Food Sci. Biotechnol. 19(1): 115-119 (2010)
DOI 10.1007/s10068-010-0016-z

RESEARCH ARTICLE

Characteristics of *Cheonggukjang* Produced by the Rotative Fermentation Method

Nam Keun Lee, Il Jae Cho, Joung Whan Park, Byung Yong Kim, and Young Tae Hahm

Received: 2 August 2009 / Revised: September 24 2009 / Accepted: 18 November 2009 / Published Online: 28 February 2010

Abstract This study was to develop a processing method for improving the quality of a fermented soybean food, *cheonggukjang*. *Bacillus* sp. AS-2 isolated from traditionally fermented *cheonggukjang* was selected for the production of *cheonggukjang* with the rotative fermentation method (RFM). The pH value of *cheonggukjang* made by RFM (cRFM) was 6.1±0.25. The amount of crude protein (329.0±0.92 mg/g) and amino-type nitrogen (120.6±21.81 mg%) were similar to those in traditionally fermented *cheonggukjang* (cTFM). The amount of ammonium-type nitrogen was 173.0±1.72 mM/g and nearly 4 times lower than that in cTFM. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging values in the traditional product were between 1.62±0.08 and 1.27±0.05, while with the RFM, the values were between 1.38±0.02 and 0.78±0.20. The antioxidative activity of the sauce-type cRFM was approximately 1.7 times higher than that of cTFM. The sensory scores for cRFM were uniformly higher than those for cTFM.

Keywords: fermented soybean food, *cheonggukjang*, rotative fermentation method, *Bacillus* sp., antioxidative activity

Introduction

Cheonggukjang is one of the traditionally fermented soybean foods in Korea. It has been made through natural fermentation at 37-40°C for 2 to 3 days with raw rice straw, which is the source of fermenting microorganisms. The physicochemical and functional properties of *cheonggukjang* depend on the fermenting microorganisms, temperature, moisture, and so on (1-3). Bioactive materials in *cheonggukjang* are generally produced during the fermentation. In particular, isoflavones and phenolic acids increase or are newly formed during the fermentation of soybeans. In terms of isoflavones, genistein, daidzein, and glycitein are converted aglycone into glycone by the enzyme activity, mostly β-glucosidase originated from *Bacillus* sp. (4,5). Caffeic and ferulic phenolic acids are also generated during the process (6). The functional properties of *cheonggukjang* include immunopotentiation (7-9), anticancer and antioxidation (10), thrombolysis (11-13), antidiabetes (14,15), antiinflammatory activity (16), and antihypertension effect (17). For this reason, researches have focused on the functional properties of fermented soybean foods such as *natto*, *douche*, and *tempeh* as well as *cheonggukjang* (18-23).

When *cheonggukjang* is produced with natural fermentation as traditional method, it is difficult to control and uniformize its quality. To solve these problems, starter culture strains should be used to produce *cheonggukjang* under the control of fermentation process. In addition, unpleasant odor, the major factor that many people hesitate to consume *cheonggukjang*, is able to be diminished or even removed. In our previous works, various *Bacillus* sp. as starter culture were isolated from the traditionally produced *cheonggukjang* in the region of Gyeonggi and Gangwon provinces, Korea, identified using 16S rDNA sequence analysis, and their enzyme activities were analyzed (24,25). In this study, *cheonggukjangs* were fermented with the selected *Bacillus* sp. AS-2 and RFM. The produced *cheonggukjangs* were analyzed with their physicochemical and functional properties.

Nam Keun Lee, Il Jae Cho, Joung Whan Park, Young Tae Hahm (✉)
Department of Biotechnology and BK21 Program, Chung-Ang University, Anseong, Gyeonggi 456-756, Korea
Tel: +82-670-3064; Fax: +82-31-670-0406
E-mail: ythahm@cau.ac.kr

Byung Yong Kim
Department of Food Science and Biotechnology, Kyung-Hee University, Yongin, Gyeonggi 446-701, Korea

Materials and Methods

Strain and culture medium *Bacillus* sp. AS-2, which was isolated from traditionally fermented *cheonggukjang* in Anseong, Korea, 2006 (24), was used to produce *cheonggukjangs*. Luria-Bertani (LB) broth medium (Difco, Detroit, MI, USA) was used for incubation of *Bacillus* sp. AS-2.

