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Phenolic profiles and antioxidant activities of commercial beers

Haifeng Zhao [a,*], Wenfen Chen [a], Jian Lu [b], Mouming Zhao [a]

[a] College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, PR China
[b] School of Biotechnology, Jiangnan University, Wuxi 214122, PR China

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ABSTRACT

The phenolic profiles and corresponding antioxidant activities of 34 commercial beers in Chinese markets were evaluated. Results found remarkable variations in total and individual phenolic contents as well as antioxidant activity across beer brands. Gallic and ferulic acids were the dominant phenolic compounds identified in the tested beer samples and both of them accounted for >50% of the total phenolic compounds. Results from Pearson correlation analysis suggested that five antioxidant activity assays positively correlated well ($p < 0.01$) with each other and showed significant positive correlations ($p < 0.05$) with (+)-catechin, protocatechuic, and caffeic acids contents. Stepwise linear regression further demonstrated that different phenolic components responsible for beer antioxidant activity were dependent on the method used, and that ferulic acid, syringic acid, (+)-catechin, caffeic acid, protocatechuic acid and (−)-epicatechin together made 55.0–88.1% of contribution to the antioxidant activity of beer.

1. Introduction

Flavour stability, one of the important characteristics in beer, is challenging brewers, and is one of the most important factors in determining the shelf-life of packaged beer. It has been widely accepted that the main factor responsible for beer flavour instability is oxidation during brewing, although there is no agreement on the main precursor of staling substances (Narziss, Miedaner, Graf, Eichhorn, & Lustig, 1993). As a consequence, a number of efforts have been made to avoid oxygen pick-up during brewing and packaging (Narziss et al., 1993). The level of total packaged oxygen might be as low as 0.1 mg/l with modern filling equipment, but oxidative staling of beer remains difficult to control (Bamforth, 2000). Researchers are seeking alternative ways to solve this problem by increasing the endogenous antioxidant activity of beer itself. Although both natural and synthetic antioxidants such as flavonoids, sulfites and ascorbate could be used in the brewing industry to improve beer flavour stability, there has been a trend towards minimising the use of additives in brewing because of consumer demand and stiffening regulations.

There are many endogenous antioxidants such as phenolic compounds, Maillard reaction products, and sulfite present in beer (Vanderhaegen, Neven, Verachtert, & Derdelinckx, 2006). Among these antioxidants, phenolic compounds are of particular interest to brewers because they play a key role in the brewing process by delaying, retarding, or preventing oxidation processes (Guido, Boivin, Benismail, Gonçalves, & Barros, 2005). Phenolic compounds identified in beer include phenolic acids, flavonoids, proanthocyanidins, tannins, and amino phenolic compounds (Gorinstein, Caspi, Zemser, & Trakhtenberg, 2000; Montanari, Perretti, Natella, Guidi, & Fantozzi, 1999), all of which have been reported to possess antioxidant and antiradical properties as well as other biological effects (Brand-Williams, Cuvelier, & Berset, 1995; Gaulejac, Provost, & Vivas, 1998). Our previous study demonstrated that phenolic profiles and antioxidant activity in barley varied considerably across varieties and changed significantly during malting (Lu et al., 2007; Zhao et al., 2008). Thus, the differences of raw materials and brewing process would lead to significant differences in phenolic profiles and antioxidant activities of beer. Moreover, there is controversy concerning the relevance of phenolic compounds and antioxidant activity of beer (Vanderhaegen et al., 2006). Therefore, investigating the phenolic profiles and antioxidant activities of commercial beers, clarifying the relationships between them and quantifying the contribution of phenolic compounds to beer antioxidant activity would be helpful to better understand the beer flavour stability.

There have been several studies on the antioxidant activity and phenolic content of beer (Lugasi, 2003; Lugasi & Hóvári, 2003; Montanari et al., 1999). However, these researches focused on the relationship between antioxidant activity and total phenolic contents, limited data are available on phenolic profiles and their contribution to antioxidant activity for commercial beers (Kaneda, Kobayashi, Furusho, Sahara, & Koshino, 1995). Moreover, it is difficult to compare data within the literature due to the lack of agreement on the appropriate method for analysing phenolic compounds and antioxidant activity evaluation. As a consequence, information in the literature on the levels and species of phenolic

* Corresponding author. Tel.: +86 20 22236089; fax: +86 20 87113914.
E-mail address: hfzhao@scut.edu.cn (H. Zhao).

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compounds is not enough and also contradictory. Beer is a complex mixture of natural compounds, activities and mechanisms of antioxidants present in beer would largely depend on the composition and conditions of the test system. Different antioxidant activity evaluation methods based on different reaction mechanisms might give various evaluation results (Frankel & Meyer, 2000; Gaulejac et al., 1998). To the best of our knowledge, there were no detailed investigations in terms of phenolic profiles and antioxidant activities of commercial beers. The correlations among different antioxidant activity evaluation methods and total and individual phenolic contents of beers have not been fully elucidated as well. Furthermore, the contribution of phenolic compounds, especially individual phenolic compounds to beer antioxidant activity measured by different methods has not been quantified successfully due to the complexity of phenolic compounds in beer, which was of extreme importance for practical beer production.

Therefore, the first objective of this study was to investigate and compare phenolic profiles and antioxidant activities of commercial beers. The second objective was to analyse the correlations among individual and total phenolic contents, and beer antioxidant activity evaluation indices. The last objective was to quantify the contribution of individual phenolic compounds to beer antioxidant activity.

2. Materials and methods

2.1. Beer samples

Three bottles of 34 beer samples including 27 domestic and 7 imported beers were purchased from local markets (Guangzhou, Guangdong province). The detailed characteristics of these beers were presented in Table 1. All samples were stored in a refrigerator at 4 °C and analysed within 48 h.

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), vanillic acid, (+)-catechin, (−)-epicatechin, protocatechuic acid, syringic acid, *p*-coumaric acid, trans-ferulic acid, gallic acid, caffeic 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. Total phenolic content (TPC) determination

The TPC of beer was determined according to the Folin–Ciocalteu spectrophotometric method (Singleton & Rossi, 1965) with some modifications. Briefly, 0.5 ml of diluted beer sample 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 deionised water. After 1 h of reaction at room temperature, the absorbance at 760 nm was determined. The measurement was compared to a calibration line of prepared gallic acid (GA) solution,

Table 1
Characteristics of 34 commercial beers.

