The Characteristics of *Cheonggukjang*, a Fermented Soybean Product, by the Degree of Germination of Raw Soybeans

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Abstract This study was conducted to observe the quality characteristics of *cheonggukjang* fermented with new small-seed soybeans ('Agakong') according to the degree of germination of the raw soybeans. The sprouting rate was 8.6±5.6% after 12 hr of germination, but at 24 hr it increased rapidly to 85.4±4.7%. We confirmed that the total isoflavone content immediately after soaking was 273.9 mg%, which was at least 3 times greater than for common soybeans; content increased at the start of germination, and increased to 338.4 mg% by 24 hr of germination, but then decreased. The quantity of viscous substance of the *cheonggukjang* increased in proportion to the degree of germination of the raw soybeans. The levels of amino acids in the *cheonggukjang* made from non-germinated soybeans, and soybeans germinated 48 hr, were 12.45 and 10.06%, respectively. The isoflavone levels in the *cheonggukjang* were determined by the degree of germination of the raw soybeans. There were no significant differences between the odor, sweet taste, savory taste, bitter taste, and overall acceptability of the *cheonggukjang* with different germination times of 0 to 36 hr.

Keywords: germination, soybean, cheonggukjang, organic acid, amino acid, isoflavone

Introduction

Soybeans and soy foods are widely consumed in the Orient as main dietary sources of protein and fat (1). It has been shown that the functional compounds of soybeans are dietary fiber, oligosaccharides, isoflavones, phytic acid, Bowman-Birk protease inhibitor, saponins, and peptides (2-6).

Epidemiological evidence from several studies has demonstrated that isoflavones in soybean are associated with anticarcinogenic effects for breast, esophageal, skin, liver, prostate, and colorectal cancers (7-9). It was reported that the isoflavones in soybeans may protect from circulatory organ diseases like arteriosclerosis and hypertension (10) and from osteoporosis (11). Among the isoflavones, the functionalities of genistin, genistein, daizin, and daidzein have been demonstrated, and isoflavone contents are greatly variable among soybeans varieties, growing environments, and parts of the soybean (12-14).

Germination may cause changes in nutrients, including functional substances, through aerobic respiration and biochemical metabolism. Previously, it was reported that non-protein nitrogen increased during germination (15), while protein and lipids decreased (16). Germination also affected oligosaccharides by significantly reducing levels 60-100% (17-19). Finally, isoflavones were shown to

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Received January 9, 2007; accepted April 28, 2007

increase within a short germination period (6-24 hr), and then gradually decreased thereafter (20).

In a previous study, we selected a new small-seed recombinant inbred line (RIL: 'Agakong') derived from an interspecific cross between 'Eunhakong' (*Glycine max*) × KLG10084 (*Glycine soja*), and verified the agronomic characteristics (21, 22).

In this study, we observed the changes in quality characteristics, including isoflavones, based on the degree of germination, as well as the properties of the *cheonggukjang* fermented with the germinated new small-seed recombinant inbred line (RIL: 'Agakong'), as basic research for the development of various fermented foods using 'Agakong'.

Materials and Methods

Materials The soybean variety used for this research was 'Agakong', developed from the interspecific cross between a cultigen ('Eunhakong') and KLG10084 (22, 23) a wild soybean line which has green seed coat color, and was acquired from Soyventure Co., Ltd. (Daegu Korea). The microorganism used for the fermentation of *cheonggukjang* was *Bacillus licheniformis* B1 which is known to have good fermentation ability and produces large amounts of viscous substance during fermentation (24)

Germination The washed soybean seeds were soaked in 20°C water for 4 hr and transferred into culture containers

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(25×25×30 cm). The culture containers were placed in a thermally-controlled dark house. The soaked soybean seeds were cultivated for 60 hr under a top-irrigation system that is used for commercial soybean sprout production. Irrigation was given for 3 min every 3 hr with subterranean water. The average temperature inside the culture house during the experiment was 20 to 25°C.

Measurement of germination characteristics The total yield was expressed as the percentage of harvested sprout weight relative to the dry seed weight. The germination ratio was expressed as the percentage of the number of germinated soybeans relative to the number of total soybeans. Twenty germinated soybeans were selected randomly by germination time, and sprout length was measured with a vernier caliper (5/100 m, Mitutoyo, Gawasaki, Japan).

