Food Sci. Biotechnol. Vol. 15, No. 1, pp. 96 ~ 101 (2006)

Food Science and Biotechnology

Isoflavone Distribution and β-Glucosidase Activity in *Cheonggukjang*, a Traditional Korean Whole Soybean-Fermented Food

SeungOk Yang, Pahn-Shick Chang and JaeHwan Lee*

Department of Food Science and Technology, Seoul National University of Technology, Seoul 139-743, Korea

Abstract Isoflavone distribution and β-glucosidase activity in *cheonggukjang*, a traditional Korean whole soybean-fermented food prepared with or without addition of *Bacillus subtilis*, were analyzed every 6 hr for 36 hr. Thermal cooking of raw-soaked soybeans significantly increased β-glucoside isoflavone level by 57.1% and decreased malonyl-β-glucosides by 57.6% ($p<0.05$). Consistent changes of isoflavone profiles in *cheonggukjang* without *B. subtilis* addition (COB) and samples with addition of *B. subtilis* (CWB) were not observed during 36 hr fermentation. β-Glucosides of isoflavones are major forms in both COB and CWB. β-Glucosidase activity in *cheonggukjang* decreased significantly compared to that of soaked soybeans due to thermal denaturation, while recovery of enzyme activity in COB was observed. Two new unidentified peaks were detected, and their relative peak areas in CWB were significantly larger than those in COB with increasing fermentation period ($p<0.05$), which indicates both peaks could be associated with fermentation metabolites.

Keywords: *cheonggukjang*, isoflavone, β-glucosidase activity, *Bacillus subtilis*

Introduction

Consumptions of soybeans and soy-containing foods have been asserted to reduce the risk of various cancers, several chronic inflammatory diseases, and osteoporosis (1-3). Bioactive and health beneficial functions of soybeans are closely related with isoflavones, which have structural similarity to estrogen and are thus called as phytoestrogen (4, 5).

Isoflavones in soy foods are found in four chemical structures including aglycones (genistein, daidzein, glycitein), and their corresponding β-glucosides, acetyl-β-glucosides, and malonyl-β-glucosides. Chemical structure and concentration of isoflavones in soy foods are dependent on many factors including genotypes, crop year, crop location, storage period, thermal processing conditions, processing types, and presence of microorganisms (6-9). Thermal processing at high temperature such as baking and frying results in the conversion of malonyl-β-glucosides into the corresponding acetyl-β-glucosides by decarboxylation. Aglycones of isoflavone are reported to be absorbed faster and higher amounts than their corresponding glucoside forms (1, 4, 10), because isoflavone glucosides are not directly transported across the gastrointestinal tract (11). Incorporation of β-glucosidase in microorganisms and/or natural products including almond powder has been attempted to increase the content of isoflavone aglycones in soy foods (12, 13).

Cheonggukjang is a traditional Korean food made of cooked whole soybeans fermented with microorganisms including *Bacillus natto* and/or *B. subtilis* within 2-3 days fermentation period (14, 15). It is regarded as a good source of protein, hydrolyzed peptides, and lipids, and has been consumed commonly among Koreans for its health-beneficial functions such as reducing arterial stiffness. *Cheonggukjang* is also characterized as its' unique flavor with sticky and fibrous poly-γ-glutamate formed during fermentation (15-17). Several studies have been reported on the changes of nitrogen concentration, monitoring volatile compounds, types of microorganisms involved, and processing effects in *cheonggukjang* (14-20). However, characterization of all 12 isoflavone profiles, monitoring of β-glucosidase activity during fermentation, and detection of any new metabolic compounds related with fermentation in *cheonggukjang* have not been reported in the literature.

The objectives of this study were to determine the changes of isoflavone distribution and β-glucosidase activity in *cheonggukjang* with or without addition of *B. subtilis*.

*Corresponding author: Tel: 82-2-970-6739; Fax: 82-2-976-6460
E-mail: jhlee@snut.ac.kr
Received October 11, 2005; accepted January 6, 2006

Materials and Methods

Materials Soybeans were purchased from a local market (Seoul, Korea), and *B. subtilis* MYCO10001 was donated by MYCO (Kyungju, Korea). Three standard isoflavones including daidzein, genistein, and β-genistin were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and six standard compounds including glycitein, β-glycitin, acetyl-β-daidzin, acetyl-β-genistin, acetyl-β-glycitin, and malonyl-β-genistin were purchased from LC Laboratories (Woburn, MA, USA). HPLC-grade methanol, acetonitrile, HCl, and acetic acid were purchased from Fisher Scientific (Fairlawn, NJ, USA). *p*-Nitrophenol-β-D-glucopyranoside (*p*NPG), *p*-nitrophenol (*p*NP), formononetin, sodium acetate, and sodium carbonate were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

