

RESEARCH NOTE

Change of Isoflavone Content during Manufacturing of *Cheonggukjang*, a Traditional Korean Fermented Soyfood

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Abstract *Cheonggukjang*, a popular Korean traditional fermented soyfood, was manufactured by fermenting steamed soybeans in a temperature-controlled room by traditional methods in which steamed soy was exposed to rice straw naturally rich in *Bacillus* species. *B. subtilis* and *B. licheniformis* were found to be the major microorganisms present in *cheonggukjang* made by the traditional method. We analyzed the composition of 12 kinds of isoflavones and their glycosides present in *cheonggukjang* collected at various fermentation times. Total isoflavone content in raw soybeans was 2,867 mg/kg and this decreased by about 50% during cooking prior to *cheonggukjang* preparation. However, total isoflavone content changed slightly during 45 hr of fermentation. Total content of isoflavone glycosides, consisting mainly of daidzin, glycitin, and genistin, decreased by about 40% during 45 hr of fermenting cooked soybeans. The contents of free isoflavones including daidzein, glycitein, and genistein showed a dramatic increase during fermentation in *cheonggukjang* preparation, with a 2.9-, 54.0-, and 20.6-fold increase in concentration, respectively, by the end of fermentation (45 hr). In conclusion, short-term fermentation of cooked soybeans with *Bacillus* species caused conspicuous changes in the composition of isoflavone derivatives, and its implication in terms of health benefits deserves further study.

Key words: *cheonggukjang*, isoflavones, fermentation, soybean

Introduction

A number of epidemiological studies have suggested that consumption of soybeans and soy foods is associated with lowered risks for several cancers including breast, prostate, and colon (1-3), cardiovascular diseases (4-7), and bone health (8). Isoflavones appear to be the major components responsible for the bioactive functions of soy according to reports published so far (1-3, 9). Isoflavonoids from legumes, such as genistein and daidzein, exist mainly in glycoside forms and are then hydrolyzed in the gut into aglycones that are easily absorbed into intestinal epithelial cells (10). Soy isoflavones act as weak estrogens or anti-estrogens depending on their concentration in the medium (11). The physiological function of isoflavones appears to be mediated by a variety of mechanisms including estrogenic activity, inhibition of topoisomerase and protein kinase, cell cycle arrest, and so on (6, 9-11). There are 12 chemical forms of isoflavones in soybeans and soy foods. Genistein, daidzein, and glycitein are the aglycones, with three possible glucoside forms, a β -glucoside, a 6''-*O*-malonyl-glucoside, and a 6''-*O*-acetyl-glucoside (12, 13). The concentrations of these forms will vary in soy foods depending upon how they are processed (14). Evidence in the literature suggests that the biological effects of soy

isoflavones do not depend upon the glucoside form (15).

Cheonggukjang has been a Korean traditional food since the Chosun dynasty (1600 AD) or earlier (16). This soy product is prepared by soaking the soybeans, then steaming and fermenting them in a closed, humid space maintained at about 40°C for 2-4 days. In the traditional method, rice straw rich in *Bacillus* species are used instead of isolated *Bacillus subtilis*. At the end of 2004, a 97,312 m² area in Sunchang, Jeonbuk Province, Korea was designated as 'Sunchang fermented soy products valley' by the Korean government. This area has been producing various kinds of fermented soy products such as soy paste, *kochujang* (fermented hot pepper-soybean paste), and *cheonggukjang* by traditional methods. We investigated the change of isoflavone composition during preparation of traditional *cheonggukjang* according to the methods used in the 'Sunchang fermented soy products valley'.

Materials and Methods

Samples *Cheonggukjang* prepared by traditional methods was obtained from a manufacturer in the 'Sunchang fermented soy products valley'. The procedure for manufacturing *cheonggukjang* was as follows; soybeans cultivated in the Sunchang area were soaked in water at 20°C for 18-20 hr, and steamed for 60 min at 121°C. The steamed soybeans were put into a room in which the temperature was maintained at 42°C, allowing the soybeans to be fermented by microbes present in the room.

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The bottom of the fermentation room was covered with rice straw naturally rich in *Bacillus* species. Samples were taken at various fermentation times, freeze-dried, and subjected to solvent extraction for isoflavone analysis.

Isoflavone standards Isoflavones standards were obtained as follows: genistein, daidzein, and glycitein from Sigma (St. Louis, MO, USA), genistin, daidzin, glycitin from Indofine (Hillsborough, NJ, USA), and malonyl genistein, malonyl daidzin, malonyl glycitein, acetyl genistein, acetyl daidzin, acetyl glycitein from LC Labs (Woburn, MA, USA).

