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The Antioxidant Properties of Four Korean Barley Cultivars at Different Harvest Times and Profiling of Major Metabolites

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Abstract

Young barley leaves (YLB) have been no studies comparing the antioxidant activities of various cultivars, or differences the antioxidant activity at different harvest times. The objective of this study was to evaluate the differences in the antioxidant activities of YLB by different cultivars and growth stages. The 4 Korean YLB, Nakyoung, Dahyang, Saegang, and Boanchal, were sprouted, and their total polyphenols, total flavonoids, and ABTS radical cation ($ABTS^{\bullet+}$) scavenging activity were measured. The 4 cultivars exhibited high total polyphenol contents (TPC) and total flavonoid contents (TFC), with a range of 1047.8–1263.2 GAE mg·100 g^{-1} and 443.7–550.7 CE mg·100 g^{-1}, DW, respectively. In addition, they exhibited high levels of $ABTS^{\bullet+}$ (RC_{50} value range of 41.8–80.8 μg·mL^{-1}) scavenging activity. After sprouting, the antioxidant activity of the 4 cultivars increased. That is, in these YLB, the TPC, TFC and radical scavenging ability were enhanced by sprouting. Lutonarin (LN) and saponarin (SN) are the major compounds of YLB. LN exhibited stronger radical scavenging activity than SN. Over time, LN contents increased and SN contents decreased. As a result, the antioxidant ability of YLB was improved with increased growing time.

Keywords: barley sprouts, polyphenols, antioxidants

1. Introduction

Signs of oxidative stress have prompted speculation that antioxidant imbalances may be involved in a number of diseases. Factors that cause oxidative stress, such as free radicals and other reactive oxygen species (ROS), are continuously formed in the human body. The effects of free radicals have been implicated in the pathology of several human diseases, including cancer, atherosclerosis, malaria, rheumatoid arthritis, and neurodegenerative diseases (Aruoma, 1998). The effects of oxidative stress are prevented or mitigated by the removal of free radicals. In addition, the presence of antioxidants can prevent free radical-derived oxidative stress. Plants produce antioxidants as secondary metabolites, in particular, as a form of defense (Scandalios, 1990). Because of this, plants have received considerable attention as potential dietary sources of biologically active ingredients such as antioxidants (Diplock, 1991). Therefore, many researchers have studied the phytochemicals found in fruits, vegetables, cereals, and sprouts. Several studies have reinforced the case for the inclusion of the young leaves of grains such as oat, quinoa, buckwheat, and wheat in the diet in order to promote health (Liu et al., 2008; Randhir et al., 2008; Alvarez-Jubete et al., 2010). Barley (*Hordeum vulgare* L.) is a widely consumed grain because of its dietary health benefits, ready availability, reasonable cost, and processing properties, which make it a good choice for use in a number of products (Moe & Adlernissen, 1994). Our previous study showed that young barley leaves (YLB) contain various phytochemicals, such as polyphenolic compounds and flavonoids (Park et al., 2014). Polyphenols are ubiquitous secondary metabolites in plants, and flavonoids are a class of polyphenol. Polyphenols possess a wide spectrum of biochemical properties such as antioxidant, antimutagenic, and anticarcinogenic properties (Didzhiapetrene Ia et al., 1988; Rice-Evans et al., 1995). Numerous studies

confirm the antioxidant activity of plant-derived polyphenolic flavonoids, which act against radicals generated in the aqueous phase (Rice-Evans et al., 1995). Flavonoids such as the saponarin (SN) and lutonarin (LN) produced by YLB (Benedet et al., 2007) have antioxidant properties. The aim of this study was to investigate the antioxidant properties of YLB, and to profile any changes in the antioxidant activities of the barley varieties at different harvest time. Furthermore, correlations between the antioxidant properties of YLB from four Korea cultivars and the major flavonoids of YLB, LN and SN were elucidated by principal component analysis (PCA).