***Cheonggukjang* preparation** Whole soybeans (300 g) were washed and soaked with three time volume of tap water for 10 hr at room temperature. Subsequently, water was decanted, and soybeans were mixed with ten time volume of tap water and cooked for 2 hr. Cooked whole

soybeans were let stand for 1 hr at room temperature to cool down and divided into two sets. One set was inoculated with 10%(w/w) *B. subtilis* (2.7×10^7 CFU/mL) and the other set was without addition of *B. subtilis*. Cooked whole soybeans prepared with or without addition of *B. subtilis* were designated as CWB and COB, respectively, and fermented for 36 hr at 40°C in a *cheonggukjang* incubator (Namyang Co., Kimpo, Korea) and sampled at 0, 6, 12, 18, 24, 30, and 36 hr.

Isoflavone extraction Isoflavone analysis method was adapted from that of Lee *et al.* (21). One gram of *cheonggukjang* was ground using a mortar and a pestle for 1 min, mixed with 2 mL of 100 mmol/L HCl, 7 mL acetonitrile, and 3 mL deionized water in a 50-mL centrifuge bottle (Nalgene Co., Rochester, NY, USA), and vortex-mixed for 1 min. Bottles were shaken for 2 hr at room temperature using a shaker (Jeio Tech, Seoul, Korea) and centrifuged at 2,208×g for 10 min (Hanil, Incheon, Korea). One milliliter of supernatant was transferred to a 20-mL glass bottle and dried under nitrogen gas flow at room temperature. Dried samples were stored at -40°C in the dark until use. Formononetin, a 4-*O*-methylated form of daidzein, was added as an internal standard to confirm the recovery of isoflavone during extraction procedures.

HPLC analysis Isoflavones in *cheonggukjang* extracts were separated and isolated using a high performance liquid chromatograph (HPLC) equipped with an ultraviolet detector (Waters Associated, Milford, MA, USA). Isoflavone separation was achieved using a 4-μm Waters Novapak C_{18} reversed-phase HPLC column (150 mm × 3.9 mm I.D.) with a Novapak C_{18} stationary phase guard column and a 0.5-μm pre-column filter from Vydac (Hesperia, CA, USA). Samples were re-solubilized with 1 mL methanol and vortex-mixed before filtration using a 0.2-μm syringe filter (Alltech Associates Inc., Deerfield, IL, USA). Mobile phase was a mixture of 1%(v/v) acetic acid in water (solvent A) and 100% acetonitrile (solvent B) at a flow rate of 0.6 mL/min. The gradient of mobile phase was 85% solvent A from 0 to 5 min, followed by decrease of solvent A up to 65% from 5 to 44 min, increase of solvent A up to 85% from 44 to 45 min, and re-equilibration of solvent A at 85% for 5 min. Injection volume was 10 μL, and isoflavones were detected at 260 nm (21). Isoflavones in the eluents were identified based on the retention times of standard compounds and results of the authors' previous reports (13, 21). Isoflavones were quantified using calibration curves prepared from HPLC peak areas of each isoflavone.

Calibration curve preparation and quantification of isoflavones One milligram of daidzein, genistein, glycitein, β-genistin, β-glycitin, acetyl-β-daidzin, acetyl-β-genistin, acetyl-β-glycitin, and malonyl-β-genistin were dissolved in 100% methanol to prepare the stock solutions. Each stock solution was serially diluted with 100% methanol, and the concentration of diluted solutions was determined using the Beer-Lambert Law with UV absorbance reading in the range of 240-360 nm by a UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan) and their molar extinction coefficients were adapted from previous reports (21, 22). Aliquots of serially diluted solutions of each isoflavone were injected into the HPLC, and the peak area of each isoflavone in the chromatogram was determined. The relationship between HPLC peak area and concentration of isoflavones from the UV-Vis spectrophotometer was calculated and used for the quantification of the isoflavones. The correlation coefficient (r) of all standard curves for isoflavones was over +0.99. The concentrations of three isoflavones without standard compounds including β-daidzin, malonyl-β-glycitin, and malonyl-β-daidzin were calculated by adapting the results of the corresponding acetyl-β-glucosides (21).

β-Glucosidase activity The β-glucosidase activity in *cheonggukjang* was determined using a modified method of Zhang *et al.* (13). Ground *cheonggukjang* (0.5 g) was mixed with 15 mL distilled water, vortex-mixed for 1 min, and centrifuged at 8,832×g at 4°C for 20 min using a refrigerated centrifuge (Ivan Sorvall, Inc., Norwalk, CO, USA). The supernatant was collected and filtered through a 0.48-μm filter before analysis. One unit (U) of β-glucosidase activity in *cheonggukjang* was defined as enzymes required to release 1 μmol *p*NG from *p*NPG per min under the above assay conditions (13).

