(c) The Korean Society of Food Science and Technology

Analysis of the Taste Components and Antioxidant Properties of Cheonggukjang Containing Korean Red Ginseng

Eun Jung Kim, Ju Yeon Hong¹, Seung Ryeul Shin¹, Yong-Sun Moon², and Kyung Young Yoon*

Department of Food and Nutrition, Yeungnam University, Gyeongsan, Gyeongbuk 712-749, Korea ¹Faculty of Herbal Cuisine and Nutrition, Daegu Hanny University, Gyeongsan, Gyeongbuk 712-715, Korea ²Department of Horticulture, Yeungnam University, Gyeongsan, Gyeongbuk 712-749, Korea

Abstract This study was performed to investigate the taste composition and antioxidant properties of *cheonggukjang* containing Korean red ginseng (RGC), as compared to either general *cheonggukjang* (GC) or non-fermented boiled soybeans (BS). Amylase activity was the highest (576.7 unit/g) in RGC, whereas protease activity was the highest (326.0 unit/g) in GC. The total soluble sugar contents of BS, GC, and RGC were 2,027.5, 905.5, and 837.5 mg/100 g, respectively. RGC had the highest amount of total amino acids (2,127.4 mg/100 g) and essential amino acid (50.9%) among the samples. The ratio of sweet to bitter components was higher in RGC than in GC. Although the extracts of RGC had higher radical scavenging activity for 1,1-diphenyl-2-picrylhydrazyl (DPPH) than BS or GC, regardless of the extract concentration, the ethanol extract of RGC showed the highest scavenging ability (92.4%) at 2.0 mg/mL. The chloroform extracts from GC and RGC showed their greatest superoxide dimutase-like activities at 17.2 and 19.7% at a concentration of 2 mg/mL, respectively. Regardless of the samples, the nitrite scavenging ability was positively correlated to the extract concentration, and RGC had highest ability among samples under the same extract concentrations.

Kewwords: cheonggukjang, Korean red ginseng, fermented food, antioxidant, taste component

Introduction

Cheonggukjang is a soybean-based fermented food that is very popular in Korea. It is made traditionally with whole boiled soybeans by fermenting them with Bacillus without salt (1). Cheonggukjang is rich in essential amino acids, vitamins B₁ and B₂, niacin, and pantothenic acid, as well as various enzymes. In addition, cheonggukjang contains a variety of phytochemical substances such as dietary fiber, isoflavones (genistein, daidzein, etc), phenolic acids, saponins, and phytic acid. It has many beneficial physiological effects (2). In particular, it contains fibrinolytic enzymes, which may have a role in removing blood clots (3,4). In addition, cheonggukjang has positive effects against arteriosclerosis, hypertention, diabetes, coronary heart disease, osteoporosis, as well as antimutagenic, anticancer, and antimicrobial effects (5). Nevertheless, its unpleasant smell during cooking is a primary obstacle in increasing cheonggukjang consumption. In Korea, there have been attempts to develop cheonggukjang products with added ingredients such as Artmisia asiatica Nakai, kiwi, radish, green tea, and yucca, to improve consumption (6-8).

Korean red ginseng is made by steaming and drying fresh ginseng, which causes its chemical transformation by heat (9). It has a strong scent as well as a sweet and roasted flavor (10). Korean red ginseng has been used for more than 2,000 years in traditional oriental medicine as a general tonic, and has broad beneficial effects against hypertension, diabetes, nociception, pulmonary vascular

obstruction, and cancer (11-13).

Korean red ginseng's extract was added to *cheonggukjang* to reduce its unpleasant taste and improve its physiological activity, which could increase *cheonggukjang* consumption. In this study, the antioxidant activities of extracts from *cheonggukjang* with or without Korean red ginseng were investigated, including radical scavenging ability on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, superoxide dismutase (SOD)-like activity, and nitrite scavenging ability. The enzyme activities, soluble sugars, and free amino acids related to *cheonggukjang*'s taste were also examined.

Materials and Methods

Preparation of Korean red ginseng extract The soybeans and red ginseng were purchased at a local market in Daegu, Korea. After grinding the Korean red ginseng using a laboratory mill (LM-3600; Perten, Stockholm, Sweden), 300 g of sample was cooked with 3,000 mL of distilled water in a pressure cooker for 50 min, and then centrifuged (SUPRA 24K; Hanil, Seoul, Korea) at 23,240×g for 15 min. The supernatant was filtered and diluted by an equal volume of water, for use in the *cheonggukjang* containing Korean red ginseng.