No.	Brand	Type	Original gravity (°P)	Alcohol content (%, v/v)	Raw materials	Country of origin
1	Peng Cheng	Lager	8.0	3.1	Malt, rice, hop	China
2	Tian Mu Hu	Lager	8.8	3.6	Malt, hop	China
3	Jin Ling	Lager	10.0	3.3	Malt, rice, hop	China
4	Siwo	Lager	8.0	3.1	Malt, rice, starch, hop	China
5	Snow	Lager	9.0	3.6	Malt, rice, syrup, hop	China
6	Tai Hu Shui	Lager	9.5	3.6	Malt, rice, syrup, hop	China
7	Cheerday	Lager	8.0	2.8	Malt, rice, hop	China
8	BBOSS	Lager	9.0	3.6	Malt, rice, hop	China
9	Yan Jing	Lager	8.0	3.1	Malt, rice, hop	China
10	Tiger	Lager	11.8	4.7	Malt, rice, hop	China
11	Tsing Tao	Lager	10.0	4.0	Malt, rice, hop	China
12	Budweiser	Lager	10.5	4.3	Malt, rice, hop	China
13	Blue Lion	Lager	11.0	4.0	Malt, rice, hop	China
14	Asahi	Lager	11.2	4.3	Malt, rice, corn, hop	China
15	Harbin	Lager	10.0	3.6	Malt, rice, hop	China
16	Suntory	Lager	10.0	3.6	Malt, rice, hop	China
17	King Benefit	Lager	11.0	3.7	Malt, rice, hop	China
18	Heineken	Lager	11.4	4.7	Malt, hop	China
19	Sanmiguel	Lager	11.0	4.8	Malt, rice, hop	China
20	Reeb	Lager	10.0	4.0	Malt, rice, hop	China
21	Pearl River	Lager	8.0	3.1	Malt, rice, hop	China
22	Beck's	Lager	10.1	4.1	Malt, hop	China
23	Blue Ribbon	Lager	10.0	3.6	Malt, rice, hop	China
24	Carlsberg	Lager	11.1	4.3	Malt, hop	China
25	Coors Light	Lager	8.1	3.9	Malt, hop	China
26	Sedrin	Lager	11.0	4.0	Malt, rice, hop	China
27	Huang He	Lager	10.0	3.6	Malt, rice, hop	China
28	Blue Girl	Lager	11.5	5.0	Malt, millet, hop	South Korea
29	Grolsch	Lager	11.5	5.0	Malt, hop	Holland
30	Corona	Lager	11.0	4.6	Malt, hop	Mexico
31	Duvel	Lager	16.9	8.5	Malt, hop	Belgium
32	Leffe	Lager	12.0	6.6	Malt, hop	Belgium
33	Samuel	Lager	13.1	4.9	Malt, hop	USA
34	Bitburger	Lager	11.3	4.8	Malt, hop	Germany

and the results were expressed as milligrams of gallic acid equivalents (GAE) per liter of beer (mg GAE/l).

2.4. Pretreatment of beer samples and extraction of phenolic compounds

For each beer sample, beers of three bottles were combined, homogenised and degassed with intensive stirring for 30 min at room temperature under protection of nitrogen gas prior to analysis. For HPLC analysis, 20 g of NaCl was added in 50 ml of the degassed beer sample. The resulting sample was extracted three times by shaking vigorously with ethyl acetate (3 × 50 ml), phase separation being assisted where necessary by centrifugation (10,000*g* for 10 min). The pooled ethyl acetate extracts were then evaporated to dryness under reduced pressure at 35 °C. Each residue was redissolved in 2 ml of methanol (HPLC grade) and then filtered through a 0.45 μm membrane (Sartorius, Goettingen, Germany). The filtrates were analysed by HPLC.

2.5. Determination of individual phenolic compounds in beer

HPLC analyses were performed using a Waters 1525 pump (Waters, Milford, MA) equipped with a Waters 717 plus autosampler coupled with a Waters 2478 dual λ absorbance detector at 280 and 254 nm according to an established protocol (Zhao et al., 2006). Separation was performed with a Symmetry C18 (5 μm, 3.9 mm × 150 mm) column (Waters, Milford, MA) at room temperature. Elution was carried out by using a gradient procedure with a mobile phase containing solvent A (0.1% acetic acid in water) and solvent B (0.1% acetic acid in methanol) as follows: 0 min, 5% B; 15 min, 20% B; 35 min, 40% B; 42 min, 65% B; 50 min, 80% B; 52 min, 5% B; 60 min, 5% B. Runtime was 60 min, the solvent flow rate was 0.8 ml/min, and the injection volume was 10 μl. Phenolic compounds were identified by comparison of their retention times and spectral parameters with those of standards. The concentrations of individual phenolic compounds in beer were calculated using calibration lines. Results were expressed as milligrams per liter of beer (mg/l).

2.6. DPPH radical scavenging activity

DPPH radical scavenging activity of beer was determined according to the method of Brand-Williams et al. (1995) with minor changes. Every diluted beer sample (0.1 ml) was added to 2.9 ml of 6×10^{-5} mol/l DPPH solution (dissolved in 50% methanol solution). The absorbance at 517 nm was measured after the solution had been allowed to stand in the dark for 60 min. The Trolox calibration curve was plotted as a function of the percentage of DPPH radical scavenging activity. The final results were expressed as millimoles of Trolox equivalents (TE) per liter of beer (mmol TE/l).

2.7. ABTS radical cation scavenging activity

The radical scavenging activity of beer against the ABTS radical cation was measured using the method of Re et al. (1999) with some modifications. ABTS was dissolved in water to a concentration of 7 mmol/l. The 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 a 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 diluted beer sample (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 millimoles of Trolox equivalents (TE) per liter of beer (mmol TE/l).

2.8. Superoxide anion radical scavenging activity

Superoxide anion radical scavenging activity of beer was performed using a HPX (hypoxanthine)/XOD system following a procedure described by Takao, Kitatani, Watanabe, Yagi, and Sakata (1994) with some modifications. Briefly, NBT, EDTA, XOD and HPX were prepared with 0.05 mol/l phosphate buffer (pH 7.4), respectively. Each beer sample (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.

2.9. Reducing power

The determination was carried out as described by Zhao et al. (2006). Briefly, 1 ml of diluted beer sample 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 (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 deionised water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. The measurement was compared to a calibration line of prepared ascorbic acid (AA) solution, and the final results were expressed as millimoles of ascorbic acid equivalents (AAE) per liter of beer (mmol AAE/l).

2.10. Metal chelating activity

The chelating activity of the beer for ferrous ions was measured following the ferrozine method with minor modifications (Dinis, Madeira, & Almeidam, 1994). The reaction mixture contained 0.5 ml of beer 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 deionised water. 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 liter of beer (μmol EDTAE/l).

