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Evolution of Phenolic Compounds and Antioxidant Activity during Malting

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Two barley varieties, Gan4 and Hamelin, were malted to investigate the evolution of phenolic compounds and antioxidant activity during malting. The antioxidant activity was evaluated with DPPH radical scavenging activity, ABTS radical cation scavenging activity, reducing power, and metal chelating activity. Results showed that malting had significant influences on individual and total phenolic contents as well as antioxidant activities of two barley varieties. The contents of some phenolic compounds and the antioxidant activities decreased significantly during steeping and the early stages of germination and then increased remarkably during the later stages of germination and subsequent kilning. The most phenolic compounds identified in barley were (+)-catechin and ferulic acid, which both changed significantly during malting. Moreover, results from the Pearson correlation analysis showed that there were good correlations among DPPH radical scavenging activity, ABTS radical cation scavenging activity, reducing power, total phenolic content and sum of individual phenolic contents during malting.

KEYWORDS: Barley; malting; antioxidant activity; phenolic compounds; Pearson correlation analysis

INTRODUCTION

Flavor stability is an important quality attribute of beer and increasingly becoming the limiting factor in shelf life of beer. *trans*-2-Nonenal, formed by lipid oxidation in beer and raw material, is considered to be one of the main compounds responsible for a stale taste in beer, as it has a flavor threshold of as little as 0.1 μg/L and may cause a cardboard, paperlike, off-flavor of beer (*1*). In recent years, significant efforts have been made to control the lipid content of raw materials and oxygen levels during wort production, fermentation, and packaging (*2, 3*). However, flavor staleness of beer still occurs. Therefore, the flavor stability of beer primarily depends on the oxygen content of the package beer, but it is now clear that lipid oxidation takes place in each step of the brewing process, from raw material to packaging (*4*). Antioxidants in barley and malt can play a significant role in malting and brewing through their antioxidant properties. These antioxidants are likely to delay the staling of beer and limit the formation of *trans*-2-nonenal by inhibition of lipoxygenase activity and nonenzymatic lipid peroxidation (*5, 6*). Thus attention is now increasingly shifting toward increasing the antioxidant activity of beer itself by protection of endogenous antioxidants of barley and development of new antioxidants from the malting process.

Malt and barley have shown antioxidant activity mainly due to the presence of phenolic compounds, especially flavonoids and hydroxycinnamic acids (*7, 8*). These endogenous antioxidants in barley and malt may actively contribute to the control of oxidative reactions and protect against beer aging by acting as free radical scavengers, reducing agents, and metal ions chelators. It was generally thought that malt had higher antioxidant activity than corresponding unmalted barley (*9*), which suggested that the malting process was of significance for antioxidant activity increase and phenolic compound development. Therefore, the endogenous antioxidants present in barley, which naturally inhibit oxidative deterioration and improve flavor stability of beer, could be protected and promoted by well manipulating malting technology.

There were some individual reports about the changes in phenolic compounds and antioxidant activity of cereals brought about by germination (*10–13*). However, the evolution of phenolic compounds and antioxidant activity during malting of barley was very limited (*9, 14, 15*). Moreover, this research focused on a certain stage of malting, particularly kilning. Other stages, such as steeping and germination, which would have

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significant effects on phenolic compounds and antioxidant activity of malt were neglected in previous studies. To our knowledge, the changes of phenolic compounds, especially individual phenolic compounds of barley during malting have not been studied thoroughly. Furthermore, the correlations among different antioxidant activity evaluation indices and total and individual phenolic content during malting have also not been fully elucidated. Therefore, one objective of the present study was to clarify the evolution of phenolic compounds and antioxidant activity during the whole malting process, including steeping, germination, and kilning by different methods. The other objective was to reveal the correlations among antioxidant activity assays, individual and total phenolic contents during malting. Through this extensive study, we attempted to achieve malts rich in antioxidants by screening barley variety and optimizing malting technology.

MATERIAL AND METHODS

Barley Samples. Two barley varieties, Gan4 and Hamelin, harvested in 2006 were obtained from China and Australia, respectively. Gan4 was two-row regular-hulled winter barley, and Hamelin was two-row regular-hulled spring barley. Both of them were sealed in polyethylene bags until ready for malting.

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). 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) was 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).

Malting. Thin barley kernels (those passing through a 1.98 by 19.05 mm slotted sieve) were removed prior to malting. Malts from Gan4 and Hamelin barley were micromalted employing 36 h of steeping with varying wet and dry periods (6 h wet, 9 h dry, 7 h wet, 9 h dry, and 5 h wet) at 15 °C, 5 days of germination at 16 °C, and a 22 h kilning procedure (45 °C for 8 h, 55 °C for 8 h, 65 °C for 3 h, and 85 °C for 3 h). Samples were taken after steeping, every day of germination, and each step during kilning. All samples taken from each stage were freeze–dried.

Preparation of Extracts from Barley and Malt. Samples were 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 (10000*g*, 10 min), the supernatant was collected for antioxidant activity determination. For HPLC analysis of phenolic compounds, after acetone was removed, each hydrous residue was extracted with ethyl acetate (20 mL) three times. The combined organic phase was evaporated to dryness under vacuum at 35 °C. Each residue was then redissolved in 2 mL of methanol (HPLC grade) and was filtered through a 0.45 μm membrane (Satorious, Germany). The filtrates were analyzed by HPLC. To avoid oxidation, all extracts were stored in the dark at −20 °C and analyses were performed within 24 h.