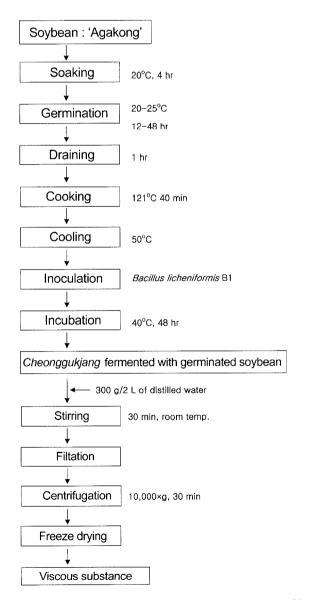


Fig. 1. The procedure for preparation of *cheonggukjang* fermented with germinated soybean and separation of viscous substance from *cheonggukjang*.

Preparation of *cheonggukjang* The manufacturing process for *cheonggukjang* is shown in Fig. 1. To describe the process in detail, each carefully selected soybean was immersed for 4 hr at a temperature of 20°C. After each soybean had sprouted, after 12, 24, 36, and 48 hr, the water was eliminated and each soybean was placed on a stainless vessel. The sprouted soybeans were allowed to develop over a period of 40 min at a temperature of 121°C. Then, the beans were cooled to 50°C and inoculated with *Bacillus licheniformis* B1, which was cultivated with the source soybeans to become 2%, for 48 hr at 40°C.

Separation of the viscous substance The separation of the viscous substance (25) is demonstrated in Fig. 1. Here, 300 g of *cheonggukjang* composed of the soybeans germinated for different times, were mixed with 2 L of pure water and agitated for 30 min at 200 rpm. After filtration, 10,000×g of the substance was centrifuged; the upper portion was freeze-dried and finally weighed.

pH and color measurements The pH measurements were carried out with a pH meter (G-P Combo w/RJ; Corning, Switzerland) using 5 g of *cheonggukjang* sample mixed with 5 mL of distilled water. The color measurements of the *cheonggukjang* were performed with a Chromameter (CR 300; Minolta, Osaka, Japan) calibrated with a white standard plate (L=97.51, a= -0.18, b=+1.67).

Free amino acids The *cheonggukjang* was homogenized and extracted with 1% picric acid. A Pass Dowex 2×8 (Clform, 100-200 mesh) column was used to remove and evaporate the picric acid. After adding sodium citrate buffer (pH 2.2), the *cheonggukjang* was filtered by a membrane filter (0.45 μm) and injected into an automatic amino acid analyzer (Biochrom 20; Uppsala, Sweden) and quantified. The analysis conditions were as follows: a buffer flow rate of 20 mL/hr and a ninhydrin flow rate of 20 mL/hr, a temperature gradient of 35-74-80-37°C, a wavelength from 570 to 440 nm, a column length of 46×250 nm, and an injection volume of 20 μL.

Quantification of isoflavones by HPLC The isoflavone analysis was performed by the method described by Wang et al. (6), with slight modification (26). Twenty uL of filtrate was injected into an HPLC system equipped with a Bondapack C18 column after the system had been equilibrated at ambient temperature. Also, the UV detector was stabilized with a mobile phase (methanol:1 mM ammonium acetate, 6:4) at a flow rate of 1 mL/min for 30 min. The effluent was detected at 254 nm and a chromatogram was recorded for 20 min. The isoflavones were identified by their retention times of standard addition, and their contents were calculated by comparing their peak areas with those of standards.

Sensory evaluation To evaluate the quality of the produced *cheonggukjang*, 17 well-trained panelists performed a 7-point sensory test in terms of external appearance, odor, taste, and overall acceptance. The panel members were aware of the experiment's objective and were trained to evaluate the taste of the *cheonggukjang*. The grading was on a scale of 7 (best) to 1 (worst), and the average score was 4. In each

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sensory test, 5 samples of 50 g of *cheonggukjang* were placed onto a dish and given to each panel member; a randomly assigned 3-digit number was given to each sample. Analysis of variance (ANOVA) and Duncan's multiple range tests were performed using the Minitab program.