Preparation of *cheonggukjang* Figure 1 shows the scheme for the *cheonggukjang* preparation. Two kg of soybeans were cleaned and soaked for 2 hr at room temperature. Two L of distilled water was added and boiled for 15 min at 121°C (KMC-1221-60; Vision, Seoul, Korea). The boiled soybeans were cooled down to 40°C and separated 2 portions for the boiled soybean (BS) sample and preparation of the general *cheonggukjang* (GC). Next, to prepare *cheonggukjang*, rice straws containing *Bacillus*

Received April 14, 2008; Revised June 3, 2008;

Acceepted June 3, 2008

^{*}Corresponding author: Tel: +82-53-810-2878; Fax: +82-53-810-4768 E-mail: yoonky2441@ynu.ac.kr

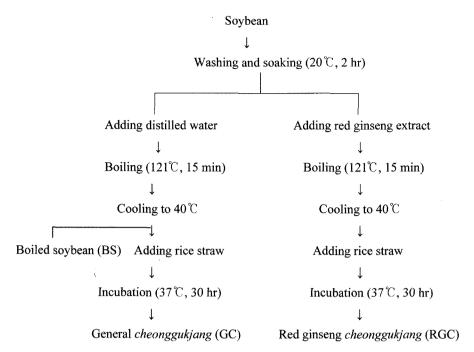


Fig. 1. The scheme for cheonggukjang preparation.

subtilis were added to the cooled soybeans and kept for 30 hr at 37°C for fermentation. Korean red ginseng cheonggukjang (RGC) was made in the same manner as described above except for adding 2,000 mL of Korean red ginseng extract instead of distilled water.

Assay of amylase and protease Ten g of each sample (BS, GC, RGC) was homogenized with 90 mL of 0.01 M phosphate buffer (pH 6.8) and centrifuged for 30 min at 16,270×g and 4°C. The filtered supernatant was increased in volume up to 100 mL.

For the amylase activity assay, an aliquot of the crude enzyme extract was incubated with 1% soluble starch in 10 mM phosphate buffer (pH 6.8) for 10 min at 40°C. Boiled crude enzyme extract was used as the control. Reducing sugar content was determined by the dinitrosalicylic acid method (14). One unit of amylase activity was defined as the amount of enzyme that produced 1 µg of maltose/min at 40°C.

One mL of crude enzyme extract was mixed with 0.5% Hammarsten casein in 5 mL of 10 mM phosphate buffer (pH 6.8) for the protease activity assay. After 10 min of incubation at 40°C, the reaction was stopped by adding 5 mL of 0.4 M trichloroacetic acid. The mixture was filtered and determined by the Casein-Folin method (15), using tyrosine as the standard. One unit of protease activity was defined as 1 µg of tyrosine released/min under the conditions described above.

Assessment of taste components The soluble sugar contents were analyzed according to the method of Kim *et al.* (16) 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 (N-1000; 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 high performance liquid chromatography (HPLC) analysis (Model 600; Waters, Milford, MA, USA) under the following conditions: a carbohydrate analysis column (3.9×300 mm) 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, 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; Phamarcia, Buckinghamshire, Sweden) using the ninhydrin method under the following conditions: a nihydrin 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.

Extract preparation for the determination of antioxidant activity The sample extracts (BS, GC, RGC) were obtained by different extraction solvents such as water, ethanol, and chloroform. For the water extract, 100 g of each sample was soaked with 800 mL of distilled water for

3 hr at 80°C with shaking, followed by centrifugation for 30 min at 16,270×g. The supernatant was filtered and lyophilized directly. The powder was dissolved in assay buffer for the 'water extract', and the final concentrations were 0.5, 1, and 2 mg/mL. The residue was then used for the ethanol extraction. The pellet was re-extracted with 800 mL of ethanol with shaking for 3 hr at 60°C and centrifuged for 30 min at 16,270×g. The supernatant was condensed with a rotary evaporator (N-1000; Eyela) under reduced pressure at 60°C after filtration. It was dissolved into water and lyophilized for the 'ethanol extract', and the pellet was used for the chloroform extract under the same conditions as described above. The final supernatant was filtered and condensed. It was also lyophilized for the 'chloroform extract'. The powders of both the 'ethanol extract' and 'chloroform extract' were dissolved in assay buffer at various concentrations (0.5, 1, and 2 mg/mL) to analyze their antioxidant effectiveness.

