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# Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties

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**Abstract**

Fourteen typical malting barley varieties from China were evaluated for their DPPH radical, ABTS radical cation and superoxide anion radical scavenging activities, reducing power, metal chelating activities, and total phenolic contents (TPC). All barley samples exhibited significant antioxidant activities determined by different assays, and contained significant levels of phenolic compounds. Gan4 and Wupi1 barley exhibited the highest DPPH radical scavenging activity, ABTS radical cation scavenging activity and reducing power. Gan4 and Humai16 barley showed the highest TPC, whereas the highest superoxide anion radical scavenging activity and metal chelating activity were found in Huaimai19 and Ken3 barley, respectively. The Pearson correlation analysis revealed that the TPC showed strong correlations with DPPH radical scavenging activity, ABTS radical cation scavenging activity, and reducing power ($P < 0.01$), whereas its correlations with superoxide anion radical scavenging activity and metal chelating activity were poor ($P > 0.05$). Moreover, DPPH radical scavenging activity, ABTS radical cation scavenging activity and reducing power were well positively correlated with each other ($P < 0.01$). Principal component analysis (PCA) was applied to understand the interrelationships among the measured antioxidant activity evaluation indices, and to gain an overview of the similarities and differences among the 14 barley varieties.


*Keywords:* Malting barley; Variety; Antioxidant activity; TPC; Pearson correlation analysis; PCA

## 1. Introduction

Flavor stability is not only an important quality attribute of beer, but also the most important factor in determining the shelf-life of packaged beer. The flavor stability of beer primarily depends on the oxygen content of the packaged beer. However, the individual stages of beer production such as malting, mashing, brewing and packing can influence the flavor stability (Narziss, 1986). Prolonging shelf-life by delaying flavor staling is one of the greatest challenges facing the brewer today. Many efforts have been made to avoid the oxygen pick-up during brewing process, the level of total packaged oxygen might be as low as 0.1 mg/l, but oxidative staling of beer is still noticeable (Bamforth, 2000). Therefore, attention is now increasingly shifting towards increasing the antioxidant activity of beer itself.

Antioxidants are generally thought to play a significant role in malting and brewing due to their ability to delay or prevent oxidation reactions and oxygen free radical reactions. Antioxidants such as sulfites, formaldehyde or ascorbate, can be added into the brewing process to improve beer flavor stability (Van Gheluwe, Valyi, & Dadic,

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1970). However, the effectiveness of some of these compounds is in doubt. Furthermore there has been a trend toward minimizing the use of additives in brewing because of consumer demand and stiffening regulations. As a result, attention needs to be focused on the protection of endogenous antioxidant in beer and in its raw materials, that is, barley and hop. About 80% of phenolic compounds present in beer are derived from barley malt, and the remaining come from hop (Goupy, Hugues, Boivin, & Amiot, 1999). Those phenolic compounds in malting barley including phenolic acids (benzoic and cinnamic acid derivatives), flavonoids, proanthocyanidins, tannins, and amino phenolic compounds (Bonoli, Marconi, & Caboni, 2004; Hernanz et al., 2001), all of which are known to inhibit nonenzymatic lipid peroxidation and widely recognized as having important antioxidant and antiradical properties. Therefore, optimizing of the natural antioxidants in malting barley and screening of malting barley variety with the highest level of radical scavengers seems more important to produce beers with high levels of antioxidant activity.

There have been some studies on the antioxidant activity and phenolic content of barley (Bonoli et al., 2004; Bonoli, Verardo, Marconi, & Caboni, 2004; Goupy et al., 1999; Maillard & Berset, 1995; Maillard, Soum, Boivin, & Berset, 1996). However, no attempt has been undertaken to study the antioxidant activity of Chinese malting barley. Moreover, it is difficult to compare data within the literature, owing to the different methods used by various researchers. Furthermore, antioxidant compounds present in barley extracts are complex, and their activities and mechanisms would largely depend on the composition and conditions of the test system. Many authors had stressed the need to perform more than one type of antioxidant activity measurement to evaluate the antioxidant activity of plant (Frankel & Meyer, 2000; Wong, Leong, & Koh, 2006). Our previous study demonstrated that extraction solvent had significant impacts on barley antioxidant activity evaluations, and 80% acetone (v/v) was recommended as antioxidants extraction solvent from malting barley for antioxidant activity evaluation (Zhao et al., 2006). Therefore, in present study, 80% acetone (v/v) was selected as antioxidant extraction solvent, and DPPH radical scavenging activity, ABTS radical cation scavenging activity, superoxide anion radical scavenging activity, metal chelating activity, and reducing power were used to evaluated the antioxidant activity of Chinese malting barley. Additionally, total phenolic contents were also determined in this study because phenolic compounds were considered to be a major group of compounds that contributed to the antioxidant activity of barley.