2. Method

2.1 Plant Material

Barley (*Hordeum vulgare* L.), four Korean curtivars, Nakyoung, Dahyang, Boanchal andSaegang, were harvested in 2014 at the experimental field of the National Institute of Crop Science, Rural Development Administration, Miryang, Korea. Young leaves of barley (YLB) were prepared with literature procedure (Seo et al., 2013). The collected leaves were freeze dried immediately after sampling. Prior to extract, leaves were pulverized to 100 mesh. All sample masses were based on dry weight.

2.2 Determination of Total Polyphenolic and Flavonoid Contents

Total polyphenol contents (TPC) and total flavonoid contents (TFC) of YLB were measured by colorimetric methods. TPC of YLB were determined by using the Folin-Ciocalteu assay (Choi et al., 2006). An aliquoit (10 μL) of extract or standard solution of gallic acid (20, 40, 60, 80, and 100 mg/L) was added to 96 well plate. A reagent blank using methanol was prepared. 200 μL of 2% Na_2CO_3 solution was added to the well plate and shaken. After 3 min, 10 μL of Folin-Ciocalteu's phenol reagent was added to the mixture. After incubation for 27 min at room temperature, the absorbance was determined against prepared reagent blank at 750 nm with an UV-Vis spectrometer. Total phenolic contents of YLB were expressed as mg gallic acid equivalents (GAE)/100 g fresh weight. The total flavonoid contents were measured by the aluminum chloride colorimetric assay (Csepregi et al., 2013). An aqliquot (25 μL) of extracts or standard solution of catechin hydra (20, 40, 60, 80, and 100 mg/L) was added to 96 well plate. To the plate were added 100 μL H_2O and 7.5 μL 5% $NaNO_2$. After 5 min, 15 μL 10% $AlCl_3 \cdot 6H_2O$ was added. After 6 min, 50 μL 1M NaOH was added and incubated for 1 min at room temperature. The absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid contents of YBL were expressed as mg (+)-catechin hydrate equivalents (CE)/100 g fresh mass.

2.3 Measurement of $ABTS^{\bullet+}$ Scavenging Activity

In vitro ABTS radical cation ($ABTS^{\bullet+}$) scavenging assay was commonly used to evaluate the free radical scavenging effectiveness of various antioxidants. The $ABTS^{\bullet+}$ radical scavenging activity of the YLB was measured as previously indicated method (Re et al., 1999). The antioxidant potential to scavenge the radical cation $ABTS^{\bullet+}$ was compared to a standard (Trolox). The pre-formed $ABTS^{\bullet+}$ is prepared by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and is stabilized in the dark at room temperature for 12 h. The $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.75 (±0.02) at 734 nm and equilibrated at 30 °C.The $ABTS^{\bullet+}$ scavenging ability of each compound was calculated by determining the decrease in absorbance at different concentrations using the following equation;

$$ABTS^{\bullet+} \text{ scavenging activity } (\%) = (A_{blank} - A_{sample})/A_{blank} \times 100 \quad (1)$$

2.4 UPLC-PDA Analysis

Using ultra performance liquid chromatography ultra performance equipped with photodiode array detector (UPLC-PDA, Waters AQUITY, MA, USA) data analysis, the contents of lutonarin and saponarin (LNC and SNC, respectively) were monitored. The separations of YLB extract were performed on a 2.1 × 100 mm, 1.7 μm ACQUITY BEH C18 chromatography column with maintained at 35 °C. The mobile phases A and B comprised water with 0.1% TFA acetonitrile with 0.1% TFA, respectively. The injection volume 2 μL and the gradient solvent system was programmed: 0 min, 0% B; 0-3 min, 3% B; 3-10 min, 3-15% B; 10-13 min, 15-30% B; 13-15 min, 30-50% B; wash to 18 min with 90% B; and a 2 min recycle time. The flow rate was 0.5 mL/min. The peak detection was observed with a PDA detector 335 nm. For the quantitative analysis, the curves of LN and SN calibration with the clearly resolved each peak were used with different concentrations of 1.25, 2.5, 5, 10, 50, 100 and 200 μg/mL. The identities of the LN and SN peaks were made by comparing the retention times between the standard peaks and the BS extract peaks. The concentrations of the compounds were expressed as mg per 100 g of YLB samples.