Results and Discussion

The sprouting characteristics of the small-seeded recombinant inbred line ('Agakong') Compared to the dried soybeans, the total yield of the soybeans ('Agakong') after soaking was 192±8.3%. Then, as germination progressed, the yield slowly increased and by the 60th hr reached 301±6.8% (Fig. 2A). This was similar to the results of Kwon *et al.* (27) who reported that the harvest rate of small seeds was higher than that of large seeds, during attempts to screen high quality varieties in establishing an index of selection characteristics while growing soybean sprout varieties. Kim *et al.* (28) reported that in cultivating soybean sprouts, irrigation with 0.3 ppm of ozone water showed a 10-17% higher harvest rate than irrigation with general tap water. The changes in root

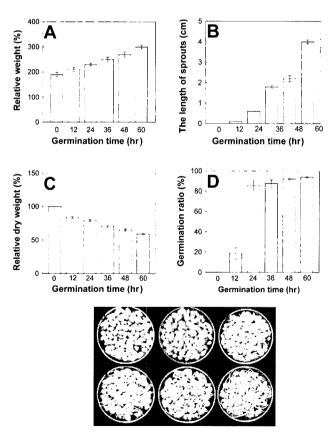


Fig. 2. The sprouting characteristics of the small seeded recombinant inbred line ('Agakong'). A, germination ratio; B, relative weight; C, length of soybean sprouts; D, relative dry weight; E-J, the photograph of sprouted soybean according to germination time. (E, soaked for 4 hr at 20°C; F, germinated for 12 hr after soaking; G, germinated for 24 hr after soaking; H, germinated for 36 hr after soaking; I, germinated for 48 hr after soaking; J, germinated for 60 hr after soaking.)

length during soybean germination are shown in Fig. 2B, with photographs in Fig. 2E-J. At the 12th hr, the embryo had already separated and the root was starting to grow, and it then showed rapid growth. By the 24th hr it reached 0.6 ± 0.0 cm, and by the 60^{th} hr reached 4.0 ± 0.1 cm. These growth results were twice as fast as those in a report where the embryo separated, and the root appeared by the 34th hr of germination for 'Dawonkong', 'Taekwangkong', and 'Myeongju-namulkong'. The reason for this phenomenon was that the growth speed of the bean sprout roots became slower as the weight of the beans became heavier (29). The changes in relative dry weight during soybean germination are shown in Fig. 2C. Compared with the control, the relative dry weight after 12 hr of germination was 73.0±1.1%. It gradually decreased with germination and by the 60th hr reached 62.2±0.7%. Previously, it was reported that the soaking time required to supply the necessary water for germination during cultivation, was slightly different depending on the soybean variety; however, when irrigating more than 6 times a day, no problems with germination after a 3 hr soaking time were observed, and the optimum temperature after soaking was 20-25°C (30). For this reason, our soybeans were soaked for 4 hr and placed in a constant temperature-controlled room, which was kept at 25°C. The germination rate, measured at an interval of 12 hr by adding water for 2 hr, is shown in Fig. 2D. After 12 hr of germination, the germination rate was 8.6±5.6%, but after 24 hr it had increased rapidly to 85.4±4.7%. By the 60th hr, the germination rate was 93.9±0.5%.

The changes in isoflavones content during soybean germination are shown in Fig. 3. The isoflavone levels were in the order of genistein > daidzein > glycitein throughout the progression of germination. We confirmed the total isoflavone content right after soaking to be 273.9 mg%, which were at least 3 times (30) more than for the common soybeans. Isoflavone content increased at the beginning of germination, and by the 24th hr reached 338.4 mg%, but decreased thereafter. The isoflavone content 60

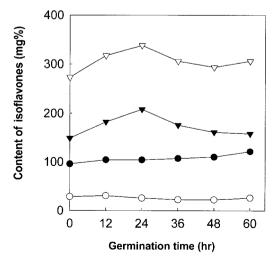


Fig. 3. Changes in the content of isoflavones of soybean ('Agakong') during germination. - ▽ - total isoflavone; - ▼ - genistein; - ○ - glycitein; - ● - daidzein.

hr after germination was 306.2 mg%. The isoflavone contents according to the germination period, using 'Myeongju-namulkong', 'Taekwangkong' and 'Dawonkong', have previously have been reported (29). Total isoflavone content increased 20-50% after 24 hr of germination, and aglycone types like daidzein and genistein, in particular, increased, showing similar results to this study. Furthermore, after producing soy milk with germinated soybeans, isoflavone levels significantly increased, and according to the sensory evaluation results, the most preferred soy milk was produced by soybeans germinated for 12 hr (32).

