



Research Article

# Physicochemical properties and physiological activity of bean sprouts extract containing *Hovenia dulcis* Thunb concentrates

Ji-An Heo<sup>1</sup>, Wool-Lim Park<sup>1</sup>, Hye-Ji Min<sup>1</sup>, Jeong-Ho Kim<sup>2</sup>, Yeong-Seon Won<sup>3</sup>, Kwon-Il Seo<sup>1\*</sup>

<sup>1</sup>Department of Food Biotechnology, Dong-A University, Busan 49315, Korea

<sup>2</sup>Suncheon Research Center for Bio Health Care, Suncheon 57922, Korea

<sup>3</sup>Immunoregulatory Material Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Jeongseup 56212, Korea

**Abstract** Reactive oxygen species are the byproducts of metabolic processes in the body. However, excessive amount may cause side effects such as cancer. Therefore, to reduce the production of these species, but their long-term administration at high doses may induce side effects. Hence, natural materials with antioxidant activities are attracting attention. Two of these natural materials are soybean sprouts and *Hovenia dulcis* Thunb. fruits, but few studies have evaluated the effects of their combination. Thus, we prepared a soybean sprout extract containing 1.5% *H. dulcis* Thunb. fruit concentrate (BHM) to develop a functional food material derived from natural products and then confirmed its physicochemical properties and physiological activity. Among the organic acids detected in BHM, malic acid exhibited the highest content of 1,451.03 ppm, and the main free sugars were glucose (645.48 ppm) and fructose (738.11 ppm). Taurine was the most abundant free amino acid at a concentration of 11.95 ppm, followed by those of arginine (10.97 ppm) and glutamic acid (10.16 ppm). Analyses of the mineral components revealed large amounts of Zn and Fe in BHM, and the respective total polyphenol and flavonoid contents in BHM were 957.16 and 601.93 ppm. The DPPH radical and H<sub>2</sub>O<sub>2</sub> scavenging activities and reducing power indicated excellent antioxidant efficacy compared to the positive controls. Furthermore, blood alcohol and acetaldehyde concentrations were measured to confirm the hangover-relieving effects of BHM, with both significantly decreased ( $p < 0.05$ ). BHM displays potential for development as a functional food, and the results of this study may be used as basic data in further research.

**Keywords** soybean sprouts, *Hovenia dulcis* Thunb., antioxidant, alcohol, acetaldehyde



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**\*Corresponding author**

Kwon-Il Seo

Tel: +82-51-200-7565

E-mail: kseo@dau.ac.kr

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## 1. Introduction

Reactive oxygen species (ROS) are the byproducts of metabolic processes in the body. They include H<sub>2</sub>O<sub>2</sub>, hydroxyl radicals, singlet oxygen, and superoxide anions. The production of an adequate amount of ROS is critical in cell functions from the activation of white blood cells to intracellular signaling. However, an

excessive amount of ROS may cause various adverse conditions, such as cancer, inflammation, and aging, by damaging cells and tissues (Benzi and Moreti, 1995; Fiers et al., 1999; Fukuzawa and Takasoshi, 1990; Halliwell et al., 1992; Rhim and Choi, 2010). To reduce ROS production, synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxytoluene (BHT), have been used therapeutically. However, legal restrictions have been enacted regarding their methods of use and dosages, as their long-term administration at high dosages may induce side effects, such as cancer (Lee et al., 2012; Shin, 1999; Yen and Hsieh, 1998). Thus, to overcome the instabilities of synthetic antioxidants, an increasing number of studies have been reported recently regarding natural materials with antioxidant activities (Masaki et al., 1995), ranging from various fruits (Cho et al., 2005; Kim et al., 2021) to vegetables (Lee et al., 2009; Nam and Kim, 2017) and herbal medicines (Lee and Ryu, 2019; Lim et al., 2017).

Soybean, which originates from the Manchurian region, has been cultivated in Korea since the period of the Three Kingdoms. It is a food with an outstanding amino acid composition and protein content (Rhim et al., 2003; Shon et al., 2009). Via soybean germination, soybean sprouts are obtained as an excellent nutritional food with an enhanced rate of digestion, as starch is degraded to monosaccharides and the contents of vitamins, proteins, and fibrins increase (Collins and Sand, 1976; Kim et al., 1993). Soybean sprouts also facilitate the production of nicotinamide adenine dinucleotide in the body to promote the synthesis of alcohol dehydrogenase, and thus, they exhibit a remarkable hangover-relieving effect (Park, 1994). Furthermore, soybean sprouts contain isoflavone, which improves menopausal symptoms and prevents osteoporosis, in addition to

its anticancer and antioxidant effects, with a role in immunity, along with vitamin C, and a preventive effect on cardiovascular disease, along with fatty acids (Choi et al., 2000; Kim et al., 2003a). Thus, previous studies reported the hypoglycemic effect of a methanol extract of soybean sprouts in rats with streptozotocin-induced diabetes (Kim et al., 2003b) and the effect of a beverage containing an extract of soybean sprouts cultivated in high-concentrated oxygen water on alcohol degradation (Sung et al., 2014). Another study reported the changes in the physicochemical and sensory properties of a soybean sprout extract caused by stir-frying (Hong et al., 2020). Ongoing studies are investigating processed foods containing soybean sprouts as their main ingredients.