Enzyme activity assay To select proper starter strain, enzyme activity assay was carried out. In analyses of protease, amylase, cellulase, and fibrinolytic enzyme activity, the isolated *Bacillus* sp. was incubated in 5 mL of LB medium at 37°C for 24 hr and centrifuged at 12,000×g for 5 min. The acquired supernatant was used as a crude enzyme solution. Their activities were analyzed with substrates of 1% casein, 1% soluble starch, 1% carboxymethyl cellulose (CMC), and 0.6% fibrinogen, respectively (26-28). The selected strain was analyzed with API ZYM kit, according to the procedure described by the manufacturer (BioMérieux, Lyon, France).

Production of *cheonggukjangs* by the RFM *Glycine max* L. cv. Baektae, one of the varieties of Korean soybean, was purchased from a grocery store in Anseong, Korea, 2007. To prepare the sauce- and bean-type *cheonggukjang* by the RFM, 10 g of soybeans were washed and soaked in 30 mL of water at 4°C for 24 hr. They were autoclaved at 121°C for 30 min, and then cooled at 40°C. One mL of the selected *Bacillus* sp. AS-2 (10^7-10^8 CFU/mL) was inoculated into the boiled soybean without removing water. They were fermented on rotary shaker (sauce-type: 250 rpm, bean-type: 120 rpm) at 37°C for 48 hr (Fig. 1). Paste-type *cheonggukjang* was produced at 250 rpm with an equal volume of water. Traditional *cheonggukjang* was fermented at 37°C for 48 hr without shaking. *Cheonggukjangs* were freeze-dried and stored at 4°C for further analysis.

Physicochemical properties of *cheonggukjang* The pH was measured with a pH meter (420A; Orion Research Inc., Boston, MA, USA) and the amount of crude protein was determined with the Bradford kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The content of amino-type ammonia-type nitrogen was measured according to the Formal titration method (18). The content of ammonia-type nitrogen was analysed with the modified phenol-hypochloride method (18). One g of *cheonggukjang* was dissolved in 10 mL of water and then kept in a 100°C water bath for 1 min. The sample was centrifuged at 13,000×g at 4°C for 5 min and filtrated with a membrane filter (0.45-μm). A volume of 1 mL of the filtrate and 2 mL of the reaction solution were mixed together and reacted at 37°C for 20 min. The changes in absorbance were measured at 630 nm with a spectrophotometer (Optizen 3220UV; Mecasys Co., Ltd., Deajeon, Korea), and the value of ammonia-type content was determined on the basis of the standard curve of $(NH_4)_2SO_4$.

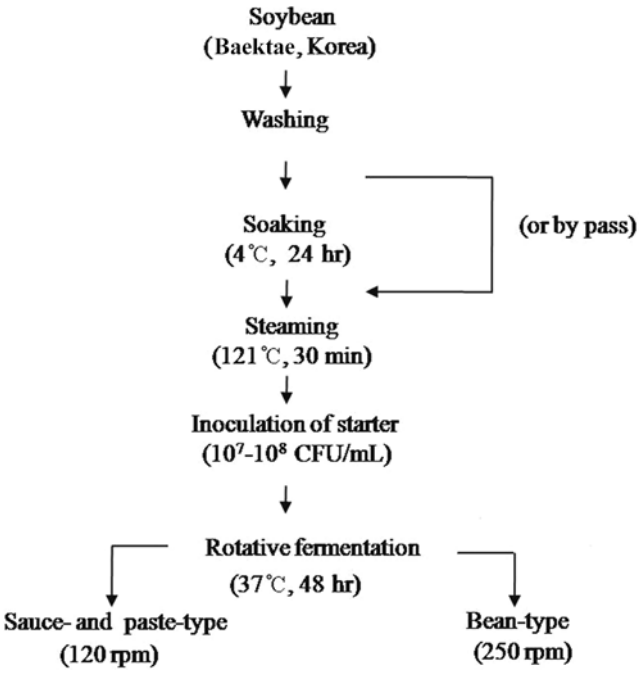

Fig. 1. The flow chart of the RFM for manufacture of *cheonggukjang*.

