JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Physicochemical and Sensory Characteristics of a Medicinal Soy Yogurt Containing Health-Benefit Ingredients

YOUNG-HEE PYO*,† AND SUN-MI SONG§

Department of Food and Nutrition, Sungshin Women's University, Seoul, Korea, and Traditional Food Research Division, Korea Food Research Institute, Gyeonggi-do, Korea

Medicinal soy yogurt (sogurt) containing high levels of γ-aminobutyric acid (GABA), free amino acids (FAAs), statins, and isoflavone aglycones was developed using lactic acid bacteria (1:1 mixture of *Lactobacillus delbrueckii* subsp. *latis* KFRI 01181 and *Lactobacillus plantarum* KFRI 00144) and *Monascus*-fermented soybean extract (MFSE, 1.5%, w/v). Changes in the content of some functional components (GABA, FAAs, statins, isoflavones) and physical (pH, titratable acidity, water-holding capacity), biological (viable cell counts), and sensory characteristics of sogurts during fermentation and cold storage were examined. The medicinal sogurt contained significantly ($p < 0.05$) high levels of FAAs (2011.2 ± 8.1 mg/100 g of dry weight of sogurt), GABA (45.5 ± 1.9 mg), statins (100.1 ± 7.5 μg), and isoflavone aglycones (56.4 ± 4.6 mg) compared with the control sogurt (1167.1 ± 8.1 mg, 32.1 ± 2.5 mg, not detected, and 19.2 ± 1.9 mg, respectively) after fermentation for 24 h at 35 °C. During cold storage for 30 days at 4 °C, medicinal sogurt displayed higher water-holding capacity and titratable acidity and total bacterial cells and lower pH than the control sogurt ($p < 0.05$). Overall sensory acceptability of medicinal sogurt supplemented with MFSE was higher than that of the control sogurt prepared without MFSE. The results indicate that the addition of the appropriate MFSE concentrations (1.5%, w/v) improved the physicochemical properties as well as sensory characteristics of soy yogurt, resulting in enhanced health-benefit ingredients and consumers' preferences.

KEYWORDS: Soy yogurt; free amino acids; GABA; statins; isoflavones; sensory characteristics; physicochemical properties

INTRODUCTION

Soy foods have attracted much attention for their possible effects on human health because of their phytochemical content, mainly isoflavones (*1*). Soybean isoflavones having both weak estrogenic and antiestrogenic activities may partly be responsible for the cholesterol -lowering and cardioprotective effects (*2, 3*). It has been suggested that the biologically active estrogen-like isoflavones are the aglycones (*2, 4*). Several researchers have tried to ferment soy milk using various organisms from different sources to enhance the levels of bioactive isoflavone aglycones in it (*5, 6*).

Soy-milk-based yogurts, namely, sogurt, have emerged as a popular alternative to traditional dairy-based yogurts due to their reduced level of cholesterol and saturated fat and because they are free of lactose (*1, 7*). Furthermore, the incorporation of probiotic bacteria as dietary adjuncts has given rise to increased consumption of probiotic products in Asia and Europe (*8, 9*). Probiotics have been used to promote the growth and activity of beneficial microorganisms in vitro (*9*) and in the large intestine (*10*).

γ-Aminobutyric acid (GABA) is a ubiquitous nonprotein amino acid that is produced primarily by the α-decarboxylation of glutamic acid catalyzed by the enzyme glutamate decarboxylase (*11*). Glutamic acid is one of the most abundant amino acids found in legumes such as soybean, red bean, and mung bean (*12*). The consumption of GABA-enriched foods such as milk, soybean, and gabaron tea has been reported to depress the elevation of systolic blood pressure in spontaneously hypertensive rats (*12, 13*)

Natural statins such as mevinolin (also known as lovastatin, monacolin K, mevacor) are a group of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors that prevent cardiovascular disease (CVD) and mortality in patients and reduce the relative risk of major coronary events and major cerebrovascular events in the population without CVD (*14*−*16*). HMG-CoA reductase inhibitors (statins) are generally classified according to their origin. Lovastatin and pravastatin are first-generation statins and are fungal derivatives or fermentation

* Address correspondence to this author at the Department of Food and Nutrition, Sungshin Women's University, 249-1 Dongsun-Dong Sungbuk-Gu, Seoul 136-742, Republic of Korea (telephone +2-920-7588; fax +2-920-2076; e-mail rosapyo@sungshin.ac.kr or rosapyo@hotmail.com).

