

Taste Composition and Biological Activities of *Cheonggukjang* Containing *Rubus coreanum*

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Abstract This study was performed to investigate the taste composition and biological activities of *cheonggukjang* containing *Rubus coreanum* to improve *cheonggukjang*'s flavor and consumption. In *R. coreanum cheonggukjang* (RCC), the total content of soluble sugars, including glucose, fructose, maltose, and sucrose, was 1,052.1 mg/100 g. Glutamic acid, phenylalanine, leucine, cystine, and tyrosine were the major amino acids, and the ratio of sweet to bitter components was higher in RCC than in general *cheonggukjang* (GC). The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities of the extracts, in decreasing order, were found to be: ethanol extract > water extract > chloroform extract, at all concentrations. The water extract had the highest SOD-like activity (10.2%) at a concentration of 0.5 mg/mL, whereas the chloroform extract showed the highest SOD-like activity (19.1%) at a concentration of 2 mg/mL. The nitrite scavenging ability was higher at pH 1.2 than at pH 3.0 or 6.0, and had a positive correlation with the extract concentration. The chloroform extract had the highest nitrite scavenging ability (84.6%) at a concentration of 2 mg/mL and pH 2.0.

Keywords: *cheonggukjang*, fermented food, biological activity, taste component

Introduction

Soybeans and processed soybean products (*cheonggukjang* and *doenjang*) have been eaten for a long time in Asia. *Cheonggukjang* is made traditionally with whole cooked soybeans fermented with *Bacillus* without the addition of salt, and is very popular in Korea (1). *Cheonggukjang* is full of essential amino acids, vitamin B₁, B₂, niacin, and pantothenic acid, as well as various enzymes. In addition, *cheonggukjang* contains various phytochemical substances such as dietary fiber, isoflavones (genistein, daidzein, etc.), phenolic acids, saponins, trypsin inhibitor, phytic acid, and so forth, offering many beneficial physiological effects (2). In particular, it has a fibrinolytic enzyme that may play a role in removing blood clots (3). *Cheonggukjang* is effective against arteriosclerosis, hypertension, diabetes, coronary heart disease, and osteoporosis, and also has antimutagenic, anticancer, and antimicrobial effects (4,5). Nevertheless, consumers tend to avoid *cheonggukjang* due to the nasty smell produced during its preparation. In Korea, there have been attempts to develop *cheonggukjang* with added ingredients such as *Artemisia asiatica* Nakai, kiwi, radish, green tea, and Yucca to improve its consumption (6-8).

Rubus coreanum (Korean mountain berry) is a perennial shrub distributed in the southern part of Korea. Its unripe fruit has been used as a traditional herbal medicine for the management of impotence, spermatorrhea, enuresis, asthma,

and allergic diseases, and it is also used as a stomachic and tonic in Korea (9). *R. coreanum* is rich in minerals, organic acids, vitamin C, and other functional constituents. It contains major phytochemical substances, including polyphenols, sanguine, gallic acids, etc. (10,11). It is reported that *R. coreanum* has anticarcinogenic, antioxidative, and antimicrobial effects, as well as the inhibition of *Helicobacter pylori* urease (9,12-14).

In order to improve its flavor and consumption, for this study we prepared *cheonggukjang* supplemented with *R. coreanum* containing various phytochemicals. We also investigated the taste components of *cheonggukjang* containing *R. coreanum* as well as the physiological effectiveness of extracts made from *R. coreanum cheonggukjang* (RCC) at scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, exhibiting superoxide dismutase (SOD)-like activity, and nitrite scavenging ability.

Materials and Methods

Materials Soybeans and *R. coreanum* were purchased at a local market in Daegu, Korea. Prior to use, *R. coreanum* was powdered using a laboratory mill (LM-3600; Perten Instruments Inc., Lahti, Finland). A 300 g amount of the ground *R. coreanum* was extracted with 3,000 mL of distilled water in a pressure cooker for 50 min, centrifuged (SUPRA 21K; Hanil, Korea) at 23,240 × g for 15 min, and filtered. The filtrate was then diluted with distilled water by 2 times its volume.

