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Antioxidant Activity and Biological Activity of Specialty Barley (*Hordeum vulgare* L.) Extracts

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ABSTRACT Specialty barley extracts were prepared and investigated for its antioxidant activity and biological activity. Hunter L^* values of the Iksan 86 extracts had higher than that of the Iksan 87 and Zasoojeongchal extracts. The extraction yields of Iksan 86, Iksan 87, and Zasoojeongchal was 8.08, 6.62, and 7.30%, respectively. The contents of total phenolic compounds of the Iksan 86, Iksan 87, and Zasoojeongchal extracts were 16.24, 15.51, and 13.95 GAE mg/g of sample, respectively. The DPPH radical scavenging activity of Iksan 86, Iksan 87, and Zasoojeongchal extracts were 50.00, 33.27, and 7.56% at a 500 ppm, respectively. The samples showed an inhibition of xanthin oxidase. ACE inhibition effect of specialty barley extracts, Iksan 86, Iksan 87, and Zasoojeongchal, was 39.81, 41.06, and 27.78%, respectively. Tyrosinase inhibition rates (%) of Iksan 86, Iksan 87, and Zasoojeongchal extracts were 26.21, 24.57, and 20.00%, respectively. Results indicated that specialty barley extracts possesses various biological activities including antioxidative capacity, xanthin oxidase inhibition activity, angiotensin converting enzyme inhibition activity, and tyrosinase inhibition activity.

Keywords : specialty barley extracts, antioxidant activity, biological activity

Cereal grains contribute significant amounts of energy, protein, selected micronutrients to the human diet and contain a large variety of biologically active substances. Also dietary intake of whole grain foods is associated with a decreased risk of chronic diseases such as diabetes, obesity and heart disease (Hill & Path, 1998; Bondia-Pons *et al*., 2009).

Barley (*Hordeum vulgare* L.) is a well-known cereal grain, cultivated throughout the world and a widely consumed cereal among the most ancient cereal crops. Almost 80-90 % of barley production is for malt and animal feeds, but now barley is gaining renewed interest as an ingredient of production of functional foods due to their concentration of bioactive compounds such as β-glucan, arabinoxylan, and tocols (Jadhav *et al*., 1998; Peterson, 1994). In resent years, specialty barley such as colored, high lysine, high β-glucan has been developed. Also, barley is rich in a wide range of biological compounds such as phenolic acid derivatives, proanthocyanidins, quinones, and flavonoids (Bonoli *et al*., 2004; Kim *et al*., 2007). There have been some studies on the phytochemical, arabinoxylans, tocol, β-glucan, phenolic content, and antioxidant activity of barley (Zhao *et al*., 2008; Panfili *et al*., 2008; Madhujith & Shahidi, 2006; Höije *et al*., 2005; Andersson *et al*., 2008).

In recent years, the demand for natural, functional, and healthy foods has tended to increase. Phytochemical rich plants have played a significant role in diet based therapies to prevent and cure various ailments (Bonoli *et al*., 2004). The whole grain contains a large variety of substances, especially those that are biologically active and demonstrate antioxidant properties, which include free radical scavenger, reducing agents, potential complexes of prooxidant metals, and quenchers of the formation of singlet oxygen (Zieliński, 2002). Rice bran extracts showed antioxidant capability in inhibition linoleic acid peroxidation (57%), scavenging DPPH radicals (93%), reducing power (78%), and Fe^{2+} chelating activity (up to 1300 μg EDTA equivalent/g) (Lai *et al*., 2009). Biological composition such as phenolics can vary in plants from organ to organ, but other factors, such as cultivar and geographical conditions, can also participate in their varia-

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bility in plants (Oliveira *et al*., 2007).

The objective of this study was to investigate the antioxidant activity and biological activities of specialty hull-less whole barley (*Hordeum vulgare* L.) extracts.

MATERIALS AND METHODS

Sample preparation

Specialty barley cv. Iksan 86, Iksan 87, and Zasoojeongchal, waxy type hull-less barley, were supplied from Department of Rice and Winter cereal Crop, National Institute of Crop Science, Korea. Barley was propagated during the 2008-2009 season at Iksan region in Chonbuk province, Korea. The whole barley was grinding using a rotor mill (ZM 100; Retsch GmbH, Haan, Germany) equipped with a 0.5 mm screen and then barley flour were put into plastic bags and stored at -20°C until used. Extraction was performed by using a 70% ethanol solution for 24 hr at room temperature. The ratio of sample and solvent was 1:10 (w/v). After extraction, the ethanol extracts were filtered through a 110 mm filter paper (No 2, Advantec, Japan) twice using 70% ethanol and transferred to a 2-L round-bottom container. The solvent of the extracts were removed by an evaporator (EYELA new Rotary Vacuum Evaporator; Rikakikai Co., Ltd, Tokyo, Japan) immediately and then lyophilized using a lyophilizer (EYELA FD1; Rikakikai Co., Tokyo, Japan). The freeze dried sample was weighed and the yield was calculated. The samples were stored at -20°C for a further investigation.