2.11. Statistical analysis

All of experiments were carried out in triplicate. Data were reported as means ± standard deviation (SD) for triplicate determinations. Analysis of variance and significant difference tests were conducted to identify differences among means by one-way ANOVA using SPSS software (version 13.0 for Windows, SPSS Inc., Chicago, IL). Moreover, correlation coefficients were calculated using Pearson product moment correlation. Stepwise regression

was used to evaluate how much variability could be explained by each independent variable (phenolic compounds) for the dependent variable (antioxidant activity) by Statistical Analysis System (version 9.1, SAS Institute, Cary, NC).

3. Results and discussion

3.1. Total phenolic content

Phenolic compounds play critical roles both in flavour stability and colloidal stability of beer. Phenolic compounds are also generally considered as one of very important antioxidant sources in beer (Vanderhaegen et al., 2006). Therefore, TPC of 34 beer samples studied were examined by the Folin–Ciocalteu assay and the results are presented in Fig. 1. Thirty-four beer samples investigated exhibited considerable differences in their TPC values, varying from 152.01 mg GAE/l for Reeb beer to 339.12 mg GAE/l for Carlsberg beer. Grolsch, Heineken and Bitburger beers also had relatively higher TPC (>290 mg GAE/l). The results were lower than the data from Lugasi (2003) but higher than those from Shahidi and Naczk (1995) (270–600 mg/l and 60–100 mg/l, respectively). This might be due to the differences between beer samples and TPC evaluation methods used in these studies. Moreover, it is important to mention that the Folin–Ciocalteu method, although widely used for beverages or plant extracts, is not specific for phenolic compounds and does suffer interference from other compounds (Dávalos, Gómez-Cordovés, & Bartolomé, 2003). Thus the results from Folin–Ciocalteu method for beer TPC evaluation might not only reflect the levels of phenolic compounds, but also the contents of Maillard reaction products, sulfite or other substance with reducing activity. Therefore, separation and identification of individual phenolic compounds are of importance to reveal the real differences in phenolic profiles present in beers.

3.2. Individual phenolic compounds

To remedy the limitation of Folin–Ciocalteu method for phenolic content determination, nine phenolic compounds including gallic acid, protocatechuic acid, (+)-catechin, vanillic acid, caffeic acid, syringic acid, (−)-epicatechin, *p*-coumaric acid and ferulic acid were identified among different beer samples and the results are summarised in Table 2. All these phenolic compounds mentioned above with different concentrations in diverse beer samples were confirmed in previous studies (Gorinstein et al., 2000; Montanari et al., 1999). Table 2 shows that gallic and ferulic acids are the most phenolic constitutes identified in beer and representing >50% of the total content of individual phenolic compounds present in all beer samples. Moreover, the tested beers in the current study exhibited relatively high levels of (+)-catechin, vanillic and *p*-coumaric acids, while the values were much lower for (−)-epicatechin and syringic acid. Considerable variations were found in phenolic profiles among different beer samples. In all beer samples tested, the levels of gallic acid, protocatechuic acid, (+)-catechin, vanillic acid, caffeic acid, syringic acid, (−)-epicatechin, *p*-coumaric acid and ferulic acid were in the ranges of 1.81–10.39 mg/l, 0.02–1.30 mg/l, 0.03–4.00 mg/l, 0.22–2.98 mg/l, 0.08–1.22 mg/l, 0.06–0.99 mg/l, 0.02–0.73 mg/l, 0.01–1.12 mg/l and 0.51–3.13 mg/l, respectively. The results agreed with the reports of Gorinstein et al. (2000). Blue Lion, Duvel, Harbin, Jin Ling and Blue girl beers exhibited the highest levels of gallic acid, (+)-catechin, vanillic acid, *p*-coumaric acid and ferulic acid, respectively. While the highest levels of protocatechuic acid and caffeic acid, syringic acid and (−)-epicatechin were found in Samuel and Leffe beers, respectively. It should be noted that some individual phenolic compounds in certain beers were not detected due to their low contents. The sum of individual phenolic contents (SPC) in different beer samples varied considerably, ranging from 4.47 mg/l (Reeb beer) to 15.50 mg/l (Blue Lion beer). The great variations in phenolic profiles for different beers might be due to the differences in raw materials, brewing process and original gravity. Moreover, significant differences in total phenolic content determined by Folin–Ciocalteu and HPLC methods were found in the present study, which also verified the non-specific of Folin–Ciocalteu method. Therefore, the measurement of phenolic profiles by HPLC method could give more information about their chemical characteristics and antioxidant activities.

3.3. DPPH radical scavenging activity

The DPPH radical scavenging activities of 34 beer samples are shown in Table 3. All beer samples selected exhibited strong DPPH radical scavenging activities at the test concentration. The values of DPPH radical scavenging activities ranged from 0.24 to 1.35 mmol TE/l. Samuel beer showed the highest DPPH radical scavenging activity, whereas Peng Cheng beer had the lowest activity. The results were in agreement with the report by Lugasi (2003) that beers exhibited significant hydrogen-donating ability. All results discussed above suggest that raw material and brewing

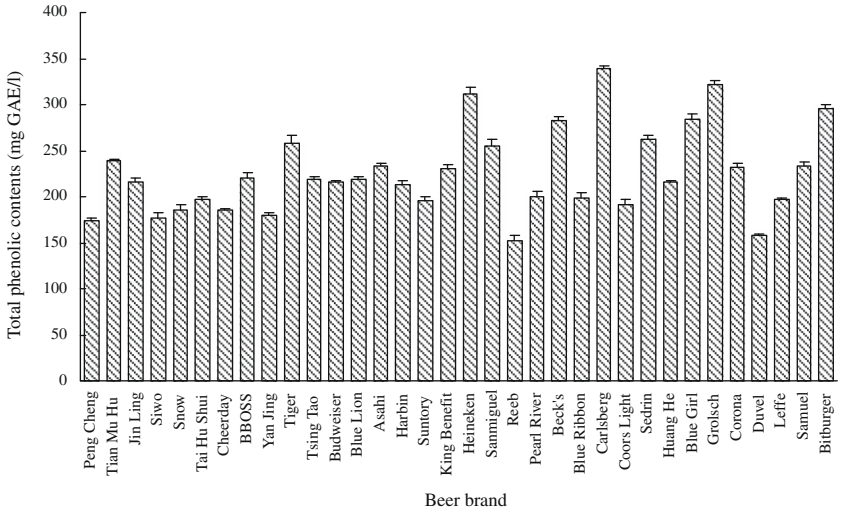

Fig. 1. Total phenolic contents (mg GAE/l) of 34 commercial beers. Vertical bars represent the standard deviation of each data point ($n = 3$).