† Sungshin Women's University.

§ Korea Food Research Institute.

10.1021/jf8026952 CCC: $40.75
Published on Web 12/08/2008

products. They differ in their molecular structures because lovastatin is administered as a prodrug in its lactonic form, whereas pravastatin, like all other statins, is administered as the active β-hydroxy acid form (*16*).

We recently found that soybean fermented with *Monascus pilosus* has a remarkable content of bioactive isoflavone aglycones (daidzein, glycitein, genistein; 1.13 mg/g of dw) and natural statins, mevinolins (2.94 mg/g of dw) (*17*). Also, it was found that MFSE have the potential for not only strong free radical scavenging effects but also antihypertensive properties (*18*). The results indicate that *Monascus*-fermented soybean has potential as a novel medicinal food or multifunctional food supplement.

There has been much research on fermentation of soy milk to make yogurt (*1, 7, 8*), but there has been little research on the medicinal sogurt with probiotics and health-promoting ingredients such as bioactive statins, isoflavones, and GABA. Therefore, our study was carried out to develop a new biosogurt enriched with functional phytochemicals by adding MFSE. In the present study, the effects of MFSE supplementation on the physicochemical and sensory characteristics during fermentation and cold storage of sogurt were investigated.

MATERIALS AND METHODS

Chemicals. Standards of daidzein, genistein, mevinolin, free amino acids (FAAs), and GABA were purchased from Sigma (St. Louis, MO). Daidzin, genistin, glycitin, and glycitein standards were obtained from Funakoshi Chemical Co. (Tokyo, Japan). General aerobic medium (MRS) was purchased from Difco Co. (Detroit, MI). All other reagents were of the highest grade available unless otherwise indicated.

Microorganisms and Media. Two bacterial strains (*Lactobacillus delbrueckii* subsp. *latis* KFRI 01181, *Lactobacillus plantarum* KFRI 00144), which have high β-glucosidase activity (*5*), were obtained from Korea Food Research Institute (KFRI, Seongnam-si, Korea). Stock cultures were maintained on agar plates containing 55 g/L of MRS broth (Difco Co.) and 20 g/L of agar. Culture for the inoculum was conducted in MRS broth medium. The initial pH of a medium was adjusted to 6.2 and sterilized in an autoclave at 121 °C for 20 min. *L. delbrueckii* subsp. *latis* KFRI 01181 and *L. plantarum* KFRI 00144 were inoculated into MRS broth (2%, v/v), and the inoculum was activated three times at 37 °C for 24 h to use as the starter for production of sogurt.

MFSE Preparation. MFSE was produced as described elsewhere (*17, 18*). In brief, whole soybeans were washed, soaked, and autoclaved. After cooling, the substrate was inoculated with nutrient broth including *M. pilosus* and incubated at 30 °C for 20 days. Samples were collected, lyophilized, and powdered. A subsample (100 g) was extracted with 1 L of 80% ethanol (v/v) for 5 h, three times, and filtered through Whatman no. 4 filter paper. The combined extracts were then rotary evaporated at 40 °C and lyophilized. The dried extract was used directly for sogurt production. Preliminary trials were conducted to determine optimum MFSE concentration (1.5%, w/v) for a medicinal production (data not shown). The process was optimized with respect to functional phytochemicals level, viable cell counts, and overall acceptance.

Preparation of Sogurt. Whole soybeans (Seoritae, products from Kangwon-do, Korea, 2005) were soaked in tap water at a beans to water ratio of 1:10 (w/v) for 13 h at room temperature. The hydrated beans were heated at 95 °C for 10 min and then drained and ground for 1 min with tap water at a ratio of 1:3 (w/v) using a blender (Dynamics Corporation of America, Greenwich, CT). Soymilk was separated from insoluble residue by filtering it through a nylon 100-mesh filter sack (Kawanishi Shoko Co. Ltd., Los Angeles, CA). Powdered MFSE (0.5−3%) were added to the prepared soy milk and homogenized in a blender for 5 min. The suspension was pasteurized at 90 °C for 15 min. The soy milk was allowed to cool to 40 °C and was aseptically inoculated with 2% of the mixed strain starter (*L. delbrueckii* subsp. *latis* and *L. plantarum*, 1:1, v/v). Inoculated soy milk with or without MFSE was then poured into 70 mL sterile transparent plastic cups with lids (30 mL per cup) and incubated at 35 °C for 48 h. Both the control sogurt without MFSE (CS) and the medicinal sogurt with MFSE (MS) were stored at 4 °C for further analysis.