One objective of this study was to determine and compare the typical malting barley varieties from China for their DPPH radical scavenging activity, ABTS radical cation scavenging activity, superoxide anion radical scavenging activity, reducing power, metal chelating activity and total phenolic contents. The other objective was to establish the relationships between antioxidant activity measured by different methods and total phenolic content of malting barley. The last objective was to classify barley variety on the basis of antioxidant activity. Results from this preliminary study will provide a better understanding of the antioxidant activity of these barley varieties and allow the screening and discrimination of the malting barley variety with higher antioxidant activity to produce beer with higher flavor stability. This research was part of our continuous efforts to improve beer flavor stability by protecting endogenous antioxidants in beer and its raw materials.

## 2. Materials and methods

### 2.1. Malting barley samples

Fourteen typical malting barley varieties cultivated in China were obtained from the same agricultural plots in Wuxi. The barley varieties that were investigated included Gan4, Gan3 and Wupi1 (cultivated mainly in northwestern China, and released in 2002, 1999 and 2002, respectively), Ken2 and Ken3 (cultivated mainly in northeastern China, and released in 1996 and 1999), Humai8, Humai16, Gangpi1, Suyin1, Huaimai19, Linnong, Nongmai, KA4B and Gang2 (cultivated mainly in eastern China, and released in 1986, 1999, 1994, 1991, 2001, 1997, 1997, 1994 and 1990, respectively). All of them were winter barley of two-row regular-hulled and harvested in 2005. All barley samples were sealed in polyethylene bag, and stored in a refrigerator at 4 °C until ready for extraction.

### 2.2. Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchroman carboxylic acid (Trolox) were purchased from Sigma–Aldrich (Steinheim, Germany). Xanthine oxidase (XOD), gallic acid and Folin-Ciocalteu's phenol reagent were obtained from Sigma–Aldrich (St. Louis, MO). 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was obtained from Wako (Osaka, Japan). 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid monosodium salt (ferrozine) and nitrotetrazolium blue chloride (NBT) were purchased from Fluka (Buchs, Switzerland). All other chemicals and solvents were of the highest commercial grade and obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

### 2.3. Preparation of extracts from barley

Thin kernels (those passing through a 1.98-by-19.05-mm slotted sieve) of barley were removed prior to grinding. Then, barley was finely ground in a laboratory mill from Bühler-Miag (Braunschweig, Germany). Five grams (dry weight) of ground samples was sonicated (40 kHz, 120 W) for 1 h with 100 ml of 80% acetone (v/v) under nitrogen at 20 °C. After centrifugation (10 000*g*, 10 min),

the supernatant was collected. To avoid oxidation, extracts were stored in the dark at −20 °C and analyses were performed within 24 h.

### 2.4. DPPH radical scavenging activity

DPPH radical scavenging activity of the barley extract was determined according to the method of Gaulejac, Provost, and Vivas (1998) with minor changes. Every barley extract (0.1 ml) was added to 2.9 ml of $6 \times 10^{-5}$ mol/l methanolic solution of DPPH. The absorbance at 517 nm was measured after the solution had been allowed to stand in the dark for 60 min. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The Trolox calibration curve was plotted as a function of the percentage of DPPH radical scavenging activity. The final results were expressed as micromoles of Trolox equivalents (TE) per gram of dry barley (μmol TE/g db).

### 2.5. ABTS radical cation scavenging activity

The radical scavenging activity of the barley extract against ABTS radical cation was measured using the method of Re et al. (1999) with some modifications. ABTS was dissolved in water to a 7 mmol/l concentration. ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mmol/l potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The ABTS radical cation solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 30 °C. An aliquot of each barley extract (0.1 ml) was mixed with 2.9 ml of diluted ABTS radical cation solution. After reaction at 30 °C for 20 min, the absorbance at 734 nm was measured. The Trolox calibration curve was plotted as a function of the percentage of ABTS radical cation scavenging activity. The final results were expressed as micromoles of Trolox equivalents (TE) per gram of dry barley (μmol TE/g db).