Detection of new peaks All new peaks formed during *cheonggukjang* fermentation for 36 hr were monitored using HPLC. Peak areas of each new peak were compared with those of total isoflavones and relative peak areas were calculated.

Statistical analysis Data were analyzed statistically by ANOVA and Duncan's multiple range test using SPSS software program (SPSS Inc., Chicago, IL, USA). A p value <0.05 was considered significant.

Results and Discussion

Isoflavone analysis in *cheonggukjang* All 12 isoflavones were isolated and identified under applied analysis conditions. A typical HPLC chromatogram and contents of isoflavones in *cheonggukjang* with or without addition of *B. subtilis* during 36 hr fermentation are shown in Fig. 1 and Table 1, respectively. Soaked soybeans (SS), cooked soybeans with microorganisms (CWB-0), and cooked soybeans without microorganisms (COB-0) contained 1754.6, 1054.8, and 1248.1 μg total isoflavones/g wet base soy, respectively. The differences in total isoflavones (TI) may come from weight changes of soybeans during soaking and cooking process, leaching loss to water, and possibly thermal degradation of isoflavones under applied experimental conditions. The weights of raw, soaked, and cooked soybeans were 300, 660, and 580 g, respectively. Raw soybeans absorbed water and swelled about 220% in weight during the soaking process, and soaked soybeans lost weight by 12.1% after cooking. Without any loss or thermal degradation during the cooking process, about 1996.6 μg total isoflavone/g cooked soybean (=660 g soaked soybean × 1754.6 μg total isoflavone/580 g cooked soybean) would have been detected in COB-0. However, 62.5% isoflavone (=1248.1/1996.6 × 100) was recovered after the cooking process. The loss of isoflavones may be

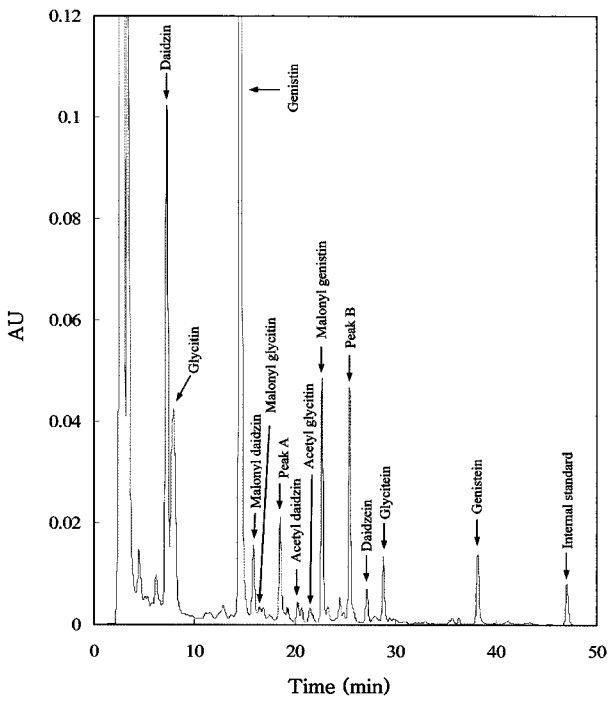

Fig. 1. HPCL chromatogram of isoflavone distribution in *cheonggukjang* with 2 new unidentified peaks and an internal standard.

mainly due to the leaching into water and possibly thermal degradation of isoflavones. It has been reported that isoflavone contents in soy-containing foods such as *tofu* and *tempeh* decrease depending on the processing conditions (22-24). Franke *et al.* (22) reported that 13% of isoflavones in raw soybeans were not recovered when cooked *tofu* was prepared. Isoflavones in *tofu* prepared using 90 and 100°C for 40 min decreased by 15 and 28%, respectively (23). Wang and Murphy (24) reported that 61 and 44% of isoflavones were lost in *tempeh* and *tofu*, respectively. Coward *et al.* (8) reported that baking and frying of soyprotein did not change total isoflavone content and converted malonyl-β-conjugates into the corresponding acetyl-β-glucosides and β-glucosides. Baking and frying process produce higher temperature than conventional cooking process; however, in terms of isoflavone loss, the presence of water appears to play more important roles in leaching or decomposing isoflavones than temperature. Although isoflavones are considered stable to heat energy, prolonged cooking process in the presence of water can greatly decrease the total isoflavone contents.

