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Enhanced Antioxidant Effect of Black Soybean by *Cheonggukjang* with Potential Probiotic *Bacillus subtilis* CSY191

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잠재적인 생균제제 *Bacillus subtilis* CSY191에 의한 검정콩 청국장의 항산화 증진 효과

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Changes in β-glycosidase activity, total phenolic and isoflavone contents, and antioxidant activities during the fermentation of Korean black soybeans (*Seoritae* and *Seomoktae*) fermented food *cheonggukjang* by the potential probiotic *Bacillus subtilis* CSY191 were investigated. The levels of total phenolic and isoflavone-malonylglycoside and -aglycone contents increased, while 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging activity and ferric reducing/antioxidant power (FRAP) increased, but the isoflavone-glycoside contents decreased after *cheonggukjang* fermentation. The content of antioxidant compounds, including isoflavone-aglycones and -malonylglycosides, was increased by fermenting-processing, whereas the content of isoflavone-glycosides was decreased in the fermented soybeans. In particular, the *Seoritae* soybean fermented at 37℃ for 48 h displayed the highest antioxidant activities, compared to those of the *Seomoktae* soybean and the fermented. The highest levels of daidzein, glycitein, and genistein were present at concentrations of 253.0 μg/g, 72.5 μg/g, and 114.1 μg/g after 48 h of *Seoritae* soybean fermentation. From those results, we suggest that the high antioxidant activity of *cheonggukjang* of black soybeans might be related to the markedly higher levels of total phenolic and isoflavone-aglycone and -malonylglycoside contents achieved during fermentation.

***Keywords*:** *Bacillus subtilis* CSY191, antioxidant activity, black soybean, *cheonggukjang*, isoflavones

Black soybeans (*Glycine max* L. Merrill) have been widely consumed as folk medicines in China, India, Japan, and Korea for hundreds of years (Jeong *et al.*, 2010) and have various biological effect such as osteoblastic cell proliferating, anticancer, and antioxidant activity (Kim *et al.*, 2011). Black soybeans are increasingly sought after in food and medicinal industries because of their various beneficial effects (Lee and Cho, 2012). Previously, numerous researchers have reported that the beneficial properties of black soybeans are due to the many phytochemicals present in the crop, including isoflavone, flavanol, flavan-3-ol, anthocyanin, and saponin (Lee and Cho, 2012). In Korea, black soybeans are used to prepare several different traditional fermented foods, including *meju* (soybean cake), *cheonggukjang* (soybean cook), *kanjang* (soybean sauce), and *doenjang* (soybean paste). In particular, *cheonggukjang* is manufactured in a traditional way in homes using different types of processes, depending on the region; thus its physicochemical and functional properties vary due to differences in soybeans, microorganisms, and fermentation time (Nam *et al.*, 2012).

In raw soybeans, isoflavones are present in four chemical forms: malonylglycosides (70-80%), acetylglycosides (5%), glycosides (25%), and aglycones (2%) (Lee and Choung, 2011). Isoflavones conjugated glycoside are converted to aglycones under acidic or alkaline conditions or by the action of β-glycosidase. The aglycone forms show greater potential for absorption in the intestine than the glycoside forms (da Silva *et al.*, 2011, FC). Incorporation of β-glycosidase has attempted to increase the content of isoflavone-aglycones in *cheonggukjang* (Yang *et al.*, 2006; Cho *et al.*, 2009, 2011). In addition, several

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researchers have reported that total phenolic and isoflavone-aglycone contents increased, depending on whether antioxidant activities increased after *cheonggukjang* fermentation (Cho *et al.*, 2009, 2011; Hu *et al.*, 2010).

The objective of this study was to compare the antioxidant activities observed in *cheonggukjang* fermentation of two Korean black soybeans, such as *Seoritae* and *Seomoktae*. In addition, we investigated the possibility that the enhancing effect of fermentation on the antioxidant activities observed during *cheonggukjang* of Korean black soybeans that may be related to the total phenolic contents and isoflavone compositions of this product.

Materials and Methods

Black soybeans, microorganism, chemicals, and instruments

Two black soybean samples (*Seoritae* and *Seomoktae*) were harvested in 2011 and were purchased from the local market in Jinju, Korea. The potential probiotic *Bacillus subtilis* CSY191 previously isolated from Korean traditional soybean paste (*doenjang*), was used as the starter organism (Lee *et al.*, 2012).