2.5 Statistical Analysis

Unless otherwise indicated, values represent the mean ± SD of data obtained from triplicate experiments.

Statistical analysis was performed using the general linear model procedure (GLM) in SAS statistical software (Version 9.1, 2002; SAS Institute; Cary, NC, USA). The principal component analysis (PCA; JMP software; SAS Institute) was done to detect clustering formation and establish relations between samples and major compounds of YLB, LN and SN, or antioxidant properties.

3. Results

3.1 Antioxidant Activities of 4 Cultivars of YLB

It was reported that YLB had excellent antioxidant activity (Moe & Adlernissen, 1994). We screened the antioxidant activity of YLB cultivars. As a result, predominant four cultivars were selected and their TPC and TFC were investigated (Table 1). ABTS radical cation ($ABTS^{\bullet+}$) scavenging assay were shown in Table 1. Nackyoung, Dahyang, Saegang, and Boanchal had high TPC and TFC with 759.7-1076.6 GAE mg/100 g and 245.2-593.4 CE mg/100 g, respectively. Among the cultivars, Nackyoung has the highest TPC with 1076.6 GAE mg/100g and Boanchal has the predominant TFC with 593.4 CE mg/100 g, respectively. In $ABTS^{\bullet+}$ scavenging assays, Nackyoung, Dahyang, Saegnag, and Boanchal exhibited excellent $ABTS^{\bullet}$ (RC_{50} value range of 41.8-142.0 μg mL^{-1}) scavenging activities.

3.2 Changing Patterns of Antioxidant Properties in 4 YLB

To investigate changes in the patterns of antioxidant capacity of 4 YLB cultivars at different growth stage, YLB were cultivated in an outdoor field and harvested at growth stages, 13, 23, 40, and 56 days after sprouting (Figure 1). The TPC of 4 cultivars (Nackyoung, Dahyang, Saegang, and Boanchal) increased from day 13 to day 40 after sprouting, (from 788.6 to 1076.6, 759.7 to 1030.1, 763.7 to 1063.2, and 794.2 to 1070.6 GAE mg 100 g^{-1}, respectively) and decreased slightly at day 56, to 1023.5, 1006.9, 922.3, and 977.5 GAE mg 100 g^{-1}, respectively. The TFC of the Nackyoung, Dahyang, and Boanchal cultivars increased from day 13 to day 56 (from 292.1 to 487.5, 245.2 to 593.4, and 282.2 to 508.1 CE mg 100 g^{-1}, respectively); however, that of the Saegang cultivar increased from day 13 to day 40, from 492.8 to 550.7 CE mg 100 g^{-1}, and decreased slightly at day 56, to 472.4 CE mg 100 g^{-1} (Table 1). In $ABTS^{\bullet+}$ assays, 4 YLB cultivars, Nackyoung, Dahyang, Saegang, and Boanchal, exhibited high levels of antioxidant activity at 40 days after sprouting. In the $ABTS^{\bullet+}$ scavenging assay, the RC_{50} values of the 4 YLB cultivars decreased between day 13 and day 40 (from 142.0 to 55.7, 80.7 to 63.8, 112.3 to 39.3, and 108.8 to 41.8 μg mL^{-1}, respectively), then increased slightly on day 56 (to 55.8, 72.4, 52.1, and 53.5 μg·mL^{-1}, respectively) (Table 1). The total radical scavenging activities of the Nackyoung and Saegang cultivars decreased slightly at day 56, whereas the activities of the Dahyang and Boanchal cultivars were slightly changed at 56 days (Table 1).