*Hovenia dulcis* Thunb. is a deciduous broad-leaved tree of the Rhamnaceae family that is cultivated in regions of East Asia, including Korea, Japan, and China (Choi et al., 2014; You et al., 2009). The *H. dulcis* Thunb. fruit is approximately 8 mm in diameter, with a delicate aroma and sweetness. *H. dulcis* Thunb. fruits contain compounds such as ampelopsin, with an outstanding hangover-relief effect, and thus, they prevent liver damage and improve liver function in contributing to recovery from fatigue (Kim et al., 2000; Kim et al., 2011; Lim et al., 2007; Park et al., 2015). Other effects include antiosteoporotic, antiarthritic, and antistress and antioxidant activities, but few studies evaluate the effects of combinations with soybean sprouts (Cha et al., 2014; Na et al., 2013).

Thus, this study investigated the synergistic effect of the combined use of soybean sprouts and *H. dulcis* Thunb. fruits by measuring the physicochemical properties and bioactivities to provide basic data regarding the potential use of both materials as ingredients in functional food products.

## 2. Materials and methods

### 2.1. Test materials and sample preparation

The soybean sprouts, clam and mussel meat, pen shell scallops, kelp, dried shiitake mushrooms, leeks, daikon, onions, and bay salt used in this study were purchased as domestic raw materials. *H. dulcis* Thunb. fruit concentrate with 60 °Brix was purchased from Himchanstep (Iksan, Korea) and stored at 4°C prior to use. The mixing ratio of the materials used in preparing the extract of the raw material mixture is shown in Table 1. Kelp and dried shiitake mushrooms were added to purified water and extracted for 1 min at 100°C. After removing the kelp, clam and mussel meat and pen shell scallops were added and extracted for 2 h, and then leeks, daikon, onions, and bay salt were added and extracted for 35 min. Soybean sprouts (33% w/w) were added to the resulting extract of the mixture (67%) and extracted 100°C for 20 min. To obtain the soybean sprout extract (BSE), the solid materials were removed via filtration. The *H. dulcis* Thunb. fruit concentrate was added to purified water to produce 1.5% w/v *H. dulcis* Thunb. fruit extract

**Table 1.** Composition of soybean sprout extract's (BSE) subsidiary ingredients

Component	Subsidiary ingredients	
	Mix ratio (%)	Quantity (g)
Manila clam meat	7	70
Pen sell scallop	2	20
Sea mussel meat	2	20
Kelp	0.1	1
Desiccated shiitake	0.07	0.7
Welsh onion	1.2	12
Dakion	1	10
Onion	0.8	8
Bay salt	0.2	2
Water	85.63	856.3

(HDE), and a soybean sprout extract (BHM) containing *H. dulcis* Thunb. fruit concentrate was prepared by adding the 1.5% w/v *H. dulcis* Thunb. fruit concentrate to the BSE. All samples used in the subsequent studies were the undiluted original solution.

### 2.2. Analyses of organic acid and free sugar contents

The methods described by Ko et al. (1998) were used. After 10 min centrifugation of the sample at 890×g, the supernatant was collected and filtered using a 0.45 μm membrane filter (Advantec MFS, Inc., Dublin, CA, USA) through a Sep-Pak C18 cartridge (Waters, Milford, MA, USA). Twenty microliters of the filtrate was analyzed using high-performance liquid chromatography (Prominence Modular HPLC, Shimadzu Co., Kyoto, Japan; Alliance, Waters Co., Milford, MA, USA).

### 2.3. Free amino acid analysis

Sulfosalicylic acid (25 mg, Sigma-Aldrich Co., St. Louis, MO, USA) was added to 10 mL of the sample, and the mixture was stored at 4°C for 4 h and then centrifuged. The supernatant was collected and filtered using a 0.2 μm membrane filter (Pall Co., Port Washington, NY, USA), and an amino acid auto-analyzer (Biochrom 20, Pharmacia Biotech, Uppsala, Sweden) was used in the analysis.

### 2.4. Mineral analysis

The sample (100 mL) was mixed with 25 mL of digestive fluid (HClO<sub>4</sub>:H<sub>2</sub>SO<sub>4</sub>:H<sub>2</sub>O<sub>2</sub> = 9:2:5, v/v/v), and the mixture was heated until it became transparent and filtered using a No. 2 filter paper (Advantec MFS, Inc., Dublin, CA, USA). The filtrate was added to distilled water to yield a final volume of 100 mL, and inductively coupled plasma (Aton scan 25, Thermo Jarrell Ash, Franklin, MA, USA) was used in the analysis.

