Physicochemical properties and in-vitro antioxidant capacity of Semen Astragali Complanati wine

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ABSTRACT
In this paper, the physicochemical properties of the Semen Astragali Complanati wine was first investigated including the pH, electrical conductivity (EC), titratable acid, alcohol content, residual sugars, chromatic characters and flavonoids, and thereafter the in-vitro antioxidant capacity and its correlation with the total phenolic and flavonoids content were systematically evaluated and analyzed for the different fractions of the Semen Astragali Complanati wine by six antioxidant assays and the Pearson product–moment correlation, respectively. On a whole, there are no significant differences between the Semen Astragali Complanati wine and the grape wine regarding most of the conventional enological parameters. The antioxidant assays showed that the Semen Astragali Complanati wine exerts great antioxidant capacity, which is attributed to the high content of polyphenols and flavonoids contained such as complanatoside A and rhamnocitrin, since high correlations exhibited between the total content of phenols and flavonoids, and the in-vitro antioxidant activity, respectively. All these results indicate that the Semen Astragali Complanati could be fermented to wine and be as a natural source of antioxidants preventing damage associated with free radicals and offer alternatives to develop high-value-added products to enhance health benefits.

1. Introduction
Reactive oxygen species play a dual role in biological systems: a positive role at a lower or moderate concentrations during normal physiological and biochemical processes and a negative role in excess, causing oxidative stress and resulting in a number of diseases (Valko et al., 2007), such as cardiovascular, cancer (Gaetano, Francesco, & Gianluigi, 2013), immune dysfunctions and neurodegenerative disorders (Huang, Ou, & Prior, 2005; Yan, Wang, & Zhu, 2013). Recently, an epidemiological survey demonstrated the efficiency of intake antioxidants (synthetic or natural) in preventing or suppressing free-radical-related diseases (Pérez-Jiménez & Saura-Calixto, 2015).

Considering the reported negative concerns of synthetic antioxidants, including butylated hydroxyanisole, butylated hydroxytoluene and tertbutyl hydroquinone (Choi, Song, Ukeda, & Sawamura, 2000), consumers are increasingly interested in consuming natural compounds rather than synthetics. In addition, the daily intake of some plant-derived natural antioxidants has been recommended to reduce the incidence of diseases from oxidative stress and benefit human health (Lin & Yen, 1999). Amongst these natural antioxidants, phenolic compounds are in the forefront due to their wide distribution in the plant kingdom.

Semen Astragali Complanati, which belongs to the seed of the Astragalus complanatus R. Br., has long been considered a...
health-keeping foodstuff in Chinese culture and was officially indexed in the *Pharmacopoeia of the People's Republic of China* (Pharmacopoeia Commission of the PRC, 2010) due to its health-promoting and medical functions, such as antioxidant (Zhang, Fan, Zhang, & Wang, 2012), antifatigue, antiasthmatic (Deng, Yao, Xie, Zhang, & Zhuang, 2013), antiviral (Qi, Liu, Wu, Gu, & Guo, 2011), arresting seminal discharge, protecting kidney, antibiosis in liver (Liu, Gu, Zhou, & Guo, 2005), antihypertensive (Xue, Li, Chai, Liu, & Chen, 2008), lipid-regulating and anti-aging (Ng et al., 2014). Regarding these health effects, the compounds that are considered to be contributors include flavonoids (Zhang et al., 2013a, 2010), organic acids, amino acids, triterpenoids (Cui, Sakai, Takeshita, Kinjo, & Nohara, 1992), myricetin, neo-planoside and complanatoside B (Zhang et al., 2013b).

Although Semen Astragali Complanati contains many active compounds, it is not suitable for direct consumption due to the sharp mouthfeel of astringency, which may also explain the very limited development as foodstuff to date. In order to modify the astringent mouthfeel, an alcoholic beverage was developed for the first time by fermenting the *Semen Astragali Complanati* with sugar. By transferring the bioactive compounds of *Semen Astragali Complanati* to a fermenting solution, a new palatable beverage could be developed; the health-promoting effect can also be achieved by the regular consumption of this beverage, which may be demonstrated by the moderate intake of Chinese white wine (with an alcoholic content of approximate 50% by volume) macerated with *Semen Astragali Complanati* for therapy or health in Chinese culture.