DPPH radical scavenging activity assay A 0.1 g of *cheonggukjang* and 1 mL of 70% methanol were mixed together in a 1.5-mL Eppendorf tube. The mixture was kept at 60°C for 1 hr, cooled at –20°C for 30 min, and then centrifuged at 13,000×g at 4°C for 10 min. The analysis of the antioxidative activity of the supernatant was conducted as follows: 50 μL of the acquired supernatant and 200 μL of 0.04% 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution were mixed and then changes in the mixture's absorbance were measured at 517 nm for 20 min (29). The scavenging activity on hydroxyl radicals was calculated by the following equation:

$$\text{Scavenging activity (\%)} = [(A_{\text{control 517 nm}} - A_{\text{sample 517 nm}})/A_{\text{control 517 nm}}] \times 100$$

Sensory evaluation Eight panelists performed a sensory evaluation, assessing taste (bitterness and savouriness), odor, the acceptability of the color, and overall acceptability according to the 5-point scale method (30). A score of 5 meant that the feature assessed was very strong or very good, and a score of 1 meant that it was very weak or very poor.

Statistical analysis All values of experimental data were

obtained in triplicate and analysed with the SPSS software package (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL, USA). Multiple comparisons were also performed for all of the data according to Duncan's multiple range tests at $p \leq 0.05$.

Results and Discussion

Selection of *Bacillus* sp. as starter culture The extracellular protease activity of *Bacillus* sp. was important in determining the quality of *cheonggukjang* (31). In terms of the enzyme activities of various *Bacillus* sp. isolated from traditionally fermented *cheonggukjangs*, protease, amylase, cellulase, and fibrinolytic activity of *Bacillus* sp. AS-2 were 0.86±0.06, 3.07±0.55, 1.63±0.25, and 1.34±0.45 units/μg, respectively. Because of high protease activity of *Bacillus* sp. AS-2 among *Bacillus* sp. isolated from traditionally fermented *cheonggukjangs* (24), it was selected for the production of *cheonggukjang* using the RFM.

For identification of the selected *Bacillus* sp. AS-2, the 16S rDNA sequences were analysed by using the Blast Search from the NCBI database; this analysis showed a homology of above 97% with the 16S rDNA sequence of *Bacillus subtilis* (24). In the enzyme assay with API ZYM kit, *Bacillus* sp. AS-2 showed the enzymatic activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phospatase, naphtol-AS-BI-phosphahydroase, and α-galactosidase (Table 1).

Table 1. Enzyme activities of *Bacillus* sp. AS-2 as determined by API ZYM kit

Enzyme	*Bacillus* sp. AS-2
Control	0[1)]
Alkaline phosphatease	5
Esterase (C4)	5
Esterase lipase (C8)	5
Lipase (C14)	0
Leucine arylasmidase	0
Valine arylasmidase	0
Crystine arylamidase	0
Trypsin	0
α-Chymotrypsin	0
Acid phospatase	5
Naphtol-AS-BI-phosphohydrolase	3
α-Galactosidase	0
β-Galactosidase	0
β-Glucuronicdase	0
α-Glucosidase	3
β-Glucosidase	0
N-Acetyl-β-glucosaminidase	0
α-Mannosidase	0
α-Fucosidase	0

[1)]Enzyme activity was determined by using color-strength values: 0, negative control; 1-2, negative reaction; 3, weak positive reaction; 4-5, strong positive reaction. The data represent the means of 3 independent experiments.

