fig. 1 and Fig. 2, due to the higher concentration of the antioxidant, there was a rapid improvement in the free radical scavenging capacity of the sample at the beginning of the reaction. In the late stage of the reaction, the reaction rate decreased and gradually stabilized.



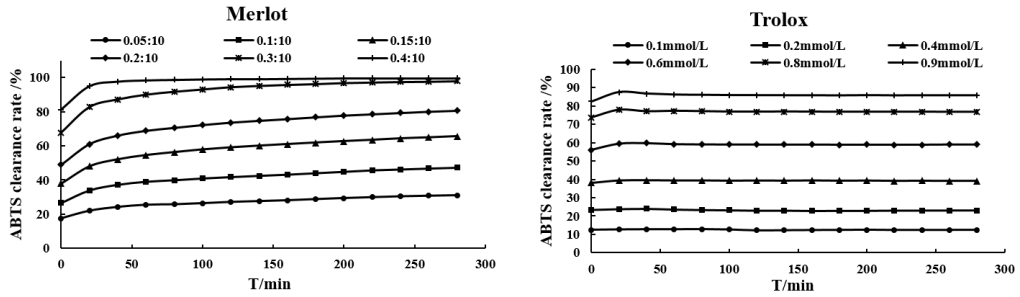


Figure 1. Changes in the inhibitory percentage of ABTS radical with red wines and Trolox

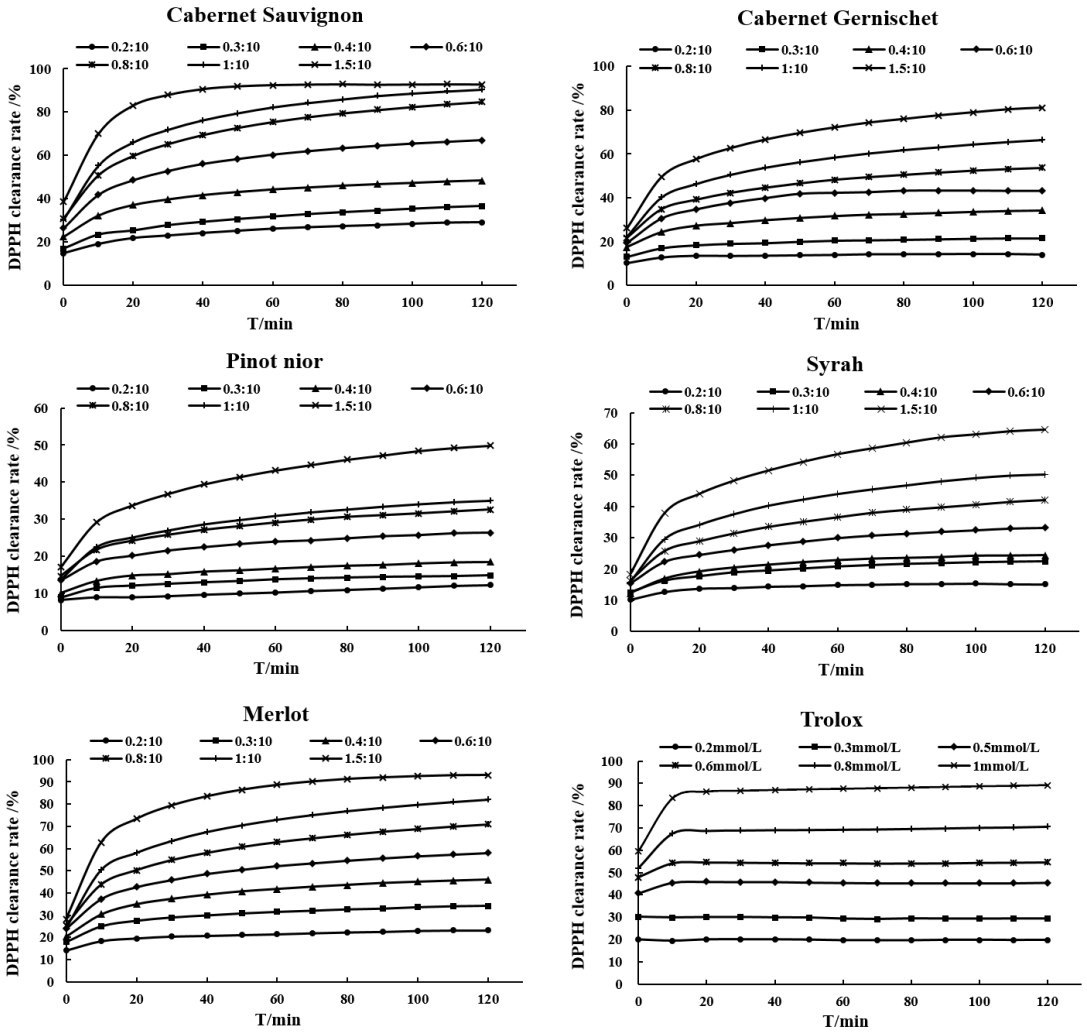


Figure 2. Changes in the inhibitory percentage of DPPH radical with red wine and Trolox