### 2.5. Determination of total phenol and flavonoid contents (TPC and TFC)

The TPC was measured using the Folin-Ciocalteu method (Gao et al., 2000). Distilled water (8.4 mL), 0.5 mL of 2 N Folin-Ciocalteu reagent (Sigma-Aldrich Co.), and 1 mL of 20% w/v Na<sub>2</sub>CO<sub>3</sub> (Junsei Chemical Co., Ltd, Tokyo, Japan) were added to 0.1 mL of the 10-fold diluted sample. The mixture was stored for 2 h, and its absorbance was then measured at 725 nm using an ultraviolet-visible (UV/Vis) spectrophotometer (U-1800, Hitachi Ltd., Tokyo, Japan). To quantify the TPC of the extract, a calibration curve was plotted using a diluted solution of gallic acid (Sigma-Aldrich Co.), and the TPC was expressed in  $\mu\text{g}$  gallic acid equivalent (GAE)/g.

The TFC was measured using the method described by Abdel-Hameed (2009) with modifications. NaNO<sub>2</sub> (5%, 0.15 mL, Sigma-Aldrich Co.) was added to 1 mL of the 10-fold diluted sample and reacted for 6 min at 25°C. AlCl<sub>3</sub> (10%, 0.3 mL, Sigma-Aldrich Co.) was then added, and the mixture was stored for 5 min, followed by the addition of 1 mL of 1 N NaOH (Sigma-Aldrich Co.). The absorbance was measured at 510 nm using the UV/Vis spectrophotometer (U-1800, Hitachi). To quantify the TFC of the extract, a calibration curve was plotted using a diluted solution of quercetin (Sigma-Aldrich Co.), and the TFC was expressed in  $\mu\text{g}$  quercetin equivalent (QE)/g.

### 2.6. Measurements of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) scavenging activities and reducing power

To measure the DPPH radical scavenging activity, the method described by Blois (1958) based on the reducibility of DPPH was used. DPPH (0.4 mM, 3 mL, Sigma-Aldrich Co.) was added to 1 mL of the sample. The mixture was vortexed for 5 sec and

then allowed to react in darkness for 30 min. The absorbance was measured at 517 nm using the UV/Vis spectrophotometer (U-1800, Hitachi), and 0.1% BHT and 0.1%  $\alpha$ -tocopherol were used as controls.

To measure the reducing power, the method described by Yildirim et al. (2001) was used after modification. Phosphate buffer (pH 6.6, 0.2 M, 2.5 mL) and 2.5 mL of K<sub>3</sub>[Fe(CN)<sub>6</sub>] (1% w/v, Junsei Chemical Co., Ltd., Tokyo, Japan) were added to 1 mL of the sample, and the mixture was reacted at 50°C for 30 min. Trichloroacetic acid (2.5 mL, 10% w/v, Sigma-Aldrich Co.) was added to the mixture to stop the reaction, and the solution was then centrifuged at 3,000 rpm for 10 min. One milliliter of the supernatant was collected and mixed with 1 mL of distilled water and 0.2 mL of FeCl<sub>3</sub> (0.1% w/v, Sigma-Aldrich Co.). The absorbance was measured at 700 nm using the UV/Vis spectrophotometer (U-1800, Hitachi).

To measure the H<sub>2</sub>O<sub>2</sub> scavenging activity, the method described by Muler (1985) was used. The sample (100  $\mu\text{L}$ ) and 20  $\mu\text{L}$  of H<sub>2</sub>O<sub>2</sub> (Junsei Chemical Co., Ltd, Tokyo, Japan) were added to a 96 microwell plate and allowed to react in an incubator for 5 min at 37°C. Subsequently, 30  $\mu\text{L}$  of 1.25 mM 2,2'-azino-bis (3-ethylbenzothiazoline-6- sulfonic acid) (Sigma-Aldrich Co.) and peroxidase (1 unit/mL, Sigma-Aldrich Co.) were added and allowed to react in the incubator for 10 min at 37°C. The absorbance was measured at 405 nm using the UV/Vis spectrophotometer (U-1800, Hitachi).

### 2.7. Animal study

The experimental animals were 24 male 5-week-old Sprague-Dawley rats purchased from Hyochang Science (Daegu, Korea). The animal study was conducted with the approval of the Institutional

Animal Care and Use Committee at Dong-A University, Busan, Korea (DIACUC-20-19). The animal breeding conditions were set at constant levels of temperature ( $22\pm 2^\circ\text{C}$ ), humidity ( $50\%\pm 5\%$ ), and 12 h light-dark cycles, and the rats were provided with liberal amounts of food and water. Via randomization, six rats were assigned to each of the following four groups: alcohol-only (Con), BSE, HDE and BHM. Each sample was orally administered at 7 mL/kg, and after 30 min, 5 mL/kg of 40% alcohol was orally administered. Blood was collected from the caudal vein after 30, 60, and 90 min to measure the level of alcohol and after 3, 6, and 9 h to measure the level of acetaldehyde. The collected blood was stored at  $25^\circ\text{C}$  for 30 min and then centrifuged at  $14,240 \times g$  for 10 min to isolate the plasma.

### 2.8. Measurement of blood alcohol and acetaldehyde concentrations

The blood alcohol and acetaldehyde concentrations were measured utilizing commercial kits for use in ethanol and acetaldehyde analyses (K-ETOHLQR and K-ACHYD, Megazyme, Bray, Ireland).

