

## The Hypocholesterolemic Effects of Soymilk Fermented with *Bacillus subtilis* Compared to Soymilk with *Cheonggukjang* Powder in Apolipoprotein E Knockout Mice

Kyoung Chun Seo<sup>1</sup>, Mi Jin Kim<sup>1</sup>, Sun Hee Hong<sup>1</sup>, So Yeon Cha<sup>1</sup>, Jeong Sook Noh<sup>1</sup>,  
Mi Jeong Kim<sup>2</sup>, and Yeong Ok Song<sup>1†</sup>

<sup>1</sup>Department of Food Science and Nutrition and Kimchi Research Institute, Pusan National University, Busan 609-735, Korea

<sup>2</sup>Department of Food and Nutrition, Silla University, Busan 617-736, Korea

### Abstract

The cholesterol-lowering effects of soymilk fermented with *Bacillus subtilis* KCCM42923 were studied in apolipoprotein E knockout (ApoE KO) mice and compared to the cholesterol-lowering effects of soymilk to which *cheonggukjang* powder had been added. ApoE KO mice were divided into 3 groups (n=7/group). Animals were fed either an atherogenic diet only (AD, control group), an AD supplemented with fermented soymilk containing *Corni fructus* (FSM group), or an AD supplemented with soymilk to which *cheonggukjang* (CGJ) powder had been added at 5% (w/v) (CPS group) for 8 weeks. The amount of FSM or CPS supplementing the AD was 20.8 mL/kg BW. There were no differences in either body weight gain or organ weights among three groups. In the FSM group, the concentration of plasma total cholesterol (TC) and LDL cholesterol (LDLC) were significantly decreased by 26.2% and 30.3% compared with the values of the control group (p<0.05). However, the triglyceride (TG) and HDL-cholesterol (HDL-C) levels were not affected. These beneficial effects of FSM on suppressing the increase in plasma cholesterol level by AD were greater than those of CPS, which revealed 15.4% and 16.4% inhibition for TC and LDLC, respectively. However, these differences between FSM and CPS groups were not significantly different. A preventative effect of FSM or CPS on the accumulation of hepatic TC, but not on TG, was observed. FSM and CPS did not demonstrate any effects on fecal lipid excretion. In conclusion, the cholesterol-lowering effects of the soymilk fermented with *Bacillus subtilis* KCCM42923 were comparable to CGJ powder-added soymilk. These results suggest that drinking FSM might provide beneficial effects on controlling plasma cholesterol levels.

**Key words:** *Bacillus subtilis*, fermented soymilk, *Corni fructus*, hypocholesterolemic effects, *cheonggukjang*

### INTRODUCTION

The lower incidence of heart disease and cancer in Asian countries than in Western countries has been attributed to the higher consumption of soybean or soy products by Asians (1). In Korea, for example, tofu, doenjang, and *cheonggukjang* (CGJ), all of which are made of soybeans, are frequently consumed in the daily diet. People with hypercholesterolemia, lactose-intolerance, or gluten sensitivity, as well as vegetarians, generally include soybean products in their diet to address these health concerns (2). CGJ, which is similar to natto, is a traditional Korean soybean product fermented with *Bacillus (B.) subtilis* (3). Health benefits of CGJ and natto on atherosclerosis (4,5), cancer (6), and diabetes (7) are well documented. However, unlike natto, which is consumed raw, CGJ is eaten as part of a stew. Regardless of the known health benefits of CGJ, Koreans, especially the younger generations, do not want to cook CGJ stew

at home because of its distinctive smell. As a consequence, there has been a demand for a new CGJ product that has less of the heavy aroma and more easy applications. Powder or tablet-type CGJ has been introduced in the market (8,9) however they still have the inconvenience of requiring a liquid for consumption. To solve these problems, our group has tried to ferment soymilk with *B. subtilis* to develop a CGJ beverage.