Table 2
Individual phenolic contents in 34 commercial beers.[a]

No.	Brand	Gallic acid	Protocatechuic acid	(+)-Catechin	Vanillic acid	Caffeic acid	Syringic acid	(−)-epicatechin	*p*-Coumaic acid	Ferulic acid	SPC
1	Peng Cheng	3.64 ± 0.05	0.20 ± 0.01	0.43 ± 0.06	1.89 ± 0.10	0.27 ± 0.03	0.07 ± 0.02	0.28 ± 0.03	0.50 ± 0.05	1.32 ± 0.07	8.60
2	Tian Mu Hu	3.59 ± 0.07	0.19 ± 0.02	1.17 ± 0.08	0.68 ± 0.03	0.31 ± 0.02	0.07 ± 0.02	0.08 ± 0.02	0.72 ± 0.02	1.85 ± 0.05	8.66
3	Jin Ling	6.35 ± 0.10	0.02 ± 0.02	0.57 ± 0.03	0.44 ± 0.05	0.24 ± 0.03	0.08 ± 0.02	0.03 ± 0.01	1.12 ± 0.05	1.60 ± 0.05	10.45
4	Siwo	8.63 ± 0.15	0.04 ± 0.01	1.56 ± 0.09	0.35 ± 0.05	0.22 ± 0.04	0.06 ± 0.01	0.41 ± ± 0.01	0.56 ± 0.06	1.35 ± 0.06	13.18
5	Snow	4.93 ± 0.03	0.19 ± 0.05	1.44 ± 0.10	0.35 ± 0.06	0.20 ± 0.02	0.12 ± 0.03	0.34 ± 0.02	0.88 ± 0.04	1.83 ± 0.04	10.28
6	Tai Hu Shui	3.65 ± 0.05	0.16 ± 0.02	1.62 ± 0.03	0.53 ± 0.07	0.19 ± 0.01	0.15 ± 0.02	0.29 ± 0.03	0.88 ± 0.02	2.55 ± 0.06	10.02
7	Cheerday	5.74 ± 0.08	0.03 ± 0.01	1.27 ± 0.06	0.48 ± 0.07	0.28 ± 0.02	0.10 ± 0.04	0.38 ± 0.01	0.58 ± 0.03	1.98 ± 0.04	10.84
8	BBOSS	5.14 ± 0.04	0.27 ± 0.02	1.47 ± 0.04	0.43 ± 0.03	0.19 ± 0.03	0.10 ± 0.01	0.36 ± 0.01	0.77 ± 0.03	1.18 ± 0.04	9.91
9	Yan Jing	3.74 ± 0.07	0.17 ± 0.02	0.44 ± 0.08	0.42 ± 0.06	0.16 ± 0.05	0.10 ± 0.02	0.18 ± 0.03	0.58 ± 0.04	1.33 ± 0.06	7.12
10	Tiger	5.62 ± 0.09	0.03 ± 0.01	0.96 ± 0.05	0.64 ± 0.05	0.21 ± 0.03	0.09 ± 0.03	0.11 ± 0.03	0.43 ± 0.04	1.13 ± 0.05	9.22
11	Tsing Tao	1.81 ± 0.11	0.19 ± 0.03	0.15 ± 0.05	0.59 ± 0.08	0.24 ± 0.01	0.12 ± 0.02	0.10 ± 0.03	0.59 ± 0.03	1.51 ± 0.07	5.30
12	Budweiser	6.9 ± 0.10	0.05 ± 0.02	0.29 ± 0.03	0.37 ± 0.02	0.36 ± 0.03	0.12 ± 0.02	0.05 ± 0.02	0.43 ± 0.03	1.78 ± 0.06	10.35
13	Blue Lion	10.39 ± 0.09	0.22 ± 0.04	0.93 ± 0.07	0.57 ± 0.03	0.25 ± 0.03	0.11 ± 0.01	0.18 ± 0.04	0.76 ± 0.05	2.09 ± 0.07	15.50
14	Asahi	6.11 ± 0.05	0.06 ± 0.01	1.00 ± 0.07	0.55 ± 0.05	0.24 ± 0.04	0.21 ± 0.01	0.24 ± 0.02	0.85 ± 0.05	1.57 ± 0.05	10.83
15	Harbin	4.75 ± 0.06	0.32 ± 0.05	1.66 ± 0.10	2.98 ± 0.07	0.27 ± 0.04	0.14 ± 0.02	0.41 ± 0.05	0.88 ± 0.04	2.87 ± 0.05	14.28
16	Suntory	5.50 ± 0.03	0.29 ± 0.08	0.74 ± 0.09	0.57 ± 0.02	0.18 ± 0.02	0.09 ± 0.01	0.38 ± 0.04	0.45 ± 0.04	1.72 ± 0.06	9.92
17	King Benefit	4.60 ± 0.04	0.17 ± 0.04	0.36 ± 0.02	0.50 ± 0.03	0.17 ± 0.01	0.13 ± 0.02	0.07 ± 0.01	0.60 ± 0.05	1.80 ± 0.03	8.40
18	Heineken	6.11 ± 0.08	ND	1.35 ± 0.02	0.78 ± 0.03	0.26 ± 0.02	0.10 ± 0.01	0.03 ± 0.01	0.28 ± 0.02	1.24 ± 0.04	10.15
19	Sanmiguel	4.13 ± 0.02	0.95 ± 0.07	0.13 ± 0.02	0.72 ± 0.06	0.12 ± 0.03	0.13 ± 0.01	ND	0.52 ± 0.01	1.64 ± 0.03	8.34
20	Reeb	2.82 ± 0.02	0.26 ± 0.02	0.13 ± 0.05	0.22 ± 0.07	0.15 ± 0.01	0.07 ± 0.03	0.04 ± 0.01	0.27 ± 0.01	0.51 ± 0.03	4.47
21	Pearl River	4.38 ± 0.05	0.09 ± 0.01	0.08 ± 0.02	0.48 ± 0.05	0.20 ± 0.01	0.10 ± 0.01	0.02 ± 0.01	0.45 ± 0.02	1.57 ± 0.02	7.37
22	Beck's	5.84 ± 0.06	0.24 ± 0.02	0.74 ± 0.02	0.57 ± 0.04	0.26 ± 0.03	0.13 ± 0.01	0.13 ± 0.01	0.35 ± 0.02	1.51 ± 0.05	9.77
23	Blue Ribbon	1.94 ± 0.03	0.19 ± 0.03	0.87 ± 0.04	0.45 ± 0.03	0.13 ± 0.03	0.10 ± 0.02	0.11 ± 0.02	0.51 ± 0.03	1.01 ± 0.02	5.31
24	Carlsberg	5.52 ± 0.07	0.31 ± 0.04	1.23 ± 0.06	0.79 ± 0.03	0.22 ± 0.02	0.17 ± 0.02	0.22 ± 0.03	0.88 ± 0.03	1.96 ± 0.03	11.30
25	Coors Light	2.98 ± 0.09	0.25 ± 0.05	0.17 ± 0.08	0.35 ± 0.02	0.08 ± 0.02	0.10 ± 0.02	0.08 ± 0.01	0.22 ± 0.01	0.83 ± 0.03	5.06
26	Sedrin	4.32 ± 0.10	0.36 ± 0.05	0.03 ± 0.01	0.62 ± 0.02	0.41 ± 0.05	0.17 ± 0.01	0.67 ± 0.04	0.77 ± 0.01	1.72 ± 0.05	9.07
27	Huang He	4.11 ± 0.05	0.15 ± 0.04	0.19 ± 0.05	0.42 ± 0.03	0.28 ± 0.03	0.09 ± 0.02	0.03 ± 0.01	0.48 ± 0.05	1.37 ± 0.02	7.12
28	Blue Girl	3.64 ± 0.04	0.24 ± 0.06	0.81 ± 0.03	1.36 ± 0.04	0.22 ± 0.02	0.25 ± 0.03	0.14 ± 0.01	0.89 ± 0.03	3.13 ± 0.04	10.68
29	Grolsch	3.41 ± 0.08	ND	1.64 ± 0.07	0.87 ± 0.05	0.23 ± 0.02	0.13 ± 0.02	0.23 ± 0.02	0.35 ± 0.04	2.04 ± 0.05	8.90
30	Corona	3.18 ± 0.06	0.29 ± 0.07	0.52 ± 0.07	0.48 ± 0.03	0.20 ± 0.01	0.16 ± 0.02	0.08 ± 0.01	0.68 ± 0.05	1.58 ± 0.04	7.17
31	Duvel	2.89 ± 0.07	0.29 ± 0.05	4.00 ± 0.15	0.70 ± 0.02	0.27 ± 0.03	0.12 ± 0.01	0.49 ± 0.05	0.01 ± 0.01	1.16 ± 0.05	9.93
32	Leffe	4.83 ± 0.02	0.37 ± 0.03	1.03 ± 0.05	0.80 ± 0.04	0.60 ± 0.04	0.99 ± 0.03	0.73 ± 0.02	0.40 ± 0.01	1.32 ± 0.06	11.07
33	Samuel	5.02 ± 0.03	1.30 ± 0.05	1.85 ± 0.08	1.06 ± 0.09	1.22 ± 0.05	0.36 ± 0.04	ND	0.77 ± 0.03	2.81 ± 0.04	14.39
34	Bitburger	2.88 ± 0.06	0.27 ± 0.01	0.74 ± 0.05	0.71 ± 0.02	0.31 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.55 ± 0.02	1.47 ± 0.05	7.11