Malonyl-β-genistin showed the highest concentration in soaked beans (SS), followed by malonyl-β-daidzin and β-genistin (Table 1). On the other hand, after cooking process, β-genistin showed the highest concentration, followed by β-daidzin and malonyl-β-genistin in cooked soybeans (COB-0). Relative percentages of aglycones, β-glucosides, acetyl-β-glucosides, and malonyl-β-glucosides in SS and COB-0 were 4.3, 7.2, 3.9, and 84.6%, respectively, and 6.0, 64.3, 2.7, and 27.0%, respectively, which indicates that cooking process caused significant changes in isoflavone profiles. Malonyl-β-glucosides and acetyl-β-glucosides decreased by 57.6 and 1.2%, respectively, while β-glucosides and aglycones increased by 57.1 and 1.7%, respectively. Therefore, thermal conversion of malonyl-β-isoflavones into the corresponding β-glucosides is the major change in isoflavone profiles during the cooking process. Grun *et al.* (23) reported that moist heat generally increases β-glucoside forms, while dry heat converts malonyl-β-glucosides into acetyl-β-glucosides. Thermal cooking for 2 hr produced high β-glucoside forms of isoflavones in cooked soybeans.

Averages of total isoflavone contents in CWB and COB for 36 hr were 870.2 and 1317.4 μg/g *cheonggukjang*, respectively (Table 1). The difference in total isoflavone contents between CWB-ave and COB-ave may come from 10%(w/w) dilution effect of *B. subtilis* addition and increase in fermentation products by *B. subtilis* in CWB; added *B. subtilis* generated more fermentation products such as poly-γ-glutamates in CWB. Although 1 g fermented soybeans was sampled for isoflavone analysis, CWB had less soy matrix containing isoflavones and more fermentation products per gram sampling in wet weight base. TI in CWB-ave was reduced compared to those in CWB-0, which indicates that *B. subtilis* in CWB may play a role in converting isoflavones into other metabolites through fermentation. Microorganisms in COW after 36 hr were not isolated in this study, while the presence of microorganisms was evident due to the formation of characteristic features of *cheonggukjang* such as formation of poly-γ-glutamate and unique odor. TI in COB was not significantly different from 0 to 30 hr fermentation, while there were increasing trends observed ($p>0.05$). TI of COB-36 was significantly higher than that of COB-0 ($p<0.05$), which could be due to the fermentation activity on the releasing isoflavones entrapped in the soy matrix. TI in CWB during fermentation was not consistent, and wide variations were observed, which may be due to uneven fermentation process or localization of microorganisms.

Wide ranges of variations were observed in the percentage changes of aglycones, β-glucosides, acetyl-β-glucosides, and malonyl-β-glucosides in CWB and COB during 36 hr fermentation (Table 1). In particular, aglycone percentages were not increased in both CWB and COB, which may be due to the lack of β-glucosidase activity. On the other hand, Sohn *et al.* (19) reported that contents of aglycones such as daidzein and genistein in *cheonggukjang* increased as fermentation time increased. They prepared *cheonggukjang* using cooked soybeans with 30 min-treatment at 113°C in an autoclave, which may have caused this difference in aglycone contents. Kim and Yoon (25) reported that aglycone contents in *meju* and *deonjang*, which are traditional Korean soy-fermented foods with several month fermentation periods, were higher than those in soybean. These findings suggest isoflavone concentrations and profiles in fermented soy foods vary depending on the thermal processing conditions, fermentation period, and addition of microorganisms.

β-Glucosidase activity β-Glucosidase activity during *cheonggukjang* fermentation is shown in Fig. 2. After cooking process, β-glucosidase activity decreased significantly due to the denaturation of β-glucosidase in soybeans ($p<0.05$). β-Glucosidase activity in COB began

Table 1. Distribution of isoflavones in *cheonggukjang* with or without addition of *Bacillus subtilis* for 36 hr fermentation