Soymilk is an aqueous extract of whole soybeans (10) and is used as a substitute for cow's milk for those with milk allergies or sensitivities, or just concerns about their own health, as well as vegetarians (2). Soy proteins and isoflavones in soymilk are thought to have similar anti-atherogenic effects as other soybean products (11). Following the report by Mann and Spoerry about the cholesterol-lowering properties of fermented milk (yogurt) (12), extensive studies have been carried out with different strains of lactic acid bacteria in the hopes of developing yogurts with greater effects on controlling plasma cho-

†Corresponding author. E-mail: yosong@pusan.ac.kr  
Phone: +82-51-510-2847, Fax: +82-51-583-3648

lesterol (13,14). The idea of increasing the health benefits of milk by fermentation with probiotic bacteria has been adapted to soymilk, which has subsequently succeeded in elevating the functional properties of soymilk (15). The soymilk fermented with probiotic bacteria has been shown to exhibit antioxidant activity (16), hypocholesterolemic activity (17), anticancer potential (18), and immunogenic activity (19).

In our previous study, we tried to use *B. subtilis* as the starter culture instead of using probiotic bacteria for the fermentation of soymilk (15). *B. subtilis* is the microorganism responsible for making CGJ. The physical properties of the soymilk fermented with *B. subtilis* were a thick liquid with a weak smell of CGJ (15). The distinctive smell of CGJ produced during the fermentation of the cooked soybean with *B. subtilis* was greatly reduced when the soymilk was fermented with the same starter culture (15). Antiradical activities of beverage type of soymilk fermented with *B. subtilis* were greater than that of unfermented soymilk (20). When different strains of *B. subtilis* were tested, soymilk fermented with *B. subtilis* KCCM 42923 demonstrated the highest anti-radical activity among the microorganisms tested (20). In order to improve the sensory properties of CGJ beverage for commercial production, *Corni fructus* was added to the soymilk before fermentation (15). A 1% v/v addition of *Corni fructus* had the most favorable sensory evaluation. The lipid lowering effects of *Corni fructus* have been well documented (21). In addition, the anti-radical activities of *Corni fructus*-containing CGJ beverage were the highest among the three samples of either unfermented soymilk, soymilk fermented with *B. subtilis*, or soymilk with CGJ powder added (15). In this study, we examined the cholesterol-lowering properties of the CGJ beverage *in vivo*. The hypocholesterolemic effects of *Corni fructus*-containing soymilk fermented by *B. subtilis* KCCM 42923 was studied with apolipoprotein E knockout (ApoE KO) and their effects were compared with CGJ powder-added soymilk.

## MATERIALS AND METHODS

### Fermentation of soymilk

Soymilk was fermented with *B. subtilis* using a previously described procedure (15). Soymilk (Donghwa Food Co., Ltd., Yangsan, Korea) containing 4.0% soy protein was dispersed into 500 mL bottles and 1% glucose (w/v) were added and heat-treated at 121°C for 15 min. Each bottle was then inoculated with *B. subtilis* (KCCM 42923 total 7~8 log CFU/mL) and *Corni fructus* (1% v/v) was added before incubation at 40°C for about 8 hr. Fermentation was terminated when the pH

of the soymilk reached  $5.5 \pm 0.2$ .

### *Corni fructus*

Water extract of *Corni fructus* was used (CH Food Company, Busan, Korea). The concentration of *Corni fructus* added to the soymilk was 1% of 10°brix extracts. Based on previous results, *Corni fructus* was added to the soymilk before fermentation (15).

### Animals

ApoE KO mice were purchased from Central Lab-Animal Inc. (Seoul, Korea). The animals were acclimated for one week and then were divided into 3 groups according to the diet given (Table 1). The control group (n=7) was given atherogenic diet (AD) only, the fermented soymilk group (FSM group, n=7) was fed AD supplemented with FSM, the CGJ powder-added soymilk group (CPS group, n=7) was provided AD supplemented with CGJ. Animals were kept individually in plastic mouse cages in a room with controlled temperature (20~23°C and lights (12 hr light/dark cycles) during the experiment. After 8 weeks, the mice were anesthetized with ether following a 12 hr fast. Blood samples were drawn from the inferior vena cava and heart. Feces were collected for 48 hr before sacrifice. Feces were freeze-dried and powdered. All samples were stored at -70°C for the further use.