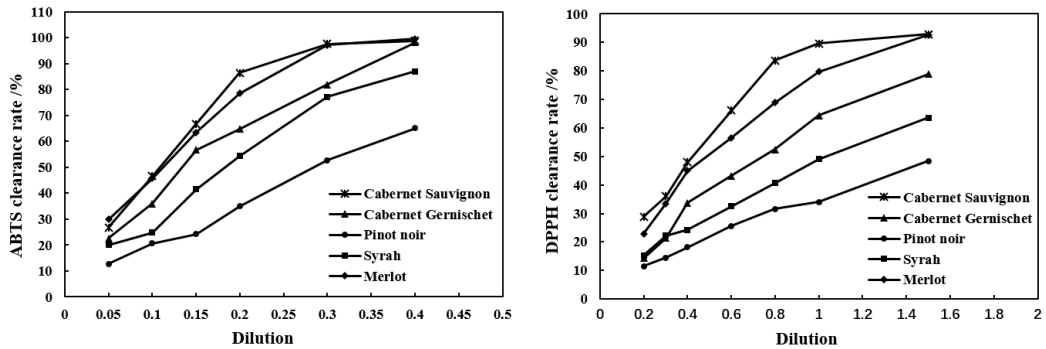


Figure 3. Influence of wine samples dilution on free radical scavenging activities.

Determination of optimal reaction conditions on ABTS assay was based on the slightly modified method of Li, et al. (2009) and Hu, *et al*. (2018) [21], [22]. For ABTS, as can be seen from Fig. 1, when the reaction progressed to 200~240min, the Lineal correlation coefficients of the free radical scavenging rate of Cabernet Sauvignon, Cabernet Gernischet, Merlot, Pinot noir and Syrah was 0.9999, 0.9970, 0.9970, 0.9972 and 0.9967, respectively, which means the ABTS free radical scavenging rate is tending towards stability. Meanwhile, slight difference in TEAC between the reaction time at 200, 220 and 240 min was found. As a result, 200~240 min is regarded as the optimal reaction time for the determination of antioxidant activity of wine. In addition, as can be seen from Fig. 3, there is a good dose-effect relationship between the concentration and the free radical scavenging rate within a dilution multiple of 0.15:10~0.3:10 (0.15~0.3 in Fig. 3) in the reaction time at 220 min, the Lineal correlation coefficients of Cabernet Gernischet, Pinot noir and Syrah was 0.9998, 0.9976 and 0.9992, respectively. In contrast, the Lineal correlation coefficients of Cabernet Sauvignon and Merlot was 1 and 0.9991 within the dilution multiple of 0.15:10~0.2:10 (0.15~0.2 in Fig. 3). Therefore, in order to meet the test requirements of all above five wine varieties, the dilution multiple of 0.15:10~0.2:10 (0.15~0.2 in Fig. 3) was selected.

For DPPH, the optimization results of the reaction time were shown in Fig. 2. It can be seen that the DPPH scavenging rate reached the reaction equilibrium time at 90~110 min during the reaction gradually increased with the extension of the reaction time. Furthermore, the results in Fig. 3 showed that the DPPH scavenging rate showed an excellent dose-effect relationship with the dilution multiple in the range of 0.4:10~0.8:10 (0.4~0.8 in Fig. 3). The Lineal correlation coefficients of the free radical scavenging rate of Cabernet Sauvignon, Cabernet Gernischet, Merlot, Pinot noir and Syrah was 0.9998, 0.9999, 0.9997, 0.9949 and 1, respectively in the reaction time at 100 min. Thus, in order to raise experiment efficiency, the optimal reaction time of 90~110 min and the dilution multiple of 0.4:10~0.8:10 (0.4~0.8 in Fig. 3) were selected. The specific reaction conditions can be selected according to the actual situation.

C. Comparison on Antioxidant Capacity of Wines from three Regions of China

According to many authors, antioxidant activity of grape berries and wines results mainly from their phenolic compounds. The high scavenging property may be due presence of large contents of phenolic compounds, the hydroxyl groups present in the molecules can provide the necessary component as a radical scavenger [23]. Generally, it is established that an oxidation process is involved in the initial development steps of cancer and cardiovascular disease, antioxidants have ability to keep free radicals from attacking the body's cells and contributing to destructive processes inside the body [24]. Well, red wine is good for a man's health in a number of ways, according to many studies. Thus, different methods are necessarily used to determine the antioxidant capacity of wines.

