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Development of a Genistein-enriched *Doenjang* Using Corn β-Glucosidase

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Abstract Genistein, one of the isoflavones in *doenjang*, is generally known to prevent various cancers, osteoporosis, climacterium, and menopause symptoms, and has better bioavailability and healthful physiological effects than its glucoside, genistin. In both traditional and commercial *doenjang*s, genistein content ranged from 370 to 1,510 mg/kg, however, significant amounts of genistin also existed at the level of 190 to 350 mg/kg. After treating with corn β-glucosidase, over 84% of genistin in *doenjang* was converted to genistein. However, physiochemical characteristics such as pH, viscosity, 2-thiobarbituric acid (TBA) value, and color were not changed significantly after corn β-glucosidase treatments. Therefore, this study shows that the improved *doenjang* with the increased genistein content can be produced using corn β-glucosidase.

Keywords: *doenjang*, isoflavone, genistin, genistein, corn β-glucosidase

Introduction

Doenjang, a traditional Korean soybean fermented paste, has been known to be a nutrient rich food with a high protein content. Recently, it has gaining popularity both from the public and industries, as many studies have reported its healthful physiological effects. *Doenjang* contains a protease inhibitor, phytic acid, and isoflavones, which have antioxidation, antimutation, and anticancer activities (1). Isoflavones, estrogen-like phenolic compounds, exist as both glucosides and aglycones in soybean foods. Especially, soybean fermented foods such as *doenjang*, *cheonggukjang*, *miso*, and *tempeh* have high amount of isoflavones, which include genistin, daidzin, glycitin, and their aglycones. Genistin and genistein are major components in soybean fermented foods because they constitutes more than 50% of total isoflavones (2). Generally the isoflavone aglycones have better bioavailability than their glucoside counterparts (3). Among isoflavone aglycones, genistein shows better functional effects than any other isoflavones. Genistein has healthful functional effects on cancer, osteoporosis, and climacterium (4,5). It also affects menopause syndrome by changing the menstruation period (6).

U.S. Department of Agriculture (USDA) recommends people to take 50-100 mg of isoflavones per day to lower the risk of cancer (2). While Americans typically take about 1 mg of isoflavones from 1-3 g of bean-based foods a day, Asians take 20-50 mg of isoflavones from 20-80 g of bean-based foods a day. For people to benefit from the various physiological effects of isoflavones, they should increase their soybean food intake by a factor of 4 to10 (7,8). Another way to achieve these effects is to eat soybean foods that contain the high level of isoflavone aglycones.

Doenjang is one of popular soybean fermented foods and is a good source of isoflavones for Koreans. Many studies have focused on the analysis of isoflavone contents in various *doenjang* products and showed that most of the isoflavones in *doenjang* are the aglycones, but there is still a fair amount of isoflavone glucosides remaining (9,12, 13). In this study, the genistein-enriched *doenjang* was developed by converting existing genistin to genistein using corn β-glucosidase.

Materials and Methods

Materials Waxy corns were purchased from a Nonghyup market at Hongcheon, Korea. Corn seeds were germinated by soaking them in distilled water and preventing direct sunlight. Corn germ buds were separated from 6 day-old seedlings and used for β-glucosidase (10). The 3 commercial *doenjang*s were the products manufactured by different companies in 2005, and the 3 traditional products were homemade in the regions of Incheon, Seoul, and Changwon in 2005. Genistin and genistein standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). *p*-Nitrophenol-β-D-glucoside (PNPG) and *n*-butyl-paraben were purchased from Junsei Chemical Co. (Tokyo, Japan). All chemicals were of analytical grade and all solvents were of high pressure liquid chromatography (HPLC) grade.

Isoflavone extraction Genistin and genistein were extracted from *doenjang* as described by Wang and Murphy (7). Twenty g of *doenjang* was mixed with 100 mL of acetonitrile (ACN) and 20 mL of 0.1 N HCl in a 500 mL flask for 4 hr at room temperature and then filtered through a Whatman No. 42 filter paper (Whatman PLC, Springfield, UK). The filtration supernatant was centrifuged at 9,000×g for 10 min at 10°C. The supernatant was filtered through a 0.45-μm HDPE filter (Pall Coperation, New York, NY, USA). After removing ACN using a rotary evaporator (N-1000SW;

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Eyela, Tokyo, Japan), a concentrated *doenjang* extract was redissolved in 5 mL of 80% methanol and filtered through a 0.45-μm HDPE filter for HPLC analysis.