Winemaking is one of the most ancient techniques and produces the most commercially biotechnological product (Moreno-Arribas & Polo, 2005). Regarding the physicochemical characteristics and antioxidant activity of *Semen Astragali Complanati*, many studies have been conducted in recent decades (Zhang, Fan, Zhang, Wang, et al. 2012); however, there is little literature about the alcoholic beverage made from *Semen Astragali Complanati*, and this is what motivated us to develop this beverage. Thus, the aim of this work was to evaluate the *in-vitro* antioxidant capacity of the *Semen Astragali Complanati* wine/beverage by measuring the physicochemical property, the content of the total phenolic and flavonoids and the scavenging activity for free radicals. Furthermore, their correlation between them was also used to investigate the main contributor to the healthy function of this newly developed alcoholic beverage.

### 2. Materials and methods

#### 2.1. Materials and reagents

The species Shaanxi Dalii Semen Astragali Complanati (harvested in September 2012) was bought from the north-west market of traditional Chinese medicine, Xi’an, China. Wine that was fermented with *Semen Astragali Complanati* in 2013 was used for the present study.

The following chemicals were purchased from Sigma-Aldrich: 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), Folin–Ciocalteau reagent, gallic acid and rutin. Calycosin, myricetin, rhamnocitrin, complanatoside A and quercetin were purchased from Chengdu PufeiDe Biotech Co. Ltd., Sichuan Province, China. All other chemicals of analytical grade were purchased from TianJin TianDa Chemicals Co. Ltd., Tianjin, China. Methanol of chromatographic grade was purchased from the Fisher Company. Milli-Q water used was obtained through a Millipore filter system (Millipore Co., USA).

#### 2.2. Winemaking process and samples

Ground powder (40 mesh) of dried *Semen Astragali Complanati* was macerated into water at 50°C (1:10, g/mL) for 2 h, and then the mixture was ultrasonically treated at 40°C for 1 h in an ultrasound bath to extract the active compounds. Supplementary sugars (sucrose) were added to adjust to the level of 190 g/L in the solutions to produce the designated alcohol levels before fermentation. Liquid SO₂ was used to reach the level of 95 mg/L for preservation. The fermentation was conducted according to the procedure reported by our laboratory (Zhang, Fan, Zhao, Wang, & Liu, 2013c), to be specific, the prepared solutions including *Semen Astragali Complanati* were transported into some glass containers of 20 L, then the dried commercial yeast was activated according to the instructions (Angel Yeast Co. Ltd, Hubei province, China), after that, the activated yeast solution was added to the prepared solution by the ratio of 1:50 (mL/mL) as the fermentation starter. The alcoholic fermentation was maintained till the content of total sugars lower than 6.0 g/L, subsequently, the fresh-fermented wine was stored in dark containers under ambient conditions (18–22°C) for approximately 6 months.

Afterward, the stored raw wines were separated into nonvolatile and volatile fractions (NVF and VF, respectively) using a rotary vacuum evaporator. In order to investigate the antioxidant capacity of the polar and nonpolar compounds in the wine, ethyl acetate was used to extract the nonpolar compounds from wine (marked with non-polar fraction [NP]), and the remaining fraction was taken as the polar compounds (polar fraction [PF]). Furthermore, the samples were collected in the original state and diluted at concentrations of 0, 5, 10, 15 and 20 folds (D₀, D₅, D₁₀, D₁₅ and D₂₀, respectively).

#### 2.3. Determination of the physicochemical characteristics of the Semen Astragali Complanati wine

#### 2.3.1. pH, EC, titratable and volatile acidity, and alcohol content measurements

The EC and pH of the fermented wine were determined with a DDSJ-308F conductivity meter and a PHS-3C pH meter (Shanghai Leici Co. Ltd., China), respectively. The titratable acidity was determined using the method of OIV-MA-AS313-01. The volatile acidity was measured using the method proposed by García-Barceló (1994), as grams per liter of acetic acid. The alcoholic content was tested according to method of OIV-MA-AS312-01A: R2009, and the results were expressed as milliliters of alcohol per 100 mL of wine.

#### 2.3.2. Determination of tartaric esters, residual sugars and chromatic characteristics

The determination of tartaric esters and residual sugars was carried out according to the method reported by Cliff, King, and Schlosser (2007) and the 3,5-dinitrosalicylic acid spectrophotometric method, respectively. The chromatic characteristics of the wine, such as Hunter L* (Light), a* (red–green component) and b* (yellow–blue component) of CIE...
(Commission Internationale de L’Eclairage), were measured using a SC-80C colorimeter (Beijing Kangguang Instrument Co. Ltd., China) according to the recommendations of OIV-MA-AS2-11: R2006.