	μg Isoflavones /g wet base soy															Percentage (%)			
Samples[a]	DE	DI	ADI[b]	MDI	GE	GI	AGI[b]	MGI	GY	GYI	AGYI	MGYI	TDE	TGYE	TGE	TI	AG	GL	ACG	MAG
SS	17.0k	31.0a	56.2i	284.9i	56.8h	84.6a	tr	1166.2i	2.36a	10.6a	11.4abc	33.1h	389.3j	57.6a	1307.7k	1754.6j	4.3a	7.2a	3.9de	84.6h
COB-0	11.5g	217.2g	19.7fg	72.5f	50.2ef	522.3gh	tr	246.9efg	13.9h	62.2ef	13.6abcd	17.6e	321.1g	107.5ghi	819.5g	1248.1gh	6.0d	64.3cd	2.7bc	27.0ef
COB-6	10.3f	217.4g	14.5cdef	68.4f	49.4e	495.7fg	tr	232.7g	13.0g	64.7fg	8.5ab	17.2de	310.8g	103.5g	777.9f	1192.2fg	6.1d	65.3cd	1.9a	26.7ef
COB-12	8.1e	216.9g	14.9defg	66.3f	29.8c	519.9gh	tr	224.7fg	13.2hi	64.3fg	10.7ab	15.5e	306.4fg	103.9gh	774.5f	1184.9efg	4.3a	67.6de	2.2ab	25.9def
COB-18	14.5j	234.8gh	18.5fg	84.4g	61.8i	564.7i	tr	268.0h	14.7ij	57.8e	14.4bcd	18.0e	352.3hi	105.1gh	894.6hi	1352.0ghi	6.7bcd	63.5b	2.4a	27.4a
COB-24	12.2h	245.4h	21.0g	88.0gh	54.5gh	617.0j	tr	290.8h	16.9k	70.6g	6.79a	21.1f	366.8i	115.5i	962.3j	1444.7hi	5.8cd	64.6cd	1.9a	27.7f
COB-30	12.7hi	223.9g	20.3g	87.6gh	52.3fg	531.6h	tr	282.3h	15.1j	64.5efg	11.6abc	21.5f	344.7h	112.8hi	866.3h	1323.8ghi	6.0d	62.0c	2.4ab	29.6g
COB-36	13.2i	273.1i	29.6h	92.0h	53.2g	593.6ij	tr	281.0h	15.1j	81.2h	19.1d	24.2g	408.0k	139.8j	927.8i	1475.6i	5.5bc	64.3cd	3.3cd	26.9ef
COB-ave	11.8	232.7	19.8	79.9	50.2	549.3	tr	260.9	14.6	66.5	12.1	19.3	344.3	112.6	860.4	1317.4	5.8	64.5	2.4	27.3
CWB-0	8.6de	195.2f	26.7h	57.9de	39.7d	446.9e	tr	186.7de	12.2f	50.4d	16.4cd	13.7bc	288.5f	92.8f	673.4e	1054.8def	5.7bcd	65.7cde	4.1e	24.5cd
CWB-6	6.6bc	157.9cd	17.6efg	46.3b	35.2c	343.7c	tr	137.0dc	8.7d	47.6cd	12.2abc	13.0b	228.5c	81.6de	516.0c	826.2b	6.1d	66.5cde	3.6de	23.8bc
CWB-12	7.5d	182.6ef	11.2bcde	52.7cd	36.2d	393.9d	tr	158.0c	9.5e	59.5ef	6.32a	12.4c	254.1de	87.8ef	588.1d	930.1bcd	5.7bcd	68.4de	1.9a	24.0bcd
CWB-18	5.0a	122.0b	9.1ab	30.3a	25.4a	246.7b	tr	95.7a	5.0b	43.3bc	6.79a	9.93a	166.5a	65.0ab	367.9a	599.5a	5.9cd	68.7de	2.7bc	22.7ab
CWB-24	6.2b	189.6f	11.5abcd	61.0e	31.2b	474.3ef	tr	192.1ef	9.8e	51.5d	10.4abc	16.7de	268.2e	88.5ef	697.7e	1054.7cde	4.5a	67.8de	2.1ab	25.6cde
CWB-30	7.0c	169.0de	10.2abc	51.9c	31.8b	379.4d	tr	158.7cd	8.3d	40.9b	8.2ab	17.8e	238.2cd	75.4cd	570.0d	883.7bc	5.3b	66.7cde	2.1ab	25.9def
CWB-36	6.2b	143.6c	6.4a	34.3a	32.8b	340.6c	tr	110.0ab	6.2c	39.0b	6.7a	16.1d	190.7b	68.1bc	483.4b	742.3ab	6.1d	70.5e	1.8a	21.6a
CWB-ave	6.7	165.7	13.2	47.8	33.2	375.1	tr	148.3	8.5	47.5	9.6	14.2	233.6	79.9	556.6	870.2	5.6	67.8	2.6	24.0

[a]Abbreviations: SS, soaked soy; COB-, *cheonggukjang* without *B. subtilis* soy-fermentation time; CWB-, *cheonggukjang* with *B. subtilis* soy-fermentation time; DE, daidzein; DI, daidzin; ADI, acetyl daidzin; MDI, malonyl daidzin; GE, genistein; GI, genistin; AGI, acetyl genistin; MGI, malonyl genistin; GY, glycitein; GYI, glycitin; AGYI, acetyl glycitin; MGYI, malonyl glycitin; TDE, total daidzein; TGYE, total glycitein; TGE, total genistein; TI, sum of TDE, TGYE, and TGE; AG, aglycone percent; GL, glucoside percent; ACG, acetyl glucoside percent; MAG, malonyl glucoside percent; tr, trace amount; CWB-ave, average of CWB; COB-ave, average of COB.
[b]Compounds coelute with unknowns. Values are average of triplicates (n=3). Different letters are significant in the same column at $p<0.05$.