Color measurement

Color of the extract solutions was measured by a color difference meter (Color JS 55; Color Technology System Co., Japan). The color was measured 3 times for each samples and then averaged. The numerical value of the color was expressed by Hunter L*, a* and b* values. Hunter L* value indicates the lightness of the samples, a* value indicates the +red/-green, and b* value indicates the +yellow/-blue.

Total phenolic contents

Total phenolic contents were measured using the Folin-Ciocalteau colorimetric method (Yu *et al*., 2004). The extract (1%, 0.1 mL) was mixed with 0.2 mL of Folin-Ciocalteau reagent (Sigma Chemical Co., St. Louis, MO, USA). Then 3 mL of 5% Na_2CO_3 was added. The absorbance of the mixture at 765 nm was recorded by a spectrophotometer (HP1B; Hewlett-Packard Co., Germany) for the mixtures after 2 hr of incubation at 23°C. The total phenolic content was expressed as gallic acid equivalents.

Antioxidant activity

Antioxidnat activity was estimated with DPPH and ABT radical scavenging activity. DPPH radical scavenging capacity was estimated according to the method of Blois (1958) with some modification. The sample dissolved in 70% ethanol (500 ppm, 1 mL) was added into the 0.2 mM DPPH radical solution (1 mL) and vortexed. The mixture was reacted for 30 min at room temperature and the absorbance was measured at 517 nm with a spectrophotometer. The scavenging activity of the DPPH radicals in percentage points was calculated by the following equation: Scavenging activity (%) = $(1-A1/A0) \times 100$, where $A0$ is the absorbance of the blank and $A1$ is the absorbance of the sample.

ABTS (2,2`-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salts) radical cation was measured using the method of Zhao *et al*. (2006) with some modification. ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 hr before use. ABTS + solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 30°C. The extract (500 ppm, 0.1 mL) was mixed with 2.9 mL of diluted ABTS + solution. After reaction at 30°C for 20 min, the absorbance at 734 nm was measured.

Xanthine oxidase (XOase) inhibition effect

Xanthine oxidase (XOase) inhibition effect was determined spectrophotometically by measuring uric acid formation at 292 nm (Jeon *et al*., 2002; Nam *et al*., 2005). The reaction mixture, containing 1 mL substrate (2 mM xanthine, Sigma) in a 0.1 M potassium phosphate buffer (pH 7.4), 0.1 mL (0.4 unit/mL, Sigma) of enzyme solution and 0.1 mL of extract solution (250 ppm), was reacted at 37°C for 20 min. The control solution was prepared by adding 0.1 mL

distilled water instead of extracts. The reaction was stopped by adding 1 mL of 20% trichloroacetic acid. The formation of uric acid by reaction was measured at 292 nm using a spectrophotometer (UV 1600PC; Shimadzu, Tokyo, Japan).

Angiotensin converting enzyme (ACE) inhibition activity

Angiotensin converting enzyme (ACE) inhibition activity was assayed by the method of Cushman & Cheung (1971) with some modification. ACE solution was extracted with rabbit lung acetone powder (1g) in 0.1M sodium borate buffer (pH 8.3) containing 0.3 M NaCl (10 mL) at 4°C for 24 hr and then extracted ACE solution was centrifuged at 4,000×*g* for 20 min. A sample solution (250 ppm, 50 μL) with 50 μL of ACE solution was preincubated at 37°C for 5 min and then incubated with 50 μL of substrate (hippuryl-histidyl-leucine, Sigma) at 37°C for 30 min. The reaction was stopped by the addition of 250 μL of 1 N HCl. The resulting hippuric acid was extracted with 1 mL of ethyl acetate. After the solution was centrifuged, the 1 mL upper layer was transferred into a test tube and evaporated at 120°C for 30 min. The hippuric acid was dissolved in 3 mL of distilled water. The ACE inhibition effect (%) was calculated by observing the spectrophotometric reading at 228 nm.

Tyrosinase inhibition effect

Tyrosinase inhibition effect was performed as previously described by Jo *et al.* (2003). A sample (250 ppm, 0.2 mL) was added to a reaction mixture containing 10 mM L-3, 4-dihydroxyphenyl-alanine (L-DOPA; Sigma) solution, 1/15 M sodium phosphate buffer (pH 6.8) and mushroom tyrosinase (100 unit/mL, Sigma). The reaction mixture was incubated at 25°C for 15 min. The amount of dopachrome produced in the reaction mixture was determined at 475 nm by a spectrophotometer (model UV 1600PC; Shimadzu, Tokyo, Japan).