***Cheonggukjang* produced by the RFM** Sauce- and bean-type of *cheonggukjangs* were produced with the RFM (Fig. 2). *Cheonggukjang* is generally produced by washing, soaking, steaming, inoculating the natural microflora, and fermenting process at 37-40°C for 2 to 3 days. The cRFM is different in terms of the fermentation step. The sauce- and bean-type cRFM were obtained by shaking the fermenting product at a high speed (250 rpm) and a low speed (120 rpm), respectively. In addition, paste-type cRFM was also obtained by controlling the water content. As a result, various types of *cheonggukjang* were produced with the change of fermentative conditions. The newly produced *cheonggukjang* products could be used in various preparations of foods.

Physicochemical properties of *cheonggukjang* The physicochemical properties of *cheonggukjang* were shown in Table 2. The pH value of the sauce-type cRFM was 6.1±0.25. The amount of crude protein (329.0±0.92 mg/g) and amino-type nitrogen (120.6±21.81 mg%) were similar to those in traditionally fermented *cheonggukjang*. However, the amount of ammonium-type nitrogen (173.0±1.72 mM/g) was nearly 4 times lower than that in the cTFM. The reduction of ammonium-type nitrogen improved the quality of *cheonggukjang* because ammonium-type nitrogen was major source of the unpleasant odour in the traditional product.

Antioxidative activity of *cheonggukjang* Antioxidative activity is one of the most important properties of *cheonggukjang* as a functional healthy food. The values corresponding to this activity are given in Fig. 3. The antioxidative activity of the sauce-type cRFM was approximately 1.7 times higher than that of cTFM. The antioxidative effect of *cheonggukjang* is due to many factors; isoflavones, phenol compounds, and browning reaction during fermentation (10,29). The increased antioxidative activity in *cheonggukjangs* (cRFMs) was thought to be a superficial change to the soybean during fermentation, or possibly due to the increased oxidative stress and browning products between peptides and glucose. When the effect of oxidative stress was observed, the antioxidative activities were a little different whether or not rotative fermentation was used (data not shown). In terms of color density, the product was less brown than the traditionally fermented *cheonggukjang* (data not shown). Based on the antioxidative activity of the sauce-type cRFM was higher than that of bean-type cRFM, its higher antioxidative activity was due to the increment of the

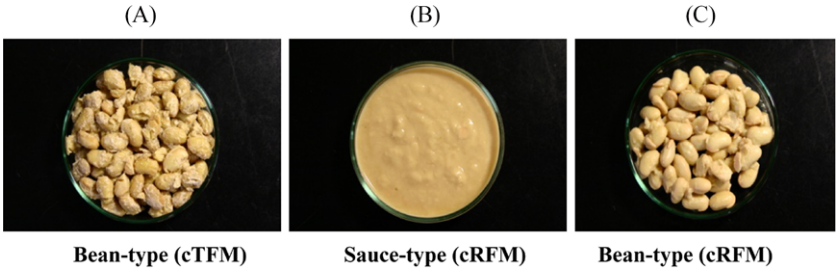

Fig. 2. Types of *cheonggukjangs*. (A) bean-type (cTFM), bean-type *cheonggukjang* produced by traditional batch-type fermentation method; (B and C) sauce-type (cRFM) and bean-type (cRFM), sauce-type and bean-type *cheonggukjangs* produced by RFM, respectively.

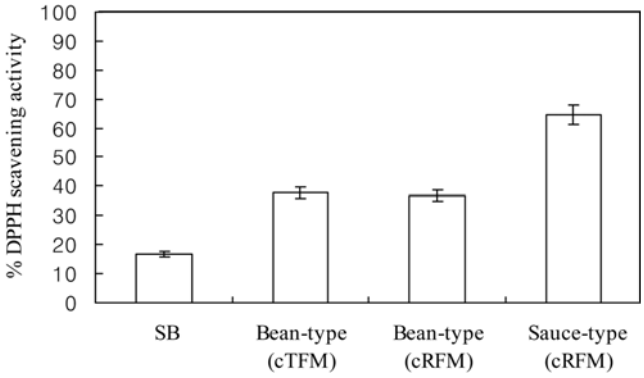

Fig. 3. Antioxidative activities of *cheonggukjangs*. SB, soybean extract; bean-type (cTFM), *cheonggukjang* produced by traditional batch-type fermentation method; bean-type (cRFM) and sauce-type (cRFM), *cheonggukjang* produced by RFM. The data represent the means of 3 independent experiments. The error bars represent standard deviations.

superficial area of product rather than the oxidative stress or browning reaction.