### 2.6. Superoxide anion radical scavenging activity

Superoxide anion radical scavenging activity of the barley extract was performed using a hypoxanthine (HPX)/XOD system following a procedure described by Lee, Kim, Kim, and Jang (2002) with some modifications. Briefly, NBT, EDTA, HPX, and XOD solution were prepared with 0.05 mol/l phosphate buffer (pH 7.4), respectively. Each barley extract (0.1 ml) was added to the reaction solution containing 0.1 ml of 30 mmol/l EDTA, 0.1 ml of 3 mmol/l HPX, and 0.2 ml of 1.42 mmol/l NBT. After the solution had been preincubated at room temperature for 3 min, 0.1 ml of 0.75 U/ml XOD was added to the mixture, and the volume was brought up to 3 ml with 0.05 mol/l phosphate buffer (pH 7.4). Then, the solution was incubated at room temperature for 40 min, and the absorbance was measured at 560 nm. The superoxide anion radical scavenging activity was calculated by using the formula given below:

$$\text{Superoxide anion radical scavenging activity } (\%) = [1 - (S - S_B)/(C - C_B)] \times 100,$$

where $S$, $S_B$, $C$ and $C_B$ are the absorbances of the sample, the blank sample, the control, and the blank control, respectively.

### 2.7. Reducing power

The determination was carried out as described by Oktay, Gülçin, and Küfrevioğlu (2003). Briefly, 1 ml of barley extract was mixed with phosphate buffer (2.5 ml, 0.2 mol/l, pH 6.6) and $K_3Fe(CN)_6$ (2.5 ml, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 ml) of trichloroacetic acid solution (10%) was added to the mixture, which was then centrifuged at 10 000*g* for 10 min. The upper layer of solution (2.5 ml) was mixed with deionized water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The measurement was compared to a standard curve of prepared ascorbic acid (AA) solution, and the final results were expressed as micromoles of ascorbic acid equivalents (AAE) per gram of dry barley (μmol AAE/g db).

### 2.8. Metal chelating activity

The chelating activity of the barley extract for ferrous ions was measured following the ferrozine method with minor modifications (Dinis, Madeira, & Almeidam, 1994). The reaction mixture contained 0.5 ml of barley extract and 0.05 ml of $FeCl_2$ (2 mmol/l). After 5 min, the reaction was initiated by the addition of 5 mmol/l ferrozine (0.1 ml), and the total volume was adjusted to 3 ml with 80% acetone solution. Then, the mixture was shaken vigorously and incubated at room temperature for 10 min. Absorbance of the solution was measured at 562 nm. The EDTA calibration curve was plotted as a function of the percentage of metal chelating activity. The final results were expressed as micromoles of EDTA equivalents (EDTAE) per gram of dry barley (μmol EDTAE/g db).

### 2.9. Total phenolic content (TPC)

The TPC of the barley extract was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton & Rossi, 1965) with some modifications. Briefly, 0.5 ml of barley extract was mixed with 2.5 ml of 10-fold diluted Folin-Ciocalteu's phenol reagent and allowed to react for 5 min. Then, 2 ml of 7.5% $Na_2CO_3$ solution was added, and the final volume was made up to 10 ml with deionized water. After 1 h of reaction at room temperature, the absorbance at 760 nm was determined. The measurement

was compared to a standard curve of prepared gallic acid (GA) solution, and the total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry barley (mg GAE/g db).

### 2.10. Statistical analysis

All tests were conducted in triplicate. Data are reported as means ± SD. Analysis of variance and significant differences among means were tested by one-way ANOVA using SPSS software (version 13.0 for Windows, SPSS Inc., Chicago, IL). The Pearson correlation analysis and principal component analysis were also performed by SPSS 13.0 for determination of the correlations among means and visualizing the differences and similarities among varieties in term of antioxidant activities, respectively.

## 3. Results and discussion

### 3.1. DPPH radical scavenging activity

Relatively stable organic radical DPPH has been used widely for the determination of antioxidant activity of pure antioxidant compounds as well as different cereal extracts (Goupy et al., 1999; Yu & Zhou, 2004). For evaluation of antioxidant activity of barley, different malting barley varieties were measured and compared with their DPPH radical scavenging activities. Results are expressed as micromoles of Trolox equivalents per gram of dry barley (μmol TE/g db) and are shown in Fig. 1. All malting barley varieties exhibited strong DPPH radical scavenging activity at the test concentration. The values of DPPH radical scavenging activity for 14 barley samples ranged from 9.33 to 11.78 μmol TE/g db. Wupi1 barley showed the highest DPPH radical scavenging activity whereas Ken2 barley had the lowest activity. Moreover, Gan4 and Humai16 barley also showed relatively high DPPH radical scavenging activity. The significant differences in DPPH radical scavenging activity for different barley varieties suggested that variety might have significant influences on the antioxidant activity of malting barley. This finding was supported by the observation that three wheat varieties differed significantly in their antioxidant properties (Yu et al., 2002).