Isoflavone analysis Freeze-dried *cheonggukjang* was powdered in a Food Mixer (FM-909T; Hanil, Seoul, Korea), and 2 g samples were extracted in 10 mL of acetonitrile, 2 mL of 0.1 N HCl, and 7 mL of water in a 125 mL screw-top Erlenmeyer flask with stirring for 2 hr at room temperature according to Murphy's procedure (17). The residues were dissolved in 80% HPLC grade methanol. An aliquot was filtered through a 0.45 μ m nylon filter (Nunc, Rochester, NY, USA) and analyzed by HPLC. A Jasco chromatograph with a Model AS 2055 autosampler, a Model PU 1580 dual pump, and a Model UV-2077 UV-visible detector was used to analyze each sample. A Phenomenex Gemini C18 column (5 μ m, 150 \times 2.00 mm) was employed for chromatographic separations. A linear gradient composed of A (0.1% phosphoric acid in water) and B (acetonitrile) was used. After injection of a 10 μ L sample, the system was increased from 10 to 35% B over 40 min, returned to 10% in 5 min, and maintained at 10% B for another 10 min. The system was recycled to 10% B at the end of 55 min. The flow rate was 0.8 mL/min. The UV absorbance was monitored at 280 nm. UV spectra were recorded and peak areas were integrated using Young-Lin Autochro 2000 software (Young-Lin, Anyang, Korea). Analyses were repeated three times and data were expressed as the mean \pm standard deviation ($n=3$). To determine the recovery of isoflavones, stock solutions of authentic isoflavone standards and 2,4,4'-trihydroxydeoxybenzoin (THB) were added to *cheonggukjang* powder before isoflavone extraction (17).

Results and Discussion

In this study we investigated changes of isoflavone content during the preparation of *cheonggukjang*. As shown in Fig. 1, twelve isoflavone standards could be effectively separated from each other. The total amount of isoflavones in raw soybeans was 2,825 mg/kg, which decreased by about 50% during the soaking and steaming processes. Also, total isoflavone content of cooked soybeans showed significant reduction during fermentation in the preparation of *cheonggukjang*, with 1,413 mg/kg dry matter at 0 hr and 1,347 mg/kg after 45 hr of fermentation. The percent of isoflavone glycosides decreased from 96.9 to 74.7% during 45 hr of fermentation, while free form isoflavones showed a dramatic increase from 3.1 to 25.3% during the same period. That is, the total amount of isoflavone glycosides in cooked soybeans gradually decreased from 1,370 mg/kg at 0 hr to 1,006 mg/kg at during 45 hr of fermentation. Malonyl- and acetyl-glycitein,

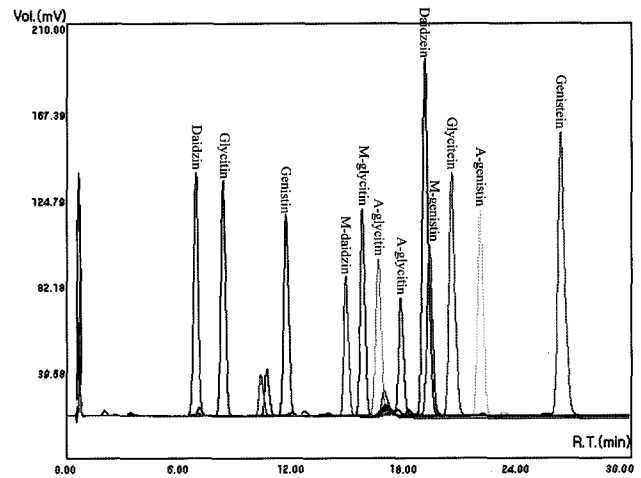
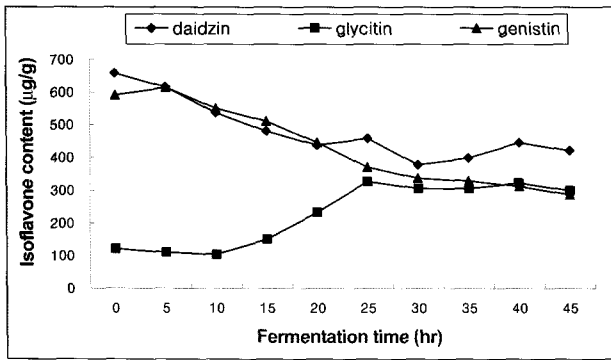