Total Phenolic Content (TPC). The TPC of the barley or malt extract was determined according to the Folin-Ciocalteu spectrophotometric method (*16*) with some modifications. Briefly, 0.5 mL of barley or malt 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 weight (mg of GAE/g of dw).

Analysis of Individual Phenolic Compounds by Analytical HPLC. HPLC analyses were performed using a Waters 1525 pump (Waters, Milford, MA) equipped with a Waters 717 plus autosampler (Waters) coupled with a Waters 2478 dual λ absorbance detector (Waters) at 280 and 254 nm according to an established protocol (*17*). Separation was performed with a Symmetry C18 (5 μm, 3.9 mm × 150 mm) column (Waters) 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. The concentrations of individual phenolic compounds in barley and malt extracts were calculated using standard curves. Results were expressed in micrograms per gram of dry weight (μg/g of dw).

DPPH Radical Scavenging Activity. DPPH radical scavenging activity of the barley or malt extract was determined according to the method of Gaulejac et al. (*18*) with minor changes. Every extract from barley or malt (0.1 mL) was added to 2.9 mL of 6×10^{-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 weight (μmol TE/g of dw).

ABTS Radical Cation Scavenging Activity. The radical scavenging activity of the barley or malt extract against ABTS radical cation was measured using the method of Re et al. (*19*) 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 extract from barley or malt (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 weight (μmol of TE/g of dw).

Reducing Power. The determination was carried out as described by Oktay et al. (*20*). Briefly, 1 mL of the barley or malt 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 TCA (10%) was added to the mixture, which was then centrifuged at 10000*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 weight (μmol of AAE/g of dw).

Metal Chelating Activity. The chelating activity of the barley or malt extract for ferrous ions was measured following the ferrozine method with minor modifications (*21*). The reaction mixture contained 0.5 mL of barley or malt 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 weight (μmol EDTAE/g dw).

Statistical Analysis. Malting of two barley varieties was carried out in triplicate. Data were reported as mean ± standard deviation (SD) for triplicate determinations. Analysis of variance and significant difference tests were conducted to identify differences among means

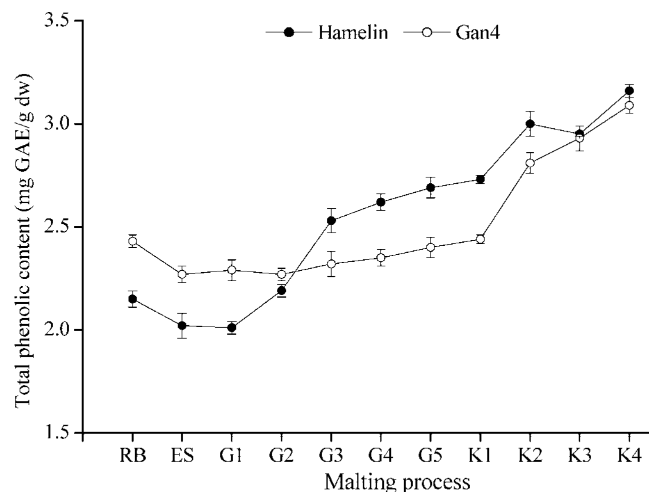

Figure 1. Evolution of total phenolic contents (milligrams of GAE per gram of dry weight) during malting of Gan4 and Hamelin barley. Vertical bars represent the standard deviation ($n = 3$) for each data point. RB, raw barley; ES, end of steeping; G1–G5, first–fifth day of germination; K1, 1−8 h of kilning (45 °C); K2, 9−16 h of kilning (55 °C); K3, 17−19 h of kilning (65 °C); K4, 20−22 h of kilning (85 °C).

by one-way ANOVA using SPSS software (version 13.0 for Windows, SPSS Inc., Chicago, IL). Moreover, the Pearson correlation test was conducted to determine the correlation among means.

RESULTS AND DISCUSSION

Total Phenolic Content. Two barley varieties, Gan4 and Hamelin, were malted and monitored for changes in TPC, and the results are shown in **Figure 1**. The TPCs in raw barley of Gan4 and Hamelin were 2.43 and 2.15 mg GAE/g of dry weight, respectively. Steeping resulted in significant decreases of 6.6% and 6.0% in TPC for Gan4 and Hamelin, respectively. The results are in accordance with the report that the TPC in two sorghum types decreased significantly after steeping (*10*). After 5 days of germination, there was no significant ($p > 0.05$) change in TPC for Gan4 barley, whereas a significant increase of 25% for Hamelin barley was found in the present study. This was in agreement with the observation that the effects of germination on TPC of sorghum partly depended on variety (*11*). Although their behaviors had some discrepancies during germination, TPC for the two barley varieties generally increased progressively with the proceeding of germination. The reason for the decrease in TPC during steeping could be due to leaching of phenolic compounds, which are primarily located in the pericarp and testa, or due to formation of insoluble complexes with proteins (*11, 22, 23*). Subsequently, the TPC in the two barley varieties increased significantly during the whole kilning process, especially at the 9−16 h of kilning at 55 °C with 15.2% and 9.9% increases for Gan4 and Hamelin barley, respectively. Overall, the TPC of Gan4 and Hamelin barley samples increased by 28.8% and 17.5% over the 22 h of kilning, respectively. The increase in TPC during kilning might be better extraction and more release of phenolic compounds (*14*). Because kilning could lead to more friable tissue, some phenolic compounds, primarily present in outer layers of the grain, were better extracted. Moreover, some phenolic compounds bound to cellular structures were also released due to the synthesis of some hydrolytic enzymes (*9*).