### Diet preparation

The atherogenic diet was prepared to contain 1.25% cholesterol (Table 1) (22). The amount of FSM and CPS supplementing the AD was 28.6 mL/kg BW, which is 10 times greater than RNI of milk for adults (200 mL/70 kg BW/day). The amount of CGJ added to the soymilk

**Table 1.** Composition of the experimental diet

Ingredients	Group		
	Control	FSM <sup>1)</sup>	CPS <sup>2)</sup>
Casein	20	20	20
Sucrose	44	44	44
Cholesterol	1.25	1.25	1.25
Lard	10	10	10
Cellulose	5	5	5
DL-Methionine	0.3	0.3	0.3
Mineral mixture	3.5	3.5	3.5
Vitamin mixture	1	1	1
Choline bitartrate	0.2	0.2	0.2
Corn starch	15	15	15
FSM <sup>1)</sup>		28.6 mL <sup>3)</sup>	
CPS <sup>2)</sup>			28.6 mL <sup>3)</sup>

<sup>1)</sup>FSM: 1% *Corni fructus* added to the soymilk followed fermentation by *Bacillus subtilis* 2829 PNU 015.

<sup>2)</sup>CPS: 5% *cheonggukjang* powder added soymilk.

<sup>3)</sup>The amount of sample added to the diet was 10 times of the one serving of soybean milk (200 mL) consumed by adults (70 kg).

was 5% (w/v).

#### Determination of lipid analysis

The plasma total cholesterol (TC) and high density lipoprotein cholesterol (HDLC) concentration were measured enzymatically using a commercial kit (AM 202-K, AM 203-K, ASAN, Korea) based on a modification of cholesterol oxidase method (23). The plasma triglyceride (TG) concentration was determined using a kit (AM 157S-K, ASAN, Korea), with modified lipase-glycerol phosphate oxidase method (24). Low density lipoprotein cholesterol (LDLC) concentration was calculated by Friedewald formula (25). Hepatic TC and TG were also determined using commercially available kits with lipid extracted from liver homogenates (Forch method).

#### Thiobarbituric acid-reactive substances (TBARS)

To determine the antioxidant effects of fermented soymilk, hepatic TBARS concentration was measured (26). Briefly, 2 mL TBARS solution contained 0.4% TBA, 15% TCA, and 2.5% HCl added to 1 mL liver homogenate. The reaction media were heated at 95~100°C for 20 min followed by immediate cooling in ice water. To avoid evaporation, the tube is tightly capped and maintained at a heating temperature below 100°C. The reaction mixture was centrifuged at 800×g for 10 min. The absorbance of the obtained supernatant was measured at 535 nm.

#### Statistical analysis

One-way analysis of variance (Anova) was followed by Duncan's multiple range test in order to determine the

statistical significance of measurements between groups, using the SAS software (SAS Institute, Cart, NC, USA).  $p < 0.05$  was considered as significant.

## RESULTS

#### Body weight gains and organ weight

As shown in Table 2, the body weight changes of the mice (n=7) among the three groups after 8 weeks did not differ. Also, no differences in the weights of the major organs of the mice among the three groups were observed.