To prepare *doenjang* samples treated with corn β-glucosidase, a freeze-dried powder of waxy corn germ buds (2.75 g) containing 50 unit/g of β-glucosidase was well mingled with 100 g of *doenjang* by a homogenizer. The mixture was incubated at 40°C for 5 hr.

HPLC analysis Quantitative and qualitative analyses of genistin and genistein extracted from *doenjang* and β-glucosidase-treated *doenjang* were carried out using a Young-Lin HPLC (Seoul, Korea) equipped with a C-18 reverse phase symmetry column (300×3.9 mm, Waters, Milford, MA, USA). The conditions for HPLC analysis as described by Wang and Murphy (7) were employed with a few modifications. A linear HPLC gradient was employed: solvent A was 0.1% acetic acid in H_2O, and solvent B was 0.1% glacial acetic acid in acetonitrile; following injection of 20 μL of the sample with a micrometrics 725 Auto Injector (Micrometrics Instrument Corp., Norcross, GA, USA).

When isoflavone samples were injected, solvent B was increased from the initial 15 to 35% over 20-27 min and decreased to 15% after 27 min. The mobile phase flow rate was 1 mL/min. Isoflavone quantification was calculated based on integral value from an ultraviolet (UV)/Vis detector monitored at 254 nm. With genistin and genistein standards of 5, 10, 25, 50, and 100 μg/mL, the linear relationship between the content and peak area was determined and used for quantitative analysis.

Physicochemical analysis pH was measured using a pH meter (735P model; Istek, Seoul, Korea) after *doenjang* samples were mixed with an equal amount of distilled water. Viscosity of *doenjang* samples was measured at 0.1 rpm for 30 sec using a Brookfield digital viscometer DV-1+ equipped with a No. 7 spindle (Brookfield Engineering Lab., Inc., Middleboro, MA, USA).

The antioxidative activity of *doenjang* was measured with 2-thiobarbituric acid (TBA) (11). Three g of *doenjang* was resuspended in 10 mL of benzene. The solution was then mixed with 10 mL of TBA mixture and reacted for 4 min. The aqueous layer was separated from the organic phase and further clarified by centrifugation at 6,700×g for 5 min. It was heated in a water bath at 100°C for 30 min and cooled down to room temperature. The TBA value was determined by reading the absorbance at 530 nm (11).

The color of *doenjang* was measured with a color difference meter (CL300; Minolta, Tokyo, Japan). The white standard plate (Calibration Plate CR-300; Minolta) was used for calibration of the equipment. 'L' value indicates the whiteness of a sample with 100 as white and 0 as black. 'a' and 'b' value expresses redness and yellowness, respectively.

Results and Discussion

Analysis of genistin and genistein in *doenjang* by HPLC To determine the levels of genistin and genistein in traditional and commercial *doenjang*s, isoflavone extracts were analyzed by HPLC. The representative chromatograms of isoflavone extracts of traditional and commercial *doenjangs* are shown in Fig. 1. Several peaks were observed and the peaks with the retention time of about 6.1 and 24.8 min were identified as genistin and genistein, respectively, by comparison with genistin and genistein standards (Fig. 1). The amounts of genistin in traditional and commercial *doenjang*s were similar, ranging from 190 to 350 mg/kg, with the exception of one product in which the amount was very low with 15 mg/kg (Table 1). The amounts of genistein ranged from 370 to 1,510 mg/kg *doenjang*. Isoflavone