Individual Phenolic Compounds. Individual phenolic compounds were measured by HPLC to gain a detailed overview of their evolution during malting. Nine phenolic compounds including (+)-catechin, ferulic acid, syringic acid, (−)-epicatechin, caffeic acid, vanillic acid, *p*-coumaric acid, gallic acid, and protocatechuic acid were identified and quantitated. Their changes in levels during malting are shown in **Table 1**. For the two barley varieties studied, (+)-catechin and ferulic acid were the main phenolic compounds identified in raw barley samples and corresponding malts. The contents of (+)-catechin acid and feulic acid in Gan4 barley were 59.71 and 16.64 μg/g of dw, which were higher than those in Hamelin barley, with values of 55.02 and 15.81 μg/g of dw, respectively. Steeping caused a significant ($p < 0.05$) decrease in the content of each phenolic compounds identified, especially for caffeic acid, vanillic acid, and gallic acid, which all reached the lowest content after steeping. Most of individual phenolic contents decreased during germination and increased in kilning. These findings were in accordance with the report that the ferulic acid and coumaic acid contents decreased several-fold upon malting (*12*). There were some discrepancies for each phenolic compound to reach the highest content during malting. The highest contents of (+)-catechin, syringic acid, (−)-epicatechin, and protocatechuic acid were found after 17−19 h of kilning at 65 °C. However, kilning at 85 °C for 3 h resulted in the highest content of caffeic acid, vanillic acid, *p*-coumaric acid, and gallic acid. Moreover, the highest contents of *p*-coumaric acid and ferulic acid were found in raw barley and after 9−16 h of kilning at 55 °C, respectively.

For Hamelin barley, (+)-catechin content decreased by 4.0% after steeping, 5.9% after 1 day of germination, 15.3% after 2 days of germination, 8.0% after 3 days of germination, 3.2% after 4 days of germination, then increased gradually from 1.5% after 5 days of germination to 12.0% after 17−19 h of kilning at 65 °C, and finally decreased slightly after the last stage of kilning at 85 °C. Similar changes to the contents of ferulic acid, syringic acid and (−)-epicatechin were found during malting. The contents of *p*-coumaric acid, gallic acid, and protocatechuic acid, however, only showed slight changes during malting. Although caffeic acid and vanillic acid contents also decreased significantly ($p < 0.01$) after steeping, subsequent germination and kilning stages gave them to gradually rise to 8.30 and 5.90 μg/g of dry weight, respectively. Woffenden et al. (*15*) also reported a significant increase in (+)-catechin content between 25 and 27 h of kilning. The general trends for the SPC decreased in steeping and early stages of germination; however subsequent germination and kilning brought about a gradual increase in the SPC.

All these data mentioned above indicated that malting had significant influences on both individual and total phenolic contents. With the exception of reasons mentioned in the TPC section, the conversion of phenolic compounds into other flavonoids, which is stimulated by enzymes that are produced during germination and involved in the biosynthesis of phenolic compounds, could partly explain the decreases in the content of individual phenolic compounds during steeping and early stages of germination (*24*). Increases in the contents of individual phenolic compounds during later stages of germination and kilning might be attributed in part to modifications and releases of phenolic compounds during malting, which made extraction much easier (*15*). The last stage of kilning at 85 °C resulted in decrease in contents of some phenolic compounds, which might be partly attributed to their degradation or further reaction under relatively high temperature and low moisture (*14*).

DPPH Radical Scavenging Activity. The evolution of DPPH radical scavenging activity during malting for Gan4 and Hamelin barley was monitored, and the results are shown in **Figure 2**. For Gan4 barley, the DPPH radical scavenging activity decreased slightly by 2.0% ($p > 0.05$) after steeping, followed by

Table 1. Evolution of Individual Phenolic Compounds during Malting of Gan4 and Hamelin Barley[a]