[a] Each value is the mean ± standard deviation of triplicate determinations; The levels of individual phenolic compounds are expressed as milligrams per liter of beer (mg/l); ND, not detected.

process might have significant influences on the DPPH radical scavenging activity of beer. The relative higher SPC in Samuel beer might be responsible for its high DPPH radical scavenging activity. Indeed, protocatechuic and caffeic acids with the highest levels in Samuel beer had significant DPPH radical scavenging activities because of their chemical structures (Brand-Williams et al., 1995). Antioxidants with DPPH radical scavenging activity could donate hydrogen to free radicals, particularly to the lipid peroxides or hydroperoxide radicals that are the major propagators of the chain autoxidation of lipids, and to form non-radical species, resulting in the inhibition of propagating phase of lipid peroxidation (Bamforth, Muller, & Walker, 1993). Beer with higher DPPH radical scavenging activity was therefore important to beer flavour stability because beer staling are generally considered as the formation of trans-2-nonenal and other saturated and unsaturated aldehydes due to lipid oxidation (Vanderhaegen et al., 2006).

3.4. ABTS radical cation scavenging activity

Different beer samples were also measured and compared for their free radical scavenging activity against the ABTS radical cation. Table 3 shows that all beer samples used in this study had significant ABTS radical cation scavenging activities. The values of ABTS radical cation scavenging activities of 34 beer samples were in the range of 0.55–1.95 mmol TE/l. Of the beer samples studied, the highest and the lowest ABTS radical cation scavenging activities were found in Samuel and Peng Cheng beers, respectively, which was consistent with the result from DPPH radical scavenging activity assay. In addition to protocatechin acid and caffeic acid, the higher activity of this beer might be its high levels of (+)-catechin and ferulic acid, both of these two phenolics identified in barley and malt had been found to have high antioxidant activity assessed by accelerated autoxidation of methyl linoleate (Maillard, Soum, Boivin, & Berset, 1996). These results also indicate that raw material and brewing process might have considerable impacts on the ABTS radical cation scavenging activity of beer. It is important to mention that TE values of the same beer obtained by the ABTS assay were consistently higher than those obtained by the DPPH assay. The same phenomena were found in recent studies on antioxidant activity of guava fruit reported by Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, and Byrne (2006), and that of malting barley by Zhao et al. (2008). Different reaction kinetics between phenol and the ABTS radical cation and DPPH radical over a similar range of concentrations might lead to the different results from two methods (Campos & Lissi, 1996). Actually, the ABTS radical cation scavenging activity also reflects hydrogen-donating ability. Beer with a higher ABTS radical cation scavenging activity might stabilise active oxygen radicals and have better flavour stability.

3.5. Superoxide anion radical scavenging activity

The superoxide anion radical scavenging activities of different beer samples are shown in Table 3. All beer samples considered in this study displayed significant superoxide anion radical scavenging

Table 3
DPPH radical scavenging activity, ABTS radical cation scavenging activity, superoxide anion radical scavenging activity, reducing power and metal chelating activity of 34 commercial beers.[a]