Three isoflavone aglycones, including daidzein, genistein, and glycitein, were obtained from Sigma-Aldrich Chemical Co. (USA), and three isoflavone glycosides, including genistin, daidzin, and glycitin, were purchased from Indofine (Hillsborough, USA). Three malonyl- and three acetyl-isoflavone glycosides (malonylgensitin, malonylglycitin, malonyldaidzin, acetylgenistin, acetylglycitin, and acetyldaidzin) were purchased from LC Laboratories (Woburn, USA). HPLC-grade H_2O, methanol, and acetonitrile were purchased from Fisher Scientific (Fairlawn, USA). Glacial acetic acid, Folin-Ciocalteu phenol reagent, 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, ferric chloride, sodium acetate, 2,4,6-tripyridyl-s-triazine (TPTZ), and rutin were obtained from Sigma-Aldrich Chemical Co. All other reagents were of analytical grade.

The UV spectra were measured with a Spectronic 2D spectrophotometer (Thermo Electron Co., USA). HPLC was performed using an Agilent 1200 series system (Agilent Co., Australia) equipped with a quaternary HPLC pump, a degasser, and an Agilent 1200 series diode array detector (DAD). The isoflavones were analyzed on a LiChrospher 100 RP C18 column (4.6 × 150 mm, 5 μm; Merck, Germany).

Fermentation of black soybean

Two whole black soybeans (1,000 g) were washed and soaked with three volumes a tap water at 20±2℃ for 12 h, and steamed for 15 min at 121±1℃. The steamed soybeans were left to stand for 1 h at 38±1℃ to cool down. Then, the cooked black soybeans were inoculated with 5.0% (w/w) strain CSY191 (7.65 log CFU/ml) and fermented for 24 h at 37±2℃ in an incubator and sampled at 0 and 48 h.

Viable cell number and β-glycosidase activity

Ten gram of sample was mixed with 90 ml of 0.85% NaCl solution and the diluted suspension (0.1 portions) was spread on a TSA plate. The plates were incubated at 37℃ for 24 h, after which colony counts were carried out.

The method described by Cho *et al.* (2011) was used to determine the β-glycosidase activity. Ground *cheonggukjang* (1.0 g) was mixed with 20 ml of 50 mM sodium phosphate buffer (pH 7.0), vortexed for 1 min, and centrifuged at 6000×g at 4℃ for 30 min. The supernatant was collected and filtered through a 0.45-μm filter before analysis. The β-glycosidase activity in the crude extract was assayed by determining the rate of hydrolysis of *p*-NPG. The crude extract (250 μl) was added to 250 μl of substrate (5 mM *p*-NPG) in 50 mM sodium phosphate buffer (pH 7.0). After 30 min of incubation 37℃, the enzymatic reaction was stopped by adding 500 μl of 0.2 M glycine-NaOH (pH 10.5) and the contents were immediately measured in a spectrophotometer at 405 nm. The blank solution was composed of 2.5 ml of 50 mM glycine-NaOH, 2.0 ml of substrate solution, and 0.5 ml of 50 mM citric buffer (pH 4.5) containing 0.1 M NaCl. The *p*-NP released by the action of the enzyme was determined by referring to a calibration curve prepared from the *p*-NP in concentrations that varied from 5 to 300 mM. One unit of β-glycosidase activity was defined as the amount of enzyme that liberated 1 μM of *p*-NPG.

Isoflavone extraction and analysis

The isoflavone extract and analysis were performed as previously described (Cho *et al.*, 2011). Briefly, each of the ground powders (10 g) was extracted with 100 ml of 50% methanol by shaking (320 rpm) at 30℃ for 12 h; the extracts were filtered through Whatman No. 2 filter paper (GE Healthcare Life Sci., USA) and then filtered through a 0.45-μm Millipore PVDF filter (Schleicher & Schuell, Germany). Samples of the filtrates were used for HPLC analysis. The rest of the filtrates were dried under a vacuum. The dried samples were stored at -70℃ in the dark until further use in antioxidant activity assays, and then the dried materials were redissolved in 50% methanol at 1 mg/ml.