Viable Cell Counts. Sogurt sample (1 mL) was collected every 12 h of fermentation and diluted 10-fold with sterilized physiological saline. After that, 0.1 mL was smeared on MRS plate count agar using a micropipet and incubated for 24 h at 37 °C. Visible colonies of each strain were then counted and the unit expressed as colony-forming units (cfu) per gram.

Acidity Measurement. The pH change was monitored by a pH-meter (Fisher Scientific, Pittsburgh, PA). Titratable acidity (TA) was determined by titrating a sample (5 g of sample + 45 mL of distilled water) with 0.1 N NaOH to an end point of pH 7.0. TA was calculated on the basis of lactic acid as the predominant acid and was expressed as percent lactic acid. Sample temperature was 25 °C for each analysis.

Water-Holding Capacity (WHC). A sample of about 20 g of sogurt (SO) was centrifuged for 10 min at 669*g* and 20 °C in a centrifuge (International Equipment, Needham, MA) (*19*). The whey expelled (WE, g) was removed and weighed. The WHC expressed in percent was defined as WHC (%) = 100 × (SO − WE)/SO.

Quantification of Statins and Isoflavones. The contents of isoflavones and mevinolins in sogurt were determined by HPLC with minor modifications (*20, 21*). For the extraction of the isoflavones, 0.5 g of ground sample in 10 mL of 80% ethanol was vigorously shaken and extracted at room temperature for 30 min using an ultrasonicator (Bransonic, Danbury, CT). The extract was centrifuged at 12000*g* for 15 min and the supernatant filtered through a siringe filter (0.22 μm, Waters Co., Milford, MA) prior to HPLC analysis. Reversed phase HPLC analysis was carried out with a JASCO system (Tokyo, Japan), using a YMC AM 303 ODS-A column (4.6 × 250 mm, Kyoto, Japan). The mobile phase was composed of 0.1% phosphoric acid in acetonitrile (solvent A) and 0.1% phosphoric acid in water (solvent B). Following the injection of 20 μL of sample, solvent A was increased from 15 to 35% over 50 min and then held at 35% for 10 min. The solvent flow rate was 1 mL/min, and the eluted isoflavones were detected at 254 nm. Individual isoflavone standards were used for peak identification according to elution time, UV spectra, and spiking tests. Isoflavone quantification was based on calibration curves for each of the standards. The results were adjusted for molecular weight of the correspondent glucosides and expressed as aglycone equivalents per gram of soy yogurt (dry basis) (*20*).

The statins in sogurt were analyzed using an isocratic solvent system with the mixture of 0.1% phosphate buffer (pH 7.7) and acetonitrile (65:35, v/v) as the mobile phase (*21*). The solvent flow rate was 0.8 mL/min, and eluted statins were detected at 238 nm. Quantitative data for statins were obtained by comparison to known standards.

Amino Acid Analysis and Determination of GABA. For the extraction of amino acid and GABA, 0.5 g of ground sample in 10 mL of sulfosalicylic acid was vigorously shaken and extracted at room temperature for 30 min using an ultrasonicator. The extract was centrifuged at 12000*g* for 15 min and the supernatant then filtered through a syringe filter and derivatized using phenylisothiocyanate (PITC) prior to HPLC analysis (*12*). Reversed phase HPLC analysis was carried out with an Agilent 1100 series system (Santa Clara, CA), using an Eclipse XDB-C18 column (4.6 × 150 mm, 3.6 μm, Kyoto, Japan) and guard column XDB-C18. For the analysis of GABA and FAAs, the mobile phase was composed of 40 mM Na_2HPO_4 (pH 7.8) (solvent A) and mixed solvent (acetonitrile/ methanol/water = 45:45:10, v/v/v) (solvent B). The solvent flow rate was 2 mL/min, and the eluted FAAs were detected at 338 nm. Quantitative data for free amino acids and GABA were obtained by comparison to known standards.

Sensory Evaluation. The sensory properties of the sogurt were evaluated by a trained panel of 10 assessors. The samples were served at 7−10 °C in plastic cups and were coded with three-digit numbers. Order of presentation of samples was randomized. A test form comprising four sensory attributes, namely, flavor, texture, appearance, and overall acceptability, was given to each panelist (*22*). The sensory evaluation was scored between 1 and 5 points, in which 1 is equal to worst and 5 is equal to best.