Statistical analysis

This experiment was designed as randomized block design with 3 replications. One-way analyses of variance were performed using SAS software (version 7.0; SAS Institute, Cary, NC, USA) along with Duncan's post hoc tests to compare differences among mean values. Each data entry represents the mean of three different experiments with three measurements in each experiment. Mean values and standard deviation were reported and the significance was defined at $p<0.05$.

RESULTS AND DISCUSSION

Color changes and extraction yield

The extraction yields of Iksan 86, Iksan 87, and Zasoojeongchal was 8.08, 6.62, and 7.30%, respectively (data not shown). The Hunter color value changes of the specialty barley extract solutions are shown in Table 1. The Hunter color L* values of Iksan 86 extract solutions was bright compared with Iksan 87 and Zasoojeongchal extracts. Color a* and b* values of the samples was showed similar results with L* values of the samples. Color is an important characteristic and selection criterion for food choice (Hallagan *et al.*, 1995). Also, food coloring agents such as anthocyanins have also been observed to possess potent antioxidant properties (Duangmal *et al.*, 2008).

Total phenolic compounds

Phenolic compounds were considered as a major group of compounds that contributed to the antioxidant activity of cereal (Zieliński *et al.*, 2000). Total phenolic contents of Iksan 86, Iksan 87, and Zasoojeongchal extracts were 16.24, 15.51, and 13.95 mg/g, respectively (Table 2). Cook & Samman (2006) reported that phenolic compounds are anti-

Table 1. Changes in hunter color values of specialty barley cultivar extracts[1)].

Samples	L*	a*	b*
Iksan 86	90.75±0.02[a]	-4.21±0.01[c]	17.09±0.02[a]
Iksan 87	67.52±0.09[c]	-0.30±0.01[b]	13.67±0.11[b]
Zasoojeongchal	70.36±0.05[b]	-0.27±0.01[a]	11.01±0.05[c]

[1)a-c]Different letters within the same column differ significantly ($p<0.05$).

oxidants and natural antioxidants may exhibit one or more of the following roles; free radical scavenger, reducing agent, potential producer. Phenolic compounds in cereals are either in free or bound forms. Generally, the free phenolic compounds are proanthocyanidins of flavonoids, whereas the bound phenolic compounds are ester-linked to cell wall polymers and consist mainly of ferulic acid and its oxidatively coupled dimmers (Bonoli *et al*., 2004). Most of the free phenolics are flavanols and tocopherols whereas the bound phenolics are mainly phenolic acids (ferulic acid and *p*-coumaric acid) in the barley (Holtekjølen *et al*., 2006). Kim *et al*. (2007) reported that antioxidant capacity of barley is related to the concentration of phenolic compounds such as chlorogenic acid, 3,4-dimethoxybenzoic acid, homogentisic acid, protocatechuin acid, and rutin.

Antioxidant activity of specialty barley extracts

DPPH radical scavenging capacity (%) of Iksan 86, Iksan 87, and Zasoojeongchal extracts were 50.00, 33.27, and 7.56% at a 500 ppm, respectively (Table 2). DPPH radical scavenging activity of the Iksan 86 extracts was higher than that of Iksan 87 and Zasoojeongchal extracts. ABT radical scavenging activity of Iksan 86, Iksan 87, and Zasoojeongchal extracts were 19.68, 19.86, and 1.84 μmol TE/g, respectively, at a 500 ppm level. Zhao *et al*. (2008) reported that the values of DPPH radical scavenging activity for 14 barley samples raged from 9.33 to 11.78 μmol TE/g db. The DPPH radical scavenging activities of the colored barley varied from 46.4 to 86.3% and average radical scavenging activity in the hulless barley groups (66.5%) was higher than that of hulled barley (63.5%) (Kim *et al*., 2007). Liu & Yao (2007) reported that whole barley extract has the strong scavenging activity, which showed similar scavenging activity to BHT at the amount of 200 μg. Qingming *et al*. (2010) reported that malt barley extract prevented the decrease of antioxidant enzyme activities, decreased liver and brain malondialodehyde levels and carbonyl content, and improved total antioxidant capability in D-galactose treated mice.