The isoflavones were analyzed by HPLC. A 20 μl sample of the crude 50% methanol extracts was injected onto an analytical C_{18} column with the column temperature set to 30℃. The isoflavones were detected by monitoring the elution at 254 nm using a DAD. The isoflavones in the samples were identified by comparing their retention times to those of

Table 1. Change of viable cell numbers and β-glycosidase activities during *cheonggukjang* fermentation of black soybeans by *B. subtilis* CSY191

Soybean Cultivars	Samples	Contents[1)]	
		Viable cell numbers (log CFU/g)	β-Glycosidase activity (Unit/g)
Seoritae	UFBS[2)]	5.01±0.20[b]	4.2±0.11[b]
	FBS[3)]	11.19±0.62[a]	22.8±1.11[a]
Seomoktae	UFBS	4.81±0.18[b]	4.0±0.22[b]
	FBS	11.27±0.55[a]	22.7±1.00[a]

[1)] Values indicate the mean's of three replications (n=3). All values are means of determinations in three independent experiments. Means with different lowercase letters (a and b) indicate significant differences of fermentation times by Tukey's multiple range test ($p < 0.05$).
[2)] Unfermented black soybeans
[3)] Fermented black soybeans at 37℃ for 48 h.

standards. The mobile phase was composed of 0.1% glacial acetic acid in water (solution A) and 100% acetonitrile (solution B). The gradient conditions were as follows: 0–20 min, 10% B; 30 min, 20% B; 40 min, 25% B; and 50 min, 35% B. The solvent flow rate was maintained at 1 ml/min.

Total phenolic contents (TPCs)

A method based on that of Cho *et al.* (2011), which uses gallic acid equivalents (GAE) was used to quantify the TPCs in the 50% methanol extracts. A 500 μl aliquot of each isoflavone extracts was mixed with 250 μl of 2 N Folin-Ciocalteu's reagents. After standing at room temperature for 3 min, a 500 μl aliquot of a 25% sodium carbonate (Na_2CO_3) solution was added, and that mixture was allowed to stand at room temperature for 1 h. Quantification was performed using a linear regression equation on the gallic acid standard curve. Five gallic acid standard solutions of 100, 250, 500, 750, and 1000 mg/L were prepared in deionized water, and 500 μl of each standard solution was collected and prepared using the same procedure described above, the absorbance of the solution was measured at 750 nm, and a standard curve was obtained by plotting the concentration against absorbance.

ABTS radical scavenging activity

$ABTS^{\bullet+}$ was dissolved in methanol to a finial concentration of 7 mM. This radical cation was produced by reacting the $ABTS^{\bullet+}$ stock solution with 2.45 mM potassium persulfate (final concentration) and by leaving the mixture for 12–16 h until the reaction was complete and the absorbance was stable. The $ABTS^{\bullet+}$ stock solution was diluted in ethanol to an absorbance of 0.7±0.02 at 734 nm. After adding 0.9 ml of the diluted $ABTS^{\bullet+}$ solution to 0.1 ml of the sample and mixing them, the absorbance was taken 3 min later (Choi *et al.*, 2012). This scavenging activity (%) was expressed as a percentage using the following formula: ABTS radical scavenging activity (%) = (1 – absorbance of sample / absorbance of control) × 100.

Ferric reducing/antioxidant power (FRAP) assay

The ferric reducing/antioxidant power (FRAP) assay developed by Choi *et al.* (2012) was used. Briefly, 1.5 ml of working FRAP reagent pre-warmed to 37℃ [300 mM acetate buffer (pH 3.6):10 mM TPTZ in 40 mM HCl:20 mM $FeCl_3$ = 10:1:1 (v/v/v)] was mixed with 50 μl of the test samples and standards. This mixture was vortexed, and the absorbance at 593 nm was read against a reagent blank at a pre-determined time after the sample-reagent mixing. The test was performed at 37℃, and a 0–4 min reaction time window was used.

Statistical analysis

All values are means of determinations in three independent experiments. Differences in the means of each value were determined by one-way ANOVA followed by the Tukey's multiple range tests at P<0.05 using the Statistical Analysis System software, Version 9.0 (SAS Institute, USA).