Determination of antioxidant activity The free radical scavenging effects were estimated according to the modified Blois's method (17). Two mL of the samples were prepared at various concentrations (0.5, 1, and 2 mg/mL), and 1 mL of 0.2 mM DPPH radical solution was added. After 30 min of incubation at 37°C, the scavenging activity was measured by a spectrophotometer (UV-2001; Hitachi, Tokyo, Japan) at 517 nm.

The SOD-like activity was determined by the method of Marklund and Marklund (18). Here, 0.5 mL of each sample was mixed with 3 mL of Tris-HCl buffer (pH 8.5) and 0.2 mL of 7.2 mM pyrogallol, and the mixture was kept for 10 min at 25°C. The reaction was stopped by 0.1 mL of 1 N HCl, and the optical density was measured by a spectrophotometer at 420 nm.

The analysis for nitrite scavenging ability was carried out by the method of Kato *et al.* (19). Each powder sample was added into 1 mL of 1 mM NaNO, and the pH was adjusted to 1.2, 3.0, and 6.0 with 0.1 N HCl or 0.2 N citrate buffer. It was then increased in volume up to 5 mL at 3 different concentrations, including 0.5, 1, and 2 mg/mL. After 1 hr of incubation at 37°C, a 0.5 mL aliquot was 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). The color intensity was measured by a spectrophotometer (UV-2001; Hitachi) at 520 nm after 15 min of reaction.

Statistical analysis Each experiment was repeated 3 times independently, and the statistical analysis was performed by Duncan's multiple-range tests using SPSS.

Results and Discussion

Enzyme activity The amylase activity was highest in RGC (576.7 unit/g), whereas GC showed the highest protease activity (326.0 unit/g). The amylase and protease activities in BS were much lower than in the other samples (Table 1). Generally, the taste of *cheonggukjang* is influenced by the activity of various enzymes, including amylase and protease. Amylase activity is responsible for the sugar content, contributing to the sweet taste of *cheonggukjang*. In addition, protease activity is one of the

Table 1. Enzyme activities of soybean products and cheonggukjang

Sample ¹⁾	Amylase (unit/g)	Protease (unit/g)
BS	60.0±10.9 ^{c2)}	57.5±14.4°
GC	486.7±33.3 ^b	326.0 ± 24.4^{a}
RGC	576.7 ± 13.3^{a}	200.3 ± 13.8^{b}

¹⁾BS, boiled soybean; GC, general *cheonggukjang*; RGC, *cheongguk-jang* added red ginseng extract.

²⁵Values are the mean of triplicates \pm SD; Means with different capital letters within a column are significantly different (p<0.05).

Table 2. Soluble sugar in soybean products and cheonggukjang

Soluble sugars	Sample ¹⁾			
	BS	GC	RGC	
Glucose	50.0±0.6	485.0±3.8	292.5±1.1	
Fructose	95.0 ± 0.2	227.5 ± 1.6	177.5±1.3	
Maltose	1,805.0±2.0	Tr	12.5±0.0	
Sucrose	77.5±1.5	57.5±0.9	217.5±1.2	
Arabinose	ND	135.5 ± 2.1	137.5±0.5	
Total	2,027.5±1.5	905.5±0.3	837.5±2.4	

¹⁾BS, boiled soybean; GC, general *cheonggukjang*; RGC, *cheonggukjang* added red ginseng extract; Results are the mean of triplicate determinations on a fresh weight basis±SD, and expressed as mg/100 g. Tr, trace; ND, not detected.

most important factors for the umami taste that is caused by amino acids, peptones, peptides, and polypeptides (5). Since a high protease activity could produce a much tastier *cheonggukjang*, RGC would give a sweeter but slightly lower umami taste than GC.