3.3 Changing in LN and SN, and Their Radical Scavenging Capabilities

Lutonarin (LN) and saponarin (SN) are the major compounds of YLB (Seikel & Geissman, 1957; Seikel et al., 1962), and exhibit antioxidant activity (Benedet et al., 2007). Interestingly, as the sprouts elongated, the LNC increased from 342.5–530.3 to 1042.5–1117.0 mg·100 g^{-1}, while SNC decreased from 1275.7–1411.9 to 183.9–411.3 mg·100 g^{-1} (Figure 2). As shown in Figure 3, the radical scavenging activity of LN is more stronger than that of SN. LN showed the RC_{50}values with 7.4 μM (compared to SN RC_{50} = 130.6 μM) on $ABTS^{\bullet+}$ scavenging activities.

3.4 Correlation Analysis

The total antioxidant potential of the plant extract was estimated using a correlation analysis between TPC and ABTS radical scavenging effect. The correlation coefficient of the relationship between the primary compounds of YLB and their antioxidant properties are presented in Table 2. In the ABTS assays, the RC_{50} values represent half the maximum radical scavenging concentration. In other words, negative correlations between TPC or TFC and ABTS indicated the presence of high levels of antioxidant activity. All elements were highly correlated, with r = 0.63–0.84. The TPC was correlated with TFC (r = 0.80). One major compound of YLB, LNC was positively correlated with TPC (r = 0.82) and TFC (r = 0.80), and negatively correlated with SNC (r = -0.78) and ABTS (r = -0.70). In contrast, another major compound of YLB, SNC, is negatively correlated with TPC (r = -0.84) and TFC (r = -0.82), and positively correlated with ABTS (r = 0.70).

3.5 Principle Component Analysis

In biplot principal component analysis (PCA), all antioxidant elementsincluding TPC, TFC and ABTS were separated by component 1 and 2. The first and second components explained 82.3% and 6.48% of the overall samples (n = 16) (Figure 4). The day 40 and day 56 groups with high LNC, TPC and TFC and low ABTS and SNC were easily visualized in the right biplot. In addition, in the left biplot, the day 13 and day 23 groups were exhibited low LNC, TPC and TFC, and high ABTS and SNC. The angles between LNC, TPC and TFC are very

small, indicating that these factors are positively correlated (Table 2). The vectors of ABTS and SNC point in the opposite direction, meaning that these factors are negatively correlated with LNC, TPC and TFC (Figure 4 and Table 2). The half maximal radical scavenging concentration (RC_{50}) represents the ABTS radical scavenging potency. Therefore, the materials having a low ABTS RC_{50} value can be inferred to be effective scavengers of free radicals. In addition, ABTS are closely correlated, as shown by the small angle between their vectors. This indicates that the age of YLB has an important effect on the antioxidant activities of YLB. Antioxidant elements, TPC, TFC, ABTS, were separated by component 1.

Table 1. Antioxidant properties of 4 YLB at different growth stages

Cultivars	Days	TPC (mg/100g)	TFC (mg/100g)	ABTS (RC_{50}, μg/mL)
Nackyoung	13	788.6±13.5	292.1±1.2	142.0±4.4
	23	939.9±8.8	463.0±6.8	47.4±1.3
	40	1076.6±4.2	393.5±16.4	55.7±1.4
	56	1023.5±16.1	487.5±11.0	55.8±6.1
Dahyang	13	759.7±20.6	245.2±11.1	80.7±7.5
	23	873.8±13.8	452.3±16.5	74.3±1.3
	40	1030.1±21.4	506.9±10.9	63.8±6.3
	56	1006.9±28.7	593.4±7.1	72.4±2.8
Saegang	13	763.7±13.3	273.0±8.7	112.3±0.3
	23	888.7±5.6	430.8±19.0	80.2±7.4
	40	1063.2±0.4	550.7±3.5	52.0±10.7
	56	922.3±18.3	472.4±9.5	39.3±1.2
Boanchal	13	794.2±24.4	282.2±0.9	108.8±6.2
	23	821.1±3.1	366.0±3.4	106.3±14.0
	40	1070.6±36.0	443.7±10.1	41.8±4.0
	56	977.5±5.8	508.1±9.2	53.5±4.0

Note. All are expressed as the mean ± SD of three replicates.