Statistics. Data were expressed as mean ± standard deviation (SD) from three independent parallel experiments. Significant differences

Table 1. Changes of Some Physicochemical Properties in Control Sogurt (CS) and Medicinal Sogurt (MS) during Cold Storage at 4 °C

property	day 1		day 15		day 30	
	CS	MS	CS	MS	CS	MS
pH	4.47 ± 0.56a	4.18 ± 0.23b	4.22 ± 0.33a	4.14 ± 0.21a	4.02 ± 0.32a	3.89 ± 0.21b
TA (%)	0.89 ± 0.03a	1.09 ± 0.08b	0.95 ± 0.06a	1.01 ± 0.19a	1.08 ± 0.13a	1.16 ± 1.02a
viable cell (log_{10}cfu/g)	7.3 ± 1.3a	8.2 ± 1.8b	7.8 ± 0.9a	8.9 ± 1.1b	6.2 ± 1.3a	7.7 ± 0.8b
WHC (%)	84.1 ± 5.6a	93.2 ± 6.1b	91.7 ± 3.1a	96.8 ± 2.8b	90.8 ± 4.8a	95.4 ± 6.1b
GABA (mg/100 g of dw)	32.1 ± 2.5a	45.5 ± 1.9b	34.8 ± 2.4a	46.9 ± 3.1b	35.1 ± 1.7a	50.3 ± 2.5b
FAAs (mg/100 g of dw)	1167.1 ± 8.1a	2011.2 ± 8.1b	1172.3 ± 8.4a	2961.2 ± 7.1b	1170.4 ± 6.1a	2180.3 ± 6.9b
isoflavones (mg/100 g of dw)	146.6 ± 2.9[b]	157.6 ± 5.8b	142.8 ± 6.2a	159.8 ± 4.5b	139.5 ± 7.3a	144.7 ± 2.6a
statins (μg/100 g of dw)	ND[c]	100.1 ± 7.5 (93.2)[d]	ND	85.6 ± 7.2 (81.8)	ND	77.4 ± 5.7 (75.0)

[a] All data are expressed as mean ($n = 3$). Different letters between two sogurt represent significant differences ($p < 0.05$). [b] Adjusted for molecular weight of the correspondent glucosides and expressed as aglycone equivalents per gram of soy yogurt (dry basis). [c] Not detected. [d] Concentration of hydroxy acid form.

between means were determined using least-squares means (SAS Institute, Cary, NC). Significance was established at $p < 0.05$.

RESULTS AND DISCUSSION

Physical and Bacterial Characteristics. *pH and TA.* Changes in pH and TA of sogurt during fermentation and cold storage are presented in **Table 1**. An appreciable decrease in pH and increase in TA were noted in soy milk supplemented with MFSE after 24 h of fermentation. The initial TA of medicinal sogurt was 0.51%. It gradually increased during fermentation, and the final acidity values after 24 h were significantly higher (1.09 ± 0.08%) ($p < 0.05$) than that of control sogurt (0.89 ± 0.03%). At the end of 24 h of fermentation at 35 °C, the pH and TA of the control were 4.47 ± 0.56 and 0.89 ± 0.03%, whereas those of the medicinal sogurt were 4.18 ± 0.23 and 1.09 ± 0.08%, respectively. This reduction in pH was sufficient to cause coagulation, and hence the appearance of all soy milk samples changed within 24 h of fermentation. As presented in **Table 1**, the pH in the medicinal sogurt was maintained constant during first 15 days of cold storage. By the end of the storage period, the pH of MS was significantly ($p < 0.05$) lower than the control and ranged between 4.47 ± 0.56 and 4.02 ± 0.32 and between 4.18 ± 0.23 and 3.89 ± 0.21 in CS and MS, respectively. Acidity (% TA) of CS and MS did not show any statistical difference on the 15th and 30th days. It was reported that the pH and % TA in soy-based yogurts were 3.9−4.3 and 0.97−1.43%, respectively (*22*, *23*), which is in accordance with the data of medicinal sogurt in the present study.