malting process	(+)-catechin	ferulic acid	syringic acid	(−)-epicatechin	caffeic acid	vanillic acid	*p*-coumaric acid	gallic acid	protocatechuic acid	SPC
	μg/g of dry weight									
					Gan4 Barley					
RB	59.71 ± 0.85b	16.64 ± 0.33d	11.58 ± 0.11b	15.91 ± 0.13b	7.25 ± 0.03d	4.51 ± 0.11d	1.85 ± 0.10a	2.53 ± 0.06d	1.69 ± 0.05b	121.67
ES	53.05 ± 0.16c	15.90 ± 0.14e	11.12 ± 0.15bc	15.37 ± 0.16c	7.02 ± 0.02e	4.00 ± 0.15e	1.70 ± 0.03b	2.38 ± 0.03e	1.56 ± 0.03c	112.10
G1	50.88 ± 0.55d	15.46 ± 0.05f	10.88 ± 0.05c	15.12 ± 0.37c	7.08 ± 0.02e	4.25 ± 0.06e	1.61 ± 0.08c	2.57 ± 0.09d	1.38 ± 0.08d	109.23
G2	49.23 ± 0.32d	15.07 ± 0.19g	10.25 ± 0.06e	14.78 ± 0.10d	7.15 ± 0.03d	4.52 ± 0.13d	1.55 ± 0.07c	2.65 ± 0.06cd	1.34 ± 0.02d	106.54
G3	45.97 ± 1.28e	14.97 ± 0.10g	10.15 ± 0.08e	14.68 ± 0.16d	7.30 ± 0.05cd	4.60 ± 0.08d	1.50 ± 0.04c	2.78 ± 0.05c	1.45 ± 0.06d	103.40
G4	45.05 ± 0.90e	14.69 ± 0.21g	10.45 ± 0.05d	15.05 ± 0.24c	7.54 ± 0.09c	4.82 ± 0.05c	1.50 ± 0.08c	2.89 ± 0.04c	1.40 ± 0.04d	103.39
G5	48.91 ± 0.47d	15.17 ± 0.28fg	10.50 ± 0.09d	15.25 ± 0.08c	7.68 ± 0.06c	4.95 ± 0.07c	1.58 ± 0.07c	3.25 ± 0.09ab	1.58 ± 0.03c	108.87
K1	52.07 ± 0.87c	16.65 ± 0.13d	10.73 ± 0.12c	15.83 ± 0.06b	7.77 ± 0.07bc	5.17 ± 0.09bc	1.64 ± 0.03bc	3.30 ± 0.06a	1.60 ± 0.05bc	114.76
K2	58.46 ± 0.26b	19.92 ± 0.34a	11.47 ± 0.10b	16.08 ± 0.09ab	7.98 ± 0.10b	5.32 ± 0.07b	1.66 ± 0.05b	3.10 ± 0.05b	1.72 ± 0.02b	125.71
K3	64.31 ± 0.50a	17.34 ± 0.25c	11.84 ± 0.07a	16.43 ± 0.14a	7.84 ± 0.02b	5.84 ± 0.10a	1.79 ± 0.07ab	3.15 ± 0.03b	1.85 ± 0.04a	130.39
K4	63.28 ± 0.82a	18.26 ± 0.12b	10.98 ± 0.03c	16.23 ± 0.25a	8.90 ± 0.08a	5.88 ± 0.14a	1.82 ± 0.08a	3.40 ± 0.08a	1.69 ± 0.05b	130.44
					Hamelin Barley					
RB	55.02 ± 0.58b	15.81 ± 0.21c	8.24 ± 0.09b	10.18 ± 0.28ab	5.51 ± 0.05e	4.93 ± 0.12bc	2.02 ± 0.06a	1.68 ± 0.06c	1.58 ± 0.02b	104.97
ES	52.82 ± 0.24c	13.76 ± 0.15d	8.05 ± 0.03c	10.00 ± 0.21b	4.87 ± 0.03f	4.17 ± 0.09e	1.99 ± 0.07a	1.15 ± 0.05d	1.59 ± 0.03b	98.40
G1	51.78 ± 0.19d	13.34 ± 0.36d	7.88 ± 0.15c	9.79 ± 0.12bc	4.95 ± 0.06f	4.24 ± 0.08e	1.93 ± 0.06a	1.04 ± 0.07d	1.52 ± 0.02b	96.47
G2	46.59 ± 1.88e	10.29 ± 0.27e	6.72 ± 0.11e	9.50 ± 0.05cd	5.23 ± 0.17e	4.52 ± 0.10d	1.89 ± 0.03ab	1.72 ± 0.09c	1.57 ± 0.04b	88.03
G3	50.62 ± 0.54d	10.71 ± 0.13e	6.85 ± 0.05e	9.43 ± 0.04d	5.97 ± 0.10d	4.77 ± 0.05c	1.80 ± 0.02b	1.83 ± 0.04c	1.63 ± 0.09b	93.61
G4	53.27 ± 0.44c	14.29 ± 0.26d	6.90 ± 0.16e	9.67 ± 0.05c	6.02 ± 0.09d	4.85 ± 0.14c	1.72 ± 0.11b	1.96 ± 0.08bc	1.69 ± 0.06ab	100.37
G5	55.83 ± 0.29b	15.12 ± 0.20c	7.18 ± 0.04d	9.84 ± 0.11b	6.75 ± 0.02c	4.90 ± 0.07bc	1.85 ± 0.02b	2.32 ± 0.03a	1.65 ± 0.03ab	105.44
K1	55.90 ± 1.12b	16.64 ± 0.11b	7.20 ± 0.13d	9.90 ± 0.22b	6.83 ± 0.07c	5.17 ± 0.16b	1.92 ± 0.03a	2.25 ± 0.04a	1.70 ± 0.01a	107.51
K2	60.46 ± 1.09a	18.27 ± 0.09a	7.65 ± 0.06c	10.12 ± 0.23ab	6.88 ± 0.04c	5.23 ± 0.09b	1.98 ± 0.08a	2.13 ± 0.02ab	1.77 ± 0.03a	114.49
K3	61.64 ± 0.52a	16.82 ± 0.22b	8.54 ± 0.10a	10.44 ± 0.15a	7.22 ± 0.08b	5.78 ± 0.14a	1.93 ± 0.04a	1.96 ± 0.07bc	1.79 ± 0.04a	116.12
K4	59.39 ± 1.98a	16.49 ± 0.34b	7.98 ± 0.08c	10.05 ± 0.18b	8.30 ± 0.08a	5.90 ± 0.13a	2.01 ± 0.06a	2.08 ± 0.03b	1.70 ± 0.02a	113.90

[a] Each value is the mean ± standard deviation of triplicate determinations. Means with different letters in the same column for each barley variety are significantly different ($p < 0.05$). RB, raw barley; ES, end of steeping; G1–G5, first–fifth day of germination; K1, 1−8 h of kilning (45 °C); K2, 9−16 h of kilning (55 °C); K3, 17−19 h of kilning (65 °C); K4, 20−22 h of kilning (85 °C).