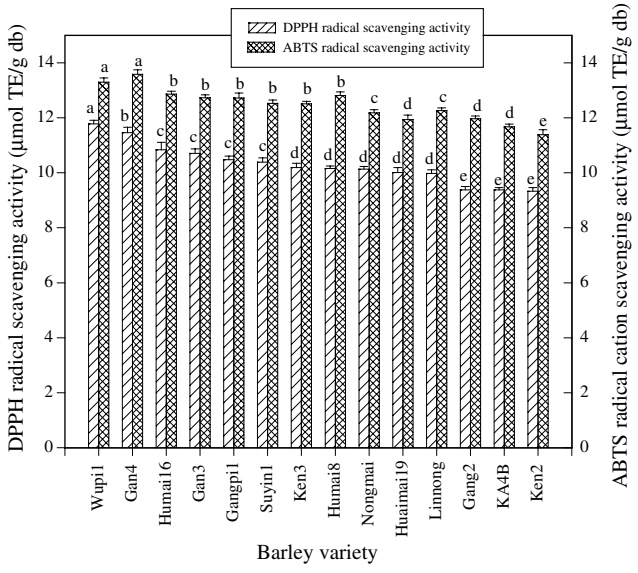


Fig. 1. DPPH radical scavenging activities (μmol of TE per g of db) and ABTS radical cation scavenging activities (μmol of TE per g of db) of different malting barley varieties. Vertical bars represent the standard deviation of each data point ($n = 3$). Locations for each barley variety and each assay marked by the different letter are significantly different ($P < 0.05$).

### 3.2. ABTS radical cation scavenging activity

ABTS radical cation is another common organic radical that has been used to determine the antioxidant activity of single compounds and other complex mixtures (Zhou, Laux, & Yu, 2004). Malting barley extracts were also measured and compared for their free radical scavenging activities against ABTS radical cation. Results are expressed as micromoles of Trolox equivalents per gram of dry barley (μmol TE/g db) and are presented in Fig. 1. All malting barley varieties used in this study showed significant ABTS radical cation scavenging activity. The values of ABTS radical cation scavenging activity for 14 barley samples ranged from 11.39 to 13.58 μmol TE/g db. The results obtained by ABTS method had some discrepancies with those of the DPPH method. Gan4 barley had the highest ABTS radical cation scavenging activity whereas the lowest was observed in Ken2 barley. Wupi1 barley also exhibited relatively higher ABTS radical cation scavenging activity than the other barley varieties. Moreover, TE values of barley extracts obtained by ABTS assay were consistently higher than those obtained by DPPH assay. Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, and Byrne (2006) also reported the same results when antioxidant activity of guava fruit was evaluated by both ABTS and DPPH assays. The different results from two methods might be due to different reaction kinetics between phenol and DPPH radical as well as ABTS radical cation over a similar range of concentrations (Campos & Lissi, 1996). This was also probably due to various phenolic compounds present in the extracts prepared from barley had different responses to various kinds of free radicals. All of these results suggested that the variety might have significant influences on the antioxidant activity of malting barley when the antioxidant activity was estimated by ABTS assay.

### 3.3. Superoxide anion radical scavenging activity

The superoxide anion radical scavenging activities of different barley varieties are shown in Fig. 2. All barley samples exhibited some superoxide anion radical scavenging activity under experimental conditions, the values of superoxide anion radical scavenging activity for 14 barley

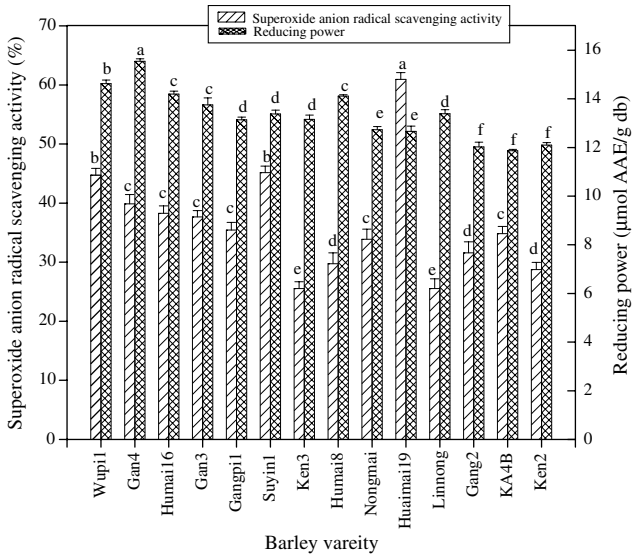


Fig. 2. Superoxide anion radical scavenging activities (percent) and reducing power (μmol of AAE per g of db) of different malting barley varieties. Vertical bars represent the standard deviation of each data point ($n = 3$). Locations for each barley variety and each assay marked by the different letter are significantly different ($P < 0.05$).

samples ranged from 25.54% to 60.93%. Huaimai19 barley had the strongest scavenging activity against superoxide anion radical among all barley varieties tested. The lowest activity was found in Linnong barley. These differed from the DPPH radical and the ABTS radical cation scavenging activity. It should be noted that superoxide anion is a major source of many free radicals, such as peroxyl, alkoxyl, hydroxyl, and nitric oxide, which are formed from superoxide anion through Fenton reaction and/or lipid oxidation or nitric oxidation (Ambrosio & Flaherty, 1992). Therefore, it is important for malting barley to have the ability to scavenge superoxide anion radicals, which can improve beer flavor stability by reducing the production of many free radicals with high reactive activity.