***Cheonggukjang* preparation** To prepare the RCC, 2,000 g of soybeans were cleaned and soaked in water at

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room temperature for 2 hr. The soaked soybeans were combined with 2,000 mL of *R. coreanum* extract and boiled at 121°C for 15 min (KMC-1221-60; Vision, Korea). The boiled soybeans were cooled to 40°C and rice straw containing *Bacillus subtilis* was added. The soybeans were then allowed to ferment at 37°C for 30 hr. General *cheonggukjang* (GC) for use as a control was prepared using the same procedure described above except for the addition of 2,000 mL of distilled water instead of *R. coreanum* extract.

Assessment of taste components The soluble sugar contents were analyzed according to the method of Park *et al.* (15) with some modification. Approximately 10 g of sample was extracted with 60 mL of 80% ethanol. This suspension was shaken for 45 min at room temperature and centrifuged for 10 min at 16,270×g. The supernatant was concentrated at 60°C under reduced pressure and defatted 3 times with 10 mL of ethyl ether. After being concentrated by a rotary evaporator (Eyela, Tokyo, Japan) at 40°C, the solid residues were dissolved in deionized water to a final volume of 10 mL. An aliquot of the aqueous extract was filtered using a 0.45 µm membrane filter and passed through a Sep-pak C₁₈ filter prior to HPLC analysis (model 600; Waters, Milford, MA, USA) under the following conditions: a carbohydrate analysis (3.9×300 mm) column with a Waters Associates Differential Refractometer RI 410; a column temperature of 85°C; a mobile phase of acetonitrile : deionized water (75 : 25) and at a flow rate of 0.4 mL/min. Commercialized soluble sugars were used as standards (Sigma-Aldrich Chemical Co., St. Louis, MO, USA).

To determine the composition of free amino acids, a 5 g portion of each sample was homogenized using a homogenizer (Nissei AM8; Nihonseiki Kaisha Ltd., Tokyo, Japan) with 20 mL of ice-cold distilled water in a 50 mL centrifuge tube for 2 min on ice. The homogenized samples were then incubated for 30 min on ice before centrifugation for 15 min at 16,270×g. This step was repeated twice. The supernatants from the first and second extractions were combined and filtered through Whatman filter paper (No. 4). Finally, the samples were analyzed using a lithium high resolution PEEK column in a amino acid analyzer (Biochrom 20; Pharmacia, Uppsala, Sweden) using the ninhydrin method under the following conditions: a ninhydrin flow rate of 0.3 mL/min, retention time of 45 min, a buffer consisting of 5 lithium citrate buffer + hydroxide solution, a buffer flow rate of 0.35 mL/min, a column temperature range of 20-99°C, a reaction coil temperature range of 40-145°C, and detection at 550 nm.

Preparation of extracts for the determination of biological activities in *cheonggukjang* A water extract of each sample was obtained as follows. A 100 g portion of the sample was extracted with 800 mL of distilled water with shaking at 80°C for 3 hr. The extract was centrifuged at 16,270×g for 30 min, filtered, and the filtrate was lyophilized. Ethanol and chloroform extracts were obtained via the same procedure, with the exception of the extraction temperature which was 60°C. The extracts were pooled and condensed in a rotary evaporator (Eyela) under reduced pressure at 60°C, and then the condensed extracts were

lyophilized. The lyophilized extracts were used to prepare solutions of various concentrations that were then analyzed for their biological activities as described in the following section. The ethanol extract of GC containing the highest biological activity (data not shown) was used as a control.

Determination of biological activities Free radical scavenging effects were estimated according to the method of Blois (16) with some modification. Two mL of each sample prepared at various concentrations (0.5, 1, or 2 mg/mL) were added to 1 mL of 0.2 mM DPPH radical solution. The mixture was shaken and left to stand for 30 min at 37°C, and then measured at 517 nm with a spectrophotometer (UV-2001; Hitachi, Tokyo, Japan).