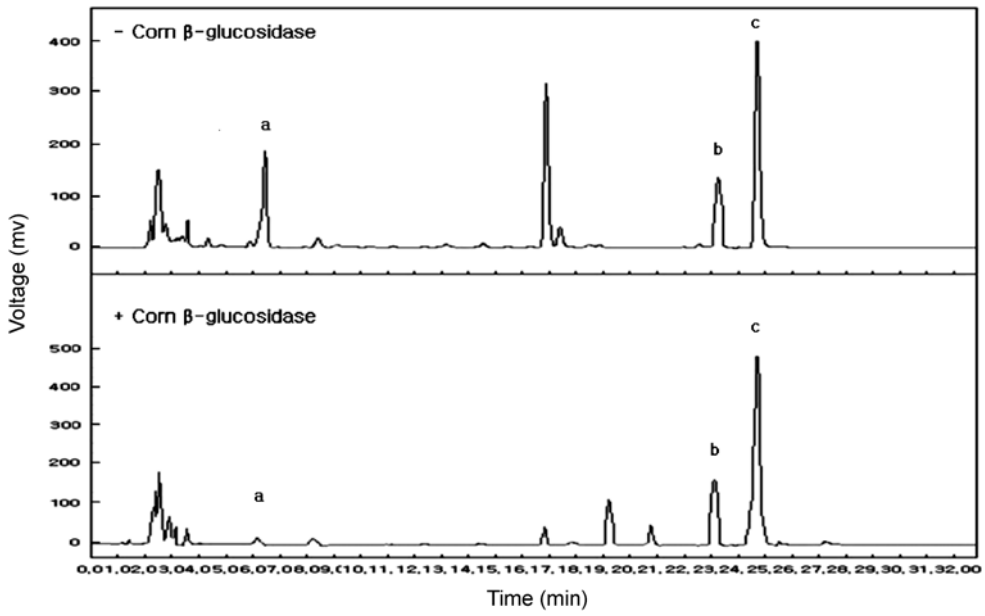

Fig. 1. HPLC chromatograms of isoflavones in *doenjang* treated with corn β-glucosidase. *N*-Butyl-paraben was used as an internal standard (peak b), and genistin (peak a), and genistein (peak c) was confirmed by spiking with commercial genistin and genistein standards.

Table 1. Genistin and genistein contents in *doenjang* treated with corn β-glucosidase

Sample	Genistin		Genistein		Conversion ratio (%)[1]
	β-Glucosidase		β-Glucosidase		
	-	+	-	+	
Traditional *doenjang* A	19.1±0.4[2]	3.0±0.3	72.7±0.8	83.6±1.7	84.4
Traditional *doenjang* B	35.7±0.6	2.9±0.3	129.1±2.3	144.1±1.2	91.8
Traditional *doenjang* C	23.2±0.4	1.4±0.1	151.9±0.5	169.9±1.0	93.9
Commercial *doenjang* A	1.5±0.1	0.1±0.0	58.0±1.2	63.3±1.2	91.7
Commercial *doenjang* B	28.4±0.2	1.6±0.2	109.5±0.4	133.9±1.0	94.4
Commercial *doenjang* C	23.6±0.2	0.1±0.0	37.0±0.2	51.1±0.2	99.5

[1]Conversion ratio was calculated by genistin difference before and after treatment with β-glucosidase divided by original amount of genistin. All values are mean±SD of triplicates.
[2]Mean±SD, mg/100 g *doenjang*.

content in *doenjang* differs in the reports of various researchers; however, our results are comparable to the previous reports, in which the amounts of genistein were 479-586 (12), 230-460 (9), and 538 mg/kg (13). Soybean variety and processing methods may be attributing factors in the variation in genistein content in *doenjang*.

In fermented soybean foods, most isoflavones are aglycone forms, in contrast to soybean in which most isoflavones are glucosides (13,14). Glucosides are converted to aglycones by microbial β-glucosidase during fermentation (15). In *doenjang* analyzed in this study, the portion of genistein was over 80%, but there was still a significant amount of genistin that could be converted to genistein by further treatments (Table 1).