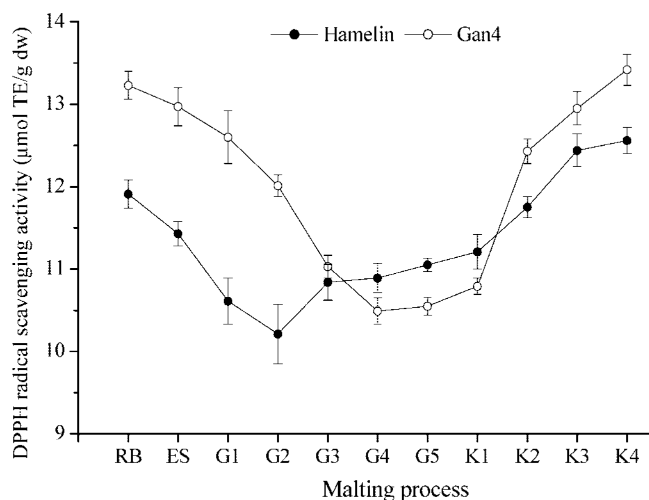

Figure 2. Evolution of DPPH radical scavenging activity (micromoles of TE per gram of dry weight) during malting of Gan4 and Hamelin barley. Vertical bars represent the standard deviation ($n = 3$) for each data point. RB, raw barley; ES, end of steeping; G1–G5, first–fifth day of germination; K1, 1−8 h of kilning (45 °C); K2, 9−16 h of kilning (55 °C); K3, 17−19 h of kilning (65 °C); K4, 20−22 h of kilning (85 °C).

a continued decrease of 20.7% ($p < 0.01$) up to the fourth day of germination. Significant increases in DPPH radical scavenging activity were observed from the fifth day of germination to the last 3 h of kilning, with values from 10.55 to 13.42 μmol TE/g of dry weight. However, there was no significant ($p > 0.05$) difference in DPPH radical scavenging activity between malted barley and raw barley for Gan4 barley. For Hamelin barley, its DPPH radical scavenging activity was lower than that of Gan4 barley. Steeping caused a significant decrease (by 4.0%, $p < 0.05$) in DPPH radical scavenging activity, with the first two days of germination resulting in a further significant ($p < 0.01$) decrease of 10.7% compared with steeping barley. Then the DPPH radical scavenging activity began to increase until 85 °C of kilning; there was no significant ($p > 0.05$) difference in DPPH radical scavenging activity between 17−19 h of kilning at 65 °C and 20–22 h of kilning at 85 °C. Compared with raw Hamelin barley (11.90 μmol TE/g of dry weight), malted barley (12.56 μmol TE/g of dry weight) had significant ($p < 0.05$) higher DPPH radical scavenging activity, which suggested that malting had important effects on DPPH radical scavenging activity of barley. These observations were in accordance with the results of Maillard et al. (*9*) and indicated that germination resulted in a decrease and kilning caused a significant increase in antioxidant activity. These results also suggested that the effect of malting on the DPPH radical scavenging activity might be partly dependent on variety.

ABTS Radical Cation Scavenging Activity. ABTS radical cation scavenging activity was used to determine the evolution of antioxidant activity during malting, and results are presented in **Figure 3**. Compared with the DPPH radical scavenging activity, the ABTS radical cation scavenging activity of barley samples was affected similarly by malting. For Gan4 barley, steeping resulted in a significant ($p < 0.05$) decrease of 4.0% in ABTS radical cation scavenging activity, which was followed by a further significant ($p < 0.01$) decrease of 11.4% after 4 days of germination. The fifth day of germination and kilning resulted in a gradual increase in ABTS radical cation scavenging activity and the highest value of 14.79 μmol of TE/g of dry weight was reached after 85 °C of kilning. The ABTS radical cation scavenging activity of Hamelin barley decreased until the second day of germination and then gradually increased up to 85 °C of kilning. The 9−16 h of kilning at 55 °C brought about a significant ($p < 0.01$) rise in ABTS radical cation scavenging activity for both barley varieties. The ABTS radical cation scavenging activities of Gan4 and Hamelin barley were 14.02 and 12.76 μmol of TE/g of dry weight, respectively.