The SOD-like activity was determined by the method of Marklund and Marklund (17), where 0.5 mL of each sample, 3 mL of Tris-HCl buffer (pH 8.5), and 0.2 mL of 7.2 mM pyrogallol were mixed and incubated at 25°C for 10 min. The reaction was stopped with 0.1 mL of 1 N HCl, and the absorbance was measured by spectrophotometry at 420 nm.

The nitrite-scavenging ability of each sample was carried out by the method of Kato *et al.* (18). Each sample extract at 3 different concentrations (0.5, 1, or 2 mg/mL) was mixed with 1 mL of 1 mM NaNO₂ and adjusted to various pH levels (pH 1.2, 3.0, and 6.0) using 0.1 N HCl and 0.2 N citrate buffer. The volume was adjusted to 5 mL and incubated at 37°C for 1 hr. A 0.5 mL aliquot of sample was then combined with 2.5 mL of 2% acetic acid and 0.2 mL of Griess reagent (1% sulfanilic acid and 1% naphthylamine in a methanol solution containing 30% acetic acid). After 15 min, the color intensity was measured by a spectrophotometer at 520 nm.

Statistical analysis Each experiment was performed 3 times and the data are expressed as means±standard deviations. Statistical analyses were performed by Duncan's multiple range tests using SPSS 12.0 software. The level of statistical significance was set at $p < 0.05$.

Results and Discussion

Soluble sugars Soluble sugars are key components of taste along with amino acids, and contribute to the sweet factor of *cheonggukjang* (19). *B. subtilis* produces various enzymes such as amylase and protease, and amylase

Table 1. Soluble sugar contents in *R. coreanum cheonggukjang*

Soluble sugar	Sample ¹⁾	
	GC	RCC
Glucose	485.0±3.84 ²⁾	615.0±2.12
Fructose	227.5±1.56	412.5±1.25
Maltose	tr ³⁾	2.1±0.03
Sucrose	57.5±0.89	22.5±1.18
Total	770.0±0.89	1,052.1±2.35

¹⁾GC, general *cheonggukjang*; RCC, *cheonggukjang* added 50% *R. coreanum* extract.

²⁾Results are the mean±SD of 3 determinations on a wet weight basis expressed as mg/100 g.

³⁾Trace.

hydrolyzes starch to soluble sugars during fermentation (2). The total soluble sugar contents of GC and RCC were 770.0 and 1,052.1 mg/100 g, respectively (Table 1), which is consistent with RCC having a sweeter taste than GC. The contents of glucose, fructose, maltose, and sucrose in RCC were found to be 615.0, 412.5, 2.1, and 22.5 mg/100 g, respectively. Kim *et al.* (20) reported that traditional *cheonggukjang* contained high amounts of sucrose and galactose, which disagrees with the present results. This difference could be related to whether or not an additive is present when the *cheonggukjang* is prepared.

Free amino acids Proteases produced by *B. subtilis* hydrolyze proteins to peptones, polypeptides, dipeptides, and amino acids during fermentation of *cheonggukjang*, and the hydrolysates contribute a particular texture, taste, and flavor to the product (21). The total content of free amino acids in RCC was 1,599.0 mg/100 g. Also, the total amounts of essential and nonessential amino acids in RCC were 608.9 and 990.1 mg/100 g, respectively (Table 2). The major amino acids were cystine (122.1 mg/100 g), glutamic acid (440.7 mg/100 g), leucine (125.6 mg/100 g), phenylalanine (195.8 mg/100 g), and tyrosine (112.3 mg/100 g), whereas alanine and tryptophan were not found in RCC. Table 3 shows several classes of free amino acids based on their taste characteristics as described by Mau *et al.* (22) and Solms (23). The concentrations of the bitter components (767.8 mg/100 g) and MSG-like components (475.4 mg/100 g) were high in RCC, whereas the concentration of the sweet components (154.5 mg/100 g)