Conversion of genistin to genistein by treatments with corn β-glucosidase Genistin is an isoflavone containing glucose by β-glycosidic linkage and can be converted to genistein by releasing glucose. Therefore, genistin can be converted to genistein with β-glucosidase treatments. To search for an appropriate enzyme source, corn and leguminous species were tested for β-glucosidase activity. Germ buds of waxy corn after a 6-day germination period showed the highest β-glucosidase activity with 18.03 unit/g (10). In addition, β-glucosidase derived from germ buds of waxy corn showed specificity to genistin, converting it to genistein. In the HPLC chromatogram of isoflavones extracted from *doenjang* treated with corn β-glucosidase, the genistin peak almost disappeared but the genistein peak increased substantially (Fig. 1). The remaining genistin was at most 30 mg/kg (Table 1). Therefore, the treatments of *doenjang* with corn β-glucosidase converted most of genistin to genistein with conversion ratio of over 84%, yielding a genistein-enriched *doenjang*.

A genistein-enriched *doenjang* would have greater healthy effects than regular one. Glucoside isoflavones can be converted to isoflavone aglycones by intestinal microorganisms (15). However, intestinal bacteria also metabolize and degrade isoflavones (16). Therefore, it is important to intake isoflavones in aglycone forms that are easily absorbed in intestine.

Physicochemical characteristics of *doenjang* treated with corn β-glucosidase Corn β-glucosidase could react with other substances and thus affect the quality of *doenjang*. To determine effects of corn β-glucosidase treatments on physicochemical characteristics, pH, viscosity, TBA value, and color were measured after corn β-glucosidase treatments.

The pH of *doenjang* is generally acidic with 4.5-5.5 (17). The pH of *doenjang* used in this study was around 4.7 and increased to 4.9 after treatments of corn β-glucosidase (Table 2). The viscosity of *doenjang* was 4.3×10^3 cp and increased to 5.0×10^3 cp after treatments of corn β-glucosidase (Table 2). This viscosity increase seems to be due to the decrease in total water contents by the addition of the powder of corn germ buds rather than by direct effects of β-glucosidase activity. The viscosity of *doenjang* is generally variable by sources and an increase of 0.7×10^3 cp will not affect the quality of *doenjang* significantly.

Antioxidant substances in *doenjang* inhibit the oxidation of lipids and thus make *doenjang* a safe food. The average TBA value of 6 *doenjang*s was about 525 μg and decreased to about 500 μg after treatments of corn β-glucosidase. Antioxidant substances in *doenjang* are isoflavones and melanoidin produced by reaction of amino acids and sugars (18). Therefore, corn β-glucosidase did not affect the total amounts of isoflavones and melanoidins.

The color of *doenjang* is an important factor not only because it contributes to the organoleptic quality but also because it reflects health functionality. The major component of the brown color of *doenjang* is melanoidin derived from sugars and amino acids. In addition to color, melanoidin is

Table 2. Physicochemical characteristics of *doenjang* treated with corn β-glucosidase[1]

β-Glucosidase	pH	Viscosity (×1,000 cp)	TBA value (×100 μg)	Hunter's value		
				L	a	b
-	4.67±0.03	4.32±0.41	5.04±0.03	34.13±0.32	7.09±0.38	12.44±0.37
+	4.88±0.05	4.90±0.43	5.02±0.02	34.40±0.35	7.19±0.18	12.31±0.28

[1]All values are averages of triplicates with a commercial *doenjang* C sample and mean±SD.

known to have several functions in *doenjang*. It improves the sugar tolerant ability of the human body and has trypsin inhibitor activity, which acts to prevent or improve diabetes (18). It decreases the production of nitrosoamine and promotes the growth of lactic acid bacteria (19). It also prevents the binding of excess iron with cellular components and the diffusion of cell membrane by free radicals (20). The Hunter's value was L (luminocity): 34.13, a (red): 7.09, b (yellow): 12.44 and L: 33.56, a: 7.00, b: 12.27 before and after treatments of corn β-glucosidase, respectively, suggesting that corn β-glucosidase affected the color of *doenjang* little (Table 2).

In this study genistein-enriched *doenjang* was developed by treating corn β-glucosidase in the final product of traditional and commercial *doenjang*s. Most of the remaining genistin was converted to genistein. Considering the health functionality of genistein, genistein enrichment of *doenjang* by corn β-glucosidase will be a simple and economical way to improve the quality of *doenjang*, long considered to be healthy functional food.

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