Table 2. Correlation coefficients between major compound of YLB and antioxidant properties

	1	2	3	4	5
1. LNC	1.00				
2. SNC	-0.78	1.00			
3. TPC	0.82	-0.84	1.00		
4. TFC	0.80	-0.82	0.80	1.00	
5. ABTS	-0.70	0.70	-0.78	-0.70	1.00

Note. Pearson's correlation analysis was conducted using averaged values of each varieties (n = 16).

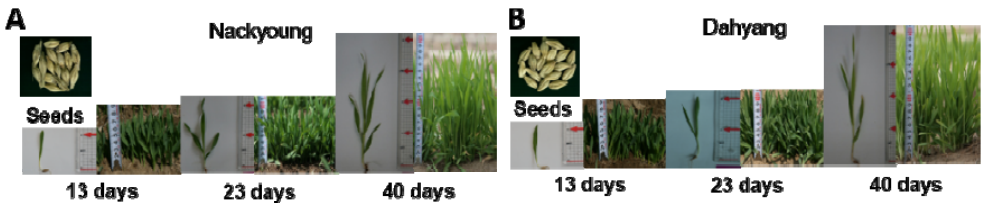

Figure 1. The pictures from 4 Korean barley sprouts varieties at different growth stages (13, 23, 40, and 56 days, 56 days after sprouting of BS were not presented. (A) Barley sprouts 'Nackyoung', (B) Barley sprouts 'Dahyang', (C) Barley sprouts 'Saegang', and (D) Barley sprouts 'Boanchal'. Barley (Hordeumvulgare L.) was harvested in 2014 at the experimental field of the National Institude of Crop Science, Rural Development Administration, Miryang Korea

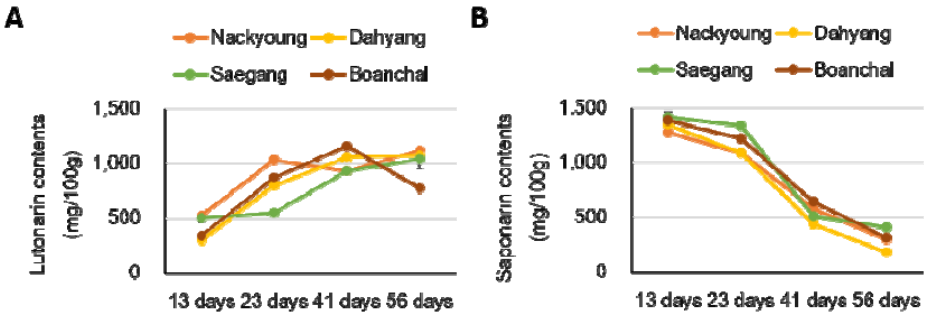

Figure 2. Changes of lutonarin (A) and saponarin (B) contents as affected by cultivation period in 4 barley sprouts