### 2.9. Statistical analysis

The mean and standard deviation were obtained using one-way analysis of variance for significant changes in each sample. The significance was evaluated by Duncan's multiple range test using the Statistic Analysis System software 9.4, SAS institute Inc., Cary, NC, USA) ( $p < 0.05$ ).

## 3. Results and discussion

### 3.1. Organic acid, free sugar, and free amino acid contents in BHM

Considerable amounts of organic acids occur in foods, and they markedly affect food flavor (Joo et

al., 1996). Table 2 shows the measured organic acid contents in BHM. Malic acid, with the highest content among those of the detected organic acids, enhances food flavor (Woo et al., 2012), and the respective detected amounts in BSE, HDE, and BHM are 559.32, 919.84, and 1,451.03 ppm. Thus, *H. dulcis* Thunb. fruit concentrate addition to BSE should enhance the flavor via changes in the organic acid composition.

Table 3 shows the analyzed contents and composition of free sugars in BHM. The respective sucrose contents in BSE, HDE, and BHM are 60.59, 294.60, and 375.09 ppm. The respective glucose contents are 215.03, 368.45, and 645.48 ppm, and the respective fructose contents are 257.18, 432.31,

**Table 2. Organic acid contents of BHM**

Organic acid	Contents (ppm)		
	BSE <sup>1)</sup>	HDE	BHM
Citric acid	118.07 $\pm$ 0.05 <sup>b2)</sup>	13.11 $\pm$ 0.01 <sup>c</sup>	124.68 $\pm$ 0.03 <sup>a</sup>
Malic acid	559.32 $\pm$ 0.03 <sup>c</sup>	919.84 $\pm$ 0.08 <sup>b</sup>	1,451.03 $\pm$ 0.04 <sup>a</sup>
Lactic acid	ND <sup>3)</sup>	4.36 $\pm$ 0.05	ND
Acetic acid	ND	ND	7.00 $\pm$ 0.02

<sup>1)</sup>BSE, soybean sprouts extract; HDE, 1.5% *Hovenia dulcis* Thunb. extract; BHM, soybean sprouts and 1.5% *Hovenia dulcis* Thunb. mixture.

<sup>2)</sup>Values are mean $\pm$ SD (n=3). <sup>a-c</sup>Means with different letters within the same row are significantly different ( $p < 0.05$ ).

<sup>3)</sup>Not detected.

**Table 3. Free sugars contents of BHM**

Free sugars	Contents (ppm)		
	BSE <sup>1)</sup>	HDE	BHM
Sucrose	60.59 $\pm$ 0.07 <sup>c2)</sup>	294.60 $\pm$ 0.02 <sup>b</sup>	375.09 $\pm$ 0.01 <sup>a</sup>
Glucose	215.03 $\pm$ 0.05 <sup>c</sup>	368.45 $\pm$ 0.01 <sup>b</sup>	645.48 $\pm$ 0.03 <sup>a</sup>
Fructose	257.18 $\pm$ 0.02 <sup>c</sup>	432.31 $\pm$ 0.05 <sup>b</sup>	738.11 $\pm$ 0.02 <sup>a</sup>

<sup>1)</sup>BSE, soybean sprouts extract; HDE, 1.5% *Hovenia dulcis* Thunb. extract; BHM, soybean sprouts and 1.5% *Hovenia dulcis* Thunb. mixture.

<sup>2)</sup>Values are mean $\pm$ SD (n=3).

<sup>a-c</sup>Means with different letters within the same row are significantly different ( $p < 0.05$ ).

and 738.11 ppm. The results are consistent with the results of Park and Kim (2005) identification of glucose (7.25%) and fructose (5.38%) as the main free sugars in the *H. dulcis* Thunb. fruit. The results are also consistent with the free sugar contents reported by Kim (2004), where the degradation of sucrose during heating increased the contents of the products, glucose and fructose. Thus, heating during BSE production likely increases the contents of glucose and fructose, in addition to the increases in the sugar contents upon HDE addition.

Free amino acids react rapidly with other compounds in food materials. Notably, as they encounter sugars, reactions such as browning may occur to enhance the color and flavor or reduce the quality of food (Kim and Ahn, 2014). Table 4 shows the analyzed contents and composition of free amino acids in BHM. The most abundant free amino acids in BSE and BHM are taurine, arginine, and glutamic acid, with proline the most abundant in HDE. However, the content of proline is higher in BHM (2.92 ppm) than BSE (1.78 ppm) and HDE (1.18 ppm). The result is consistent with that reported by Oh et al. (1990): the extracts of the fruits of *Lycium barbarum*, *Angelica gigas*, *Eleutherococcus senticosus*, and *Schisandra chinensis* displayed variations in the contents and types of eluted free amino acids. The result is also consistent with that reported by Im and Lee (2019): the treatment conditions of an extract of burdock tea led to changes in the contents of the detected free amino acids. In addition, Satoh et al. (2002) reported that proline was crucial in free radical scavenging. Therefore, our result suggests that the free amino acid contents may vary due to differences in sample type, processing method, and mixing ratio. Additionally, our result suggests that further studies should verify the synergistic effect