#### Plasma lipid concentrations

As shown in Table 3, plasma TC and LDLC concentrations of the control group reached 1013.74 and 930.3 mg/dL, respectively, indicating that hypercholesterolemia was successfully induced by AD. Total cholesterol concentrations of both the FSM and CPS groups were decreased significantly compare to that of the control group ( $p < 0.05$ ). Approximately 26 and 15% reductions in the TC concentration were observed for the FSM and CPS groups, respectively, compared to the control group. The increase in LDLC concentrations by AD were significantly suppressed by supplementing AD with FSM and CPS (Table 3), but no differences in HDLC and TG concentrations among the three groups were observed. When the cholesterol-lowering effects of FSM and CPS were compared, TC and LDLC concentrations for the FSM group were lower by 11 and 14%, respectively than those for the CPS group. But the differences were not statisti-

**Table 2.** Body weight gain and relative organ weights of apoE KO mouse fed atherogenic diet for 8 week

Experimental group <sup>1)</sup>	Body weight gain (%) <sup>2)</sup>	Relative organ weight (mg/100 g body weight)					
		Liver	Heart	Lung	Kidney	Spleen	Testis
Control	15.71 ± 3.11 <sup>NS</sup>	1.45 ± 0.24 <sup>NS</sup>	0.14 ± 0.02 <sup>NS</sup>	0.20 ± 0.03 <sup>NS</sup>	0.37 ± 0.07 <sup>NS</sup>	0.15 ± 0.07 <sup>NS</sup>	0.17 ± 0.01 <sup>NS</sup>
FSM	18.07 ± 6.99	1.61 ± 0.37	0.15 ± 0.04	0.18 ± 0.03	0.38 ± 0.06	0.13 ± 0.05	0.18 ± 0.02
CPS	20.50 ± 5.88	1.47 ± 0.23	0.17 ± 0.03	0.20 ± 0.02	0.43 ± 0.07	0.15 ± 0.03	0.16 ± 0.02

Values are mean ± SD. <sup>NS</sup>Not significant.

FSM: 1% *Corni fructus* added to the soymilk followed by fermentation with *Bacillus subtilis*.

CPS: 5% *cheonggukjang* powder was added to the soymilk.

<sup>1)</sup>Control group: mouse fed atherogenic diet (1.25% cholesterol diet), FSM group: mouse fed AD supplemented with FSM (28.6 mL/kg BW), CPS group: mouse fed AD supplemented with CPS (28.6 mL/kg BW).

<sup>2)</sup>(final weight - initial weight) / final weight × 100.

**Table 3.** Plasma cholesterol concentration of apoE KO mouse fed atherogenic diet for 8 weeks

Experimental group <sup>1)</sup>	Concentration (mg/dL)			
	TC	HDLC	LDLC	TG
Control	1013.7 ± 228.6 <sup>a</sup>	43.6 ± 9.0 <sup>NS</sup>	930.3 ± 225.6 <sup>a</sup>	228.1 ± 92.8 <sup>NS</sup>
FSM	748.3 ± 107.3 <sup>b</sup>	61.0 ± 24.4	648.5 ± 124.3 <sup>b</sup>	193.8 ± 37.2
CPS	861.3 ± 105.7 <sup>ab</sup>	44.3 ± 10.0	778.1 ± 102.9 <sup>ab</sup>	194.4 ± 72.0

Values were mean ± SD. <sup>NS</sup>Not significant.

<sup>a,b</sup>Data were significantly different with one-way ANOVA followed by Duncan's multiple range test at the 0.05 level of significance.

<sup>1)</sup>See the legend of Table 1.

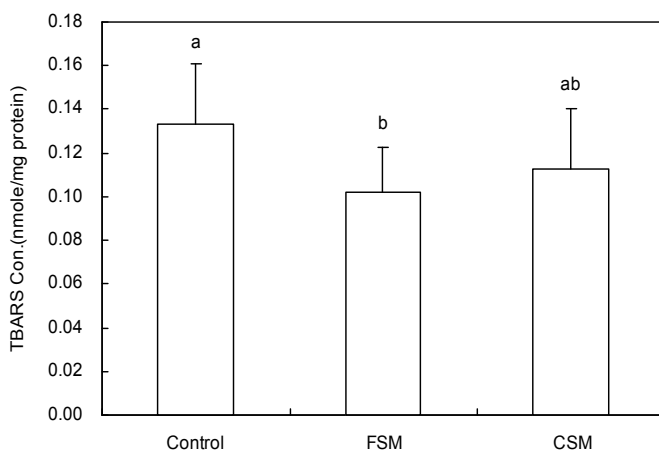
**Table 4.** Hepatic lipid concentrations of apoE KO mouse fed atherogenic diet for 8 weeks