Sensory evaluation In the sensory evaluation of cRFMs, bitter and savoury taste, odor, color, and overall acceptability were evaluated (Table 3 and Fig. 4). The sensory scores for the cRFMs were uniformly lower than those for cTFM, with the exception of overall acceptability. The savouriness of *cheonggukjang* was mostly determined by taste and odour, which come from the glutamic acid and ammonia (2,32). Although the savouriness of the cRFMs was weak, the overall acceptability was better than that of the cTFM. It may be due to the decrement of bitter taste and odour occurred from ammonium-type nitrogen.

In conclusion, the goal of our study was to improve the quality of *cheonggukjang* produced by the rotative processing method with the selected starter strain, which was isolated from traditionally fermented *cheonggukjang*. As a result, the quality and functional properties of the final product obtained from the RFM were significantly better than those of the traditional product. In particular, the improvement in terms of the unpleasant odor of *cheonggukjang* was noticeable. Various types of the product such as dressing

Table 2. Properties of *cheonggukjang* produced with traditional and RFM

Property	Bean-type (cTFM)[1)]	Sauce-type (cRFM)[2)]
pH	7.3±0.25[3)]	6.1±0.25
Crude protein (mg/g)	350.0±2.44	329.0±0.92
Amino-type nitrogen (mg%)	125.3±13.40	120.6±21.81
Ammonia-type nitrogen (mM/g)	634.0±6.84	173.0±1.72

[1)]Bean-type *cheonggukjang* produced with traditional batch-type fermentation method
[2)]Sauce-type *cheonggukjang* produced with RFM
[3)]Values given are the means of results in triplicate±SD.

Table 3. Sensory quality of *cheonggukjang* produced with traditional and RFM

Type[1)]	Bitter taste[2)]	Odor[2)]	Savory taste[2)]	Brown color acceptability[3)]	Overall acceptability[4)]
Bean-type (cTFM)	3.25±0.71[5)]	3.13±0.35	2.63±0.52	3.13±0.35	2.75±0.71
Sauce-type (cRFM)	1.50±0.76	1.88±0.64	1.86±0.35	2.38±0.52	3.50±0.76
Bean-type (cRFM)	1.63±0.74	2.00±0.53	2.38±0.52	2.38±0.52	3.63±0.52

[1)]cTFM, *cheonggukjang* produced with traditional batch-type fermentation method; cRFM, *cheonggukjang* produced with RFM
[2)]Sensory scores were evaluated from very low (1) to very strong (5).
[3)]Sensory scores were evaluated from very light (1) to very dark (5).
[4)]Sensory scores were evaluated from very poor (1) to very good (5).
[5)]Superscripts indicate a statistically significant difference at the $p \leq 0.05$ level by Duncan's multiple range test.

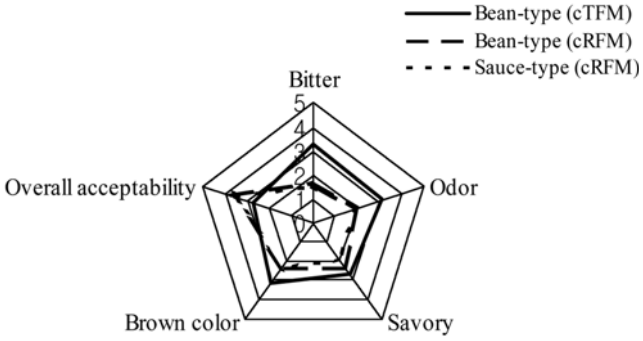

Fig. 4. Comparison of sensory characteristic of *cheonggukjangs* produced with traditional and RFM. Bean-type (cTFM), bean-type *cheonggukjang* produced by traditional batch-type fermentation method; sauce-type (cRFM), sauce-type *cheonggukjang* produced by RFM; bean-type (cRFM), bean-type *cheonggukjang* produced by RFM

sauces-, paste-, and bean-type were also obtained according to the different fermentation conditions, especially incubating speeds at which it was shaken. From these positive results, further studies should be conducted with various soybean varieties, mixture of strains, and other fermentation conditions by using the RFM.