Results and Discussion

Change of viable cell numbers and β-glycosidase activities during *cheonggukjang* fermentation of black soybeans

The viable cell numbers and β-glycosidase activities increased with fermentation (Table 1). As the results, the viable cell numbers of bacteria in *cheonggukjang* fermentation of black soybeans range from 5.01 and 4.81 log CFU/ml to 11.19 and 11.27 log CFU/ml, while the levels of β-glycosidase activity increased from an initial (0 h) 4.2 and 4.0 unit/g to 22.8 and 22.7 unit/g after 48 h, respectively. Several studies reported that the viable cell numbers of *Bacillus* spp. increased depending on β-glycosidase activities increased during *cheonggukjang* fermentation (Yang *et al.*, 2006; Cho *et al.*, 2009, 2011).

Change of total phenolic and isoflavone contents during *cheonggukjang* fermentation of black soybeans

The change in the TPCs during *cheonggukjang* fermentation of two black soybeans (*Seoritae* and *Seomoktae*) is shown in

Table 2. Distributions of 12 isoflavone contents during *cheonggukjang* fermentation of black soybeans by *B. subtilis* CSY191

Soybean Cultivars	Samples	Isoflavone contents[1] (μg/g)												Total
		Glycosides			Malonylglycosides			Acetylglycosides			Aglycones			
		Daidzin	Glycitin	Genistin	Daidzin	Glycitin	Genistin	Daidzin	Glycitin	Genistin	Daidzein	Glycitein	Genistein	
Seoritae	UFBS[2]	603.2±30.11^{a}	217.5±10.18^{a}	913.1±45.65^{a}	160.5±8.00^{a}	22.7±1.01^{c}	274.9±13.74^{b}	nd[4]	nd	tr	111.1±5.65^{c}	101.7±51.21^{a}	63.5±3.22^{ab}	2463.3
	FBS[3]	303.7±14.22^{c}	368.0±18.32^{a}	516.5±25.32^{c}	166.7±7.56^{a}	90.5±4.65^{a}	254.4±10.16^{bc}	nd	tr[5]	tr	253.0±12.66^{a}	72.5±3.06^{b}	114.1±5.67^{a}	2139.2
Seomoktae	UFBS	470.3±23.65^{b}	306.3±15.63^{ab}	747.9±37.20^{b}	125.6±6.93^{b}	26.2±1.53^{c}	229.9±11.49^{c}	nd	tr	tr	93.0±4.26^{d}	82.5±4.31^{b}	42.4±2.31^{b}	2124.2
	FBS	279.0±13.81^{c}	353.5±16.26^{a}	489.8±24.34^{c}	179.2±8.99^{a}	56.6±2.38^{b}	330.7±13.23^{a}	nd	nd	tr	155.0±7.47^{b}	46.4±2.33^{c}	57.9±2.49^{ab}	1948.1

[1] Values indicate the mean's of three replications (n=3). All values are means of determinations in three independent experiments. Means with different lowercase letters (a, b, and c) indicate significant differences of fermentation times by Tukey's multiple range test (P<0.05).
[2] UFBS, Unfermented black soybeans.
[3] FBS, Fermented black soybeans at 37℃ for 48 h.
[4] nd, not detected.
[5] tr, trace < 0.002 μg/g.

Fig. 1. The *cheonggukjang* of *Seoritae* and *Seomoktae* at 37℃ for 0 and 48 h showed 15.05 (*Seoritae*) and 13.33 (*Seomoktae*) mg GAE/g dry weight and 20.37 and 17.93 mg GAE/g dry weight, respectively (Fig. 1). The phenolics are secondary plant metabolites that are present in all plants. Phenolics are usually found in conjugated forms through hydroxyl groups with sugars and glycosides in plant materials (Juan and Chou, 2010). Catalyzing the release of the total phenolic contents from the soybean substrate during fermentation may thus lead to an increase in the content of those compounds, as shown in Fig. 1. Similar previous studies reported that the total phenolic content increased during soybean fermentation in foods, such as *cheonggukjang* and *natto* (Shon *et al.*, 2007; Cho *et al.*, 2009, 2011; Juan and Chou, 2010).