Differences in the pH and color of *cheonggukiang* Table 1 shows the pH level and color differences of cheonggukjang fermented with germinated soybeans at different germination times, every 12 hr. The pH level was between 7.7 and 7.2 regardless of the degree of germination of the raw material. The L-value of the cheonggukjang fermented using non-germinated raw material was 51.4; large differences among the germination times were not shown, with values ranging 49.8-51.4. The a-value was 0.9 in the control, and as germination time of the raw soybeans increased, the a-value of the cheonggukjang also increased. But changes based on the germination rate were insufficient. The b-values didn't show large differences according to the degree of germination of the raw materials. In addition, the a/b values and ΔT (48.8-50.4) showed only slight differences.

Differences in viscous substance content for cheonggukjang according to the degree of germination of the raw soybeans Figure 4 shows the differences in viscous substance contents for cheonggukjang according to the degree of germination of the raw soybeans. The viscous substance amount increased in proportion to the degree of germination of the raw soybeans. We confirmed that the *cheonggukjang* made from soybeans germinated 48 hr had 1.08±0.01%, which was approximately 1.3 times greater than the *cheonggukjang* made from the nongerminated soybean's (0.85±0.03%). Low molecular weight viscous substances show strong radical scavenging activities for hydroxyl and superoxide anion radicals, as assessed by electron spin resonance (33). Also, it was

Table 1. The pH and color of whole soybean-cheonggukjang fermented with germinated soybeans according to the germination time

		Germination time (hr)				
	_	0	12	24	36	48
pН		7.7	7.6	7.4	7.3	7.2
	L	51.4	50.9	50.1	49.8	50.1
	a	8.5	8.3	8.6	8.8	8.3
Color	b	23.8	24.7	24.8	24.9	25.4
	a/b	0.35	0.34	0.35	0.37	0.33
	$\Delta T^{\rm l)}$	57.3	57.2	56.3	56.0	56.8

 $^{^{1)}\}Delta T = \sqrt{(L^2 + a^2 + b^2)}$.

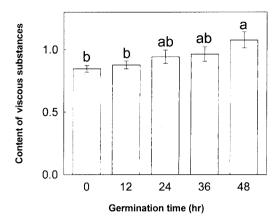


Fig. 4. The composition of viscous substance in the *cheonggukjang* fermented with germinated soybean according to germination time. Means followed by the same letter are not significantly different at the 5% level.

demonstrated that the ingestion of viscous substances from natto led to the inhibition of low-density lipoprotein (LDL) oxidation, and that the fraction formed direct antioxidants in the body (34). In addition, greater inhibitory effects of angiotensin converting enzyme were observed in the viscous fraction than in the bean extract (35). Therefore, the efficacy of *cheonggukjang* for inhibiting LDL oxidation and angiotensin converting enzyme, as well as its radical scavenging activities for hydroxyl and superoxide anion radicals, are predicted to be stronger when using germinated soybeans; however, further research related to such results needs to be completed.

Differences in free amino acids for cheonggukjang according to the degree of germination of the raw soybeans The levels of free amino acids cheonggukjang according to the degree of germination of the raw soybeans are shown in Table 2. The level of organic amino acids in the cheonggukjang made from the non-germinated soybeans was 12.45%. For the cheonggukjang made of soybeans germinated 48 hr, the content was 10.06%. Among the free amino acids, threonine, serine, glycine, alanine, and lysine represent a sweet taste; aspartic acid, glutamic acid, and cystein a savory taste; and methionine, isoleucine, and leucine a bitter taste (36). Weak differences between the sweet taste components (28.8-29.2%), savory taste components (28.7-30.3%), and bitter taste components (15.2-15.4%) were discovered by the germination of the raw soybean.