### 2.3.3. Calycosin, Myricetin, Rhamnocitrin, Quercetin and Complanatoside A determinations by high performance liquid chromatography (HPLC)

Flavonoids were analyzed by an Elite HPLC system (Dalian Elite Analytical Instrument Co. Ltd., China) that was equipped with a P230II binary pump, a Rheodyne injector (loop, 20 µL) and a UV230II detector (Elite). Chromatograms were recorded by the EC2006 software (Elite). Samples were separated on a column of TC-C18 (5 µm, 4.6 mm × 250 mm, Agilent, USA). All of the mobile phases were ultrasonically degassed for 25 min and filtered through a 0.45-µm membrane prior to use. The HPLC working parameters were as follows: 1.0 mL/min flow rate, 30°C column temperature, 20 µL injection volume, H2O containing 0.1% formic acid as the mobile phase A and methanol containing 0.1% formic acid as mobile phase B. The following linear gradient program was used: 0–45 min, 5–60% B; 45–55 min, 60–100% B and 55–60 min, 100–5% B. The detector was set at 266 nm, and the corresponding standards were injected to perform the peak identification and to compare the retention time and the UV–Vis spectrum.

### 2.4. Total Phenolic Content and Total Flavonoid Content of the Semen Astragali Complanati wine

The total phenolic content was measured following the Folin–Ciocalteu method with minor modifications (Singleton, Orthofer, & Lamuela-Raventos, 1999). Briefly, 1.0 mL of sample, 5.0 mL of deionized water and 1.0 mL of Folin–Ciocalteau’s reagent (tenfold dilution) were vigorously mixed. Three minutes later, 3.0 mL of 7.5% Na2CO3 was added, and the mixture was incubated for 2 h at room temperature. The absorbance at 765 nm was measured by a UV–Vis spectrophotometer of TU-1810 (Beijing Purkinje General Instrument Co., Ltd., China). The standard gallic acid (0–0.8 mg/mL, R2 = 0.9990) was used to plot the calibration curve, and the milligram of gallic acid equivalents per liter of wine was used to express the results.

The total flavonoid content was estimated following a previously described method (Xue et al., 2008). Briefly, 0.2 mL of sample and 0.3 mL of 0.5 M NaNO2 were mixed and allowed to stand for 6 min, followed by the addition of 0.3 mL of AlCl3 (0.1 M) and 4 mL of NaOH (0.4 M). After 15 min of incubation, the absorbance at 510 nm was measured. The flavonoid content was expressed as milligram of rutin equivalents per liter of wine (mg RE/L).

### 2.5. Antioxidant activity assays

#### 2.5.1. Antioxidant activity in a hemoglobin-induced linoleic acid emulsion system

The antioxidant activity of wine was principally evaluated by the inhibition rate of the hemoglobin-catalyzed peroxidation of linoleic acid assay (Kuo, Yeh, & Pan, 1999). Briefly, 10 mL of sample was added to 0.37 mL of phosphate buffer (0.05 M, pH 7.0), with 0.05% Tween-20 and linoleic acid (100 mM, 20 µL) and then incubated at 37°C for 3 min. Then the linoleic acid peroxidation was triggered by the addition of 20 µL of hemoglobin (0.035%) and incubated at 37°C for 10 min. Then, 5 mL of ethanol with HCl (0.6%) was added to terminate the peroxidation. The amount of peroxide value, in hydroperoxide formed, was determined at the absorbance of 480 nm (A0). A0 was the blank absorbance, and A1 was the control absorbance (containing all of the reagents except for the test sample). The degree of linoleic acid oxidation was directly proportional to the absorbance values. Gallic acid (4 mg/L) and rutin (20 mg/L) were used as positive controls. Thus, the inhibition (%) of the sample in linoleic acid oxidation was calculated as follows:

\[
I% = \left(1 - \frac{A_c - A_0}{A_c - A_0}\right) \times 100
\]

where A1 is the sample absorbance at 593 nm, and A0 is the control absorbance at 593 nm.

#### 2.5.2. Scavenging activity on Hydroxyl radical (·OH)

The scavenging activity of wine on the ·OH was measured following previous literature (Zhang et al., 2013c). Gallic acid (4 mg/L) and rutin (20 mg/L) were used as the positive controls. The scavenging activity was estimated (S%) using the following equation:

\[
S% = \left(1 - \frac{A_c - A_1}{A_c - A_0}\right) \times 100
\]

where A1 is the sample absorbance at 300 nm, and A0 is the control absorbance at 300 nm.

#### 2.5.3. Scavenging activity on superoxide anion (O2·−)

The scavenging activity of wine on O2·− was measured using the method of Lu and Foo (2001). Gallic acid (4 mg/L) and rutin (20 mg/L) were used as standards. The scavenging capability of the superoxide radical (S%) was calculated as follows:

\[
S% = \left(1 - \frac{A_c - A_1}{A_c - A_0}\right) \times 100
\]

where A1 is the sample absorbance at 530 nm, and A0 is the control absorbance at 530 nm.