Table 2. Free amino acid contents in *R. coreanum cheonggukjang*

Free amino acid	Sample ¹⁾	
	GC	RCC
L-Alanine	12.7±2.4 ²⁾	ND ³⁾
L-Arginine	87.2±3.5	73.6±2.5
L-Aspartic acid	39.5±2.2	34.7±1.2
L-Cystine	115.4±3.7	122.1±9.5
L-Glutamic acid	463.2±4.8	440.7±5.9
Glycine	25.5±1.2	27.6±3.2
L-Histidine	95.6±2.1	68.6±0.3
L-Isoleucine*	45.6±5.2	50.6±1.8
L-Leucine*	118.5±2.8	125.6±8.9
L-Lysine*	49.1±4.2	62.3±3.2
L-Methionine*	81.8±1.7	51.3±0.6
L-Phenylalanine*	148.7±0.5	195.8±6.7
L-Proline	65.6±2.6	95.4±3.8
L-Serine	16.1±1.9	15.5±0.5
L-Threonine*	14.5±0.4	16.0±1.1
L-Tryptophan*	ND	ND
L-Tyrosine	108.0±5.8	112.3±8.9
L-Vaine*	120.7±2.8	106.9±7.2
Total	1,607.7±3.4	1,599.0±5.6
Essential AA	578.9 (36%)	608.9 (38%)

¹⁾GC, general *cheonggukjang*; RCC, *cheonggukjang* added 50% *R. coreanum* extract.

²⁾Results are the mean±SD of 3 determinations on a fresh weight basis expressed as mg/100g.

³⁾Not detected. *Essential amino acid.

Table 3. Contents and taste characteristics of free amino acids in *R. coreanum cheonggukjang*

Taste characteristics ¹⁾	Sample ²⁾	
	GC	RCC
MSG-like	502.7±3.7 ³⁾	475.4±5.6
Sweet	134.4±0.5	154.5±2.8
Bitter	763.7±5.8	767.8±3.6
Tasteless	157.1±2.3	174.6±2.1

¹⁾MSG like (monosodium glutamate-like), Asp+Glu; Sweet, Ala+Gly+Pro+Ser+Thr; Bitter, Arg+His+Ile+Leu+Met+Phe+Pro+Trp+Val; Tasteless, Lys+Tyr.

²⁾GC, general *cheonggukjang*; RCC, *cheonggukjang* added 50% *R. coreanum* extract.

³⁾Results are the mean±SD of 3 determinations on a wet weight basis expressed as mg/100g.

was low. Most previous papers have reported that *cheonggukjang* contains high amounts of glutamic acid, leucine, and phenylalanine (19,20,24). These results agree well with the present results, even though the minor amino acid content was slightly different due to the kinds of additives, raw materials, microbes, and fermentation methods used. The ratio of sweet components/bitter components in RCC was slightly higher than that of GC, and it is expected that the sweet taste of RCC is somewhat stronger than that of GC. Considering these results, the characteristic taste of *cheonggukjang* containing *R. coreanum* appears to be due to a combination of the umami taste of glutamic acid, the bitter taste of leucine and valine, and the sweet taste of alanine and lysine.

DPPH radical scavenging activity DPPH is a free radical compound widely used to test the free radical scavenging ability of various samples (25,26). The DPPH scavenging effects of the water, ethanol, and chloroform extracts of RCC were investigated and the results are shown in Fig. 1. The DPPH scavenging activities of the water and chloroform extracts of RCC were dose-dependent, whereas the scavenging activity of the ethanol extract was not significantly different despite increases in concentration. The DPPH scavenging activity of GC ethanol extract used as a control was 18-85% at 0.5-2 mg/mL. The activities of RCC extracts were, in decreasing order: ethanol extract > water extract > chloroform extract at all concentrations. In particular, the activity of RCC ethanol extract was higher than that of the control at the same concentration. In addition, the RCC ethanol and water extracts had higher DPPH scavenging activity than that of ascorbic acid. In contrast, Shon *et al.* (27) reported that the DPPH scavenging effects of a methanol extract of *cheonggukjang* made with black soybean was much lower than that of ascorbic acid. The radical scavenging effects of this study may be due to the influence of phytochemicals extracted from *R. coreanum*. Yoon *et al.* (13) found that 3,4,5-trihydroxybenzoic acid had the highest DPPH radical scavenging activity among the antioxidative compounds identified in *R. coreanum*.