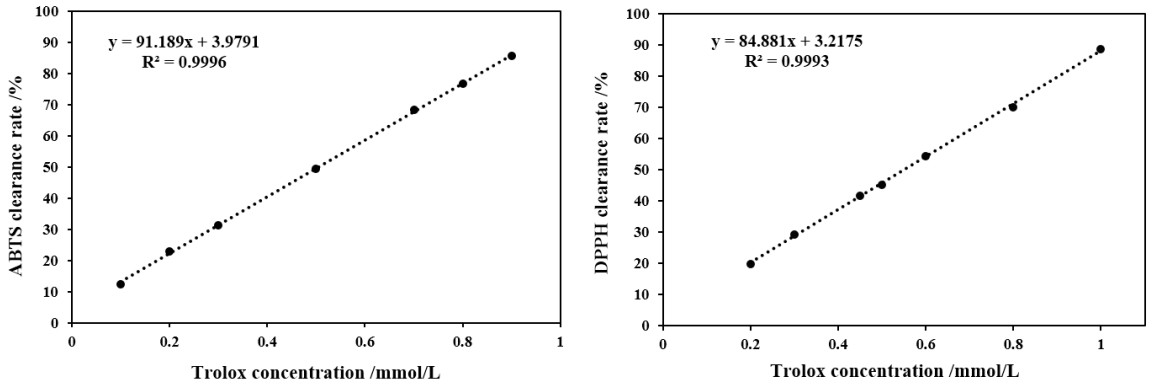


Figure 4. Linear fitting curve for ABTS and DPPH free clearance rate by Trolox

The antioxidant capacity of all wine samples was determined in the optimal reaction condition of 220 min, 0.2:10 for ABTS method and 100 min, 0.6:10 for DPPH method. As shown in Fig. 4, the ABTS and DPPH clearance rate and the concentration of Trolox had a good dose-effect relationship, the results were expressed as mM Trolox equivalent antioxidant capacity and were shown in Table IV. Among the five red wine grapes from the three regions evaluated, Cabernet Sauvignon grown in Ningxia and Xinjiang had the best antioxidant capacity than that the other varieties, followed by Merlot grown in Ningxia and Xinjiang, Syrah grown in Ningxia and Cabernet Sauvignon grown in Yantai. On the other hand, five red wine grapes from the three regions were all showed better antioxidant capacity in Ningxia than the other two regions. As for Cabernet Gernischet wine grapes from the two regions, grapes grown in Ningxia ranked first, followed by wine grapes grown in Yantai. In addition, Syrah and Merlot grown in Xinjiang were of relatively lower antioxidant capacity than that in Ningxia, respectively. Pinot nior grown in Xinjiang region also showed a lower value in antioxidant capacity than other wines varieties, this result is agreed with R. Van Leeuw *et al*. (2014) [25].

Owing to being landlocked, Ningxia and Xinjiang have the characteristics of large diurnal temperature

variation, few and concentrated rainfall, long sunshine time, strong solar radiation, which allows sufficient accumulation of sugars and phenols and exhibits a characteristic of "high oxidation resistance". By contrast, Yantai has the characteristics of smaller Dryness index and heavy rainfall, resulting in thin phenols content in grape berries and lower antioxidant activity.

TALBE IV. ANTIOXIDANT PROPERTIES OF WINES TESTED

Cultivars	Location	ABTS (TE)	DPPH (TE)
Cabernet Sauvignon	Xinjiang	0.872±0.080	0.986±0.039
	Ningxia	0.894±0.038	0.987±0.020
	Yantai	0.783±0.078	0.889±0.093
Merlot	Xinjiang	0.831±0.076	0.896±0.107
	Ningxia	0.858±0.016	0.962±0.011
Cabernet Gernischet	Ningxia	0.789±0.032	0.879±0.062
	Yantai	0.501±0.117	0.572±0.132
Syrah	Xinjiang	0.721±0.026	0.683±0.005
	Ningxia	0.793±0.005	0.891±0.003
Pinot noir	Xinjiang	0.517±0.123	0.605±0.126

Note: Values represent means ± SD. DPPH, ABTS expressed as mM Trolox equivalents (TE).

D. DPPH and ABTS

Analysis of ABTS+ and DPPH+ scavenging activity are the two most widely used and stable chromogen compounds to evaluate the antioxidant activity of biological material. In the DPPH+ scavenging assay, antioxidants reacting with DPPH+ produce yellow α, α-diphenyl-β-picrylhydrazine. The degree of discoloration indicates the radical scavenging activity of the antioxidant [26].