In the case of *cheonggukjang* of *Seoritae* soybean, the isoflavone-malonylglycoside and -aglycone contents increased throughout fermentation to approximately 1.3- and 1.8-fold relative to their starting amounts at 48 h (23.9% and 20.6%, respectively), but the isoflavone-glycoside contents decreased from 70.2 to 55.5% at the end of fermentation (48 h). In the *cheonggukjang* of *Seomoktae* soybean, the levels of isoflavone-malonylglycoside and -aglycone increased throughout fermentation to approximately 1.6 and 1.2-fold relative to their starting amounts at 48 h (29.1% and 13.3%, respectively), but the isoflavone-glycoside contents decreased to 71.2 to 57.6% at the end of the fermentation time (48 h) (Fig. 2). In particular, daidzin of the glycoside type decreased from 603.2 μg/g to 303.7 μg/g, and the corresponding daidzein of the aglycone type increased to a maximum of 253.0 μg/g at 48 h during the fermentation process of *Seoritae* soybean (Table 2). The isoflavone-glycosides decreased, while the isoflavone-aglycones increased during *cheonggukjang* fermentation (Fig. 3A–3D).

The content and composition of these isoflavones vary in soybean foods depending on the soybean varieties and processing techniques used, such as fermentation. It has been reported that the isoflavone levels in soybean-containing foods, such as *tofu*, *douchi*, and *cheonggukjang*, decrease depending on the processing conditions (Coward *et al.*, 1998; Jang *et al.*, 2006; Prabhakaran *et al.*, 2006; Yang *et al.*, 2006; Cho *et al.*, 2009, 2011). Jang *et al.* (2006) reported that the total isoflavone content in raw soybeans was 2.87 μg/g, and this content decreased by approximately 50% during cooking prior to

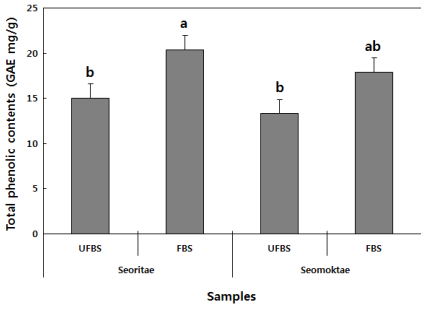

Fig. 1. Change of total phenolic contents during *cheonggukjang* fermentation of brown soybeans by *B. subtilis* CSY191. All values are means of determinations in three independent experiments. Means with different lowercase letters (a and b) indicate significant differences of fermentation times by Tukey's multiple range test (P<0.05).

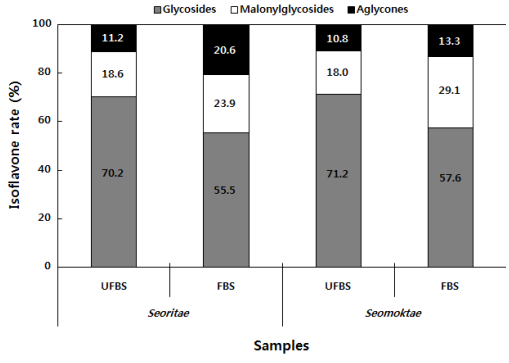

Fig. 2. Change of isoflavone-β-glycoside, -malonyl-β-glycoside, and -aglycones and total isoflavone contents during *cheonggukjang* fermentation of brown soybeans by *B. subtilis* CSY191. All values are means of determinations in three independent experiments.