Group <sup>1)</sup>	Concentration (mg/g tissue)	
	TC	TG
Control	6.1 ± 1.0 <sup>a</sup>	47.5 ± 12.5 <sup>NS</sup>
FSM	4.9 ± 0.6 <sup>b</sup>	38.7 ± 12.6
CPS	4.9 ± 0.8 <sup>b</sup>	36.7 ± 6.3

Values were mean ± SD. <sup>NS</sup>Not significant.

<sup>a,b</sup>Data were significantly different with one-way ANOVA followed by Duncan's multiple range test at the 0.05 level of significance.

<sup>1)</sup>See the legend of Table 1.



**Fig. 1.** Hepatic TBARS concentration of apoE KO mouse fed AD diet supplemented with FSM<sup>1)</sup> or CPS<sup>2)</sup> for 8 week. <sup>a,b</sup>Data were significantly different with one-way ANOVA followed by Duncan's multiple range test at the 0.05 level of significance. <sup>1)</sup>FSM: 1% *Corni fructus* added to the soymilk followed by fermentation with *Bacillus subtilis*. <sup>2)</sup>CPS: 5% *cheongguk-jang* powder was added to the soymilk.

cally significant. The cholesterol-lowering effect of FSM was found to be comparable to that of CPS.

#### Inhibition of hepatic lipid accumulation

The hepatic TC concentrations of FSM and CPS groups compared to that of the control group were lower by 19.7 and 19.4%, respectively (Table 4,  $p < 0.05$ ). But TG concentrations among three groups were not significantly different. The antioxidant effects of FSM and CPS on hepatic lipid oxidation determined as a TBARS concentration were examined. As shown in Fig. 1, the TBARS concentration of the FSM group was the lowest, followed by the CPS group and then the control group ( $p < 0.05$ ).

#### Fecal excretion of lipid

AS shown in Table 5, both TC and TG excretion in the feces of FSM and CPS group were only slightly increased compared to the control group. No significant differences in lipid excretion were observed among three groups.

**Table 5.** Fecal lipids concentrations of apoE KO mouse fed atherogenic diet for 8 weeks

Group <sup>1)</sup>	Concentration (mg/g feces)	
	TC	TG
Control	65.9 ± 6.1 <sup>NS</sup>	7.5 ± 1.3 <sup>NS</sup>
FSM	70.1 ± 14.1	8.2 ± 2.8
CPS	71.5 ± 6.7	9.0 ± 1.4

Values were mean ± SD. <sup>NS</sup>Not significant.

<sup>1)</sup>See the legend of Table 1.

## DISCUSSION

Recently, a powder or tablet type of CGJ which is consumed without additional cooking has been introduced in the market for a convenient usage (8,9). However, these products still require a beverage for consumption. To address this issue, we successfully created a CGJ-containing beverage by fermenting soymilk with *B. subtilis*. Antiradical activity was observed in this CGJ beverage *in vitro* (20). In this present study, the hypocholesterolemic effects of the *B. subtilis* fermented soymilk were examined and compared to the effects seen with soymilk to which CGJ powder was added. These experiments were run in ApoE KO mice fed AD.