The free radical-scavenging activities found by ABTS and DPPH assays in the red wine varieties differed significantly. For ABTS, the values varied from 0.501 ± 0.117 to 0.894 ± 0.038 mM. For DPPH, the values varied from 0.572 ± 0.132 to 0.989 ± 0.039 mM for the whole red wine varieties. High TEAC value indicates that the mechanism of antioxidant action of wine sample was as a hydrogen donor and it could terminate the oxidation process by converting free radicals to the stable forms. In the case of individual wine varieties under the same conditions, the DPPH and ABTS of the wines decrease in the order: Cabernet Sauvignon > Merlot > Syrah > Cabernet Gernischet> Pinot nior. Moreover, the antioxidant capacity of four single varietal wines in Ningxia region was respectively in a higher value than in Yantai and Xinjiang region. The results of investigation show that the higher the concentration of antioxidant, the lower is the amount of remaining DPPH and ABTS, and the higher is the free radical-scavenging activity. The same is observed for ABTS radical cation.

These differences observed between assays are related to the individual molecular structure of each compound. It must be borne in mind that each assay is a measure of the antioxidant activity but using different radicals [27]. This paper presents the practical approaches to evaluate the antioxidant capacity of the wine varieties have advantages of highly sensitive, accurate, high efficiency and simple.

E. Correlation between Polyphenolic Composition and Antioxidant Capacity

In order to determine the contribution of individual phenolic compounds to the antioxidant capacity, correlation analysis between total phenols, flavonoids, flavanols, anthocyanins content and total antioxidant activity were analysed for the 5 grape varieties from three regions of China to explore the relationships amongst the different antioxidant variables measured for all the wine samples.

As can be observed, the values of Cabernet Sauvignon and Merlot wines with the highest polyphenol contents in Ningxia region were higher than those of the other regional samples in every antioxidant test used. The present study reveals a strong correlation between total antioxidant activity and total phenols (R^2 = 0.959, $p < 0.05$). As the results shown in Table V, the TP, TFO and TFA content of wine samples almost exhibited the strongest correlation ($p < 0.05$) with antioxidant properties. These results were well in accordance with recent reports in the literature, which as the positive correlation indicates that the higher total phenolic content resulted in a higher total antioxidant activity [6], [28]. In contrast, total anthocyanins content exhibited weaker correlation with ABTS and DPPH assay (R^2 = 0.445 and R^2 = 0.539, respectively). Regarding different methods, the significant correlation between methods was confirmed with ABTS and DPPH methods (R^2 = 0.961, $p < 0.05$), indicated that every one of them can be considered as a relevant and reliable characteristic of the antioxidant capacity of wines.

TABLE V. LINEAL CORRELATION COEFFICIENTS (R) BETWEEN POLYPHENOLIC COMPOSITION AND ANTIOXIDANT CAPACITY.

	ABTS	DPPH	TP	TFO	TFA	TA
ABTS	1	0.961	0.929	0.922	0.855	0.445
DPPH		1	0.959	0.961	0.928	0.539
TP			1	0.923	0.851	0.571
TFO				1	0.926	0.428
TFA					1	0.303
TA						1

IV. CONCLUSIONS

Our results have found that significant differences in phenolic content can exist among grape varieties. Cabernet Sauvignon wines are an excellent source of phenolics and antioxidants. In addition, there are also significant differences between three regions. Among the wines tested, grapes grown in Ningxia region have higher phenolic content levels than other wine species, followed by Xinjiang and Yantai region, the same result is obtained for antioxidant capacity. The amounts of phenolic materials and antioxidant activity vary considerably in different types of wines, depending on the grape variety, environmental factors of wine growth and the wine processing techniques. The research on the anti-oxidation ability of wine can evaluate the quality of wine, provide reference for consumers, and provide theoretical basis for the reform of wine technological measures. Therefore, further studies need to be carried out.

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Meanwhile, she gained a lot by actively participating in school activities and academic seminars. Efforts are under way to study on the antioxidant properties of wine and hope to make an achievement.

Ms. Chang is still a graduate student in Yantai University. Prof. Zhao Yuping, as her graduate study supervisors, have been in close contact with Prof Li Jiming from Technology Center Changyu Group Co., Ltd. Our experimental groups have been made certain scientific achievements in the field of wine and brandy. About Wine, we have just successfully applied for a National Quality Curriculum, have been loved and welcomed by the vast majority of students. That's what we need, some new blood in the team.