No.	Brand	DPPH radical scavenging activity[b]	ABTS radical cation scavenging activity[b]	Superoxide anion radical scavenging activity[c]	Reducing power[d]	Metal chelating activity[e]
1	Peng Cheng	0.24 ± 0.02	0.55 ± 0.04	15.71 ± 1.20	1.04 ± 0.08	0.64 ± 0.05
2	Tian Mu Hu	0.52 ± 0.03	0.89 ± 0.08	37.14 ± 2.30	1.41 ± 0.05	5.53 ± 0.10
3	Jin Ling	0.49 ± 0.02	0.82 ± 0.07	35.71 ± 3.00	1.32 ± 0.10	3.48 ± 0.12
4	Siwo	0.60 ± 0.02	0.88 ± 0.06	41.42 ± 3.50	1.24 ± 0.09	7.12 ± 0.15
5	Snow	0.50 ± 0.04	0.80 ± 0.07	34.29 ± 2.50	1.30 ± 0.11	2.49 ± 0.18
6	Tai Hu Shui	0.47 ± 0.02	0.76 ± 0.04	34.29 ± 2.90	1.24 ± 0.07	7.52 ± 0.15
7	Cheerday	0.44 ± 0.05	0.74 ± 0.05	25.71 ± 4.60	1.24 ± 0.09	1.17 ± 0.10
8	BBOSS	0.55 ± 0.02	0.87 ± 0.09	40.00 ± 3.40	1.42 ± 0.12	7.85 ± 0.08
9	Yan Jing	0.39 ± 0.03	0.62 ± 0.05	35.71 ± 1.50	1.08 ± 0.08	9.31 ± 0.20
10	Tiger	0.58 ± 0.02	0.95 ± 0.05	35.71 ± 1.00	1.45 ± 0.11	6.66 ± 0.18
11	Tsing Tao	0.57 ± 0.02	0.85 ± 0.03	34.29 ± 1.80	1.36 ± 0.13	4.34 ± 0.12
12	Budweiser	0.43 ± 0.01	0.73 ± 0.04	22.86 ± 3.50	1.39 ± 0.07	6.20 ± 0.06
13	Blue Lion	0.58 ± 0.03	0.90 ± 0.07	45.71 ± 3.10	1.30 ± 0.08	5.56 ± 0.13
14	Asahi	0.71 ± 0.01	0.97 ± 0.04	41.43 ± 4.50	1.61 ± 0.14	1.76 ± 0.15
15	Harbin	0.57 ± 0.02	0.88 ± 0.04	32.86 ± 4.00	1.27 ± 0.11	12.55 ± 0.25
16	Suntory	0.49 ± 0.03	0.75 ± 0.05	24.89 ± 4.60	1.27 ± 0.12	3.09 ± 0.20
17	King Benefit	0.62 ± 0.03	0.84 ± 0.06	35.71 ± 3.50	1.33 ± 0.12	2.36 ± 0.14
18	Heineken	0.75 ± 0.04	1.13 ± 0.07	48.57 ± 3.40	1.72 ± 0.10	3.68 ± 0.09
19	Sanmiguel	0.69 ± 0.02	0.90 ± 0.05	31.42 ± 2.00	1.57 ± 0.06	2.49 ± 0.06
20	Reeb	0.43 ± 0.01	0.57 ± 0.05	35.53 ± 1.60	1.01 ± 0.09	0.35 ± 0.05
21	Pearl River	0.49 ± 0.02	0.73 ± 0.03	19.74 ± 3.50	1.23 ± 0.10	0.22 ± 0.07
22	Beck's	0.65 ± 0.01	0.96 ± 0.03	40.79 ± 1.70	1.59 ± 0.07	10.82 ± 0.25
23	Blue Ribbon	0.51 ± 0.02	0.69 ± 0.03	38.16 ± 2.80	1.12 ± 0.07	5.13 ± 0.10
24	Carlsberg	0.82 ± 0.06	1.14 ± 0.05	40.79 ± 3.00	1.96 ± 0.15	13.09 ± 0.34
25	Coors Light	0.54 ± 0.01	0.74 ± 0.04	43.42 ± 1.00	1.37 ± 0.12	0.52 ± 0.23
26	Sedrin	0.75 ± 0.07	1.03 ± 0.06	40.79 ± 2.60	1.74 ± 0.10	1.12 ± 0.11
27	Huang He	0.40 ± 0.03	0.71 ± 0.06	15.79 ± 4.20	1.28 ± 0.08	6.74 ± 0.14
28	Blue Girl	0.70 ± 0.01	0.99 ± 0.08	47.37 ± 2.40	1.57 ± 0.07	5.54 ± 0.17
29	Grolsch	0.85 ± 0.05	1.20 ± 0.07	50.00 ± 1.80	1.73 ± 0.06	26.34 ± 0.13
30	Corona	0.49 ± 0.03	0.79 ± 0.03	38.16 ± 1.60	1.39 ± 0.10	0.12 ± 0.16
31	Duvel	0.89 ± 0.06	1.27 ± 0.08	52.63 ± 3.30	1.84 ± 0.11	24.13 ± 0.24
32	Leffe	0.91 ± 0.03	1.33 ± 0.05	55.26 ± 4.20	2.08 ± 0.15	14.3 ± 0.28
33	Samuel	1.35 ± 0.02	1.95 ± 0.05	57.89 ± 3.50	2.84 ± 0.18	54.57 ± 0.36
34	Bitburger	0.71 ± 0.06	1.1 ± 0.06	38.16 ± 2.60	1.62 ± 0.16	22.06 ± 0.16

[a] Values are the means of three replications ± standard deviation.
[b] Values expressed as mmol TE/l.
[c] Values expressed as percent.
[d] Values expressed as mmol AAE/l.
[e] Values expressed as μmol EDTAE/l.

activities under experimental conditions. The values of superoxide anion radical scavenging activity for beers were between 15.71% and 57.89%. Comparison of the results obtained by DPPH and ABTS methods, similar results was observed when antioxidant activity of beer was evaluated by superoxide anion radical scavenging activity. The highest and the lowest activities were also found in Samuel and Peng Cheng beers, respectively. Therefore, superoxide anion radical scavenging activity of beer was influenced significantly by the raw materials and brewing process. It is also important for beer to have higher superoxide anion scavenging activity, because the superoxide anion was a major source of many free radicals, such as peroxyl, alkoxyl, hydroxyl, and nitric oxide, which were formed from the superoxide anion through a Fenton reaction and/or lipid oxidation or nitric oxidation (Ambrosio & Flaherty, 1992). Thus, beer with a higher superoxide anion radical scavenging activity could reduce the production of many free radicals, which improve beer flavour stability by protecting beer components from the free radical attacks.

3.6. Reducing power

As shown in Table 3, there were significant variations in reducing power for different beer samples. The reducing power of 34 beer samples tested in this investigation ranged from 1.01 mmol AAE/l in the case of Reeb beer to 2.84 mmol AAE/l detected in Samuel beer. The results were partly different with the results evaluated in the three assays mentioned above, and this might be due to the different reaction mechanisms among antioxidant activity evaluation assays. Lugasi (2003) also found significant reducing power in dark and lager beers, whereas no significant difference was found between the two types of beers. These observations suggest that raw materials and brewing process have some influences on the reducing power of beer. Reducing power is generally associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they could reduce the oxidised intermediates of lipid peroxidation processes and act as primary or secondary antioxidants.