Soluble sugars Soluble sugars are key components of taste along with amino acids, and contribute to the sweet factor of cheonggukjang (20). B. subtilis produces various enzymes such as amylase and protease, and amylase hydrolyzes starch to soluble sugars during fermentation (2). The total soluble sugar contents of BS, GC, and RGC were 2,027.5, 905.5, and 837.5 mg/100 g, respectively (Table 2), which is consistent with GC having a sweetest taste caused by soluble sugars among samples. Maltose is the main soluble sugar in BS, and its content was 1,805.5 mg/100 g. In contrast, GC and RGC contained a very small amount of maltose, and it was supposed that B. subtilis might uptake the maltose and used it as an energy source during fermentation. GC and RGC were plenty of glucose and fructose. RGC had higher amount of sucrose than GC because of influx of sucrose contained Korean red ginseng. The intensity of sweet taste from soluble sugars might be similar between GC and RGC. Kim et al. (21) 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 (22). The total contents of free amino acids in BS, GC, and RGC were 1,631.1, 2,127.4,

Table 3. Free amino acids in soybean products and cheonggukjang

Free amino acids	Sample ¹⁾			
Free amino acids	BS	GC	RGC	
L-Alanine	116.2	62.7	180.8	
L-Arginine	662.1	287.2	155.2	
L-Aspartic acid	130.5	39.5	33.1	
L-Cystine	142.0	115.4	148.3	
L-Glutamic acid	286.1	463.2	701.8	
Glycine	35.2	25.5	66.1	
L-Histidine	31.4	95.6	237.7	
L-Isoleucine*	14.9	45.6	111.3	
L-Leucine*	19.0	118.5	425.4	
L-Lysine*	31.7	149.1	357.9	
L-Methionine*	. 14.9	81.8	188.4	
L-Phenylalanine*	38.1	248.7	605.8	
L-Proline	15.3	65.6	126.9	
L-Serine	25.7	16.1	32.8	
L-Threonine*	16.1	14.5	55.8	
L-Tyrosine	30.0	178.0	313.2	
L-Valine*	24.6	120.7	321.9	
Total	1,631.3	2,127.4	4,062.4	
Essential amino acid (%)	9.8	36.6	50.9	

¹⁾BS, boiled soybean; GC, general *cheonggukjang*; RGC, *cheonggukjang* added red ginseng extract; Results are expressed as mg/100 g; *Essential amino acid.

and 4,062.4 mg/100 g, respectively. Also, the percentage of essential amino acids in BS, GC, and RGC were 9.8, 36.6, and 50.9%, respectively (Table 3). RGC had the highest amount of amino acids and essential amino acids among the samples even though protease activity in RGC was lower than that in GC. These high amounts of free amino acids in RGC is supposed to inflow of free amino or protein and synergistic effects caused by compounds contained red ginseng extract during fermentation. So it is needed more experiments about co-fermentation effects between soybean and red ginseng extract during fermentation. The major amino acids in RGC were glutamic acid (701.8 mg/100 g), phenylalanine (605.8 mg/100 g), leucine (425.4 mg/100 g), and lysine (357.9 mg/100 g). Table 4 shows several classes of free amino acids based on their taste characteristics as described by Mau et al. (23) and Solms (24). The concentration of the bitter components in all samples was high (820.3, 1,063.7, and 2,172.6 mg/100 g in BS, GB, and RGC, respectively), whereas the concentration of the sweet components was low (416.6, 502.7, and 734.9 mg/100 g in BS, GB, and RGC, respectively). Most previous papers have reported that cheonggukjang contains high amounts of glutamic acid, leucine, and phenylalanine (8,20,21). 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 RGC was higher than that of GC, therefore it is expected that the sweet taste caused by free amino acid of RGC is somewhat stronger than that of GC. Considering these results, the characteristic taste of cheonggukjang containing Korean red ginseng

Table 4. Contents and taste characteristics of free amino acids in *cheonggukjung* with *R. coreanum*

00 7 0			
Taste		Sample ²⁾	
characteristics ¹⁾	BS	GC	RGC
MSG-like	416.6	502.7	734.9
Sweet	327.2	279.7	700.1
Bitter	820.3	1,063.7	2,172.6
Tasteless	61.7	327.1	671.1

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⁾BS, boiled soybean; GC, general *cheonggukjang*; RGC, *cheonggukjang* added red ginseng extract; Results are expressed as mg/100 g.

appears to be due to a combination of the umami taste of glutamic acid, the bitter taste of phenylalanine and leucine, and the sweet taste of alanine and proline.