### 3.4. Reducing power

It has been reported that reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity (Oktay et al., 2003). Compounds with reducing power indicate that they are electron donors, and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Yen & Chen, 1995). For the measurements of the reducing power, the $Fe^{3+}$ to $Fe^{2+}$ reduction in the presence of barley extracts was investigated. As shown in Fig. 2, all malting barley samples with different varieties showed significant reducing power. The values of reducing power for 14 barley samples ranged from 11.87 to 15.54 μmol AAE/g db. The trend for reducing power of the 14 barley samples was similar to their ABTS radical cation scavenging activities, but dissimilar to their DPPH and superoxide anion radical scavenging activities, when a comparison among Figs. 1 and 2 was made. The same as the results obtained from the ABTS method, Gan4 barley showed the strongest reducing power, followed by Wupi1 and Humai16 barley, respectively. The lowest reducing power was found in KA4B barley. These observations suggested that variety might have some influences on the reducing power of malting barley.

### 3.5. Metal chelating activity

It has been well recognized that transition metal ions such as those of iron and copper are important catalysts for the generation of the first few free radicals to initiate the radical chain reaction or the radical mediates lipid peroxidation (Nawar, 1996). Chelating agents may inhibit radical generations by stabilizing transition metals, consequently reducing free radical damage. In addition, some phenolic compounds exhibit antioxidant activity through the chelation of metal ions. To better estimate the potential antioxidant activities of the different barley varieties, chelating activity of each barley sample was evaluated against $Fe^{2+}$. As showed in Fig. 3, all barley varieties exhibited metal chelating activities at test concentration. The values of the metal chelating activity ranged from 1.15 to 2.06 μmol EDTAE/g db. Ken3 barley showed the highest metal chelating activity whereas Linnong barley had the lowest activity among the selected malting barley varieties. There were no significant differences in metal chelating activity among Huaimai19, Suyin1 and Linnong barley varieties. These data also suggested that variety might have some influences on the metal chelating activity of

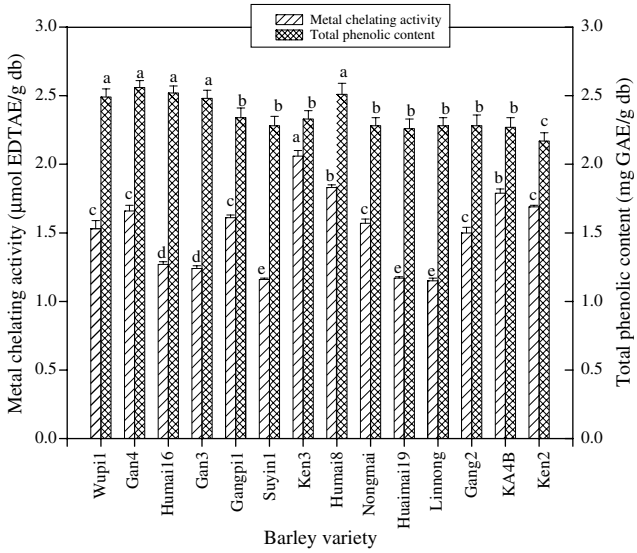


Fig. 3. Metal chelating activities (μmol of EDTAE per g of db) and total phenolic contents (mg of GAE per g of db) of different malting barley varieties. The concentrations of all barley extracts are on a same dry weight basis. Vertical bars represent the standard deviation of each data point ($n = 3$). Locations for each barley variety and each assay marked by the different letter are significantly different ($P < 0.05$).

malting barley. The results were also significantly different from those obtained by the mentioned above four methods, when comparing metal chelating activity method with the DPPH, ABTS, superoxide anion, and reducing power methods, respectively. In the present study, the different observations from these methods for evaluating antioxidant activity of malting barley might be due to different mechanisms of reaction. Moreover, antioxidant properties of single compounds within a group could vary remarkably, so the same levels of phenolics was not necessarily correspond to the same antioxidant responses. Bamforth, Muller, and Walker (1993) indicated that oxygen could be activated by transition metal ions, these active oxygen participated the beer oxidation and resulted in the occurrence of off-flavor in beer. Therefore, screening of barley variety with higher metal chelating activity was important for improving the beer flavor stability.