References

1. Lee BY, Kim DM, Kim KH. Studies on the change in rheological properties of *cheonggukjang*. Korean J. Food Sci. Technol. 23: 478-484 (1991)
2. Lee MY, Park SY, Jung KO, Park KY, Kim SD. Quality and functional characteristics of *cheonggukjang* prepared with various *Bacillus* sp. isolated from traditional *cheonggukjang*. J. Food. Sci. 70: 191-196 (2005)
3. Lee MY, No HK, Kim SD, Prinyawiwatkul W. Quality of *cheonggukjangs* prepared with various *Bacillus* strains. Int. J. Food Sci. Tech. 47: 587-592 (2007)
4. Yang SO, Chang PS, Lee JH. Isoflavone distribution and β-glucosidase activity in *cheonggukjang*, a traditional Korean whole soybean-fermented food. Food Sci. Biotechnol. 15: 96-101 (2006)
5. Jang CH, Lim JK, Kim JH, Park CS, Kwon DY, Kim YS, Shin DH, Kim JS. Change of isoflavones content during manufacturing of *cheonggukjang*, a traditional Korean fermented soyfood. Food Sci. Biotechnol. 15: 643-646 (2006)
6. Lee KH, Ryu SH, Lee YS, Kim YM, Moon GS. Changes of antioxidative activity and related compounds on the *cheonggukjang* preparation by adding drained boiling water. Korean J. Food Cook. Sci. 21: 163-170 (2005)
7. Kwon HY, Kim YS, Kwon GS, Kwon CS. Isolation of immuno-stimulating strain *Bacillus pumilus* JB-1 from *cheonggukjang* and fermentational characteristics of JB-1. Korean J. Microbiol. Biotechnol. 32: 291-296 (2004)
8. Lee CH, Yang EI, Song GS, Chai OH, Kim YS. Effects of *cheonggukjang* on immune responses and gastrointestinal functions in rats. Food Sci. Biotechnol. 15: 19-23 (2006)
9. Kim YN, Heo SI, Wang MH. Antigenotoxic, fibrinolytic, and immunomodulating activity of traditionally fermented soy product, *cheonggukjang*. J. Food Process Pres. 33: 87-104 (2009)
10. Lee JJ, Cho CH, Kim JY, Kee DS, Kim HB. Antioxidant activity of substances extracted by alcohol from *cheonggukjang* powder. Korean J. Microbiol. 37: 177-181 (2001)
11. Chang JH, Shim YY, Kim SH, Chee KM, Cha SK. Fibrinolytic and immuno-stimulating activities of *Bacillus* spp. strains isolated from *cheonggukjang*. Korean J. Food Sci. Technol. 37: 255-260 (2005)
12. Kim IJ, Kim HK, Chung JH, Jeong YK, Ryu CH. Study of functional *cheonggukjang* contain fibrinolytic enzyme. Korean J. Life Sci. 12: 357-362 (2002)
13. Mine Y, Kwan-Wong AH, Jiang B. Fibrinolytic enzymes in Asian traditional fermented foods. Food Res. Int. 38: 243-250 (2005)
14. Kim JI, Kang MJ, Kwon TW. Antidiabetic effect of soybean and *cheonggukjang*. Korea Soybean Digest 20: 44-52 (2003)
15. Kim DJ, Jeong YJ, Kwon JH, Moon KD, Kim HJ, Jeon SM, Lee MK, Park YB, Choi MS. Beneficial effect of *cheonggukjang* on regulating blood glucose and pancreatic beta-cell functions in C75BL/KsJ-*db/db* mice. J. Med. Food 11: 215-223 (2008)
16. Choi YH, Lim H, Heo MY, Kwon DY, Kim HP. Anti-inflammatory activity of the ethanol extract of *cheonggukjang*, Korean fermented bean: 5-Lipoxygenase inhibition. J. Med. Food 11: 539-543 (2008)
17. Matsui T, Yoo HJ, Hwang JS, Lee DS, Kim HB. Isolation of angiotensin I-converting enayme inhibitory peptide from *cheonggukjang*. Korean J. Microbiol. 40: 355-358 (2004)