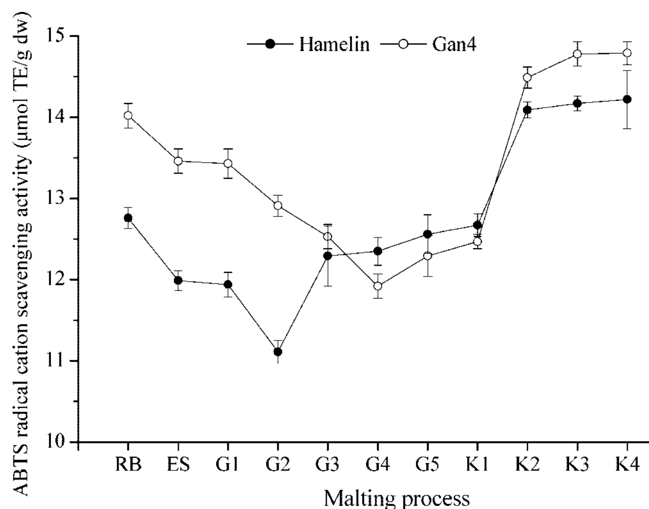

Figure 3. Evolution of ABTS radical cation scavenging activity (micromoles of TE per gram of dry weight) during malting of Gan4 and Hamelin barley. Vertical bars represent the standard deviation ($n = 3$) for each data point. RB, raw barley; ES, end of steeping; G1–G5, first–fifth day of germination; K1, 1−8 h of kilning (45 °C); K2, 9−16 h of kilning (55 °C); K3, 17−19 h of kilning (65 °C); K4, 20−22 h of kilning (85 °C).

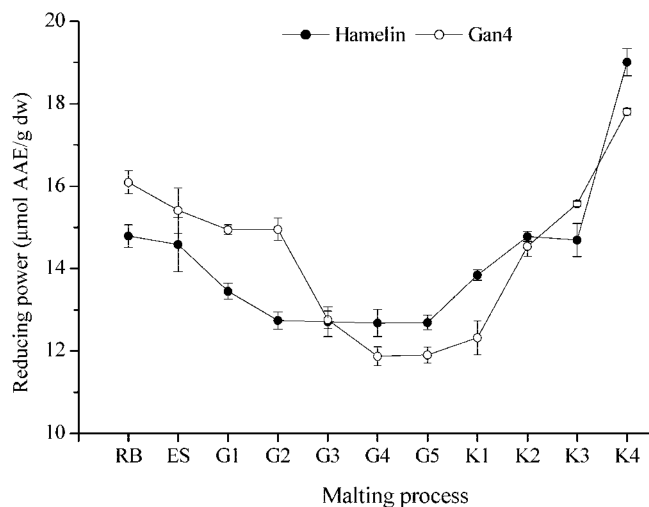

Figure 4. Evolution of reducing power (micromoles of AAE per gram of dry weight) during malting of Gan4 and Hamelin barley. Vertical bars represent the standard deviation ($n = 3$) for each data point. RB, raw barley; ES, end of steeping; G1–G5, first–fifth day of germination; K1, 1−8 h of kilning (45 °C); K2, 9−16 h of kilning (55 °C); K3, 17−19 h of kilning (65 °C); K4, 20−22 h of kilning (85 °C).

However, after malting they changed to 14.79 and 14.22 μmol of TE/g of dry weight, respectively, significantly higher than those of the raw barley. Overall, the ABTS radical cation scavenging activity of Gan4 and Hamelin barley increased by 5.5% and 11.4% over the whole malting process, respectively. These results were not same as those obtained from DPPH radical scavenging activity. It could be explained that the same type and level of antioxidants in barley samples could not give the same response for DPPH and ABTS radicals (*25, 26*).

Reducing Power. Figure 4 showed the evolution of reducing power of Gan4 and Hamelin barley during malting. The reducing powers of Gan4 and Hamelin barley were 16.09 and 14.79 μmol of AAE/g of dry weight, respectively. Steeping and germination resulted in significant ($p < 0.01$) decreases of 26.0% and 14.2% in reducing power for Gan4 and Hamelin barley, respectively. However, significant ($p < 0.01$) increases in reducing power

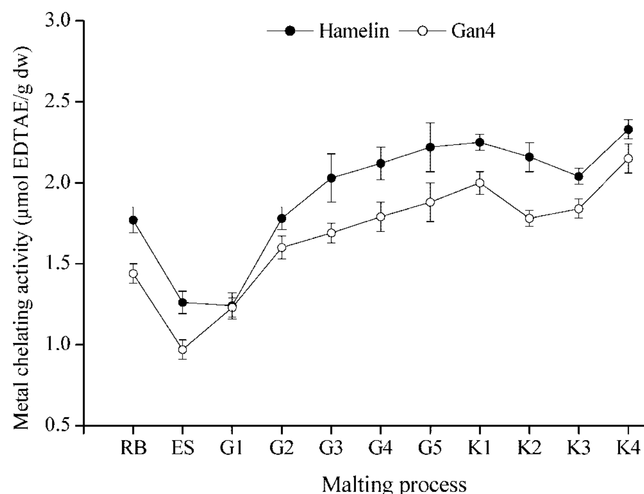

Figure 5. Evolution of metal chelating activity (micromoles of EDTAE per gram of dry weight) during malting of Gan4 and Hamelin barley. Vertical bars represent the standard deviation ($n = 3$) for each data point. RB, raw barley; ES, end of steeping; G1–G5, first–fifth day of germination; K1, 1−8 h of kilning (45 °C); K2, 9−16 h of kilning (55 °C); K3, 17−19 h of kilning (65 °C); K4, 20−22 h of kilning (85 °C).

were observed for both barley samples after kilning, with overall 10.7% and 28.5% increases. The last stage of kilning brought about a sharp rise in the reducing power for these barley samples. In addition to the phenolic compounds commonly measured in barley and malt, Maillard reaction products generated on kilning, might contribute to such sharp increase in reducing power. Previous studies indicated that Maillard reaction products in pale malt had significant reducing power (*15*). Indeed, compared with lager beer, black beer with higher content of Maillard reaction products showed higher reducing power (*27*). It should be noted that Gan4 barley had higher reducing power than Hamelin barley, whereas the latter showed higher reducing power than the former after malting. These suggested that the malting process had significant influence on the reducing power of barley. Barley with low reducing power could be improved by manipulation of the malting process. Moreover, compounds with reducing power indicate that they are electron donors and can act as primary and secondary antioxidants. Therefore, barley with higher reducing power could improve flavor stability of beer by inhibiting lipid peroxidation processes.