### 3.6. Total phenolic content

Phenolic compounds were considered as a major group of compounds that contributed to the antioxidant activity of cereal (Zieliński & Kozłowska, 2000). To better understand the relationship between the antioxidant activity and TPC, the TPC of the barley extracts were determined using the Folin-Ciocalteu phenol reagent. The results are expressed as milligrams of gallic acid equivalents per gram of dry barley and are presented in Fig. 3. Significant amounts of total phenolics were detected in all barley varieties. The TPC values of the different malting barley varieties for 14 barley samples ranged from 2.17 to 2.56 mg GAE/g db. The results were higher than those reported by Maillard et al. (1996). This might be due to the differences of the barley varieties and the extraction methods used in both studies. For TPC measured, Gan4 barley exhibited the highest amount of TPC whereas the lowest TPC was observed in Ken2 barley. Generally, barley with high amount of phenolics also showed high antioxidant activity. For example, Gan4, Wupi1, Humai16 showed high DPPH, ABTS cation radical scavenging activities and reducing power also had high TPC. Moreover, Humai8 barley had relatively high TPC and metal chelating activity, but exhibited relatively low superoxide anion and DPPH radical scavenging activities. In this study, although all malting barley varieties showed high TPC, the differences in TPC for most malting barley varieties selected were not significant ($P > 0.05$). It can be explained by the fact that Folin-Ciocalteu assay showed specific not only to just phenolics but to any other substances that could be oxidized by the Folin-Ciocalteu phenol reagent (Singleton, Orthofer, & Lamuela-Raventos, 1999; Zieliński & Kozłowska, 2000), the sensitivity of Folin-Ciocalteu method was not so good as other method used to determination of antioxidant activity of phenolic compounds. All of these data suggested that variety might also have influences on TPC of malting barley, although the specificity of this method was considered to be poor.

## 4. Pearson correlation analysis

### 4.1. Correlations of DPPH radical scavenging activity, ABTS radical cation scavenging activity, superoxide anion radical scavenging activity, reducing power and metal chelating activity with TPC

Phenolic compounds have been reported to be responsible for the antioxidant activities of grain, vegetables, and other botanical materials (Zieliński & Kozłowska, 2000). DPPH radical scavenging activity, ABTS radical cation scavenging activity, superoxide anion radical scavenging activity, reducing power, and metal chelating activity all have been used to evaluate antioxidant activity of cereal. Therefore, to make further understanding of the interrelationship between malting barley antioxidant activity and their phenolic compounds contents, 14 extracts prepared from different barley varieties were used to analyze the correlations between antioxidant activity evaluation indices and TPC, and the results were presented in Table 1. The highest correlation coefficient was found between the TPC and the ABTS radical cation scavenging activity (0.892, $P < 0.01$), and the lowest one between the TPC and the metal chelating activity (0.041, $P > 0.05$). Moreover, the TPC gave the strong positive correlations with DPPH radical scavenging activity and reducing power ($P < 0.01$), but a poor correlation with superoxide anion radical scavenging activity. Zhou et al. (2004) reported good correlations for the wheat grain and fractions when DPPH radical scavenging activity and ABTS radical cation scavenging activity were compared with TPC. Satisfactory correlations of DPPH radical scavenging activity and reducing power with TPC were also found in beers and wines (Lugasi & Hóvári, 2003). All of these data indicated that phenolic compounds in malting barley were the major contributors of ABTS radical cation scavenging activity, DPPH radical scavenging activity and reducing power. However, the poor correlations of superoxide anion radical scavenging activity and metal chelating activity with TPC in this study suggested that phenolic compounds in malting barley might be the weak chelators of ferrous ions and scavengers of superoxide anion radicals. Moreover, the

Table 1
Correlation coefficients between assays[a]

| | DSA | ASA | SSA | RP | MCA | TPC |
|---|---|---|---|---|---|---|
| DSA | 1 | 0.929** | 0.347 | 0.905** | −0.139 | 0.799** |
| ASA | – | 1 | 0.155 | 0.948** | −0.009 | 0.892** |
| SSA | – | – | 1 | 0.160 | −0.513 | 0.098 |
| RP | – | – | – | 1 | −0.075 | 0.886** |
| MCA | – | – | – | – | 1 | 0.041 |
| TPC | – | – | – | – | – | 1 |

[a] DSA, DPPH radical scavenging activity; ASA, ABTS radical cation scavenging activity; SSA, superoxide anion radical scavenging activity; RP, reducing power; MCA, metal chelating activity; TPC, total phenolic content.