3.7. Metal chelating activity

Table 3 shows the metal chelating activities of selected commercial beers. Although all beer samples exhibited metal chelating activities at test concentrations, a 450-fold difference was recorded between Corona (0.12 μmol EDTAE/l) and Samuel beers (54.57 μmol EDTAE/l), which suggested that the raw materials and brewing process might have remarkable impacts on metal chelating activity. The results obtained were partly different from the data tested by DPPH, ABTS, superoxide anion, and reducing power methods. In the present study, the different observations from these methods for evaluating antioxidant activity of beer might be due to different mechanisms of reaction. Moreover, antioxidant properties of single compounds within a group could vary remarkably, so that the same levels of antioxidants were not necessarily corresponded

to the same antioxidant responses. Some beers showed very weak metal chelating activities, which might be beers in the present study potentially contained weak-chelating phenolic compounds. Indeed, Miranda, Stevens, Ivanov, McCall, Frei and Deinzer (2000) also found that the prenylated and nonprenylated chalcones and flavanones found in beer and hops did not chelate copper ions *in vitro*. It is well known that even trace amounts of metals such as iron or copper will convert molecular oxygen to ROS (reactive oxygen species). ROS participates the beer oxidation and results in the occurrence of off-flavour in beer (Bamforth et al., 1993).

3.8. Correlations among beer antioxidant activity assays and individual and total phenolic contents

The Pearson's product moment correlation coefficients among five different beer antioxidant activity assays and individual and total phenolic contents were calculated and shown in Table 4. Significant positive correlations among five antioxidant activity assays for beer were observed (ranging from 0.552 to 0.973, $p < 0.01$), especially among DPPH radical scavenging activity, ABTS radical cation scavenging activity and reducing power, suggesting that overall antioxidant activity evaluation results for 34 beer samples using five assays were consistent although these assays involved different reaction mechanisms. These findings suggest that the compounds which could scavenge DPPH, ABTS and superoxide anion radicals in beer are capable of reducing ferric ions and chelating ferrous ions. Moreover, (+)-catechin, (−)-epicatechin, protocatechuic, caffeic and syringic acids contents exhibited strong positive correlations with five antioxidant activity assays (ranging from 0.341 to 0.688, $p < 0.05$), except for the case of syringic acid for metal chelating activity assay and (−)-epicatechin for superoxide anion radical scavenging activity and metal chelating activity. All these results suggest an association between these individual phenolic compounds and antioxidant activity, and all these phenolic compounds might make considerable contributions to the antioxidant activity of beer. The results were in agreement with the previous reports that (+)-catechin and ferulic acid were the most efficient antioxidants in beer (Waters, Heasman, & Hughes, 1997) and the decreases or increases in antioxidant activity during brewing was accompanied significantly by changes in the levels of (+)-catechin and ferulic acid (Pascoe, Ames, & Chandra, 2003). However, gallic and ferulic acids, the major phenolic compounds identified in beer, exhibited very weak correlations with antioxidant activity except for the correlation between ferulic acid–ABTS radical cation scavenging activity, and ferulic acid–metal chelating activity, which might be caused by different responses of phenolic compounds to different antioxidant activity evaluation assays. As for correlations among antioxidant activity assays and TPC and SPC, both of them gave significant ($p < 0.05$) positive correlations with DPPH radical scavenging activity, ABTS radical cation scavenging activity and reducing power, but no correlation was found between TPC and SPC. All these results indicate that TPC might not be a good predictor for beer antioxidant activity evaluated by superoxide anion radical scavenging activity and metal chelating activity, and prove the non-specificity of Folin–Ciocalteu method for total phenolic content determination (Zieliński & Kozłowska, 2000). There were also some antioxidant activities in beer that might be attributable to other unidentified substances or to synergistic interactions. There were significant positive correlations between some individual phenolic compounds, suggesting that these phenolic compounds might have the same behaviors during beer brewing. Results obtained from this study indicated that individual phenolic contents could reflect the antioxidant activity of beer more objective than TPC or SPC, which was of significance for practical beer production.

3.9. Contribution of phenolic compounds to beer antioxidant activity analysed by stepwise linear regression

It is rather difficult to isolate and characterise every compound in beer, and then to evaluate their antioxidant activities due to the diversity and complexity of the natural antioxidant compounds. However, quantification of the contribution of phenolic compounds, especially individual phenolic compounds to beer antioxidant activity was of importance for improving beer flavour stability by increasing selectively certain phenolic content in beer. Nine independent variables (individual phenolic compounds identified in beer) were used to explain variability for the dependent variable (antioxidant activity evaluated by different assays) by stepwise linear regression and results are presented in Table 5. Gallic, vanillic and *p*-coumaric acids found did not help explain the variation in beer antioxidant activity in the current study. There were some differences in phenolic species and their contributions for the different antioxidant assays although (+)-catechin,

Table 4
Correlations among beer antioxidant activity evaluation indices, individual and total phenolic contents.[a]

	DSA	ASA	SSA	RP	MCA	TPC	GA	PA	CC	VA	CA	SA	ECC	PCA	FA	SPC
DSA	1	0.973**	0.793**	0.950**	0.788**	0.433*	0.045	0.696**	0.462**	0.121	0.701**	0.502**	0.390*	−0.022	0.316	0.407*
ASA	–	1	0.750**	0.967**	0.843**	0.426*	0.092	0.671**	0.511**	0.173	0.787**	0.509**	0.405*	0.017	0.359*	0.482*
SSA	–	–	1	0.682**	0.552**	0.304	0.057	0.401*	0.506**	−0.006	0.398*	0.464**	0.294	−0.031	0.131	0.315
RP	–	–	–	1	0.777**	0.461**	0.052	0.699**	0.386*	0.115	0.790**	0.551**	0.381*	0.017	0.321	0.404*
MCA	–	–	–	–	1	0.225	−0.053	0.662**	0.543**	0.227	0.769**	0.303	0.246	−0.047	0.352*	0.373*
TPC	–	–	–	–	–	1	0.059	0.196	−0.055	0.130	0.073	0.009	−0.206	0.165	0.304	0.125
GA	–	–	–	–	–	–	1	−0.139	0.079	−0.080	0.114	0.005	0.113	0.241	0.174	0.754**
PA	–	–	–	–	–	–	–	1	0.122	0.225	0.647**	0.306	0.428*	0.020	0.299	0.205
CC	–	–	–	–	–	–	–	–	1	0.205	0.234	0.089	0.425*	−0.094	0.212	0.484**
VA	–	–	–	–	–	–	–	–	–	1	0.196	0.120	0.237	0.202	0.518**	0.389*
CA	–	–	–	–	–	–	–	–	–	–	1	0.524*	0.537**	0.119	0.374*	0.451**
SA	–	–	–	–	–	–	–	–	–	–	–	1	0.526**	0.001	0.134	0.245
ECC	–	–	–	–	–	–	–	–	–	–	–	–	1	0.046	0.142	0.440*
PCA	–	–	–	–	–	–	–	–	–	–	–	–	–	1	0.604*	0.418*
FA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	0.622**
SPC	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1