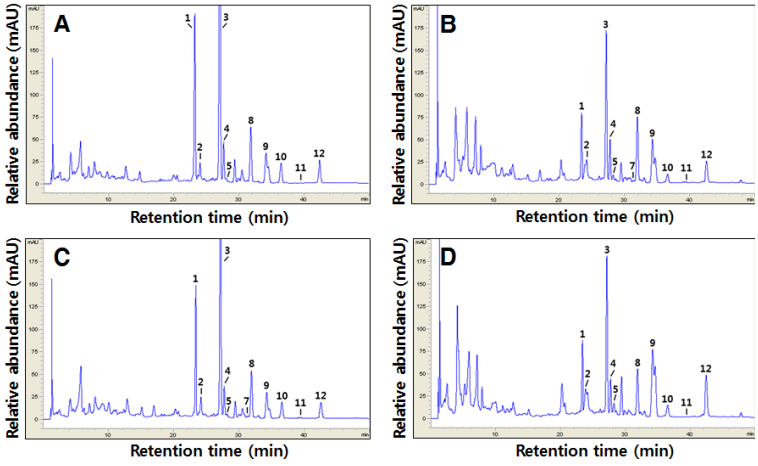

Fig. 3. Typical HPLC chromatograms of isoflavones. (A) HPLC chromatogram of isoflavone extract in *cheonggukjang* of *Seoritae* according to fermentation period (0 h); (B) HPLC chromatogram of isoflavone extract in *cheonggukjang* of *Seoritae* according to fermentation period (48 h); (C) HPLC chromatogram of isoflavone extract in *cheonggukjang* of *Seomoktae* according to fermentation period (0 h); and (D) HPLC chromatogram of isoflavone extract in *cheonggukjang* of *Seomoktae* according to fermentation period (48 h). 1, daidzin; 2, glycitin; 3, genistin; 4, malonyldaidzin; 5, malonylglycitin; 7, acetylglycitin; 8, malonylgenistin; 9, daidzein; 10, glycitein; 11, acetylgenistein; and 12, genistein.

cheonggukjang fermentation. The total isoflavone content decreased from 1,055 μg/g (0 h) to 870 μg/g (36 h) during *cheonggukjang* fermentation by *B. subtilis* (Yang *et al.*, 2006). Recently, Cho *et al.* (2011) showed that the total isoflavone content in *cheonggukjang* fermentation decreased approximately 64% from an initial 2923.21 μg/g to 1051.59 μg/g after 60 h of fermentation. In this study, the total isoflavone content decreased by approximately 13.2% and 9.0 after fermentation processing in raw soybeans from 2463.3 and 2124.2 μg/g to 2139.2 and 1948.1 μg/g at the end of fermentation (48 h), respectively (Table 2).

In general, most isoflavones in soybean are present in glycoside form, and they are converted into aglycones during fermentation by microbial β-glycosidase activity (Otieno *et al.*, 2005; Yang *et al.*, 2006; Wang *et al.*, 2007; Cho *et al.*, 2009, 2011). Cho *et al.* (2011) recently reported that the levels of isoflavone-aglycones increased, while the β-glycosidase activity and isoflavone-glycosides decreased during *cheonggukjang* fermentation by the potential probiotic *B. subtilis* CS90. In this study, we found that the starter potential probiotics *B. subtilis* CSY191 had the effect of increasing the β-glycosidase activity, and the aglycone contents increased over 48 h. In contrast, Yang *et al.* (2006) reported that the addition of *B. subtilis* had no effect on β-glycosidase activity, and the aglycone contents did not increase during *cheonggukjang* fermentation.

Change of antioxidant activities during *cheonggukjang* fermentation of black soybeans

The *cheonggukjang* of *Seoritae* soybean exhibited stronger antioxidant activities than the *cheonggukjang* of *Seomoktae* soybean during fermentation periods of 0 and 48 h (Fig. 4).

To determine the hydrogen-donating antioxidants and chain-breaking antioxidants, we measured the ABTS radical

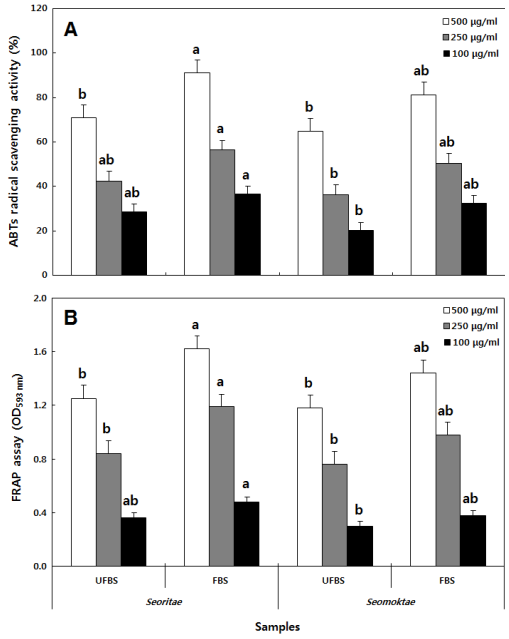

Fig. 4. Effects of antioxidant activity by *cheonggukjang* fermentation of black soybeans with *B. subtilis* CSY191. (A) Changes of ABTS radical scavenging activities during *cheonggukjang* fermentation; and (B) Changes of FRAP during *cheonggukjang* fermentation. All values are means of determinations in three independent experiments. All values are means of determinations in three independent experiments. Means with different lowercase letters (a and b) indicate significant differences of fermentation times by Tukey's multiple range test ($P<0.05$).

scavenging ability of *cheonggukjang* of *Seoritae* and *Seomoktae* soybeans. The levels of ABTS radical activity in *cheonggukjang* of *Seoritae* increased greatly from 70.81% (500 μg/ml) at 0 h of fermentation to 91.06% at 48 h of fermentation. Additionally, the ABTS radical activities in *cheonggukjang* of *Seormoktae* increased greatly from 64.87% at 0 h of fermentation to 81.12% at 48 h of fermentation (Fig. 4A).