** Significant at $P < 0.01$.

metal chelating activity of malting barley might partly depend on the functional groups and content of individual phenolic compounds in barley extracts (Wong et al., 2006). Indeed, there were numerous flavonoids, such as the prenylated, nonprenylated chalcones and flavanones found in beer and hops, that did not chelate copper ions in vitro (Miranda et al., 2000).

### 4.2. Correlations between methods used to evaluate antioxidant activity of malting barley

In general, the different methods used to determine the antioxidant activity are based on different reaction mechanisms, thus they often give different results. Moreover, the response of phenolics for antioxidant activity estimated by various methods also depends on their chemical structures. Therefore, to correlate the results obtained by the five methods, a regression analysis also was carried out. Table 1 showed that the DPPH radical scavenging activity, ABTS radical cation scavenging activity and reducing power were well positively correlated with each other ($P < 0.01$), but all of them exhibited weak correlations with superoxide anion radical scavenging activity and metal chelating activity. This suggested that the compounds which could scavenge DPPH radical in the malting barley were also able to scavenge ABTS radical cation and to reduce ferric ions. However, not all of these compounds were the chelators of ferrous ions and scavengers of superoxide anion radicals. Surprisingly, metal chelating activity showed negative correlations with all other antioxidant activity evaluation indices in the present study, indicating that malting barley with higher metal chelating activity might have lower superoxide anion radical scavenging activity and other antioxidant activities. Wong et al. (2006) reported the negative correlations between DPPH radical scavenging activity, ferric ion reducing ability, and cupric ion chelating activity in 25 plant extracts. All of these results suggested that antioxidant activity of malting barley determined by one method might be partly able to reflect the result determined using another assay. For example, the malting barley with higher metal chelating activity might have lower superoxide anion radical scavenging activity.

## 5. Principal component analysis

### 5.1. Principal component analysis of antioxidant activity evaluation indices and TPC

Principal component analysis was performed to understand the interrelationships among the measured antioxidant activity evaluation indices and TPC. The results of PCA are shown in Fig. 4. Two principal components, explaining the 87.40% of the total data variance, have been chosen on the basis of their eigenvalues (>1). The loadings in PCA loading plot express not only how well the principal components correlate with the original variables, but also correlations between antioxidant activity evaluation indices and TPC. The first principal component (PC1) correlated well with DPPH radical scavenging activity, ABTS radical cation scavenging activity, reducing power and TPC having loadings 0.959, 0.974, 0.969 and 0.917, respectively. The second principal component (PC2) was related to superoxide anion radical scavenging activity and metal chelating activity with loadings of −0.824 and 0.865, respectively. Fig. 4 also showed good correlations between DPPH radical scavenging activity, ABTS radical cation scavenging activity, reducing power and TPC. Such correlations among the antioxidant activities evaluation indices and TPC are similar to the conclusion draw from the Pearson correlation analysis. Moreover, DPPH radical scavenging activity, ABTS radical cation scavenging activity and reducing power were found to be similarly loaded on PC1, which indicated the three properties are closely related to antioxidant activity. TPC also had high loading

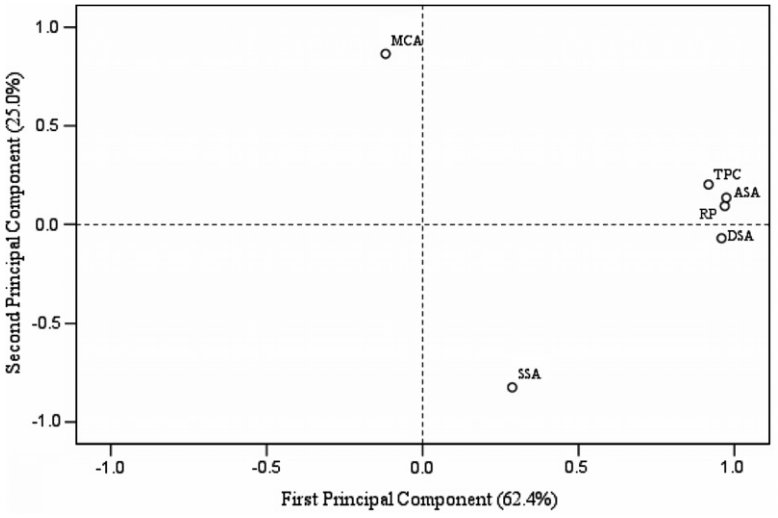


Fig. 4. Principal component analysis loading plot of antioxidant activity and TPC from different barley varieties.