Metal Chelating Activity. It has been well recognized that chelating agents may inhibit radical generation by stabilizing transition metals, consequently reducing free radical damage (*28*). In addition, some phenolic compounds exhibit antioxidant activity through the chelation of metal ions (*29*). To better estimate the evolution of antioxidant activity of barley during malting, chelating activity of each barley sample was evaluated against Fe^{2+}. As shown in **Figure 5**, the metal chelating activities of Gan4 and Hamelin barley were 1.44 and 1.77 μmol of EDTAE/g of dry weight, respectively. The metal chelating activities of both barley varieties decreased (by 32.6% and 28.8%, respectively) significantly ($p < 0.01$) after steeping and followed by significant ($p < 0.01$) increases during germination and 1−8 h of kilning at 45 °C. With the kilning proceeding, metal chelating activity decreased slightly and then increased after the last stage of kilning. Malting resulted in overall increases of 49.3% and 31.6% of metal chelating activity for Gan4 and Hamelin barley, respectively. Therefore, all of the above data indicated that malting also had significant effects on metal chelating activity of barley. It was also important for

Table 2. Correlations among Antioxidant Activity Assays, Individual and Total Phenolic Content[a]

	DSA	ASA	RP	MCA	TPC	CC	FA	SA	ECC	CA	VA	PCA	GA	PA	SPC
DSA	1	0.890**	0.853**	−0.159	0.443*	0.725**	0.648**	0.548**	0.442*	0.510*	0.316	0.158	0.284	0.327	0.787**
ASA		1	0.758**	0.168	0.739**	0.849**	0.817**	0.499*	0.415	0.687**	0.622**	0.140	0.430*	0.551**	0.912**
RP			1	0.011	0.486*	0.686**	0.488*	0.240	0.136	0.398	0.397	0.423*	0.040	0.319	0.588**
MCA				1	0.738**	0.335	0.199	−0.392	−0.308	0.352	0.779**	0.217	0.211	0.518*	0.160
TPC					1	0.748**	0.602**	0.024	0.030	0.672**	0.884**	0.225	0.378	0.720**	0.653**
CC						1	0.685**	0.136	0.040	0.388	0.682**	0.548**	0.123	0.829**	0.770**
FA							1	0.543**	0.484**	0.699**	0.557**	−0.009	0.551**	0.427*	0.892**
SA								1	0.976**	0.653**	0.060	−0.582**	0.757**	−0.142	0.695**
ECC									1	0.689**	0.055	−0.690**	0.847**	−0.193	0.643**
CA										1	0.628**	−0.399	0.870**	0.194	0.797**
VA											1	0.235	0.400	0.678**	0.626**
PCA												1	−0.659**	0.578**	−0.044
GA													1	0.014	0.670**
PA														1	0.504*
SPC															1

[a] DSA, DPPH radical scavenging activity; ASA, ABTS cation radical scavenging activity; RP, reducing power; MCA, metal chelating activity; TPC, total phenolic content; CC, (+)-catechin; FA, ferulic acid; SA, syringic acid; ECC, (–)-epicatechin; CA, caffeic acid; VA, vanillic acid; PCA, *p*-coumaric acid; GA, gallic acid; PA, protocatechuic acid; * significant at $p < 0.05$. ** significant at $p < 0.01$.

barley or malt possessing high metal chelating activity, because it could chelate transition metal ions, which could improve beer flavor stability by blocking the radical chain reaction (*30*).

Correlations among Antioxidant Activity Assays. Correlations among four different antioxidant activity assays during malting were analyzed by using the Pearson correlation test, and correlation coefficients are shown in **Table 2**. DPPH radical scavenging activity, ABTS radical cation scavenging activity, and reducing power were well positively correlated with each other (ranging from 0.758 to 0.890, $p < 0.01$), but all of them exhibited weak correlations with metal chelating activity. This suggested that the compounds which could scavenge DPPH radical in the barley samples during malting 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. Moreover, metal chelating activity showed negative correlation (−0.159) with DPPH radical scavenging activity in the present study, indicating that malting barley with higher metal chelating activity might not have higher DPPH radical scavenging activity. A negative correlation between DPPH radical scavenging activity and cupric ion chelating was found in the previous study (*31*). The different antioxidant responses and levels of phenolic compounds in different barley samples could explain the negative or positive correlations among antioxidant activity assays. Although all of these methods could be used to measure antioxidant activities of barley sample during malting, results cannot be superimposed and even indicate different behaviors in some cases. Differences in antioxidant activity assays for the same evolution process reflect differences in the nature of the compounds responding to different antioxidant assays, as well as the changing composition of the barley sample during malting.