The hypercholesterolemia induced by AD, which was over 1,000 mg/mL, was suppressed when the diet was supplemented with both FSM and CPS. Approximately 26 and 15% decrease in TC concentration by FSM and CPS compared to the control group was observed. LDLC, one of the major risk factor for atherosclerosis, is also reduced by FSM and CPS supplementation. When the TC and LDLC lowering effects FSM were compared to those for CPS, the hypocholesterolemic activity of FSM was found to be the greatest, although the differences were not significant. Concentrations for TC and LDLC in the FSM group were lower by 11 and 14%, respectively than those for the CPS group. However, neither HDLC increase nor TG decrease effect of FSM and CPS was observed. Hepatic TC, but not TG, accumulation was also significantly inhibited by FSM and CPS. Furthermore, FSM significantly reduced hepatic lipid oxidation, suggesting FSM exerts an antioxidant effect as well as hypocholesterolemic effect. The observation that antiradical effects and total antioxidant activity of soymilk are enhanced when it is fermented is strongly supported by our previous results (20). Moreover, FSM exhibited greater radical scavenging activity than fermented soymilk to which *Corni fructus* has not been added (15). However, neither FSM nor CPS enhanced lipid excretion in the feces.

Low-molecular peptide (27), soluble dietary pectin (28), and mucilage (29) produced during the fermentation process, as well as antioxidative (30) compounds in the

soymilk, likely contributed to the hypocholesterolemic effect of FSM and CPS. From these results, we could conclude that beverage form of CGJ can be used as a substitute for either CGJ powder or CGJ tablets for cholesterol lowering purposes.

In conclusion, the beverage type of CGJ was demonstrated to have cholesterol-lowering activity in ApoE KO mice fed AD. Our data suggest that, in terms of cholesterol lowering activity, the beverage form of CGJ will be as effective as CGJ powder added to soymilk.

### ACKNOWLEDGEMENT

This research was financially supported by the Ministry of Education, Science Technology (MEST) and Korea Institute for Advancement of Technology (KIAT) through the Human Resource Training Project for Regional Innovation.