Radical scavenging effects on DPPH Radical scavenging activity is known as an index of antioxidant effectiveness for phenolic compounds (25). DPPH has been used extensively as a free radical to evaluate reducing substances (26). The DPPH radical scavenging ability of BS was lower than that of GC and RGC at all extract concentrations. High scavenging activity of RGC might be attributed to the synergistic effects of isoflavons and phenolic compounds contained red ginseng. The radical scavenging activity was highly correlated with the extract concentration, and the activity varied depending on the extraction solvents. Table 5 shows the DPPH radical scavenging abilities among BS, GC, and RGC based on the extraction solvents. There were no significant differences among the extracts at the concentration of 0.25 mg/mL of the tested samples, while the chloroform extract had a much higher activity at the concentration of 0.5 mg/mL, and the ethanol extract showed a better radical scavenging activity at the 2 mg/mL concentration. In this study, the overall scavenging abilities of the RGC extracts were significantly higher than those of the GC or BS extracts. In particular, the DPPH radical scavenging ability of the RGC ethanol extract amounted to 92.4%. This result indicates that the cheonggukjang containing Korean red ginseng had stronger free radical scavenging ability than the general cheonggukjang. This is plausible since Korean red ginseng contains abundant phenolic compounds, including cis and trans-ferulic acid, which have radical scavenging effects (27). In addition, soybean-based foods contain abundant isoflavones that show antioxidant activity. Among the various commercial soybean products, higher isoflavone contents are observed in fermented soybean foods like cheonggukjang (28,29).

SOD-like activity SODs are known to convert O_2^- to H_2O_2 , and play an important role in defense mechanisms (30). The enzymes are very useful in research in terms of radical participation in reactions related to oxygen, such as autoxidation (18). In this study, SOD-like activity was measured by the amounts of intermediate products from pyrogallol, which rapidly autoxidizes in aqueous solution. The SOD-like activity of RGC was higher than that of BS and GC (Table 6). RGC and GC showed much stronger

Table 5. DPPH radical scavenging ability of extracts from boiled soybeans and cheonggukjung

Concentration (mg/mL)	Sample ¹⁾	Water extract	Ethanol extract	Chloroform extract
	BS	13.6±1.7 ^{a,C2)}	2.23±0.6 ^{b,B}	16.6±3.7 ^{a,A}
0.25	GC	$21.0\pm2.9^{\text{ns3}}$,B	18.6 ± 3.2^{A}	18.6 ± 1.2^{B}
	RGC	35.4±2.7 ^{ns,A}	28.0 ± 7.5^{A}	29.8 ± 4.9^{A}
0.5	BS	17.5±0.5 ^{b,C}	6.6±2.3 ^{e,C}	36.9±1.6 ^{a,B}
	GC	22.3±3.2 ^{b,B}	17.9±1.1 ^{c,B}	$37.4\pm0.6^{a,B}$
	RGC	$35.8 \pm 0.7^{b,A}$	$32.7 \pm 0.7^{c,A}$	$46.7\pm0.1^{a,A}$
1.0	BS	34.4±0.7 ^{b,B}	27.0±1.3 ^{c,C}	41.6±1.4 ^{a,B}
	GC	38.3±3.4 ^{c,B}	51.2±1.4 ^{a,B}	$42.9\pm0.4^{b,A}$
	RGC	$48.4 \pm 1.6^{b,A}$	56.3±3.8 ^{a,A}	$58.0\pm0.5^{a,A}$
2.0	BS	48.3±1.1 ^{b,B}	70.6±4.6 ^{a,C}	47.7±2.2 ^{b,C}
	GC	53.4±4.3 ^{e,B}	$86.4\pm1.0^{a,B}$	$60.6 \pm 0.9^{b,B}$
	RGC	68.5±2.5°,A	$92.4\pm0.41^{a,A}$	$72.4 \pm 2.6^{b,A}$

¹⁾BS, boiled soybean; GC, general cheonggukjang; RGC, cheonggukjang added red ginseng extract.

Table 6. Superoxide dismutase-like activity of extracts from boiled soybeans and cheonggukjung