#### 2.5.4. Scavenging activity on the DPPH radical

The DPPH radical, which has been extensively used to evaluate the scavenging capacity of antioxidants, is a stable commercial-free radical with a typical absorption at 517 nm. Unlike other laboratory-generated free radicals, such as ·OH and O2·−, DPPH has the advantage of being not influenced by certain side reactions, such as enzyme inhibition and metal ion chelating (Amarowicz, Pegg, Rahimi-Moghaddam, Barl, & Weil, 2004), and it evaluates the ability of the samples donating hydrogen to the free radical.

In this study, the scavenging activity of DPPH was measured following the method of Zhang et al. (2013c). Gallic acid (4 mg/L) and rutin (20 mg/L) were used as standards. The scavenging activity of DPPH (S%) was calculated as follows:

\[
S% = \left(1 - \frac{A_c - A_1}{A_c - A_0}\right) \times 100
\]

where A1 is the sample absorbance at 517 nm, and A0 is the control absorbance at 517 nm.

#### 2.5.5. Ferrous ion-chelating activity

The ferrous ion chelating (FIC) was measured by the method of Decker and Welch (1990). Briefly, 1 mL of sample was mixed with 0.05 mL of FeCl2 (2 mmol/L) and 0.2 mL of...
ferrozine (5 mmol/L), and the absorbance at 593 nm was recorded 10 min later. With the 4 mg/L of gallic acid and 20 mg/L of rutin as positive controls, the inhibition percentage of FIC (%) was calculated using the following equation:

\[ FIC = \left( \frac{A_c - A_t}{A_{control}} \right) \times 100 \]

where \( A_c \) is the absorbance of sample and \( A_t \) is the absorbance of the control.

2.5.6. Ferric-reducing antioxidant power

The ferric-reducing antioxidant power (FRAP) of the samples was determined with a slight modification of the method of Oyaizu (1988). Briefly, 1 mL of sample was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% \( K_3[Fe(CN)_6] \) solution. After 20 min of incubation at 50°C, 2.5 mL of trichloroacetic acid (10%) was added, and the reaction solution was centrifuged at 6500g for 10 min. Then, 2.5 mL of the supernatant solution was collected and mixed with 2.5 mL of water and 0.5 mL of FeCl\(_3\) solution (0.1%). Finally, the absorbance at 700 nm was recorded after 10 min of reaction.

2.6. Statistical analysis

The experimental data were expressed as means ± standard deviations. All of the determinations were conducted in triplicate. A one-way analysis of variance (ANOVA) was performed to check for differences between the means using SPSS version 10.1 (SPSS Inc.). The Pearson correlation coefficient was used to estimate the correlations between total phenolics, flavonoid content and antioxidant activities of Semen Astragali Complanati wine. The difference between means was checked by ANOVA; the level of significance was determined by a given p value.

3. Results and discussion

3.1. Physicochemical characteristics of the Semen Astragali Complanati wine

The physicochemical characteristics of wine that was produced from Semen Astragali Complanati are described as follows: pH 3.80, 1500 μS/cm electrical conductivity, 7.5 g/L titratable acid (as tartaric acid), 0.35 g/L volatile acid (as acetic acid), 9.50% ethanol and 5 g/L residual sugars, and these values are very comparable to those of moderate grape wines. The values of \( L^* \), \( a^* \) and \( b^* \) of the wine are 85, 4 and 28, respectively, which is slightly different from those of the common grape wines that were tested in our lab (80, 20 and 8), especially for the values of \( a^* \) and \( b^* \) (Zhang, Shen, Fan, & García-Martin, 2016). It may be attributed to the lower content of anthocyanins in the Semen Astragali Complanati. In general, the color of grape juice and wine is an important attribute that is related to the visual appeal and quality and that is attributed to the free anthocyanins, principally as flavylum cation (red) and quinoidal anhydro-base (blue), anthocyanin self-association and copigmentation of anthocyanins with other phenols in wines. In addition, the content of tartaric esters is 60 mg/L, which is significantly lower than the content of 250 mg/L in grape wines; this result may be explained by the lower tartaric acid content in Semen Astragali Complanati and by the yeast strain and the conditions that were used for fermentation (López-Ramírez, Martín-del-Campo, Escalona-Buendi, García-Fajardo, & Estarrón-Espinosa, 2013; Zhang et al., 2016). The concentration and relative distribution of ester are usually influenced by the yeast variety and fermentation parameters, such as pH, temperature, oxygen levels, fatty acid or sterol levels (Sudheer Kumar, Varakumar, & Reddy, 2012). Moreover, esters are often considered related to the sensory property of wine. Thus, the flavor of the Semen Astragali Complanati wine may be slightly better than that of grape wine. Overall, there is no significant difference between the Semen Astragali Complanati wine and the grape wine in most of conventional enological indexes.