Differences in isoflavone contents for cheonggukjang according to the degree of germination of the raw soybeans The isoflavone contents of the cheonggukjang according to different degrees of germination are shown in Table 3. The level of isoflavones in the cheonggukjang made from non-germinated soybeans was 281.4 mg after 48 hr of fermentation. On the other hand, for the germinated soybeans, after 12 and 24 hr each displayed levels of 329.3 and 359.1 mg, respectively. This confirms that the initial germination helped increase the level of isoflavones. However, when germination proceeded for

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Table 2. Amino acid compositions of *cheonggukjang* fermented with germinated soybean (Unit: mg%)

Amino acid		Germination time (hr)				
Am	ino acia	0	24	48		
	Thr	491.8	431.0	447.8		
Sweet taste	Ser	733.9	631.8	576.0		
	Gly	594.4	530.9	478.5		
	Ala	595.6	521.4	486.7		
	Lys	1,175.5	1,073.9	950.3		
	Subtotal	3,591.2 (28.8%)	3,189.0 (29.0%)	2,939.3 (29.2%)		
	Asp	1,228.8	1036.4	1,032.8		
Corrowr	Glu	2,347.3	1,976.2	1,883.4		
Savory taste	Cys	167.0	146.1	133.1		
	Subtotal	3,743.1 (30.1%)	3,158.7 (28.7%)	3,049.3 (30.3%)		
	Met	171.0	154.8	130.6		
D:44	Ile	619.2	545.8	518.6		
Bitter taste	Leu	1,102.4	987.2	901.0		
	Subtotal	1,892.6 (15.2%)	1,687.8 (15.4%)	1,550.2 (15.4%)		
Others	Pro	930.5	907.3	659.9		
	Val	645.6	561.8	553.4		
	Tyr	478.4	423.9	384.0		
	Phe	707.5	638.4	567.0		
	His	354.2	327.9	271.2		
	Arg	107.7	99.9	87.0		
	Subtotal	3,223.9 (25.9%)	2,959.2 (26.9%)	2,522.5 (25.1%)		
Total		12,450.8	10,994.8	10,061.4		

 $\begin{tabular}{ll} \textbf{Table 3. Isoflavones compositions of $cheonggukjang fermented} \\ \textbf{with germinated soybean} & (Unit: mg\%) \end{tabular}$

Isoflavone	Germination time (hr)					
isonavone	0	12	24	36	48	
Daidzein	99.5	105.4	109.2	112.9	116.3	
Glycitein	19.3	21.6	16.9	15.2	14.8	
Genistein	162.6	202.4	232.9	194.6	176.9	
Total isoflavones	281.4	329.3	359.1	322.7	308.0	

more than 24 hr, there was a decrease in content. These changes are similar to the patterns of isoflavone levels, according to soybean germination time, shown in Fig. 3. Thus, the level of isoflavones in the *cheonggukjang* was determined by the degree of germination of the raw soybeans. Therefore, when considering the level of isoflavones, it is more desirable to make *cheonggukjang* using soybeans germinated for 24 hr.

Table 4. Sensory evaluation of *cheonggukjang* fermented with germinated soybean¹⁾

Parameter		Germination time (hr)					
Parameter	0	12	24	36	48		
External appearance	4.0 ^b	4.8a	4.8ª	4.4 ^{ab}	4.5 ^{ab}		
Odor	4.5 ^a	4.3 ^{ab}	4.1^{ab}	3.9^{ab}	3.7^{b}		
Sweet taste	4.5 ^a	4.0^{ab}	4.1 ^{ab}	4.0^{ab}	3.5^{b}		
Savory taste	4.1 ^{ab}	4.3^{a}	4.0^{ab}	3.5^{b}	3.4^{b}		
Bitter taste	3.9^{ab}	4.5a	4.3^{a}	3.2^{b}	3.3 ^b		
Overall acceptability	4.2ab	4.5a	4.2^{ab}	3.9 ^{ab}	3.7 ^b		

¹⁾Each value indicates the average of sensory scores ranging from 1 (dislike extremely) to 7 (like extremely) recorded by 17 well-trained panelists; In a column, means followed by the same letter are not significantly different at the 5% level.

Sensory evaluation Table 4 shows the sensory evaluation results of the *cheonggukjang* made by inoculating B. licheniformis B1, and fermented with germinated soybeans of differing germination times, every 12 hr. Odor, sweet taste, savory taste, bitter taste, and overall acceptability did significant not show differences among cheonggukjangs with different germination times of 0 to 36 hr. However, the cheonggukjang made from the 48-hr germinated soybeans showed statistical results that were definitely lower than the former ones. The external appearance was better with the germinated soybeans; however, the degrees of germination among the raw soybeans did not show significant differences.

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