to increase and was significantly higher than that in CWB after 18 hr fermentation ($p<0.05$). Addition of *B. subtilis* had no effect on the increase of β-glucosidase activity. Kim and Yoon (25) reported that β-glucosidase activity in fermented soy foods such as *meju* and *deonjang* was 36 and 12% of those in soybeans, respectively. Therefore, additional sources of β-glucosidase are needed to increase aglycone contents in soy foods prepared with cooked soybeans due to the denaturation of β-glucosidase and slow recovery of β-glucosidase activity. Inactivation of β-glucosidase in cooked soybeans and ineffective roles of *B. subtilis* on the cleavage of β-glucoside linkage can explain the results of aglycone profiles in *cheonggukjang*.

Detection of new peaks Two new unidentified peaks were detected during *cheonggukjang* fermentation using the current extraction method (Fig. 1). One peak (A) appeared at 18.1 min, and the other peak (B) was eluted at 25.0 min. Relative peak areas of the two new peaks in *cheonggukjang* with or without addition of *B. subtilis* are shown in Fig. 3. Peak A was first detected at 18 hr *cheonggukjang* fermentation, irrespective of addition of *B. subtilis*, and began to increase as fermentation time increased (Fig. 3-A). Peak A from CWB was significantly higher than that from COB after 18 hr ($p<0.05$). Relative peak area of peak A from COB increased up to 5.5% at 30 hr and started to decrease, while that from CWB gradually increased up to 12.0% for 36 hr fermentation (Fig. 3-A).

Peak B was first detected from soaked and cooked soybeans, and relative peak area increased after 12 hr fermentation. Peak B showed similar pattern to peak A, and CWB had significantly higher relative peak areas than COB after 12 hr fermentation ($p<0.05$). Relative peak area of peak B from CWB increased up to 24.0% for 36 hr, while that from COB increased up to 10.2% at 30 hr and started to decrease thereafter (Fig. 3-B). These results thus suggest unidentified peaks A and B could be related to fermentation metabolites generated from fermentation activities of *B. subtilis*.

New isoflavone metabolites or isoflavonoids such as 6-*O*-acyl isoflavone glycosides in *natto* (26), which is a similar type of food of *cheonggukjang* consumed in Japan, and 8-hydroxyglycitein and 6-hydroxydaidzein in *miso* (27) were found and identified. Toda *et al.* (26) reported that daidzein 7-*O*-β-(6'-*O*-succinyl)-D-glucoside, genistein 7-*O*-β-(6'-*O*-succinyl)-D-glucoside, and glycitein 7-*O*-β-(6'-*O*-succinyl)-D-glucoside were found in *natto*. Some new isoflavonoids in fermented soy products were reported to have bone loss reducing (26) or free radical scavenging activity (27). Peaks A and B could be associated with those compounds identified in other soy fermented foods and currently, identification study of peaks A and B is ongoing.

In conclusion, β-glucosides of isoflavones are major forms in *cheonggukjang*, and aglycone content did not increase due to the inactivation of β-glucosidase during

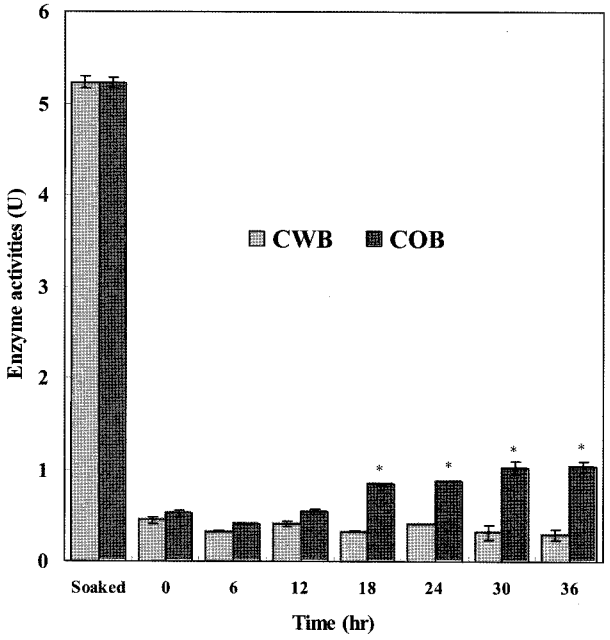

Fig. 2. β-Glucosidase activity in *cheonggukjang* with or without addition of *Bacillus subtilis* during fermentation. *indicates significant difference between COB and CWB at $p<0.05$.