Concentration (mg/mL)	Sample ¹⁾	Water extract	Ethanol extract	Chloroform extract
	BS	7.9±0.8 ^{a,B2})	5.6±1.3 ^{a,B}	4.3±1.5 ^{b,B}
0.25	GC	$8.2\pm0.6^{\text{ns3},B}$	9.6 ± 1.1^{A}	9.2 ± 1.2^{A}
	RGC	9.5±0.2 ^{ns,A}	8.6 ± 2.1^{AB}	10.6±3.7 ^A
0.5	BS	8.0±2.0 ^{ns,NS}	5.5±1.0 ^C	5.8±0.5°
	GC	10.8 ± 1.0^{b}	$12.4\pm0.5^{a,B}$	7.5±0.3 ^{c,B}
	RGC	10.2 ± 2.0^{c}	$17.1\pm0.6^{a,A}$	$12.8\pm10.8^{b,A}$
1.0	BS	8.8±0.6 ^{ab,C}	10.1±0.2 ^{a,C}	7.9±1.0 ^{b,B}
	GC	11.2±0.3 ^{ns,B}	13.2±1.4 ^B	10.9 ± 1.4^{AB}
	RGC	$13.6 \pm 0.4^{b,A}$	$16.9 \pm 1.3^{a,A}$	12.7±2.5 ^{b,A}
2.0	BS	10.8±1.5 ^{ns,B}	13.1±1.7 ^{NS}	11.2±3.2 ^B
	GC	$15.0 \pm 1.3^{\text{ns,A}}$	15.0 ± 17	17.2±2.9 ^A
	RGC	15.1±0.1 ^{b,A}	17.1 ± 2.7^{ab}	19.7±2.1 ^{a,A}

¹⁾BS, boiled soybean; GC, general *cheonggukjang*; RGC, *cheonggukjang* added red ginseng extract.

SOD-like activity than BS. The ethanol extracts had their highest SOD-like activity at concentrations of 0.5 and 1.0 mg/mL, while the chloroform extracts showed their highest activity at the concentration of 2.0 mg/mL. The SOD-like activity of the chloroform extracts from GC and RGC were 17.2 and 19.7% at the 2.0 mg/mL concentration, respectively. The isoflavones in soybeans have various antioxidant effects such as the suppression of superoxide anion production and the elimination of H_2O_2 in the body (2). It is suggested that SOD-like activity was directly related to the isoflavone content of the samples. Among the various Korean soybean products, the highest amounts of isoflavones can be found in cheonggukjang. It has 2.76 times more isoflavones than soybeans, resulting in better anticancer activity in humans due to it high radical scavenging ability and SOD-like activity (31).

Nitrite scavenging effects Nitrosamines are potent

carcinogens in humans when they are acquired from the diet or produced from endogenous biosynthesis in the body. Nitrosamines can be synthesized by nitrite or nitrous acid and amines, under acidic conditions (19,32). Nitrosamines are easily found in foods, including preserved meats, fish, cheese, and even drinking water. However, the carcinogenicity caused by nitrosamines could be prohibited by the inhibition of nitrosamines formation (19).

Table 7 shows the nitrite scavenging abilities of the extracts from the BS, GC, and RGC, at pH 1.2. The nitrite scavenging ability of all sample extracts was higher at pH 1.2, rather than at pH 3.0 or 6.0 (data not shown), and it was positively correlated to the extract concentration. Byun et al. (33) reported that Korean medicinal herbs showed their best nitrite scavenging abilities at pH 1.2 instead of 4.2. The nitrite scavenging abilities of the ethanol extracts from BS and GC were the highest, but the chloroform extract of RGC showed the best nitrite scavenging ability

²⁾Values are the mean of triplicates ±SD; Means with different capital and small letters are significantly different within a column and row, respectively (p<0.05); ns, not significant.

²⁾Values are the mean of triplicates±SD; Means with different capital and small letters are significantly different within a column and row, respectively (p<0.05); ns, not significant.

Table 7. Nitrite scavenging ability of extracts from boiled soybeans and cheonggukjung at pH 1.2

Concentration (mg/mL)	Sample ¹⁾	Water extract	Ethanol extract	Chloroform extract
	BS	6.2±3.5 ^{b,C2)}	15.5±1.7 ^{a,NS3)}	7.6±0.5 ^{b,B}
0.25	GC	$16.9 \pm 0.4^{b,B}$	16.8 ± 1.5^{a}	10.5±4.2 ^{b,B}
	RGC	22.2±1.6 ^{b,A}	$17.5 \pm 0.5^{\circ}$	$29.4\pm3.5^{a,A}$
	BS	11.1±3.5 ^{b,B}	17.8±2.1 ^{a,C}	13.3±2.9 ^{ab,B}
0.5	GC	20.9±0.8 ^{b,A}	$26.7 \pm 1.9^{a,B}$	11.1±0.6 ^{c,B}
	RGC	22.5±0.8 ^{b,A}	$34.1 \pm 4.7^{c,A}$	$33.1 \pm 0.7^{a,A}$
	BS	15.7±3.0 ^{b,B}	27.4±2.1 ^{a,B}	20.1±1.4 ^{b,B}
1.0	GC	$26.6 \pm 5.5^{\text{ns,A}}$	26.6 ± 1.9^{B}	21.2 ± 1.1^{B}
	RGC	29.9±1.4 ^{b,A}	$32.0\pm0.5^{b,A}$	$40.4{\pm}1.0^{a,A}$
2.0	BS	25.3±0.5 ^{b,B}	35.0±3.9 ^{a,B}	30.6±1.9 ^{a,B}
	GC	33.6±1.8 ^{b,A}	$43.0\pm1.4^{a,A}$	$24.8 \pm 0.7^{c,C}$
	RGC .	$35.8 \pm 2.7^{b,A}$	$44.1 \pm 3.8^{a,A}$	42.6±0.8 ^{a,A}