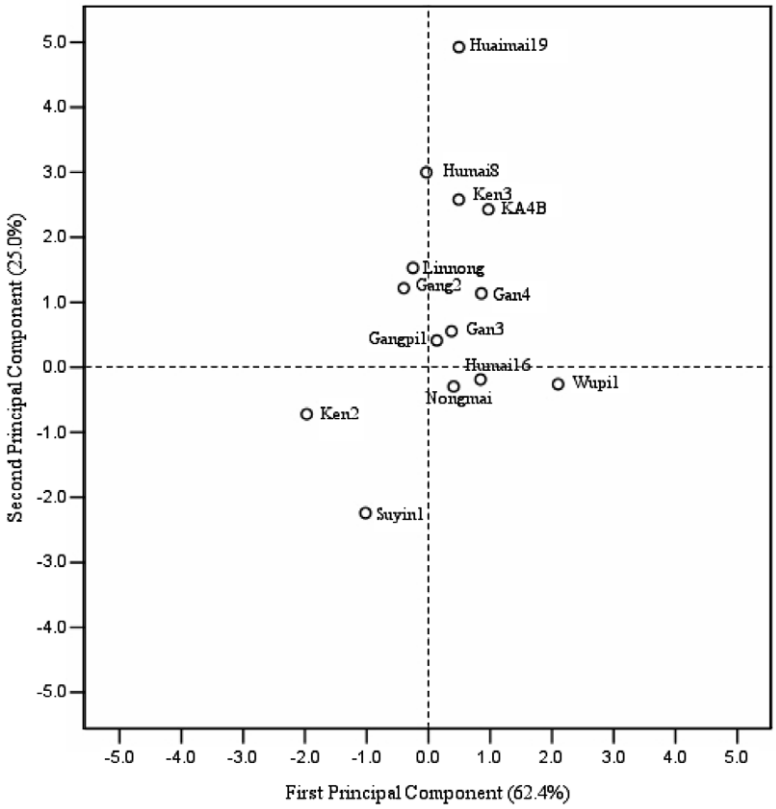


Fig. 5. Principal component analysis score plot of different barley varieties.

on PC1, which suggests phenolic compounds are good antioxidants. Along PC2, superoxide anion radical scavenging activity and metal chelating activity get high loadings, while TPC has low loading, which also indicated that phenolic compounds might not be good superoxide anion radical scavenger and metal chelators.

### 5.2. Principal component analysis of 14 barley varieties

Principal component analysis was also performed to gain an overview of the similarities and differences among the 14 barley varieties. The results of PCA are shown in Fig. 5. The sample sites on the PCA score plot are shown in Fig. 5 with most of the sites located near the origin. The results reflected antioxidant activity in most of the barley variety, as the origin represents the mean antioxidant activity of all samples. The distance between the locations of any two barley samples on the score plot is directly proportional to the degree of differences or similarity between them. PCA showed that Huaimai19 and Suyin1 barley located on the opposite side of the PC2, the superoxide anion radical scavenging activity and metal chelating activity were to be the major responsible for the difference of the two barley samples. This was because Huaimai19 barley had the highest superoxide anion radical scavenging activity, while Suyin1 barley exhibited the lowest metal chelating activity. As far as the PC1 concerned, Wupi1 barley had the largest positive score, while Ken2 barley had the largest negative score, which also highlighted their significant differences in antioxidant activity. DPPH radical scavenging activity seemed to play a significant role on the distinguishing of these two barley samples, as Wupi1 barley had the highest DPPH radical scavenging activity, while Ken2 barley exhibited the lowest activity. Therefore, PCA could be helpful to provide valuable information on classification and discrimination of barley variety and on relationships between antioxidant activity evaluation indices and TPC of barley.

## 6. Conclusions

In conclusion, the present study determined antioxidant activities and total phenolic contents of 14 typical malting barley varieties from China. Results showed that malting barley variety had influences on both the antioxidant activity and the TPC. Gan4 and Wupi1 barley exhibited the highest DPPH radical scavenging activity, ABTS radical cation scavenging activity and reducing power. Moreover, Gan4 and Humai16 barley showed the highest TPC. The highest superoxide anion radical scavenging activity and metal chelating activity were found in Huaimai19 and Ken3 barley, respectively. Some correlations between antioxidant activity evaluation indices and TPC were revealed by both Pearson correlation analysis and PCA. Moreover, PCA also discriminated some barley variety on the basis of their antioxidant activities and TPC. These data demonstrated the

importance of evaluating antioxidant activity of malting barley and screening malting barley variety with higher antioxidant activity. Our research also provided important information that might lead to improve beer flavor stability if well designed and performed. Further research is needed to characterize the major antioxidant phenolic compounds present in malting barley by HPLC and demonstrate their evolution during brewing process.

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