Regarding the flavonoids in the Semen Astragali Complanati wine, five compounds were separated and identified with HPLC by comparing the retention time and UV–Vis spectrum with those of the correspondingly injected standards, such as calycosin, myricetin, rhamnocitrin, quercetin and complanatoside A (Figure 1). The contents of the identified compounds were calculated by the corresponding curves that were constructed with standards of at least six appropriate concentrations, and the values of the five regression coefficients \( R^2 \) were greater than 0.9900 (Table 1). To be specific, the contents of calycosin, myricetin, rhamnocitrin, quercetin and complanatoside A in the wine were 130, 100, 80, 65 and 140 μg/mL, respectively. Overall, all of the identified compounds had a relatively higher content in the wine transferred from the Semen Astragali Complanati seeds during fermentation and aging, which makes this wine very healthy.

Figure 1. Liquid chromatograms of standards (a) and wine sample (b) (1. Myricetin 2. Complanatoside A 3. Calycosin 4. Quercetin 5. Rhamnocitrin).

Figura 1. Cromatogramas de líquidos de los estándares (a) y la muestra de vino (b) (1. Miricetina 2. Complanatoside A 3. Calycosin 4. Quercetina 5. Rhamnositrina).
Table 1. Regression equation, coefficient and linear range for the five-standard compounds.

| Compounds          | Regression equation | Coefficient | Linear range, μg/L |
|--------------------|---------------------|-------------|-------------------|
| Myricetin          | Y = 17467x + 2.47   | 0.9916      | 0.1167–0.7000     |
| Complanatoside A   | Y = 15704x + 19.67  | 0.9929      | 0.1042–0.2083     |
| Calycosin          | Y = 15610x – 94.72  | 0.9945      | 0.6500–6.5000     |
| Quercetin          | Y = 120000x – 71.76 | 0.9928      | 0.0281–0.3375     |
| Rhamnocitrin       | Y = 16177x – 106.80 | 0.9935      | 0.1250–0.5000     |

3.2. Contents of total phenolics and flavonoids in Semen Astragali Complanati wine

Phenolics are usually present in conjugated forms with sugars or other polyols via O-glycosidic or ester bonds (Robbins, 2003) and contribute greatly to the organoleptic properties of red wine by affecting the color, astringency and aroma. As an important processing technique, fermentation can not only catalyze the release of total flavonoids and phenolics from the conjugated forms, but also accelerate the penetrability of the cell wall, causing the leakage of cytoplasm, which might further lead to an increase of those compounds during the winemaking process (Lee, Hung, & Chou, 2008).

In this paper, the total phenolic content (TPC) and total flavonoid content (TFC) were detected and calculated from the calibrated curves of gallic acid (Y = 5.3071x + 0.0085, $R^2 = 0.9990$) and rutin (Y = 0.1x – 0.0037, $R^2 = 0.9992$), respectively. As shown in Figure 2, each fraction of the wine has a relatively higher content of TPC and TFC, which demonstrates a good correlation between the contents and the diluted ratios of wine and may suggest that the TPC and TFC are the main compounds of the Semen Astragali Complanati wine.

Regarding the TPC, there was no significant difference between the sample of $D_0$ and NVF, which implies that the total phenols may be the main nonvolatile compounds in the Semen Astragali Complanati wine, while the TPC in the PF was significantly lower than that in the fraction of $D_0$ and NVF.

Furthermore, the TPC and TFC in the NVF were significantly higher than those in the VF ($p < 0.05$), which may be attributed to the different compounds that are contained in these fractions; for instance, the nonvolatile fraction contains more sugars, organic acids and phenolic acids, while the VF may be characterized by an abundance of aldehydes, monoterpenes and sesquiterpenes rather than phenolics. In contrast, the TPC and TFC in the PF were significantly higher than those in the NPF.

3.3. Antioxidant capacity evaluation

Regarding the differences among the large numbers of antioxidant systems available, the results of one single assay can only give a limited suggestion of the antioxidant characteristics. To be specific, the chemical complexity of the Semen Astragali Complanati wine, often a mixture of many compounds with different polarities, chemical behaviors and functional groups, usually leads to some scattered results, closely depending on the assay employed. Thus, it is oversimplified and inappropriate to only select one single method to evaluate the antioxidant capacity. Therefore, to ensure comparative of the results and to cover a wide range of potential applications, six antioxidant assays using different reactive oxygen species, including both the primary and secondary steps of oxidation (Tundis et al., 2011), were used to describe a full profile of the antioxidant activity for the Semen Astragali Complanati wine.