¹⁾BS, boiled soybean; GC, general *cheonggukjang*; RGC, *cheonggukjang* added red ginseng extract.

at the low concentration of 0.25 mg/mL. However, the ethanol extracts of all samples had the highest nitrite scavenging ability at the high concentration of 2.0 mg/mL. The nitrite scavenging ability of RGC was higher than that of GC or BS, under the same extract concentrations. The nitrite scavenging abilities of the water, ethanol, and chloroform extracts from RGC were 35.8, 44.4, and 42.6%, respectively. These results indicate that the consumption of *cheonggukjang* containing Korean red ginseng may be efficacious for cancer prevention, due to a reduction of nitrosamine formation in the human stomach.

In this study, the results show that RGC contains a similar amount of soluble sugars and a higher ratio of sweet to bitter taste components than GC, and we can assume that *cheonggukjang* containing Korea red ginseng has its particular taste due to its unique combination of free amino acids and soluble sugars. The results show that *cheonggukjang*, especially *cheonggukjang* containing Korean red ginseng, had higher antioxidant effectiveness and enzyme activity than boiled soybeans. Therefore, it is expected that the *cheonggukjang* containing Korean red ginseng would have improved quality and taste. More detailed analyses of the antioxidant compounds and taste components of these extracts are needed in future experiments.

Acknowledgments

This research was supported by the Yeungnam University research grants in 2008.

References

- 1. Byun MW, Son JH, Yook HS, Jo C, Kim DH. Effect of gamma irradiation on the physiological activity of Korean soybean fermented foods, *cheonggukjang* and *doenjang*. Radiat. Phys. Chem. 64: 245-248 (2002)
- Kim SH, Yang JL, Song YS. Physiological functions of cheonggukjang. Food Ind. Nutr. 4: 40-46 (1999)
- 3. Yoo CK, Seo WS, Lee CS, Kang SM. Purification and characterization of fibrinolytic enzyme excreted by *Bacillus subtilis*

- K-54 isolated from *cheonggukjang*. Korean J. Appl. Microbiol. Biotechnol. 26: 507-514 (1998)
- Lee SK, Heo S, Bae DH, Choi KH. Medium optimization for fibrinolytic enzyme production by *Bacillus subtilis* KCK-7 isolated from Korean traditional *cheonggukjang*. Korean J. Appl. Microbiol. Biotechnol. 26: 226-231 (1998)
- Lee JO, Ha SD, Kim AJ, Yuh CS, Bang IS, Park SH. Industrial application and physiological functions of *cheonggukjang*. Food Sci. Indus. 38: 69-78 (2005)
- Lee CH, Yang Eİ, Song GS, Chai OH, Kim YS. Cheonggukjang mucilage stimulates immunohistochemical activities of gastrointestinal tract in rats. Food Sci. Biotechnol. 14: 813-817 (2005)
- Park WJ, Park HY, Yoo JH, Rhee MS. Effect of Artmisia asiatica Nakai extract on the flavor of cheonggultjang. Food Eng. Prog. 5: 115-124 (2001)
- Sohn MY, Kim MH, Park SK, Park JR, Sung NJ. Taste components and palatability of black bean *cheonggukjang* added with kiwi and radish. J. Korean Soc. Food Sci. Nutr. 31: 39-44 (2002)
- Park JD. Recent studies on the chemical constituents of Korean ginseng (*Panax ginseng* C.A. Meyer). Korean J. Ginseng Sci. 20: 389-415 (1996)
- Sohn HJ, Lee SK, Wee JJ. Flavour characteristics of Korean red ginseng. J. Ginseng Res. 24: 148-152 (2000)
- Jung NP, Jin SH. Studies on the physiological and biochemical effects of Korean ginseng. Korean J. Ginseng Sci. 20: 431-471 (1996)
- 12. Han K, Shin IC, Choi KJ, Yun YP, Hong JT, Oh KW. Korea red ginseng water extract increases nitric oxide concentrations in exhaled breath. Nitric Oxide 12: 159-162 (2005)
- Kim DJ, Lee HS, Ryu JK, Lee T, Park IS, Yoon SM, Song SU, Shu JK. Free radical-scavenging activity of Korean red ginseng for erectile dysfunction in non-insulin-dependent diabetes mellitus rats. Urology 65: 611-615 (2005)
- Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31: 426-431 (1959)
- Oda K, Murao S. Purification and properties of carboxyl proteinase in basidiomycetes. Agr. Biol. Chem. 38: 2435-2437 (1974)
- Kim EJ, Hong JY, Shin SR, Heo HJ, Moon YS, Park SH, Kim KS, Yoon KY. Taste composition and biological activities of cheonggukjang containing Rubus coreanum. Food Sci. Biotechnol. 17: 687-691 (2008)
- 17. Blois MS. Antioxidant determination by the use of a stable free radical. Nature 181: 1199-1200 (1958)
- Marklund S, Marklund G. Involvement of superoxide anion radical in the oxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 47: 469-474 (1975)