### REFERENCES

- David CK, John AE. 1996. A review of the clinical effects of phytoestrogens. *Obstetrics & Gynecology* 87: 897-904.
- Morandi S, D'Agostina, Ferrario F, Arnoldi A. 2005. Isoflavone content of Italian soy food products and daily intakes of some specific classes of consumers. *Eur Food Res Technol* 221: 84-91.
- Joo MH, Hur SH, Han YS, Kim JY. 2007. Isolation, identification, and characterization of *Bacillus* strains from the traditional Korean soybean-fermented food, chungkookjang. *J Appl Biol Chem* 50: 202-210.
- Yang JL, Lee SH, Song YS. 2003. Improving effect of powders of cooked soybean and chongkukjang on blood pressure and lipid metabolism in spontaneously hypertensive rats. *J Korean Soc Food Sci Nutr* 33: 899-905.
- Ko JB. 2006. Effect of cheonggukjang added *Phellinus linteus* on lipid metabolism in hyperlipidemic rats. *J Korean Soc Food Sci Nutr* 35: 410-415.
- Park JH, Jeong HJ, Lumen BO. 2007. *In vitro* digestibility of the cancer preventive soy peptides lunasin and BBI. *J Agric Food Chem* 55: 10703-10706.
- Kim JI, Kang MJ, Kwon TW. 2003. Antidiabetic effect of soybean and chongkukjang. *Korean Soybean Digest* 20: 44-52.
- Yang HR, Park SS, Lee JW, Lee KB, Han MK. 2009. Comparison of vitamin contents and organoleptic characteristics in powdered cheongkukjang dried by different drying methods. *Korean J Food & Nutr* 22: 192-198.
- Youn KS. 2004. Preparation and quality characteristics or tablet using cheonggukjang powder. *J East Asian Soc Dietary Life* 14: 495-500.
- Hiroko K, Norie O, Satoshi M, Emi Y, Osamu C, Rie T, Fumiyasu I. 1998. Effects of soy milk and bifidobacterium fermented soy milk on lipid metabolism in aged ovariectomized rats. *Biosci Biotechnol Biochem* 62: 1688-1692.
- Arthony MS, Clarkson TB, Hughes CL, Morgan TM, Burke GL. 1996. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal Rhesus monkeys. *J Nutr* 126: 43-50.
- Mann GV, Spoerry A. 1974. Studies of a surfactant and cholesteremia in the Maasai. *Am J Clin Nutr* 27: 464-469.
- Abd El-Gawad IA, El-Sayed EM, Hafez SA, El-Zeini HM, Saleh FA. 2005. The hypocholesterolaemic effect of milk yoghurt and soy-yoghurt containing bifidobacteria in rats fed on a cholesterol-enriched diet. *Int Dairy J* 15: 37-44.
- James W, Anderson MD, Gilliland SE. 1999. Effect of fermented milk (yogurt) containing *Lactobacillus acidophilus* L1 on serum cholesterol in hypercholesterolemic humans. *J Am Coll Nutr* 18: 43-50.
- Seo KC, Choi KH, Noh JS, Kim MJ, Song YO. 2009. Improvement of the functional and organoleptic properties of the fermented soymilk with *Bacillus subtilis* 2829PNU015 in addition of *Corni fructus* extracts. *J Food Sci Nutr* 14: 54-59.
- Wang YC, Yu RC, Chou CC. 2006. Antioxidative activities of soymilk fermented with lactic acid bacteria and bifidobacteria. *Food Microbiol* 23: 128-135.
- Liong MT, Shah NP. 2005. Bile salt deconjugation and BSH activity of five bifidobacterial strains and their cholesterol co-precipitating properties. *Food Res Int* 38: 135-142.
- Hsieh ML, Chou CC. 2006. Mutagenicity and antimutagenic effect of soymilk fermented with lactic acid bacteria and bifidobacteria. *Int J Food Microbiol* 111: 43-47.
- Reid G. 2006. Safety and efficacious probiotics: What are they? *Trends Microbiol* 14: 348-352.
- Seo KC, Kim MJ, Kwon MJ, Kim HJ, Noh JS, Song YO. 2009. Antioxidative activities of soymilk fermented with *Bacillus subtilis*. *Food Sci Biotechnol* 18: 1298-1300.
- Lee YA, Moon HY. 2004. The effect of medicinal plants extracts feeding on hypoglycemic and lipid metabolism in streptozotocin-induced diabetic rats and antioxidant activity of medicinal plants. *Life Sci Res* 3: 61-68.
- Hina PM, Verstuyft J, Paigen B. 1990. Synthetic low and high fat diets for the study of atherosclerosis in the mouse. *J Lipid Res* 31: 859-869.
- Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. 1974. Enzymatic determination of total serum cholesterol. *Clin Chem* 20: 470-475.
- McGowan MW, Artiss JD, Strandbergh DR, Zak B. 1983. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin Chem* 29: 538-542.
- Friedewald WT, Levy RI, Frederickson DS. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem* 18: 499-502.
- Buege JA, Aust SD. 1978. Microsomal lipid peroxidation. *Methods Enzymol* 52: 302-310.
- Kim JS, Yoo SM, Chang CM. 1998. Physicochemical properties of traditional Chonggukjang produced in different region. *Agric Chem Biotechnol* 41: 377-383.
- Taguchi K, Kawabata M, Ohtsuki K, Tanaka Y. 1986. Changes in dietary fiber of natto and tempeh during fermentation. *J Jpn Soc Nutr Food Sci* 39: 203-208.
- Kim SC, Lee SH, Wi SJ. 2002. The effects of natto mucilage on the components of serum lipid in rats. *J Korean Oil Chem Soc* 19: 63-67.
- Yokota T, Hattori T, Ohishi H, Hasegawa K, Watanabe K. 1996. The effect of antioxidant-containing fraction from fermented soybean food on atherosclerosis development in cholesterol-fed rabbits. *Lebensm-Wiss u-Technol* 29: 751-755.