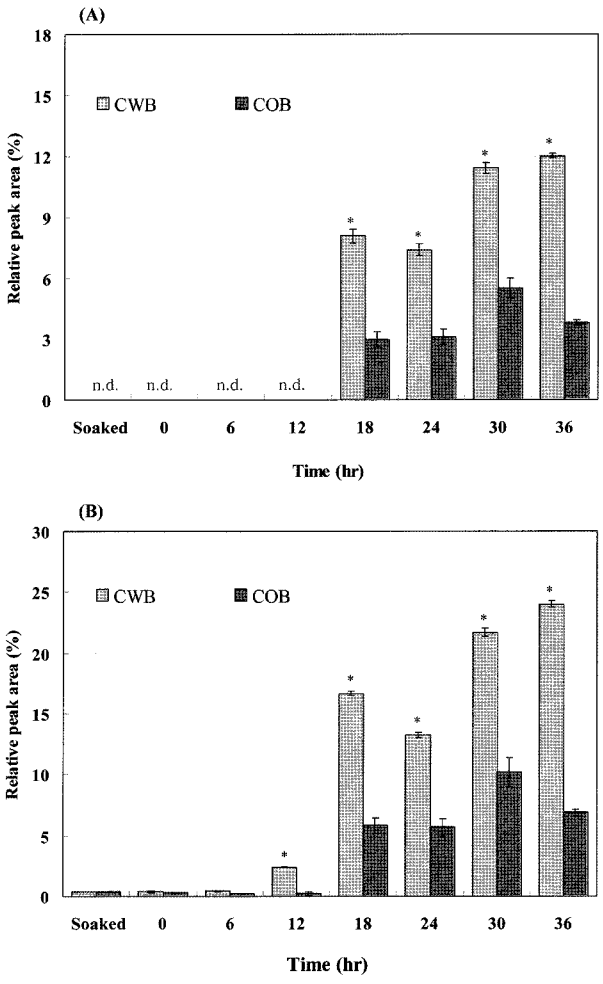

Fig. 3. Relative peak areas of peak A (A) and B (B) during *cheonggukjang* fermentation. Peak A and B are new peaks eluted at 18.1 and 25.0 min, respectively in Fig. 1. *indicates significant difference between COB and CWB at $p<0.05$. n.d.: not detected.

soybean cooking process. β-Glucosidase activity in *cheonggukjang* was only 10% of soaked soybeans and began to recover only in COB. Two new unidentified peaks were formed, and CWB produced more relative peak areas of two new peaks than COB, which indicates that both peaks could be associated with fermentation metabolites from *B. subtilis*.

Acknowledgments

This study was supported by High-Technology Development Project (project number: 304001-03-2-HD110), Ministry of Agriculture and Forestry, Republic of Korea.