18. In JP, Lee SK, Ahn BK, Chung IM, Jang CH. Flavor improvement of *cheonggukjang* by addition of Yucca (*Yucca shidigera*) extract. Korean J. Food Sci. Technol. 34: 57-64 (2002)
19. Yoo HJ, Lee DS, Kim HB. *Cheonggukjang* fermentation of mixture of barley, wormwood, sea tangle, and soybean. Korean J. Microbiol. 40: 49-53 (2004)
20. Kada S, Yabusaki M, Kaga T, Ashida H, Yoshida K. Identification of two major ammonia-releasing reactions involved in secondary *natto* fermentation. Biosci. Biotech. Bioch. 72: 1869-1876 (2008)
21. Mitsui N, Murasawa H, Sekiguchi J. Development of *natto* with germination-defective mutants of *Bacillus subtilis* (*natto*). Appl. Microbiol. Biotechn. 82: 741-748 (2009)
22. Nakajima N, Nozaki N, Ishihara K, Ishikawa A, Tsuji H. Analysis of isoflavone content in *tempeh*, a fermented soybean, and preparation of a new isoflavone-enriched *tempeh*. J. Biosci. Bioeng. 100: 685-687 (2005)
23. Han B, Kiers JL, Nout RM. Solid-substrate fermentation of soybeans with *Rhizopus* spp.: Comparison of discontinuous rotation with stationary bed fermentation. J. Biosci. Bioeng. 8: 205-209 (1999)
24. Lee NK, Jeon EH, Cho IJ, Hahm YT. Isolation, identification, and characterization of *Bacillus* spp. from the traditionally fermented *cheonggukjangs* in the Gyeonggi and the Gangwon Provinces. J. Korean Soc. Appl. Biol. Chem. 49: 276-280 (2006)
25. Hwang HA, Lee NK, Cho IJ, Hahm YT, Kwon KO, Kim BY. Selection of microorganisms and optimization of manufacture process for *cheonggukjang*. Korean J. Food Sci. Technol. 40: 406-411 (2008)
26. Kim KY, Kim HG, Song BC, Cha CJ. Screening for fermentative microorganisms that grow on brown rice with high amylase and protease activities. Korean J. Microbiol. 42: 160-163 (2006)
27. Hahm BK, Kim YK, Yu JH, Bai DH. Isolation and identification of cellulase-producing microorganism, and determination of optimal culture condition. Korean J. Food Sci. Technol. 29: 1028-1032 (1997)
28. Kil JO, Kim GN, Park IS. Production and characterization of fibrinolytic enzyme: Optimal condition for production of the enzyme from *Bacillus* sp. KP-6408 isolated from *cheonggukjang*. J. Korean Soc. Food Sci. Nutr. 27: 51-56 (1998)
29. Lee NK, Hahm YT. Antioxidative characteristics of browning reaction products of glucose-poly-γ-glutamate (Glu-PGA) obtained from amino-carbonyl reaction. Korean J. Food Sci. Technol. 37: 812-815 (2005)
30. Herbert A, Jeol LS. Sensory Evaluation Practices. 3rd ed. Elsevier Academic Press, San Diago, CA, USA. pp. 89-97 (2004)
31. Ahn YS, Kim YS, Shin DH. Isolation, identification, and fermentation characteristics of *Bacillus* sp. with high protease activity from traditional *cheonggukjang*. Korean J. Food Sci. Technol. 38: 82-87 (2006)
32. Cheo JS, Yoo SM, Kim HR, Kim JS, Chang CM. Volatile compounds of *cheonggukjang* prepared by different fermentation methods and soybean cultivars. J. Korean Soc. Agric. Chem. Biotechnol. 42: 111-115 (1999)