Superoxide dismutase (SOD)-like activity SODs are known to catalyze the conversion of O₂⁻ to H₂O₂ plus O₂ and provide protection from oxidation in which O₂⁻ appears to play an important role (28). This enzyme has

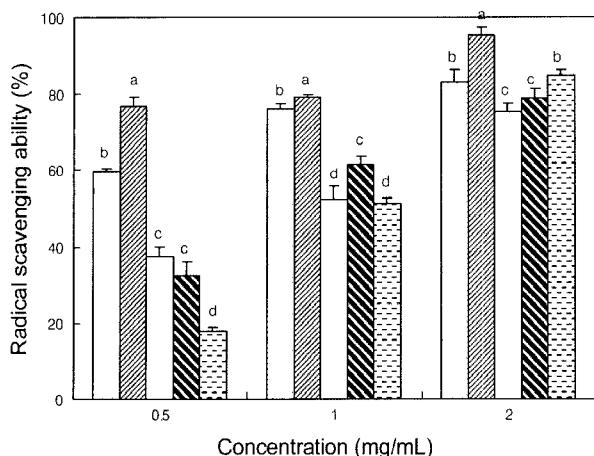


Fig. 1. DPPH radical scavenging ability of extracts of *R. coreanum cheonggukjang*. Values are the means of 3 replicates \pm SD. Means with different letters are significantly different at $p < 0.05$ by Duncan's multiple range test range. (□), Water extract; (▨), ethanol extract; (□), chloroform extract; (▩), ascorbic acid; (▤), control (ethanol extract of general *cheonggukjang*).

proven to be a useful probe for studying the participation of radicals in reactions involving oxygen such as autoxidations (17). In this study, SOD-like activity was measured by the amounts of intermediate products formed from pyrogallol, which rapidly autoxidizes in aqueous solution. Of all the samples tested, ascorbic acid showed the highest SOD-like activity with 32-82% at 0.5-2 mg/mL, and the activities of all extracts from RCC were much lower compared to that of ascorbic acid (Fig. 2). All extracts showed dosage-dependent SOD-like activity, although the activities were lower than that of vitamin C being 8-20% at concentrations of 0.5-2 mg/mL. At a 0.5 mg/mL concentration, the control showed higher SOD-like activity compared to extracts of RCC. In contrast, the SOD-like activities of the RCC extracts were higher than that of the control at a concentration of 2 mg/mL. The

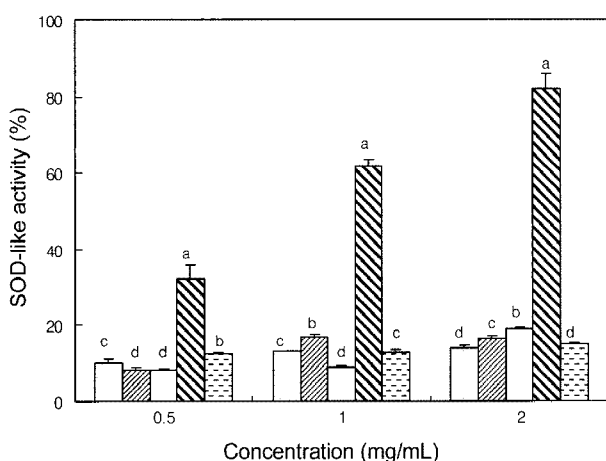


Fig. 2. Superoxide dismutase-like activity of extracts of *R. coreanum cheonggukjang*. Values are the means of 3 replicates \pm SD. Means with different letters are significantly different at $p < 0.05$ by Duncan's multiple range test range. (□), Water extract; (▨), ethanol extract; (□), chloroform extract; (▩), ascorbic acid; (▤), control (ethanol extract of general *cheonggukjang*).

activities of RCC extracts were found to be, in decreasing order: chloroform extract > ethanol extract > water extract at concentration of 2 mg/mL.