²⁾Values are the mean of triplicates±SD; Means with different capital and small letters are significantly different within a column and row, respectively (p<0.05); NS, not significant.

- Kato H, Lee IE, Chuyen NV, Kim SB, Hayase F. Inhibition on nitrosamine formation by nondialyzable melanoidins. Agr. Biol. Chem. 51: 1333-1338 (1987)
- Kim JS, Yoo SM, Choe JS, Park HJ, Hong SP, Chang CM. Physicochemical properties of traditional *cheonggukjang* produced in different regions. Agric. Chem. Biotechnol. 41: 377-383 (1998)
- 21. Kim KJ, Ryu MK, Kim SS. *Cheonggukjang koji* fermentation with rice straw. Korean J. Food Sci. Technol. 14: 301-308 (1982)
- 22. Joo HK. Studies on chemical composition of commercial cheonggukjang and flavor compounds cheonggukjang by mugwort (Artimisia asiatica) or red pepper seed oil. Korean Soybean Digest 13: 44-56 (1996)
- Mau J-L, Chyau C-C, Li JY, Tseng Y-H. Flavor components in straw mushrooms *Volariella volvacea* harvested at different stages of maturity. J. Agr. Food Chem. 45: 4726-4729 (1997)
- Solms J. The taste of amino acids, peptides, and proteins. J. Agr. Food Chem. 17: 686-688 (1969)
- Kang YH, Park YK, Lee GD. The nitrite scavenging and electron donating ability of phenolic compounds. Korean J. Food Sci. Technol. 28: 232-239 (1996)
- 26. Cotelle N, Bemier JL, Catteau JP, Pommery J, Wallet JC, Gaydou

- EM. Antioxidant properties of hydroxyl-flavones. Free Radical Bio. Med. 20: 35-43 (1996)
- Jung MY, Jeon BS, Bock JY. Free, esterified, and insoluble-bound phenolic acids in white and red Korean ginsengs (*Panax ginseng* C.A. Meyer). Food Chem. 79: 105-111 (2002)
- Nguyenle T, Wang E, Cheung AP. An investigation on the extraction and concentration of isoflavones in soy-based products. J. Pharmaceut. Biomed. 14: 221-232 (1995)
- 29. Wang HJ, Murphy P. Isoflavone content in commercial soybean foods. J. Agr. Food Chem. 42: 1666-1673 (1994)
- Nagamim H, Umakoshi H, Shinmanouchi T, Kuboi T. Variable SOD-like activity of liposome modified with Mn(II)-porphyrin derivative complex. Biochem. Eng. J. 21: 221-227 (2004)
- Kim JD, Zhung J, Row KH. Comparisons of isoflavones from Korean and Chinese soybean and processed products. Biochem. Eng. J. 36: 49-53 (2007)
- 32. Yurchenko S, MÖlder U. Volatile *N*-nitrosamines in various fish products. Food Chem. 96: 325-333 (2006)
- Byun MW, Yook HS, Sim KS, Chung CK. Effects of gamma irradiation on physiological effectiveness of Korean medicinal herbs. Radiat Phys. Chem. 54: 291-300 (1999)