References

1. Hendrich S, Wang GJ, Lin HK, Xu X, Tew BY, Wang HJ, Murphy PA. Isoflavone metabolism and bioavailability. pp. 211-230. In: Antioxidant Status, Diet, Nutrition, and Health. Papas AM. (ed). CRC press, Boca Raton, FL, USA (1999)
2. Teede HJ, McGrath BP, DeSilva L, Cehun M, Fassoulakis A, Nestel PJ. Isoflavones reduce arterial stiffness: a placebo-controlled study in men and postmenopausal women. Arterioscler. Thromb. Vasc. Biol. 23: 1066-1071 (2003)
3. Zheng G, Zhu S. Antioxidant effects of soybean isoflavones. pp. 123-130. In: Antioxidants in Human Health and Disease. Basu TK, Temple NJ, Garg ML. (eds). CABI Publishing, Wallingford, UK (1999)
4. Shin JI, Lee MS, Park OJ. Effects of soy-isoflavonoid on molecular markers related to apoptosis in mature and ovariectomized female rats, and mammalian tumor cell lines. Food Sci. Biotechnol. 14: 709-714 (2005)
5. Nam HY, Min SG, Shin HC, Kim HY, Fukushima M, Han KH, Park WJ, Choi KD, Lee CH. The protective effects of isoflavone extracted from soybean paste in free radical initiator treated rats. Food Sci. Biotechnol. 14: 586-592 (2005)
6. Wang H, Murphy PA. Isoflavone composition of American and Japanese soybeans in Iowa: effects of variety, crop year, and location. J. Agric. Food Chem. 42: 1674-1677 (1994)
7. Lee SJ, Chung IM, Ahn JK, Lee SK, Kim SH, Yoo NH. Variation in antioxidative activity of soybean (Glycine max L.) varieties with crop year and duration of storage time. Food Sci. Biotechnol. 11: 649-653 (2002)
8. 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)
9. Kim JS, Kim JG, Kim WJ. Changes in isoflavone and oligosaccharides of soybeans during germination. Korean J. Food Sci. Technol. 36: 294-298 (2004)
10. Izumi T, Piskula MK, Osawa S, Obata A, Tobe K, Saito M, Kataoka S, Kubota Y, Kikuchi M. Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. J. Nutr. 130: 1695-1699 (2000)
11. Day AJ, Dupont S, Ridley S, Rhodes MJC, Morgan MRA, Williamson G. Deglucosylation of flavonoid and isoflavonoids glycosides by human small intestine and liver β-glucosidase activity. FEBS Lett. 436: 71-75 (1998)
12. Mun SW, Park MS, An JB, Ji GE. Quality characteristics of chocolate blended with Bifidobacterium-fermented isoflavone powder. Korean J. Food Sci. Technol. 35: 1162-1168 (2003)
13. Zhang YC, Lee JH, Vodovotz Y, Schwartz SJ. Changes in distribution of isoflavones and β-glucosidase activity during soy bread proofing and baking. Cereal Chem. 8196: 741-745 (2004)
14. Joo HK. Studies on the manufacturing of Cheonggukjang. Korean J. Food Sci. Technol. 3: 64-67 (1971)
15. Kim YT, Kim WK, Oh HI. General microbiology, physiology, and metabolism: screening and identification of the fibinolytic bacteria strain from chungkook-jang. Korean J. Microbiol. Biotechnol. 23: 1-5 (1995)
16. Ashiuchi M, Kamei T, Baek DH, Shin SY, Sung MH, Soda K, Yagi T, Misono H. Isolation of Bacillus subtilis (chungkookjang), a poly-ã-glutamic acid producer with high genetic competence. Microbiol. Biotechnol. 57: 764-769 (2001)
17. Kim IJ, Kim HK, Chung HJ, Jeoung YK, Ryu CH. Study of functional Chungkukjang contain fibrinolytic enzyme. Korean J. Life Sci. 12: 357-362 (2002)
18. Kim YS, Jung HJ, Park YS, Yu TS. Characteristics of flavor and functionality of *Bacillus subtilis* K-20 Cheonggukjang. Korean J. Food Sci. Technol. 35: 475-478 (2003)
19. Sohn MY, Seo KI, Lee SW, Choi SH, Sung NJ. Biological activities of Chungkugjang prepared with black bean and changes in phytoestrogen content during fermentation. Korean J. Food Sci. Technol. 32: 936-941 (2000)
20. Choi SH, Ji YA. Changes in flavor of Chungkookjang during fermentation. Korean J. Food Sci. Technol. 21: 229-234 (1989)
21. Lee JH, Renita M, Pioritto RJ, Martin SKSt, Schwartz SJ, Vodovotz Y. Isoflavone characterization and antioxidant activity of Ohio soybeans. J. Agric. Food Chem. 52: 2647-2651 (2004)
22. Murphy PA, Barua K, Hauck CC. Solvent extraction selection in the determination of isoflavones in soy foods. J. Chromatogr. B. 777: 129-138 (2002)
23. Grun IU, Adhikari K, Li C, Li Y, Lin B, Zhang J, Fernando LN. Changes in the profile of genistein, daidzein, and their conjugates during thermal processing of tofu. J. Agric. Food Chem. 49: 2839-2843 (2001)
24. Wang HJ, Murphy PA. Mass balance study of isoflavones during soybean processing. J. Agric. Food Chem.. 44: 2377-2383 (1996)
25. Kim JS, Yoon S. Isoflavone contents and β-glucosidase activities in soybean, Meju and Deonjang. Korean J. Food Sci. Technol. 31: 1405-1409 (1999)
26. Toda T, Uesugi T, Hirai K, Nukaya H, Tsuji K, Ishida H. New 6-O-acyl isoflavone glycosides from soybeans fermented with Bacillus subtilis (natto). I. 6-O-succinylated isoflavone glycosides and their preventive effects on bone loss in ovariectomized rats fed a calcium-deficient diet. Biol. Pharm. Bull. 22: 1193-1201 (1999)
27. Hirota A, Inaba M, Chen YC, Abe N, Taki S, Yano M, Kawaii S. Isolation of 8-hydroxyglycitein and 6-hydroxydaidzein from soybean miso. Biosci. Biotechnol. Biochem. 68: 1372-1374 (2004)