Xanthin oxidase and angiotensin converting enzyme inhibition activity

Table 3 shows the xanthin oxidase (XOase) and angiotensin converting enzyme (ACE) inhibition activity of the specialty barley extracts. The order of xanthin oxidase inhibition effect for the samples was as follows: Zasoojeongchal extracts > Ikdan 87 extracts > Iksan 86 extracts. However, there was no difference between the samples (p>0.05). Xanthine oxidase catalyzed the metabolism of hypoxanthine and xanthine to uric acid (Kong *et al*., 2000). Increase of the uric acid and its accumulation in blood and bone result in gout and accumulation in the kidney, occasionally causing kidney trouble (Stroch & Ferber, 1988). Nam *et al*. (2006) reported that xanthin oxidase (XOD) inhibition assay suggest that the superoxide scavenging by rice cultivars extracts might be

Table 2. Total phenolic compounds (mg/g samples) and free radical scavenging activity of specialty barley cultivar extracts[1)].

Samples	Total phenolics (mg/g)	DPPH radical scavenging capacity (%)	ABT radical scavenging capacity (μmol TE/g)
Iksan 86	16.24±0.682[a]	50.00±2.259[a]	19.68±1.920
Iksan 87	15.51±1.686[ab]	33.27±6.718[b]	19.86±0.606
Zasoojeongchal	13.95±1.949[b]	7.56±7.762[c]	18.84±4.458

[1)a-b]Different letters within the same column differ significantly (p<0.05).

Table 3. Xanthin oxidase and angiotensin converting enzyme inhibition activity of specialty barley cultivar extracts[1)].

Samples[2)]	Xanthin oxidase inhibition activity (%)	ACE inhibition activity (%)
Iksan 86	10.31±1.48	39.81±0.904[b]
Iksan 87	12.05±1.80	41.06±4.631[a]
Zasoojeongchal	13.78±1.59	27.78±2.495[c]

[1)a-c]Different letters within the same column differ significantly (p<0.05).

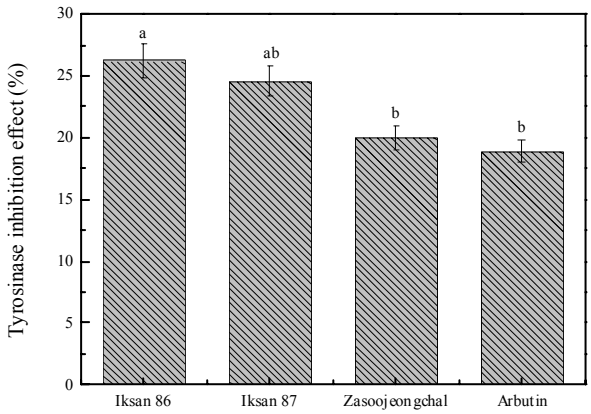

Fig. 1. Tyrosinase inhibition effect of specialty barley extracts[1)].
[1)a-b]Different letters within the same row differ significantly ($p<0.05$).

caused by a blockage of enzyme action of XOD, not by direct quenching of the radicals at concentrations of 5 mg/mL.

Angiotensin converting enzyme (ACE) is an enzyme in the rennin-angiotensin system, and converts and inactive peptide, angiotensin I to angiotensin II which has a hypertensive function. Inhibition of ACE activity therefore results in decrease of blood pressure (Murakami, 1993). ACE inhibition effect of specialty barley extracts, Iksan 86, Iksan 87, and Zasoojeongchal, was 39.81, 41.06, and 27.78%, respectively (Table 3). ACE inhibitory matter has been discovered in various food sources such as wheat, maize, soybean, milk, and gelatine (Vercruysse *et al*., 2005).

Tyrosinase inhibition activity of specialty barley extracts

Tyrosinase is a multifunctional copper-containing enzyme (mono-, and diphenolase activities) which is involved in the synthesis of melanin. The sources of tyrosinase inhibitors are found mainly in microorganisms and plants (Wu *et al*., 2003). As shown in Fig. 1, the inhibition rates (%) of the mushroom tyrosinase of Iksan 86, Iksan 87, and Zasoojeongchal extracts were 26.21, 24.57, and 20.00% at 250 ppm, respectively. Also, arbutin was a naturally occurring cosmetic vehicle and whitening agent with tyrosinase inhibitory activity (Barsoom *et al*., 2006). In our study, the tyrosinase inhibition rate of the specialty barley extract higher than that of the arbutin solution.

In conclusion, we found that Iksan 86 barley cultivar extracts shown the highest degrees of total phenolic compound, DPPH and ABT radical scavenging activity, and tyrosinase inhibition activity. In addition, Iksan 87 and Zasoojeongchal cultivar extracts also possesses various biological activities including antioxidative activity, xanthin oxidase inhibition activity, angiotensin converting enzyme inhibition activity, and tyrosinase inhibition activity. These results suggest that specialty barley cultivar extracts can be considered a potential source of a functional ingredient.

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