3.3.1. Antioxidant activity of the Semen Astragali Complanati wine in a linoleic acid oxidation system as induced by hemoglobin

In Figure 3, the results demonstrate that the PF of the Semen Astragali Complanati wine had a stronger inhibiting ability of 76.1% in linoleic acid oxidation, while in comparison, the values for the positive standards of rutin and gallic acid...
Inhibition rate on the linoleic acid oxidation by the different fractions of Semen Astragali Complanati wine.

\[ 5.85 \pm 2.92, 38.27 \pm 3.13 < 0.05. \]

Índice de barrido de radicales libres del vino de Semen Astragali Complanati

\[ 7.60 \pm 2.35, 17.70 \pm 2.72, 1.29 \pm 0.31, 22.15 \pm 3.17, 2.74 \pm 0.42 \]

(mean ± SD, (promedio ± SD, n = 3)).

Scavenging rate on free radicals of the Semen Astragali Complanati wine

\[ 83.82 \pm 2.31, 84.35 \pm 3.06, 9.67 \pm 2.84 \]

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Índice de inhibición en la oxidación de ácido linoleico mediante diferentes fracciones de vino de Semen Astragali Complanati.

| Sample | Hydroxyl radical | Superoxide radical | DPPH radical |
|--------|------------------|--------------------|-------------|
| D0     | 52.84 ± 1.12\(c\) | 83.82 ± 2.31\(e\)  | 22.15 ± 3.17\(e\) |
| D5     | 21.97 ± 4.51\(b\) | 60.81 ± 3.14\(e\)  | 8.55 ± 1.41\(i\)  |
| D10    | 9.67 ± 2.84\(a\)  | 49.31 ± 2.96\(e\)  | 6.99 ± 0.45\(b\)  |
| D15    | 5.85 ± 2.92\(a\)  | 38.27 ± 3.13\(e\)  | 2.94 ± 0.41\(d\)  |
| D20    | 2.21 ± 1.20\(a\)  | 17.70 ± 2.72\(c\)  | 1.65 ± 0.37\(d\)  |
| VF     | 0.59 ± 0.14\(a\)  | 7.67 ± 2.35\(a\)   | 9.56 ± 0.62\(c\)  |
| NVF    | 79.39 ± 3.43\(g\) | 85.53 ± 1.91\(h\)  | 29.23 ± 3.09\(f\) |
| NPF    | 0.34 ± 0.15\(a\)  | 3.61 ± 1.57\(b\)   | 1.29 ± 0.31\(c\)  |
| PF     | 59.54 ± 6.22\(g\) | 84.35 ± 3.06\(e\)  | 30.88 ± 1.24\(d\) |
| Rutin  | 13.05 ± 0.31\(a\) | 69.85 ± 2.46\(d\)  | 2.74 ± 0.42\(c\)  |
| Gallic acid | 12.54 ± 0.11\(a\) | 60.92 ± 1.37\(d\)  | 3.96 ± 0.17\(b\)  |

Values in the same column with different superscripts are significantly different at p < 0.05.

Los valores en la misma columna con diferentes superíndices son significativamente distintos a P < 0.05.

3.3.2. Scavenging activity on ·OH of the Semen Astragali Complanati wine

As shown in Table 2, all of the samples other than the fractions of VF and NPF generally were found to have a certain capacity to scavenge the hydroxyl radical and NVF of the wine fraction exhibited the highest ·OH scavenging potential (79.39%), which was significantly higher than the others, including the positive standards rutin and gallic acid. Additionally, the wine also showed a scavenging activity on ·OH in a concentration-dependent manner, as demonstrated by the dilution times. In total, the scavenging ·OH capacity of Semen Astragali Complanati wine appears to highly correlate with the inhibition of lipid peroxidation, which might be attributed to the combined effects of reducing power, hydrogen atom donation and active oxygen scavenging. These results suggest that the antioxidant activity of the Semen Astragali Complanati wine may not be exclusive due to the specific bioactive compounds, but their synergistic or antagonistic effects; moreover, compounds with the most hydroxyl groups apparently have the greatest antioxidant capacity and mainly exist in the NVF and PF parts of the wine (Loizzo et al., 2013; Rivero-Pérez, Muñiz, & González-Sanjosé, 2007).