Fig. 1. Chromatogram of 12 standard isoflavones and their glycosides.

however, were the only exceptions and tended to increase during fermentation. The content of malonyl- and acetyl-glycitein in samples at 0 hr was 10 and 9 mg/kg, respectively, and increased to 231 and 33 mg/kg after 45 hr of fermentation. The total amount of aglycone forms of isoflavones in *cheonggukjang* increased 8.0-fold during 45 hr of fermentation, from 43 mg/kg dry matter at 0 hr to 342 mg/kg at 45 hr. Free forms of glycitein and genistein showed the largest increase during 45 hr of fermentation, with respective 41.0- and 12.8-fold increases, while daidzein increased 3.5-fold. Daidzin and genistein were the most abundant isoflavones in cooked soybeans and accounted for 32.1 and 38.8%, respectively. After fermentation for 45 hr, genistin and daidzin were responsible for 15.1 and 19.2% of total isoflavones, respectively, while the three free forms of isoflavones, daidzein, glycitein, and genistein, accounted for 8.5, 12.2, and 4.8% of total isoflavones in *cheonggukjang*, respectively.

Cheonggukjang is prepared by fermenting steamed or boiled soybeans with *Bacillus* species at about 40°C for 2-4 days (16). Its consumption is hampered by its unique, unattractive flavor, but recently consumption has started to increase mainly due to a number of reports of the preventive effects of soy and its products with regard to chronic diseases such as cancers, cardiovascular diseases, and osteoporosis. *Cheonggukjang* is similar to *natto*, a Japanese soyfood, in that both are prepared by fermenting cooked soybeans with *Bacillus* species. Traditional *cheonggukjang* is prepared by fermenting cooked soy with airborne microbes or microflora present in rice straw, while Japanese *natto* is made by inoculating steamed soy with *B. subtilis* or *natto*. That is, traditional *cheonggukjang* is manufactured by using mixed species of microorganisms instead of single species. The samples used in this study appeared to contain *B. subtilis* and *licheniformis*. In general, most isoflavones in soy are present in glycoside forms and are converted into aglycones during fermentation due to β -glucosidase activity in the microorganisms. As shown in Fig. 2, a dramatic increase in aglycone content was observed between 15 and 45 hr of fermentation. This

A. Total glycosides



B. Total aglycones

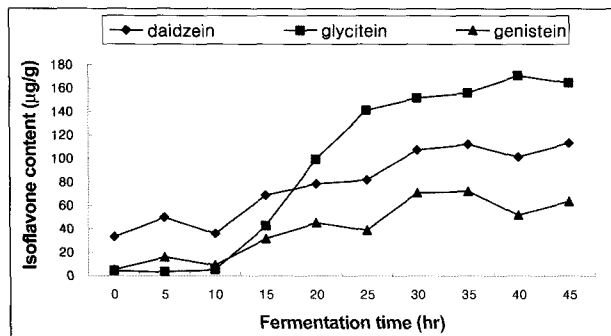


Fig. 2. Change of isoflavone composition during fermentation of cooked soybeans in cheonggukjang preparation. Total glycosides (A) represent the sum of malonyl-glycoside, acetyl-glycoside, and glycoside forms of isoflavones while total aglycones (B) indicate the sum of free forms of daidzein, genistein, and glycitein. Isoflavone contents are presented as a dry basis.

suggests that it takes more than 15 hr of fermentation for β -glucosidase from the microorganisms to become actively produced or fully active.

Though still controversial, the bioavailability of aglycone forms of isoflavones seems to be no different from that of glycoside forms, which are absorbed after being changed into aglycones by certain species of bacteria in large intestine (18). Accordingly, *cheonggukjang* is a good source of bioavailable isoflavones compared to other fermented soy foods such as soy sauce and *miso*, which are high in free forms of isoflavones but significantly low in total isoflavones. Other benefits of *cheonggukjang* include immunomodulatory activity, fibrinolytic action, and antioxidant activity (19). Furthermore, there is higher possibility that soy foods with high isoflavone glycosides may produce relatively high levels of equol because isoflavones should survive the journey to the large intestine and be exposed to microorganisms involved in the conversion of daidzein into equol, whereas aglycones will be absorbed primarily in the small intestine with only a small fraction reaching the large intestine (20).

A recent study reported that the food matrix is an important factor in the bioavailability of isoflavones (20, 21). For instance, consumption of *tempeh* (mainly isoflavone aglycones) resulted in higher serum peak levels of both daidzein and genistein compared with textured vegetable protein (predominantly isoflavone glucosides) (22).