**Table 4. Free amino acid contents of BHM**

Free amino acids	Contents (ppm)		
	BSE <sup>1)</sup>	HDE	BHM
Phosphoserine	0.63±0.01 <sup>a2)</sup>	0.23±0.10 <sup>b</sup>	0.65±0.07 <sup>a</sup>
Taurine	12.95±0.05 <sup>a</sup>	0.04±0.02 <sup>c</sup>	11.95±0.03 <sup>b</sup>
Aspartic acid	2.27±0.09 <sup>a</sup>	0.08±0.05 <sup>b</sup>	2.24±0.01 <sup>a</sup>
Threonine	3.28±0.12 <sup>a</sup>	0.03±0.01 <sup>c</sup>	3.04±0.04 <sup>b</sup>
Serine	6.40±0.05 <sup>a</sup>	0.03±0.01 <sup>c</sup>	6.01±0.28 <sup>b</sup>
Glutamic acid	10.58±0.02 <sup>a</sup>	0.31±0.07 <sup>c</sup>	10.16±0.03 <sup>b</sup>
Glycine	8.07±0.04 <sup>a</sup>	ND <sup>3)</sup>	7.44±0.07 <sup>b</sup>
Alanine	7.85±0.10 <sup>a</sup>	0.32±0.03 <sup>c</sup>	7.48±0.05 <sup>b</sup>
Valine	6.31±0.01 <sup>a</sup>	ND	5.78±0.01 <sup>b</sup>
Methionine	0.51±0.05 <sup>a</sup>	ND	0.33±0.01 <sup>b</sup>
Isoleucine	3.34±0.05 <sup>a</sup>	0.02±0.01 <sup>c</sup>	3.08±0.02 <sup>b</sup>
Leucine	2.76±0.23 <sup>a</sup>	0.03±0.02 <sup>b</sup>	2.59±0.03 <sup>b</sup>
Tyrosine	1.32±0.08 <sup>a</sup>	ND	1.32±0.01 <sup>a</sup>
Phenylalanine	7.39±0.01 <sup>a</sup>	ND	6.89±0.06 <sup>b</sup>
$\beta$ -Alanine	2.29±0.02 <sup>a</sup>	ND	1.37±0.03 <sup>b</sup>
$\gamma$ -Amino-n-butyric acid	1.73±0.07 <sup>b</sup>	0.28±0.03 <sup>c</sup>	1.96±0.01 <sup>a</sup>
Ornithine	0.09±0.08 <sup>a</sup>	ND	0.08±0.07 <sup>a</sup>
Lysine	3.11±0.03 <sup>a</sup>	ND	2.91±0.05 <sup>b</sup>
Histidine	3.27±0.05 <sup>a</sup>	ND	2.90±0.02 <sup>b</sup>
Arginine	11.82±0.01 <sup>a</sup>	0.09±0.03 <sup>c</sup>	10.97±0.21 <sup>b</sup>
Proline	1.78±0.02 <sup>b</sup>	1.18±0.14 <sup>c</sup>	2.92±0.01 <sup>a</sup>

<sup>1)</sup>BSE, soybean sprouts extract; HDE, 1.5% *Hovenia dulcis* Thunb. extract; BHM, soybean sprouts and 1.5% *Hovenia dulcis* Thunb. mixture.

<sup>2)</sup>Values are mean±SD (n=3). <sup>a-c</sup>Means with different letters within the same row are significantly different (p<0.05).

<sup>3)</sup>Not detected.

and investigate the antioxidant activities and bioactive materials in detail.

### 3.2. Mineral analysis of BHM

Table 5 shows the analyzed contents of minerals in BHM. Eight minerals, i.e., Zn, Cu, Fe, Mn, K, Ca, Na, and Mg, are detected. The respective contents of Zn in BSE, HDE, and BHM are the highest at 46.31, 16.74, and 36.28 ppm. The contents of Fe and Mn are higher in BHM than those in BSE or

**Table 5.** Mineral contents of BHM

Component	Contents (ppm)		
	BSE <sup>1)</sup>	HDE	BHM
Zn	46.31±0.03 <sup>a2)</sup>	16.74±0.01 <sup>c</sup>	36.28±0.01 <sup>b</sup>
Cu	2.04±0.11 <sup>a</sup>	0.21±0.20 <sup>c</sup>	0.79±0.03 <sup>b</sup>
Fe	16.86±0.07 <sup>b</sup>	3.77±0.03 <sup>c</sup>	20.15±0.05 <sup>a</sup>
Mn	2.09±0.23 <sup>b</sup>	1.56±0.17 <sup>c</sup>	2.84±0.02 <sup>a</sup>
K	0.67±0.01 <sup>a</sup>	0.15±0.05 <sup>c</sup>	0.33±0.04 <sup>b</sup>
Ca	0.58±0.05 <sup>a</sup>	0.08±0.03 <sup>b</sup>	0.55±0.04 <sup>a</sup>
Na	0.49±0.15 <sup>a</sup>	ND <sup>3)</sup>	0.42±0.01 <sup>a</sup>
Mg	0.66±0.03 <sup>a</sup>	0.08±0.01 <sup>c</sup>	0.60±0.02 <sup>b</sup>

<sup>1)</sup>BSE, soybean sprouts extract; HDE, 1.5% *Hovenia dulcis* Thunb. extract; BHM, soybean sprouts and 1.5% *Hovenia dulcis* Thunb. mixture.