3.3.3. Scavenging activity on O2- · of the Semen Astragali Complanati wine

In this paper, the Semen Astragali Complanati wine was demonstrated as a potent scavenger of O2- · with a dose-dependent scavenging activity. The scavenging capacity was as follows: NVF > PF > D0 > Rutin > Gallic acid > D15 > D20 > VF > NPF; the NVF and PF were found to exert the highest O2- · scavenging activities of 85.53% and 84.35%, respectively. These results indicate that the Semen Astragali Complanati wine had a notable scavenging capacity on superoxide anion free radicals compared to the values of 69.85% and 62.92% for rutin and gallic acid, respectively, which were used as positive controls of potent superoxide radical scavengers.

The perfect scavenging effect of the Semen Astragali Complanati wine on O2- · may be attributed to the active hydroxyl groups in the phenolic and flavonoid compounds, which can quench free radicals and form more stable...
macroradicals according to free radical theory (Yazdani-pedram, Lagos, Campos, & Retuert, 1992). Therefore, these active hydroxyl groups are considered the origin of the higher scavenging capacity of the Semen Astragali Complanati wine.

3.3.4. Scavenging activity on DPPH of the Semen Astragali Complanati wine

As shown in Table 2, the values for the DPPH scavenging capacity of the wines ranged from 1.29% to 30.88%. Compared to rutin and gallic acid, most of the tested samples showed an obvious DPPH scavenging activity except for the fractions of NPF, D_{15} and D_{20}. The PF and NPF had the strongest and lowest antioxidant capacity of 30.88% and 1.29% when scavenging the DPPH radical, respectively. The scavenging effects on DPPH decreased in the following order: PF > NVF > D_{0} > VF > D_{5} > D_{10} > gallic acid > D_{15} > rutin > D_{20} > NPF, which is generally in accordance with the content of total phenols (Figure 2). The bleaching activity is mainly from the presence of polyphenols and flavonoids, especially phenolics with a substitution of hydroxyl groups in the aromatic rings, thus easily demonstrating the hydrogen-donating capacity. These results demonstrate that the DPPH scavenging ability can be credibly predicted by the Folin–Ciocalteu assay, as the two methods follow a similar mechanism of the propensity donating hydrogen (Katsube et al., 2004). The Semen Astragali Complanati wine exerted a concentration-dependent manner on the DPPH scavenging activity (Table 2), which might indicate that there are some compounds being contributors on scavenging the DPPH radical.

3.3.5. Fe^{2+}-chelating ability of the Semen Astragali Complanati wine

As shown in Figure 4, the wine fractions of NVF and PF exhibited Fe^{2+}-chelating ability values of 39.47% and 35.17%, respectively, which are significantly higher ($p < 0.05$) than those of the other’s including the positive controls gallic acid and rutin. Moreover, the Fe^{2+}-chelating ability of the wine decreased with the increased diluted folds, demonstrating a highly negative correlation.

Generally, primary antioxidants can quench radicals inhibiting chain initiation and propagation, while secondary antioxidants suppress the radical formation protecting against oxidative damage. In this study, the results clearly indicate that the Semen Astragali Complanati wine not only exerts strong primary antioxidant attributes, but also acts as a moderate secondary antioxidant, since it contains many active components of chelating to metal ions. Moreover, a higher correlation between the chelating metal and the TPC and TFC might suggest that the metal chelation is mainly due to the presence of total phenols and flavonoids.

3.3.6. FRAP of the Semen Astragali Complanati wine

Research has revealed a direct correlation between the antioxidant power and the reducing activity of natural compounds (Yildirim, Mavi, & Kara, 2001). In the meantime, reducing power test is often employed to evaluate the ability of natural antioxidants donating the electron (Dorman, Peltoketo, Hiltunen, & Tikkanen, 2003).

As shown in Figure 5, the wine fractions of VF and NPF showed the lowest FRAP levels of 0.002 and 0.004, respectively. The order of the FRAP for all of the samples is as follows: rutin > gallic acid > PF > NVF > D_{5} > D_{15} > NPF > VF. On the whole, the ferric-reducing power of the Semen Astragali Complanati wine was concentration dependent. Moreover, highly significant correlation coefficients were found between the amounts of flavonoids ($r = 0.906$), phenolics ($r = 0.933$) and the iron-reducing power. Such potential FRAP might be attributed to the presence of phenolics and flavonoids in the wine (Wang et al., 2015). In fact, these results indicate that the antioxidant occurrence of the Semen Astragali Complanati wine appears to function as good as electron donor, reacting with free radicals and hence terminating the chain reaction. Although the reducing powers of Semen Astragali Complanati wine were lower than those of rutin and gallic acid.

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Figure 4. Fe^{2+}-chelating ability of the Semen Astragali Complanati wine.