The FRAP assay is a direct test of the total antioxidant power. In the case of *cheonggukjang* of *Seoritae*, the values resulting from the FRAP assay of the fermented soybeans at 37℃ for 0 and 48 h increased by 1.25 (500 μg/ml) and 1.62, respectively. In the *cheonggukjang* of *Seormoktae*, the values resulting from FRAP assay of fermented soybeans at 37℃ for 0 and 48 h increased by 1.18 and 1.44, respectively (Fig. 4B).

The total phenolic contents were measured as an overall indicator of the contents of these molecules with antioxidant properties (Slavin *et al*., 2009). Shon *et al.* (2007) reported that a methanol extract of *cheonggukjang* exhibited a radical-scavenging activity of 69–87% and total phenolic contents of 0.13–0.27 mg/g. In particular, Juan and Chou (2010) found that fermentation enhanced the total phenolic content as well as antioxidant activity of the black soybean extract. Previously, many isoflavones were reported to have low scavenging potency for DPPH free radicals with scavenging effects only half that of α-tocopherol and one-third that of epicatechin (Kao and Chen, 2006). However, isoflavones have direct free radical quenching ability, with daidzein and genistein being particularly effective (Shon *et al.*, 2007; Cho *et al.*, 2009, 2011). In addition, Kim *et al.* (2008) reported that the *cheonggukjang* extract and its constituents, genistein and daidzein, exhibited significant antioxidant activity *in vitro*. We recently reported that the radical scavenging activity increased from 53.6% to 93.9% depending on the total phenolic and isoflavone-aglycone (daidzein) contents during *cheonggukjang* fermentation by the potential probiotic *B. subtilis* CS90 (Cho *et al.*, 2011). In particular, Kwak *et al.* (2007) suggested that the stronger antioxidant activity of *cheonggukjnag* might be related to the markedly higher total phenolic contents and isoflavone-aglycones and -malonylgycosides achieved during fermentation.

In conclusion, this study has documented for the first time that changes occur in the total phenolic contents and in the contents of 12 isoflavones during *cheonggukjang* fermentation of Korean black soybeans (*Seoritae* and *Seomoktae*) by the potential probiotic *B. subtilis* CSY191. Among these changes, the total phenolic and isoflavone-aglycone contents were markedly increased, while the isoflavone-glycosides were decreased according to the β-glycosidase activities. The total phenolic and total isoflavone contents and antioxidant activities were higher in *cheonggukjang* of *Seoritae* than *cheonggukjang* of *Seomoktae* at 0 and 48 h of fermentation. These results suggest that the high antioxidant activity of *cheonggukjang* fermentation of black soybeans might be related to the markedly higher total phenolic and isoflavone-aglycone contents achieved during fermentation.

적 요

잠재적인 생균제제 *Bacillus subtilis* CSY191에 의해 제조된 국산 검정콩(서리태 및 서목태) 청국장 발효 중 β-glycosidase 활성, total phenolic와 isoflavone 함량 및 항산화 활성을 조사하였다. 청국장 발효 후 total phenolic 및 isoflavone-malonylgycoside와 -aglycone 함량은 증가하였고 이에 따라 ABTS 라디칼 소거활성 및 FRAP 활성은 증가하였으나 isoflavone-glycoside 함량은 감소하였다. 특히, 37℃에서 48시간 발효된 서리태 청국장은 서목태 원료 및 청국장보다 높은 활성을 나타내었다. 서리태 48시간 발효 후, daidzein, glycitein 및 genistein의 함량은 각각 253.0 μg/g 72.5 μg/g 및 114.1 μg/g을 나타내었다. 이 결과로부터, 검정콩 청국장의 높은 항산화 활성은 total phenolic 및 isoflavone-malonylgycoside와 -aglycone 함량 증가에 의한 것으로 추측할 수 있다.

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