Correlations among Antioxidant Activity Assays and Individual and Total Phenolic Contents. In order to clarify whether phenolic compounds make a contribution to antioxidant activity measured by different methods during malting, all data obtained from barley samples during malting were used to analyze correlations among antioxidant activity assays and individual and total phenolic contents. **Table 2** showed that (+)-catechin, ferulic acid, and syringic acid contents had significant positive correlations with DPPH radical scavenging activity (ranging from 0.548 to 0.725, $p < 0.01$). The contents of (+)-catechin, ferulic acid, caffeic acid, vanillic acid, and protocatechuic acid gave strong positive correlations with ABTS radical cation scavenging activity (ranging from 0.551 to 0.849, $p < 0.01$). Moreover, (+)-catechin and vanillic acid contents showed high positive correlations with reducing power and metal chelating activity, respectively (0.686 and 0.779, $p < 0.01$). All these observations mentioned above indicated that different phenolic compounds made considerable different contributions to different antioxidant activity assays. Phenolic compounds with significant positive correlations with certain antioxidant activity assays suggested that such phenolic compounds were mainly responsible for such antioxidant activity. In addition, negative correlations between metal chelating activity and syringic acid as well as (−)-epicatechin contents during malting were found in the present study.

DPPH radical scavenging activity, ABTS radical cation scavenging activity, reducing power, and metal chelating activity showed significant correlations with both TPC and SPC (ranging from 0.443 to 0.912, $p < 0.05$, except for the case between metal chelating activity and SPC with a correlation coefficient of 0.160). The results were supported by Bonoli et al. (*32*) that there was a significant positive correlation between TPC and DPPH radical scavenging activity when free and bound phenolic compounds extracted by different methods and their antioxidant activities were compared. A good correlation (0.653, $p < 0.01$) between TPC and SPC suggested that both methods provided accurate information on total phenolic content of barley samples during malting, although the 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 (*33, 34*). Moreover, all individual phenolic contents identified except for *p*-coumaic acid and protocatechuic acid showed significant positive correlations with SPC (ranging from 0.626 to 0.892, $p < 0.01$), and (+)-catechin, ferulic acid, caffeic acid, vanillic acid, and protocatechuic acid contents gave strong correlations with TPC (ranging from 0.602 to 0.884, $p < 0.01$), suggesting they were major phenolic compounds in barley during malting under experimental conditions. Additionally, there were significant positive correlations among (+)-catechin, vanillic acid, and ferulic acid (ranging from 0.557 to 0.685, $p < 0.01$) as well as among (−)-epicatechin, ferulic acid, syringic acid, caffeic acid, and gallic acid (ranging from 0.484 to 0.976, $p < 0.01$). However, strong positive correlations between *p*-coumaric acid and (+)-catechin as well as protocatechuic acid (0.548 and 0.578, $p < 0.01$), and significant negative correlations between *p*-coumaric acid and (−)-epicatechin, syringic acid, and gallic acid (ranging from −0.690 to −0.582, $p < 0.01$) were found during malting in the present study.

Therefore, all of data mentioned above indicated that evolution of antioxidant activity during malting might be attributed to changes in individual and total phenolic content. However, decreases in some individual and total phenolic content but increases in antioxidant activity measured by different methods were also found in some cases, especially for the case of the last stage of kilning at 85 °C during malting. The loss and degradation of phenolic compounds as well as Maillard reaction products generated on kilning at high temperature could be used to explain the results of opposite behaviors between phenolic contents and antioxidant activity.

In conclusion, the present study has clearly demonstrated the evolution of phenolic compounds and antioxidant activity estimated by DPPH radical scavenging activity, ABTS radical cation scavenging activity, reducing power, and metal chelating activity during malting. Malting including steeping, germination, and kilning had significant effects on individual and total phenolic contents as well as antioxidant activities of barley samples. The most phenolic compounds identified were (+)-catechin and ferulic acid, both of which changed significantly during malting. There were significant correlations among DPPH radical scavenging activity, ABTS radical cation scavenging activity, reducing power, TPC, and SPC during malting. Therefore, it is important for us to understand the changes in phenolic compounds and antioxidant activity during malting, which can provide useful information for us on protection and improving endogenous antioxidants in malting. The results obtained from this research also could contribute to the selection of barley variety for production malt with higher antioxidant activity. Moreover, this research was part of our continuous efforts to improve beer flavor stability by protecting endogenous antioxidants in raw materials and beer. On the basis of results obtained from current study, further work on optimizing malting process and production of malt with high antioxidant activity are in progress to improve the flavor stability of beer.

ABBREVIATIONS USED

DPPH, 1,1-diphenyl-2-picrylhydrazyl; ABTS, 2.2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical cation; TPC, total phenolic content; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-carboxylic acid; EDTA, disodium ethylenediaminetetraacetate; HPLC, high-performance liquid chromatography; TE, Trolox equivalents; GAE, gallic acid equivalents; TCA, trichloroacetic acid; EDTAE, disodium ethylenediaminetetraacetate equivalents; SPC, sum of individual phenolic contents; RB, raw barley; ES, end of steeping; G1–G5, first–fifth day of germination; K1, 1−8 h of kilning (45 °C); K2, 9−16 h of kilning (55 °C); K3, 17−19 h of kilning (65 °C); K4, 20−22 h of kilning (85 °C).

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