Meanwhile, a relatively large peak that elutes at a similar time as malonyl-glycitin was detected, but its identity was not determined. This suggests that fermentation during *cheonggukjang* preparation causes either the formation of a novel isoflavonoid or the conversion of isoflavones into compounds other than the known 12

Table 1. Isoflavone contents (µg/g dry matter) of cheonggukjang collected at various fermentation times

Isoflavone	Fermentation time (hr)										
	raw	0	5	10	15	20	25	30	35	40	45
daidzin	99±31 ¹⁾	454±17	425±24	401±25	327±28	280±5	245±22	191±5	192±7	212±7	204±10
glycitin	45±15	101±5	101±5	87±6	77±6	60±6	65±26	36±4	34±5	41±4	35±4
genistin	184±50	548±22	568±19	513±30	476±32	401±14	333±16	295±12	289±17	281±14	259±22
M-daidzin	1009±29	129±41	127±29	82±7	97±12	75±9	120±54	96±30	113±22	134±10	120±27
M-glycitin	157±2	10±5	3±0	12±5	63±5	144±3	217±1	220±0	225±7	244±5	231±1
M-genistin	1111±8	tr ²⁾	tr	tr	tr	tr	tr	tr	tr	tr	tr
A-daidzin	85±47	75±3	64±8	54±2	56±5	83±2	93±2	91±3	94±2	99±5	97±6
A-glycitin	101±174	10±2	7±1	5±4	11±5	29±12	44±7	50±10	46±9	38±12	33±12
A-genistin	tr	43±3	47±3	37±3	35±3	45±0	38±5	41±6	39±5	31±6	27±5
Total glycoside	2818	1370	1341	1191	1142	1117	1156	1020	1033	1079	1006
daidzein	tr	33±6	50±8	36±2	69±6	79±2	82±2	107±3	112±5	102±6	114±7
glycitein	tr	4±3	3±4	5±3	43±6	100±0	141±4	152±2	156±2	171±1	165±2
genistein	7±2	5±4	16±3	9±1	32±9	45±1	39±1	71±4	72±5	52±3	64±4
Total aglycone	7	43	69	51	143	223	262	330	341	325	342
Total isoflavones	2825	1413	1410	1242	1285	1340	1418	1350	1373	1404	1347

¹⁾Values are mean ± SD (n=3). ²⁾tr: trace.

isoflavones. If this compound is assumed to be an isoflavonoid, total isoflavone content would have been changed little during fermentation. In particular, the gradual increase of total glycitin and its other glycoside derivatives strongly suggests the possibility of the conversion of genistin or daidzin derivatives into malonyl and acetyl glycitin during *cheonggukjang* fermentation. In fact, a certain bacterium has been reported to contain an enzyme that transfers a methyl group to flavonoids such as daidzein and genistein (23).

Major isoflavone loss during *cheonggukjang* preparation occurred at the soaking and steaming steps. Accordingly, the development of novel processes to minimize isoflavone loss during these steps is worthwhile for improving the health benefits of *cheonggukjang*. Meanwhile, the major forms of daidzin and genistein in raw soybeans appear to be malonyl derivatives which are converted into glycosides, acetyl derivatives, and free aglycones during *cheonggukjang* preparation processes including soaking, steaming, and fermentation. In particular, malonyl genistin was extremely sensitive to pretreatment conditions (soaking and steaming at 121°C, 60 min) and was completely converted to genistin and acetyl genistin with the formation of a small amount of the free form. The concentration of free isoflavones reached a maximum level at 30 hr of fermentation. Further fermentation did not change the content of either the free or glycoside forms of isoflavones.

In conclusion, there was an extensive change in the composition of isoflavone derivatives during fermentation for *cheonggukjang* preparation. The effects of isoflavone profile modification during *cheonggukjang* preparation on health benefits and pharmacokinetics merits further evaluation.