<sup>2)</sup>Values are mean±SD (n=3). <sup>a-c)</sup>Means with different letters within the same row are significantly different (p<0.05).

<sup>3)</sup>Not detected.

HDE: respective Fe contents of 16.86, 3.77, and 20.15 ppm in BSE, HDE, and BHM, with respective Mn contents of 2.09, 1.56, and 2.84 ppm. Therefore, the antioxidant efficacy of BHM should be high, with high contents of Zn, Fe, and Mn, which are involved in the inhibition of ROS (Lee et al., 2003).

### 3.3. TPC and TFC in BHM

The TPC and TFC of BHM are shown in Table 6. The TPC in BHM is high at 957.16 ppm, followed by those in BSE and HDE at 531.47 and 500.63 ppm, respectively. A similar trend is observed for the TFC, with a high level of 601.93 ppm in BHM, followed by those of BSE (373.21 ppm) and HDE (367.07 ppm). Natural antioxidants containing vitamins, carotenoids, phenols, and other such compounds serve as effective bioactive materials in preventing oxidative stress, which causes adverse conditions, including allergies, inflammation, and arteriosclerosis (Van, 2016). Additionally, polyphenolic compounds exhibit that may readily bind to proteins and various other compounds as aromatic

**Table 6.** Total polyphenol and flavonoid contents of BHM

Sample	Contents (ppm)	
	Total polyphenol (μg GAE/g <sup>1)</sup> )	Total flavonoid (μg QE/g <sup>2)</sup> )
BSE <sup>3)</sup>	531.47± 6.42 <sup>b4)</sup>	373.21±11.95 <sup>b</sup>
HDE	500.63±5.50 <sup>c</sup>	367.07±14.42 <sup>b</sup>
BHM	957.16±10.47 <sup>a</sup>	601.93±13.80 <sup>a</sup>

<sup>1)</sup>GAE, gallic acid equivalent.

<sup>2)</sup>QE, quercetin equivalent.

<sup>3)</sup>BSE, soybean sprouts extract; HDE, 1.5% *Hovenia dulcis* Thunb. extract; BHM, soybean sprouts and 1.5% *Hovenia dulcis* Thunb. mixture.

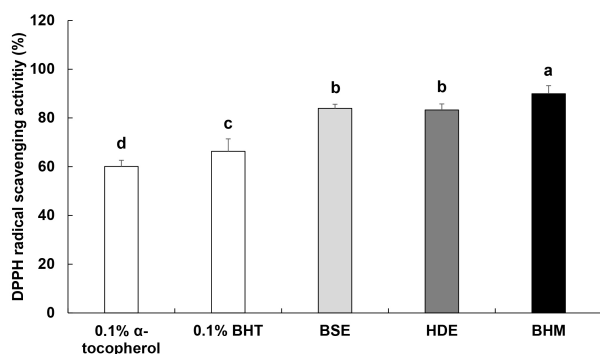
<sup>4)</sup>Values are mean±SD (n=3).

<sup>a-c)</sup>Means with different superscripts in the same column indicate significant differences (p<0.05) by Duncan's multiple range tests.

compounds, and thus, display outstanding anticancer and -oxidant and anti-inflammatory effects (Kang et al., 2002a; Lee et al., 2008). This may be the cause of the excellent antioxidant efficacy of BHM in this study, as it is enriched with polyphenols and flavonoids.

### 3.4. DPPH radical and H<sub>2</sub>O<sub>2</sub> scavenging activities and reducing power of BHM

DPPH radical scavenging activity is measured to determine the radical scavenging activity of a given antioxidant material based on the change in absorbance as the antioxidant material donates hydrogen ions or electrons to return radicals to stabilized states (Lee et al., 2007). The electron-donating capacity of BHM is shown in Fig. 1. The respective measured activities of the positive controls 0.1% α-tocopherol and 0.1% BHT are 60.93% and 66.30%, and the respective activities of BSE, HDE, and BHM are 83.94%, 83.26%, and 89.94%. The result is consistent with the DPPH radical scavenging activities of the water extracts of *H. dulcis* Thunb. leaves and fruit reported by Jeong and Shim (2000). However, the result deviates from the slightly low DPPH radical scavenging activity of