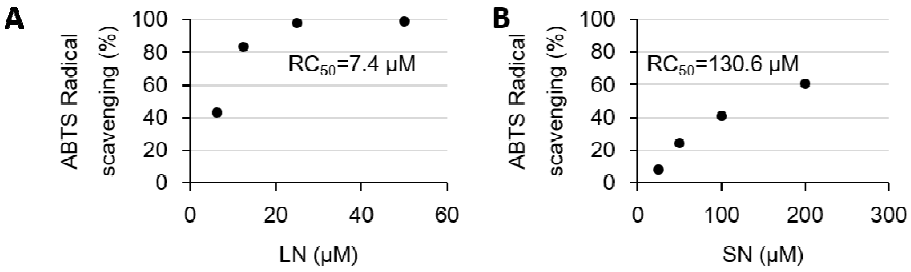

Figure 3. ABTS radical scavenging activities of lutonarin and saponarin

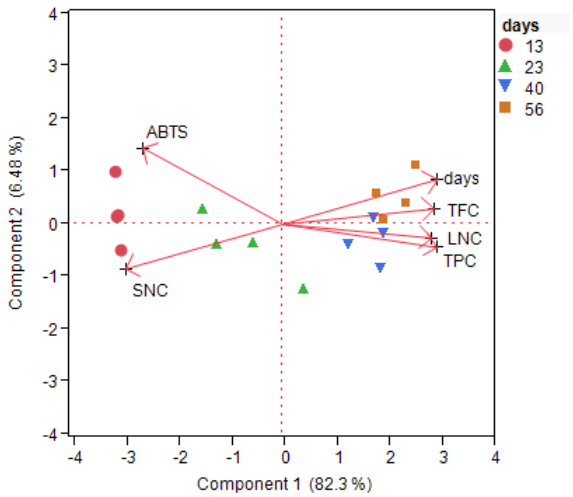

Figure 4. Biplot PCA for antioxidant properties by harvest times

4. Discussion

The patterns of antioxidant activity of 4 YLB cultivars for GS showed interesting results. TPC and TFC increased markedly from day 13 to day 40, and increased slightly at day 56 in the Nackyoung and Dahyang cultivars, but decreased slightly in the Saegang and Boanchal cultivars during the same growth period (Table 1). With respect to the ABTS radical cation ($ABTS^{\bullet+}$) scavenging activity, the results illustrated a similar tendency while growing of 4 YLB.

The major compounds of YLB, lutonarin (LN) and saponarin (SN) (Seikel & Geissman, 1957; Seikel et al., 1962) exhibit antioxidant activity (Benedet et al., 2007). The relationships between the antioxidant properties and patterns of LNC (lutonarin contents) and SNC (saponarin contents) during the different growth stages of 4 YLB cultivars (13, 23, 40, and 56 days after sprouting) were investigated. The LNC increased as glowing up of YLB, and the antioxidant capacity increased. As the plants' capacity to defend against oxidative stress increased as they grow, we hypothesize that the antioxidant properties of YLB are derived from the radical scavenging activities of LN. To prove this hypothesis, correlation analyses were performed. In correlation analysis, The TPC was positively correlated with TFC, because flavonoids are a type of polyphenol. LNC, was positively correlated with TPC and TFC, and negatively correlated with ABTS. In the other hand, SNC, another major compound of YLB, is negatively correlated with TPC and TFC, and positively correlated with ABTS. This demonstrates that high contents of LN indicate the presence of strong radical scavenging activities. In light of comprehensive data analysis, this suggests that the antioxidant properties of YLB are derived from the radical scavenging activities of LN. The biplot principal component analysis (PCA) displays the correlations between antioxidant properties and growth times after germination, and can be used to profile growth times. The 4 cultivars of YLB at different growth stages are mainly separated by principal components 1. The day 13 and day 23 groups were represented in the left biplot, and day 40 and day 56 groups were exhibited in the right biplot. These analyses elucidate changes in the antioxidant capabilities of YLB, and can help to determine optimal growth times.

This research focused in particular on the antioxidant properties of 4 Korean cultivars of YLB. 4 Korean cultivars exhibited changes in the patterns of antioxidant activity. TPC, TFC and ABTS activity demonstrated the superior antioxidant activity of 4 cultivars; Nackyoung, Dahyang, Boanchal, and Saegang. The capacity for YLB to resist oxidation stress varied according to different growth stages. As growth continued, TPC and TFC tended to increase. In addition, ABTS radical scavenging ability was increased in YLB. These phenomena seem to be related to the presence of LN, one of the flavonoids found in YLB. In the correlation and biplot PCA analyses, TPC, TFC, and ABTS were highly correlated. LNC was identified as an important parameter of antioxidant contents in determining growth stages.

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