Nitrite scavenging ability Table 4 shows the nitrite scavenging abilities of extracts at various pH levels. The nitrite scavenging ability was higher at pH 1.2 than at pH 3.0 or 6.0, and positively correlated with extract concentration. Ascorbic acid had the highest scavenging ability of all samples at the same pH and concentration. Of the extracts tested, chloroform extract had the highest nitrate scavenging ability at pH 2.0, even though the activity of this extract was approximately 55-75% of that of ascorbic acid at the same concentration. By contrast, the ethanol extract showed the highest scavenging ability for the tested extracts at pH 3.0 and 6.0, even though this activity was very low at all concentrations. The activity of extracts of RCC, especially the ethanol extract, was higher than that of the control at pH 2.0. Byun *et al.* (29) reported that the nitrite scavenging ability of Korean medicinal herbs was higher at conditions of pH 1.2 than at pH 4.2 or 6.0, which agrees well with the present results. Phenolic compounds are reported to have high nitrite scavenging effects (30), and have even higher effects in conditions of low environmental pH (31). Soybeans and *R. coreanum* contain isoflavones, phenolic acids, and phenolic compounds, which have effective antioxidative activities (2,10-12,32, 33). These results indicate that the intake of RCC with foods containing nitrites may be efficacious for cancer prevention due to the reduction of nitrosamine formation in the human stomach, which is highly acidic.

The results of this study show that RCC contains a

Table 4. Nitrite scavenging abilities of extracts of *R. coreanum cheonggukjang* at different pH conditions (%)

Sample	Concentration (mg/mL)		
	0.5	1	2
pH 2.0			
Water extract	22.4 \pm 2.2 ¹⁾	28.7 \pm 2.2 ^d	30.2 \pm 0.0 ^d
Ethanol extract	19.9 \pm 2.0 ^d	31.5 \pm 0.0 ^c	41.2 \pm 1.9 ^c
Chloroform extract	33.1 \pm 0.9 ^b	40.6 \pm 1.4 ^b	52.9 \pm 0.9 ^b
Ascorbic acid	54.2 \pm 1.6 ^a	74.9 \pm 2.1 ^a	84.6 \pm 1.3 ^a
Control ²⁾	19.5 \pm 1.3 ^d	26.9 \pm 2.6 ^d	31.5 \pm 2.2 ^d
pH 3.0			
Water extract	4.6 \pm 0.4 ^c	7.6 \pm 0.4 ^c	8.9 \pm 0.7 ^c
Ethanol extract	8.4 \pm 1.2 ^b	10.7 \pm 2.1 ^b	14.6 \pm 1.0 ^b
Chloroform extract	3.3 \pm 0.4 ^d	4.9 \pm 0.4 ^d	13.1 \pm 1.6 ^b
Ascorbic acid	45.8 \pm 0.2 ^a	60.4 \pm 0.2 ^a	63.8 \pm 0.2 ^a
Control	10.9 \pm 1.4 ^b	11.30 \pm 0.7 ^b	14.2 \pm 0.1 ^b
pH 6.0			
Water extract	2.1 \pm 0.4 ^c	2.0 \pm 0.1 ^c	2.2 \pm 0.1 ^d
Ethanol extract	3.2 \pm 0.6 ^b	3.7 \pm 0.6 ^b	5.5 \pm 0.5 ^b
Chloroform extract	2.8 \pm 0.2 ^b	3.2 \pm 0.1 ^b	3.8 \pm 0.2 ^c
Ascorbic acid	17.0 \pm 1.4 ^a	20.3 \pm 0.9 ^a	19.1 \pm 1.0 ^a
Control	1.7 \pm 0.4 ^c	4.1 \pm 1.6 ^b	5.1 \pm 1.3 ^b

¹⁾ Values are the mean \pm SD of 3 determinations; means with different letters are significantly different at $p < 0.05$ by Duncan's multiple range test range.

²⁾ The control was ethanol extract of general *cheonggukjang*.

higher amount of soluble sugars and a higher ratio of sweet to bitter taste components than GC, and we can assume that *cheonggukjang* containing *R. coreanum* has its particular taste due to its unique combination of free amino acids and soluble sugars. These results also show that RCC has higher biological activity than GC, and it is expected that adding *R. coreanum* to *cheonggukjang* will improve the overall quality of general *cheonggukjang*. Additional experiments are needed to analyze the antioxidant compounds and taste components of the samples in more detail.

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