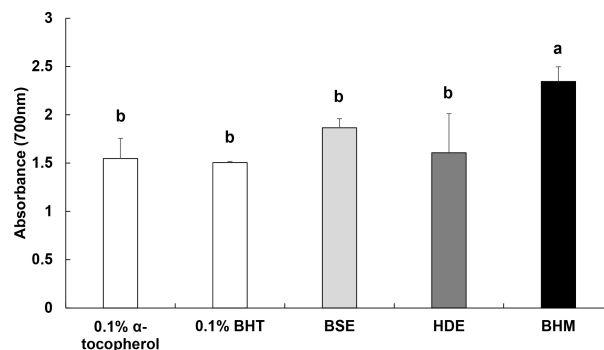


**Fig. 1. DPPH radical scavenging activity of BHM.** Values are mean $\pm$ SD (n=3). Means with different letters (a-d) indicate significant difference ( $p < 0.05$ ) by Duncan's multiple range tests. BSE, soybean sprouts extract; HDE, 1.5% *Hovenia dulcis* Thunb. extract; BHM, soybean sprouts and 1.5% *Hovenia dulcis* Thunb. mixture.

the heated extract of a mixture of mussel meat and soybean sprouts reported by Ok et al. (2014). This is likely due to the differences in the contents of antioxidant materials in each added ingredient, and thus, BSE, HDE, and BHM exhibit high DPPH radical scavenging activities in this study.

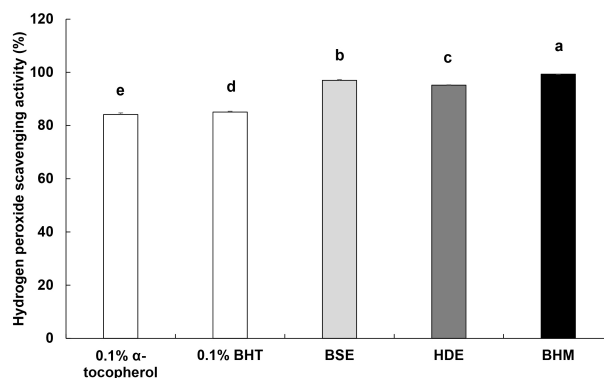
Reducing power is measured to identify the antioxidant activity based on the absorbance upon the conversion of  $Fe^{3+}$  to  $Fe^{2+}$  mediated by an antioxidant material (Kim et al., 2000). The measured reducing power of BHM is shown in Fig. 2. The respective reducing power values of BSE and HDE are 1.87 and 1.61, which do not vary significantly from those of 0.1%  $\alpha$ -tocopherol and 0.1% BHT of 1.55 and 1.51, respectively. Conversely, the reducing power of BHM of 2.35 is the highest. Shiddhuraju et al. (2002) reported that the reducing power increases as the level of coloration increases, with a strong correlation between reducing power and antioxidant activity. Hence, BHM, with the highest absorbance, likely displays an excellent antioxidant efficacy.

$H_2O_2$  exhibits effects such as aging and damages DNA, as it generates a high toxicity in the body via



**Fig. 2. Reducing power of BHM.** Values are mean $\pm$ SD (n=3). Means with different letters (a, b) indicate significant difference ( $p < 0.05$ ) by Duncan's multiple range tests. BSE, soybean sprouts extract; HDE, 1.5% *Hovenia dulcis* Thunb. extract; BHM, soybean sprouts and 1.5% *Hovenia dulcis* Thunb. mixture.

hydroxyl radicals (Kang et al., 2002b). The measured  $H_2O_2$  scavenging activity of BHM is shown in Fig. 3. The respective scavenging activities of the positive controls 0.1%  $\alpha$ -tocopherol and 0.1% BHT are 84.14% and 85.08%, and BHM exhibits the highest activity of 99.33%, followed by those of BSE and HDE at 96.50% and 95.20%, respectively. Lee et al. (2005) reported that the ROS scavenging activity and polyphenolic content correlate positively, and thus, the outstanding  $H_2O_2$  scavenging activity of



**Fig. 3. Hydrogen peroxide scavenging activity of BHM.** Values are mean $\pm$ SD (n=3). Means with different letters (a-e) indicate significant difference ( $p < 0.05$ ) by Duncan's multiple range tests. BSE, soybean sprouts extract; HDE, 1.5% *Hovenia dulcis* Thunb. extract; BHM, soybean sprouts and 1.5% *Hovenia dulcis* Thunb. mixture.



BHM is likely proportional to its high TPC, as shown in Table 6.

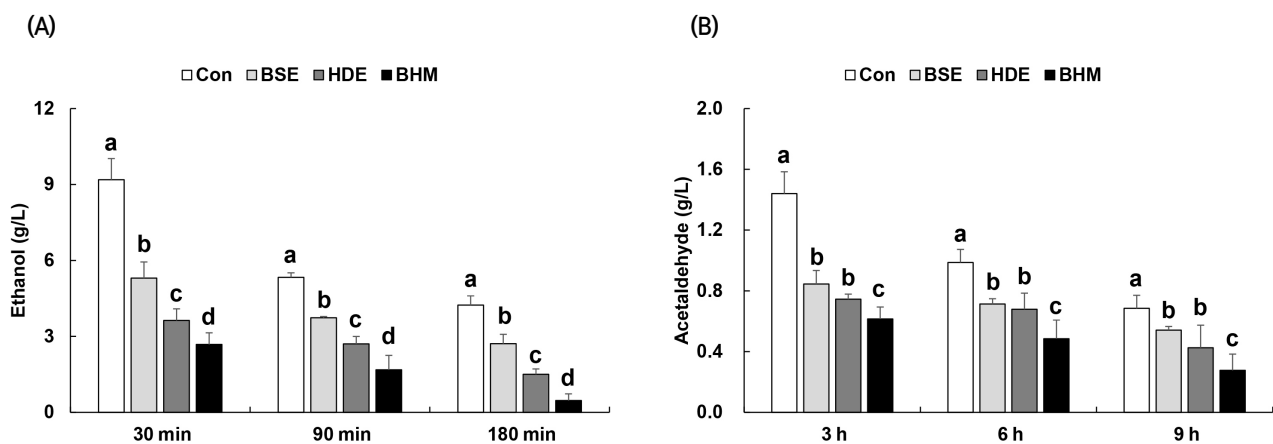
### 3.5. Hangover-relieving effect of BHM