[a] DSA, DPPH radical scavenging activity; ASA, ABTS radical cation scavenging activity; HSA, hydroxyl radical scavenging activity; SSA, superoxide anion radical scavenging activity; RP, reducing power; MCA, metal chelating activity; TPC, total phenolic content; GA, gallic acid; PA, protocatechuic acid; CC, (+)-catechin; VA, vanillic acid; CA, caffeic acid; SA, syringic acid; ECC, (−)-epicatechin; PCA, *p*-coumaric acid; FA, ferulic acid; SPC, sum of individual phenolic contents.

* Significant at $p < 0.05$.

** Significant at $p < 0.01$.

Table 5
Partial (part.) and cumulative (cum.) R^2 values from stepwise regression analysis across phenolic compounds for five antioxidant activity assays.[a]

Parameter	DSA		ASA		SSA		RP		MCA	
	Part. R^2	Cum. R^2	Part. R^2	Cum. R^2	Part. R^2	Cum. R^2	Part. R^2	Cum. R^2	Part. R^2	Cum. R^2
Ferulic acid	46.0	46.0 (1)	–	–	–	–	–	–	–	–
Syringic acid	18.5	64.5 (2)	–	–	12.0	55.0 (3)	–	–	–	–
(+)-Catechin	8.8	73.3 (3)	8.9	82.4 (3)	15.0	43.0 (2)	–	–	11.6	84.9 (3)
Caffeic acid	–	–	54.9	54.9 (1)	–	–	57.8	57.8 (1)	55.9	55.9 (1)
Protocatechuic acid	–	–	18.6	73.5 (2)	28.0	28.0 (1)	14.0	71.8 (2)	17.4	73.3 (2)
(−)-Epicatechin	–	–	–	–	–	–	–	–	3.2	88.1 (4)

[a] Data were expressed as percentages; Numbers in parentheses indicate the order in which parameters were added to the model; DSA, DPPH radical scavenging activity; ASA, ABTS radical cation scavenging activity; SSA, superoxide anion radical scavenging activity; RP, reducing power; MCA, metal chelating activity.

(−)-epicatechin, ferulic, syringic, caffeic and protocatechuic acids were observed to make significant ($p < 0.05$) contributions to antioxidant activity of beer. Stepwise linear regression showed that ferulic acid alone was able to explain 46.0% of the variation in DPPH radical scavenging activity observed in the present study. Sequential addition of the syringic and (+)-catechin increased this to 64.5% and 73.3%, respectively. As for ABTS radical cation scavenging activity, caffeic acid was also the most important factor that alone explained 54.9% of the observed variation. Sequential addition of protocatechuic acid and (+)-catechin increased the predictive value of the model to 73.5% and 82.4%, respectively. Furthermore, protocatechuic acid could explain 28.0% of the observed variation in superoxide anion radical scavenging activity. Sequential addition of (+)-catechin and syringic acid increased the predictive value of the model to 43.0% and 55.0%, respectively. However, caffeic and protocatechuic acids were able to explain 71.8% of total variation in reducing power. As for metal chelating activity, caffeic acid was the most important factor, since it alone explained 55.9% of the observed variation. Sequential addition of protocatechuic acids, (+)-catechin and (−)-epicatechin increased the predictive value of the model to 73.3%, 84.9% and 88.1%, respectively. Therefore, results from stepwise regression analysis showed that antioxidant activity of beer was mainly attributed to their phenolic constituents. The contribution of phenolic compounds identified in this study to beer antioxidant activity was between 55.0% and 88.1%, which was dependent on antioxidant activity evaluation methods. The results supported that polyphenols were mainly responsible for the endogenous reducing power of beer and partial removal of the polyphenol fraction by polyvinylpolypyrrolidone treatment diminished the reducing power by 9–38% (McMurrough, Madigan, Kelly, & Smyth, 1996). Moreover, it should be noted that (+)-catechin was important to beer antioxidant activity because it not only exhibited mainly significant positive correlations with the majority of antioxidant assays, but also made considerable contributions to antioxidant activity of beer (Tables 4 and 5). Actually, compounds with flavonoid structure like (+)-catechin generally showed higher antioxidant activity than non-flavonoid compounds, such as phenolic acids, stilbenes, lignans and coumarins. The activity of flavonoids to act as antioxidants depends upon their molecular structure. The position and number of hydroxyl groups and double bonds as well as the ortho 3′,4′-dihtdroxy moiety in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Flavonoids possessing multiple hydroxyl groups, especially 3′,4′ o-dihydroxy groups, and the 3- and 5-OH groups with 4-oxo function in A and C rings are generally the more efficient antioxidants than non-flavonoid compounds (Rice-Evans, Miller, & Paganga, 1996). Indeed, (+)-catechin had been found to have higher antioxidant activity than caffeic acid, ferulic acid, chlorogenic acid and *p*-coumaric acid assessed by the ABTS method (Rice-Evans, Miller, & Paganga, 1997). Therefore, it is efficient to improve beer antioxidant activity by increasing the levels of phenolic compounds, particularly flavonoids and some phenolic acids in beer.

4. Conclusions

There were considerable variations in phenolic profiles (both total and individual phenolic contents) and antioxidant activities of commercial beers across different brands. DPPH radical scavenging activity, ABTS radical cation scavenging activity, superoxide anion radical scavenging activity, reducing power and metal chelating activity exhibited significant positive correlations with (+)-catechin, protocatechuic, caffeic and syringic acids contents. Although the individual phenolic compounds responsible for beer antioxidant activity were different, the overall contribution of phenolic compounds to antioxidant activity of beer was between 55.0% and 88.1%. Therefore, it was important for us to characterise the phenolic species mainly responsible for beer antioxidant activity, which provide a good means for brewers to increase selectively certain phenolic content during brewing for improvement on flavour stability of final beer. Moreover, this research was part of our continuous efforts to improve beer flavour stability by protecting endogenous antioxidants in raw materials and beer. Further work on optimising brewing processes will be the improvement of beer's flavour stability through raising selectively certain phenolic contents.

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