Figura 4. Habilidad quelante Fe^{2+} del vino de Semen Astragali Complanati.
3.4. Correlation between the TPC, TFC and antioxidant activities

Many reports have revealed the correlation between the antioxidant activity and the contents of total flavonoids, phenolic, tannins and anthocyanins in plant materials, such as fruits, medicinal plants and vegetables (Amessis-Ouchemouka, Abu-Reidahb, Quirantes-Piné, Madania, & Segura-Carreterob, 2014; Loizzo et al., 2013; Rivero-Pérez et al., 2007; Wang et al., 2015). In order to elucidate whether these flavonoids/phenolics are the main free-radical-scavenging compounds in the Semen Astragali Complanati wine, a direct correlation was conducted between them by linear regression analysis (Table 3). The TPC and TFC exhibited a high correlation with the inhibition rate on linoleic acid oxidation, the scavenging activity on OH, O$_2^-$ and DPPH, and the FRAP and FIC activity. These results may indicate that the phenolics and flavonoids were the major contributors to the good free-radical-scavenging activities of the Semen Astragali Complanati wine.

In general, the sample with a high scavenging capacity of free radicals would also show a high content of phenolics, that is, phenolics are usually considered the main antioxidant components, and their total contents are directly proportional to their antioxidant activities. The mechanism of the antioxidant activity of the Semen Astragali Complanati wine might not only be attributed to the specific bioactive compounds, but also to the high molecular weight with more hydroxyl groups, the proximity of many aromatic rings and their interaction effects. Because the chemical compositions and structures are important factors governing the effects of natural compounds, the antioxidant power could be explained by not only the phenolic contents, but also their characterization (Heinonen et al., 1998). In some cases, the chemical structures of phenolic compounds might be deterministic in exerting their antioxidant capacities. Generally, phenolic compounds with para- and ortho-dihydroxylation or a methoxy and a hydroxy group are more effective in exerting antioxidant power than are simple phenolics.

![Figure 5. Ferric-reducing antioxidant power of the Semen Astragali complanati wine.](image-url)

**Table 3.** Correlation coefficients among total phenolics, flavonoids content and antioxidant activities of Semen Astragali Complanati wine (Pearson product-moment correlation).

|          | TPC  | TFC  | I-LAO | S--OH | S--O$_2^-$ | S-DPPH | FRAP | FIC  |
|----------|------|------|-------|-------|------------|--------|------|------|
| TPC      | 1**  | 0.856** | 0.795* | 0.921** | 0.765* | 0.927** | 0.730* | 1**  |
| TFC      |      | 0.856** | 0.813** | 0.934** | 0.848** | 0.809** | 0.862** | 1**  |
| I-LAO    |      |       | 1**   | 0.775* | 0.873** | 0.793* | 0.827** | 0.611 |      |
| S--OH    |      |       |       | 1**   | 0.610** | 0.951** | 0.996** | 0.458 |      |
| S--O$_2^-$|      |       |       |       | 1**   | 0.735* | 0.810** | 0.491 |      |
| S-DPPH   |      |       |       |       |       | 1**   | 0.940** | 0.641 |      |
| FRAP     |      |       |       |       |       |       | 1**   |      |      |
| FIC      |      |       |       |       |       |       |       |      |      |

**Abreviaciones:** TPC, contenido total de fenoles; TFC, contenido total de flavonoides; I-LAO, Inhibición de oxidación de ácido línoleico; S--OH/ S--O$_2^-$, Actividad de barrido del radical hidroxilo/ oxiuros de superoxido; S-DPPH, Actividad de barrido del radical DPPH; FRAP, Potencial reductor antioxidante férrico; FIC, Actividad quelante del ión férrico.

*p < 0.05, Significant correlation; **p < 0.01, very significant correlation; ***p < 0.001, extremely significant correlation.

*p < 0.05, correlación significativa; ** p < 0.01, correlación muy significativa; *** p < 0.001, correlación extremadamente significativa.
instance, in both reducing activity and DPPH assays, the antioxidant activities of phenolics mainly were attributable to hydroxyl donation, especially in ortho-phenolic hydroxyl site.

4. Conclusions
This work revealed that the TPC and TFC significantly correlated with the antioxidant activities, and the major antioxidant components might be the phenols and flavonoids in the Semen Astragali Complanati wine. That is, the remarkable free radical scavenging activity and antioxidant power were mainly attributed to the presence of the high polyphenols/flavonoids content. In summary, this study suggests that the Semen Astragali Complanati wine could be a potential source of natural antioxidant compounds and offer opportunities for developing highly value-added products to enhance the health benefits. Further studies are needed to elucidate the individual phenolic compounds in this wine, and in-vivo studies are needed to better understand their antioxidant mechanisms.

Disclosure statement
The authors declare that there is no conflict of interest.

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