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References

- Messina M. Modern applications for an ancient bean: soybeans and the prevention and treatment of chronic disease. *J. Nutr.* 125: 567S-569S (1995)
- Allred CD, Allred KF, Ju YH, Goepfing TS, Doerge DR, Helferich WG. Soy processing influences growth of estrogen-dependent breast cancer tumors. *Carcinogenesis* 25: 1649-1657 (2005)
- Kim JH, Lim HA, Lee JS, Sung MK, Kim YK, Yu RN, Kim JS. Effect of low doses of genistein and equol on protein expression profile in MCF-7 cells. *Food Sci. Biotechnol.* 14: 854-859 (2005)
- Anderson JW, Blake JE, Turner J, Smith BM. Effects of soy protein on renal function and proteinuria in patients with type 2 diabetes. *Am. J. Clin. Nutr.* 68: 1347S-1353S (1998)
- Anthony MS, Clarkson TB, Williams JK. Effects of soy isoflavones on atherosclerosis: potential mechanisms. *Am. J. Clin. Nutr.* 68: 1390S-1393S (1998)
- Bingham SA, Atkinson C, Liggins J, Bluck L, Coward A. Phytoestrogens: where are we now? *Brit. J. Nutr.* 79: 393-406 (1998)
- McVeigh BL, Dillingham BL, Lampe JW, Duncan AM. Effect of soy protein varying in isoflavone content on serum lipids in healthy young men. *Am. J. Clin. Nutr.* 83: 244-251 (2006)
- Watkins BA, Reinwald S, Li Y, Seifert MF. Protective actions of soy isoflavones and n-3 PUFAs on bone mass in ovariectomized rats. *J. Nutr. Biochem.* 16: 479-488 (2005)
- Omoni AO, Aluko RE. Soybean foods and their benefits: potential mechanisms of action. *Nutr. Rev.* 63: 272-283 (2005)
- Cornwell T, Cohick W, Raskin I. Dietary phytoestrogens and health. *Phytochemistry* 65: 995-1016 (2004)
- Ososki AL, Kennelly EJ. Phytoestrogens: a review of the present state of research. *Phytother. Res.* 17: 845-869 (2003)
- Choi YB, Rhee JS, Lee YB, Nam SY, Kim KS. Extraction of isoflavones from soybean hypocotyls using aqueous ethanol. *Food Sci. Biotechnol.* 13: 719-723 (2004)
- Murphy PA, Song T, Buseman G, Barua K, Beecher GR, Trainer D, Holden J. Isoflavones in retail and institutional soy foods. *J. Agr. Food Chem.* 47: 2697-2704 (1999)
- Coward L, Smith M, Kirk M, Barnes S. Chemical modification of isoflavones in soyfoods during cooking and processing. *Am. J. Clin. Nutr.* 68: 1486S-1491S (1998)
- Xu X, Wang HJ, Murphy PA, Hendrich S. Neither background diet nor type of soy food affects short-term isoflavone bioavailability in women. *J. Nutr.* 130: 798-801 (2000)
- Shin DH. Change in preparation methods and bioactive function of fermented soyfoods. *Korea Soybean Digest* 21: 49-63 (2004)
- Murphy PA, Song T, Buseman G, Barua K. Isoflavones in soy-based infant formula. *J. Agr. Food Chem.* 45: 4635-4638 (1997)
- Richelle M, Pridmore-Merten S, Bodenstab S, Enslin M, Offord EA. Hydrolysis of isoflavone glycosides to aglycones by beta-glycosidase does not alter plasma and urine isoflavone pharmacokinetics in postmenopausal women. *J. Nutr.* 132: 2587-2592 (2002)
- Youn KC, Kim DH, Kim JO, Park BJ, Yook HS, Cho JM, Byun MW. Quality characteristics of the *cheonggukjang* fermented by the mixed culture of *Bacillus natto* and *Bacillus licheniformis*. *J. Korean. Soc. Food Sci. Nutr.* 31: 204-210 (2002)
- Setchell KD, Clerici C, Lephart ED, Cole SJ, Heenan C, Castellani D, Wolfe BE, Nechemias-Zimmer L, Brown NM, Lund TD, Handa RJ, Heubi JE. S-equol, a potent ligand for estrogen receptor beta, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial flora. *Am. J. Clin. Nutr.* 81: 1072-1079 (2005)
- Cassidy A, Brown JE, Hawdon A, Faughnan MS, King LJ, Millward J, Zimmer-Nechemias L, Wolfe B, Setchell KD. Factors affecting the bioavailability of soy isoflavones in humans after ingestion of physiologically relevant levels from different soy foods. *J. Nutr.* 136: 45-51 (2006)
- Zubik L, Meydani M. Bioavailability of soybean isoflavones from aglycone and glucoside forms in American women. *Am. J. Clin. Nutr.* 77: 1459-1465 (2003)
- Kim BG, Jung BR, Lee Y, Hur HG, Lim Y, Ahn JH. Regiospecific flavonoid 7-O-methylation with *Streptomyces avermitis* O-methyltransferase expressed in *Escherichia coli*. *J. Agr. Food Chem.* 54: 823-828 (2006)