Sixty to ninety percent of alcohol is absorbed within 30 min of ingestion. The accumulation of acetaldehyde produced during alcohol oxidation, rather than the alcohol itself, causes liver damage and the hangover (Choi et al., 2014; Shumate et al., 1967). The concentrations of alcohol and acetaldehyde in blood, as measured to study the alcohol degradation effect of BHM, are shown in Fig. 4. The respective blood alcohol concentrations of Con, BSE, HDE, and BHM are 9.19, 5.30, 3.62, and 2.68 g/L at 30 min after ingestion, reaching the highest levels. In BHM, compared to those of Con, BSE, and HDE, the alcohol concentration is respectively decreased by 69%, 55%, and 38% at 90 min after ingestion and 89%, 83%, and 69% at 180 min after ingestion (Fig. 4(A)). A similar trend is observed for the blood acetaldehyde concentration of BHM: it is respectively decreased by 57%, 27%, and 16% at 3 h after ingestion, 50%, 31%, and 27% at 6 h after ingestion, and 59%, 47%, and 34% at 9 h after

ingestion compared to those of Con, BSE, and HDE (Fig. 4(B)). Sung et al. (2014) reported that the blood alcohol and acetaldehyde concentrations may be significantly reduced via the oral administration of a powder form of soybean sprouts extract beverage in rats following the alcohol, and Ji et al. (2001) reported that *H. dulcis* Thunb. fruit may lower the blood alcohol concentrations in mice with acute alcohol toxicity. These results are consistent with the result in this study, where the blood alcohol and acetaldehyde concentrations are reduced via the administration of BSE and HDE. Compared to the use of only BSE or HDE, the combination may more effectively lower the blood alcohol and acetaldehyde concentrations via a synergistic effect, which may produce an outstanding hangover-relieving effect.

## 4. Conclusions

To develop a functional food material based on natural products, BHM was produced in this study, and its physicochemical properties and antioxidant activity were examined. The content of malic acid was the highest of those of the organic acids in



**Fig. 4.** Effect of BHM on (A) alcohol and (B) acetaldehyde concentrations in serum in alcohol administered rats. Values are mean $\pm$ SD (n=3). Means with different letters (a–d) indicate significant difference ( $p < 0.05$ ) by Duncan's multiple range tests. Control, alcohol; BSE, soybean sprouts extract; HDE, 1.5% *Hovenia dulcis* Thunb. extract; BHM, soybean sprouts and 1.5% *Hovenia dulcis* Thunb. mixture.

BHM at 1,451.03 ppm, and the main free sugars in BHM were glucose and fructose, with respective contents of 645.48 and 738.11 ppm. Taurine was the main free amino acid in BHM, with a concentration of 11.95 ppm, followed by those of arginine and glutamic acid at 10.97 and 10.16 ppm, respectively. The most abundant minerals in BHM were Zn and Fe, and the respective TPC and TFC of BHM were 957.16 and 601.93 ppm. The antioxidant activity was measured based on the DPPH radical and H<sub>2</sub>O<sub>2</sub> scavenging activities and reducing power. Compared to those of the positive controls, BHM exhibited an outstanding antioxidant efficacy, and the blood alcohol and acetaldehyde concentrations were significantly reduced. Based on the results, BHM displays potential as a component of functional foods, and the results of this study should provide basic data for use in future research.

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#### Conflict of interests

The authors declare no potential conflicts of interest.

#### Author contributions

Conceptualization: Won YS. Methodology: Kim JH. Formal analysis: Park WL. Validation: Min HJ. Writing - original draft: Heo JA. Writing - review & editing: Seo KI.

#### Ethics approval

This research was approved by IACUC from the Dong-A University Committee for the Care and Use of

Laboratory Animals (DIACUC-20-19).

#### ORCID

Ji-An Heo (First author)

<https://orcid.org/0009-0008-5511-5506>

Wool-Lim Park

<https://orcid.org/0000-0003-3976-4852>

Hye-Ji Min

<https://orcid.org/0009-0005-7847-7372>

Jeong-Ho Kim

<https://orcid.org/0009-0003-4872-3291>

Yeong-Seon Won

<https://orcid.org/0009-0000-0950-7834>

Kwon-Il Seo (Corresponding author)

<https://orcid.org/0000-0002-2391-9146>

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