WHC. Measurements of WHC showed significant differences ($p < 0.05$) between CS and MS (**Figure 1**). The higher WHC was obtained for sogurt with MFSE added during fermentation. On the first day of cold storage, the MS was 93.2 ± 6.1%, which was 9.1% higher than that of CS (84.1 ± 5.6%). The WHC values of MS on the 15th day (96.8 ± 2.8%) and 30th day (95.4 ± 6.1%) were found to be statistically higher than those of CS at 91.7 ± 3.1 and 90.8 ± 4.8%, respectively ($p < 0.05$). These results could be attributed to the fact that the addition of MFSE in sogurt resulted in increased colloidal linkage between soy protein micelles and, hence, a more intense network of the sogurt gels. Lower WHC or whey separation is related to an unstable gel network and excessive rearrangements of a weak gel network (*23*). Thus, the addition of MFSE contributed to syneresis prevention and increased the proportion of WHC in soy yogurts.

Viable Cell Counts. The effects of MFSE addition on the increase in total bacteria counts during the fermentation of sogurts are shown in **Figure 2**. The mixed starter culture containing *L. delbrueckii* subsp. *latis* and *L. plantarum* grew well in the soy milk with added MFSE, and their populations increased in a time-dependent manner, reaching almost maximal

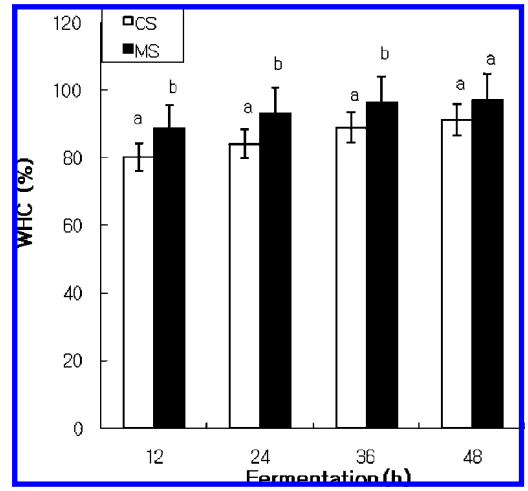

Figure 1. Changes of water holding capacity (% WHC) in control sogurt (CS) and medicinal sogurt (MS) during the fermentation at 35 °C. All data are expressed as mean ($n = 3$). Different letters between two sogurts represent significant differences ($p < 0.05$).

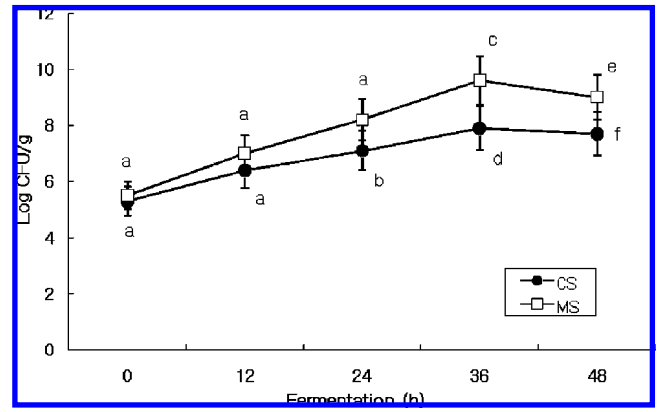

Figure 2. Changes of total lactic acid bacteria count in control sogurt (CS) and medicinal sogurt (MS) during the fermentation at 35 °C. All data are expressed as mean ($n = 3$). Different letters between two sogurts represent significant differences ($p < 0.05$).

numbers (9.0−9.6 log_{10} cfu/g) between 36 and 48 h of fermentation. There was an approximately 1 log cycle decrease in the counts of strains of the control sogurt after 30 days of storage (from 7.3 ± 1.3 to 6.2 ± 1.3 log_{10} cfu/g) (**Table 1**). However, for the yogurts supplemented with MFSE, the counts of viable cell decreased by 0.5 cycle only (from 8.2 ± 1.8 to 7.7 ± 0.8 log_{10} cfu/g). Apparently, MFSE supplementation had a significant ($p < 0.05$) effect on the sogurt culture in improving its concentration during fermentation (**Figure 2**) and maintaining its high viability throughout the cold storage for 30 days

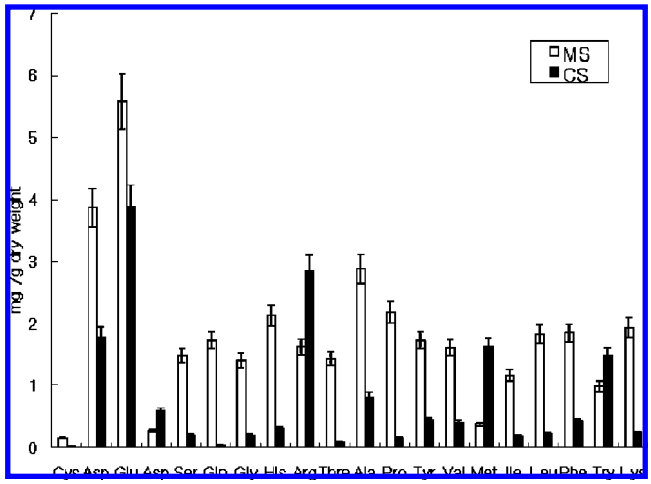

Figure 3. Changes of free amino acids in control sogurt (CS) and medicinal sogurt (MS) during the fermentation for 48 h at 35 °C. All data are expressed as mean ($n = 3$).

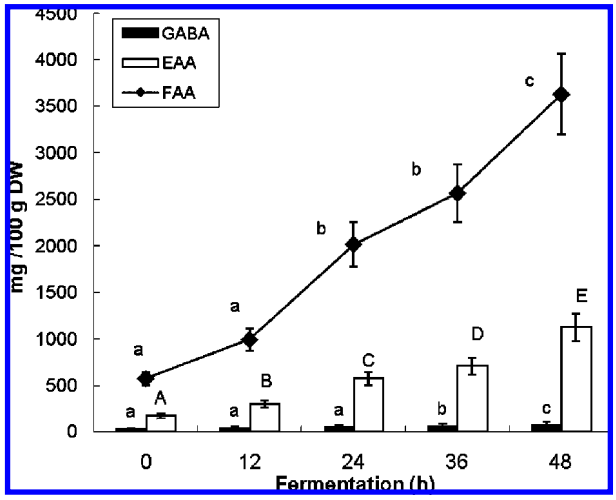

Figure 4. Effect of MFSE addition on the content of γ-aminobutyric acid (GABA) and essential amino acids (EAA) and free amino acids (FAA) in sogurt during the fermentation at 35 °C. All data are expressed as mean ($n = 3$). Different letters represent significant differences ($p < 0.05$).

compared with the control (**Table 1**). These results can be explained in terms of the better proportion of nutrients in MS. As presented in **Figures 3** and **4**, total FAAs in medicinal sogurts (3626.4 ± 24.1 mg/100 g of dw) for 48 h of fermentation was significantly ($p < 0.05$) higher compared with that assessed in the control sogurts (1601.5 ± 18.1 mg/100 g of dw). In particular, the content of essential amino acids (EAAs, 1121.6 ± 15.2 mg/100 g of dw) in the MS increased by 2.4 times compared with those of the control sogurt (469.7 ± 4.8 mg/100 g of dw) (**Figure 4**). Thus, proteolytic activity of lactic acid bacteria (LAB) in sogurt enriched with MFSE may have produced a good proportion of some EAAs and FAAs, which may have helped the LAB strain to multiply. It has been suggested that fermented dairy products require probiotic bacteria at 10^7 cfu/mL to give health effects in the gastrointestinal tract when consumed (*24*). Mital and Steinkraus (*25*) reported a count of 6.8×10^7 cfu/mL for soy milk after a fermentation time of 16−18 h, whereas Ouwehand and Salminen (*24*) obtained counts of around 2.4×10^8 cfu/mL in yogurts prepared with bovine and soy milk mixed in different proportions. Thus, it can be said that the bacteria counts found for medicinal sogurt in the present study are in the range of the results obtained by other authors.

Chemical and Sensory Characteristics. *GABA and FAAs.* The contents of GABA and FAAs in sogurts were monitored by HPLC analysis during the processing. The contents of GABA, EAAs, and FAAs in medicinal sogurts were shown to increase with the fermentation time (**Figures 3** and **4**). The profile of EAAs in MS is presented in **Figure 3**, which showed considerable amounts of EAAs except for methionine and tryptophane. Thus, the addition of MFSE in sogurt production tended to yield a finished product with higher contents of GABA, some EAAs (Thr, Val, Ile, Leu, Phe, Lys), and total FAAs than the control sogurt. It is not certain whether the LAB strain used affected the amount of GABA produced during process. However, with increases in fermentation time up to 48 h, GABA and FFAs contents increased to 68.6 ± 5.7 and 3626.4 ± 24.1 mg, respectively, from 24.6 ± 1.9 and 568.9 ± 11.2 mg/100 g of dw (**Figure 4**). It has been suggested that the glutamic acid in the soybean was effectively transformed to GABA by GAD released from the soybean and produced by LAB during the fermentation (*11*, *12*). As shown in **Figure 3**, glutamic acid was the most abundant acid in both MS and CS, representing about 15 and 24% of total FAAs, respectively. Recently, a few studies have been reported that various dietary materials or products containing GABA resulted in decreased blood pressure in SHR and in hypertensive humans (*12*, *13*). Also, FAAs and oligopeptides in foods are expected to not only improve the umami taste but also have some nutritional advantages such as rapid absorption and antioxidant activity (*26*,*27*). Therefore, it can be said that the amount of GABA and some EAAs and FAAs incorporated into the medicinal sogurt is high enough to have some functional value. However, they still need to be confirmed by animal and clinical studies.

Natural Statins. The HPLC results for statins in sogurt are shown in **Table 1**. The statin content in medicinal sogurt was only found due to MFSE addition and was not detected in the control sogurt. Moreover, statin content was shown to decrease with increase of storage time. For example, after 30 days of cold storage at 4 °C, the concentration of statin leveled off from 100.1 ± 7.5 to 77.4 ± 5.7 μg/100 g of dw.

For most people, statins are safe and well-tolerated, and their widespread use has the potential to have a major effect on the global burden of CVD (*15*). With respect to their chemical structure, the hydroxy acid forms in vivo are the active drugs to lower plasma cholesterol, whereas the lactone forms are inactive (prodrug). The lactone form of statins can be absorbed from the gastrointestinal tract and transformed to the active drugs in liver and nonhepatic tissues (*16*). As presented in **Table 1**, none of statins was detected in the control sogurt over storage. It was reported that mevinolin from *Monascus*-fermented soybean was present in the substrate predominantly in the acid form (94.8−96.7%) (*28*). Similarly, medicinal sogurt also contained bioactive statin, hydroxy acid form, contributing about 93.1−96.9% of total statins (**Table 1**).

Isoflavones. Isoflavone contents in sogurt during cold storage and fermentation are summarized in **Tables 1** and **2**, respectively. As expected, the contents of isoflavone aglycones in sogurts increased with fermentation time, resulting from hydrolysis of glucosidic forms by LAB strains, which were selected due to their higher β-glucosidase activity (*5*). These results agreed with several previous works (*5*, *6*). It was indicated that an interconversion of malonyl to acetyl forms, through decarboxylation, and of these to β-glucoside forms, through de-esterification, can be induced by microbes during fermentation (*5*, *6*). As shown in **Table 2**, the content of each isoflavone isoform in MS was measured as follows: aglycones > β-glucosides > malonyl-glucosides > acetyl-gluco-

Table 2. Effects of MFSE Addition on Isoflavone Profiles (Milligrams per 100 g of Dry Weight)[a] during Sogurt Fermentation at 35 °C

fermentation (h)	β-glucosides	malonyl	acetyl	aglycones	total
0	73.7 ± 8.1a	79.5 ± 7.4a	5.5 ± 1.2a	10.2 ± 1.3a	168.9 ± 11.7a
12	59.2 ± 5.2b	72.1 ± 8.1a	13.7 ± 2.3b	21.2 ± 5.1b	166.2 ± 10.8a
24	45.8 ± 4.5b	37.8 ± 6.3b	17.6 ± 2.8b	56.4 ± 4.6c	157.6 ± 10.4b
48	34.8 ± 7.3c	32.4 ± 8.2b	13.7 ± 3.6b	68.9 ± 7.5d	149.8 ± 8.3b

[a] Adjusted for molecular weight of the correspondent glucosides and expressed as aglycone equivalents per gram of soy yogurt (dry basis). All data are expressed as mean ($n = 3$). Different letters in the same column represent significant differences ($p < 0.05$).

Table 3. Changes of Some Sensory Properties in Control Sogurt (CS) and Medicinal Sogurt (MS) during Cold Storage at 4 °C[a]

	day 1		day 15		day 30	
attribute	CS	MS	CS	MS	CS	MS
flavor	3.2 ± 0.3a	3.8 ± 0.1b	3.0 ± 0.2a	3.3 ± 0.5a	2.7 ± 0.5a	3.0 ± 0.1a
texture	3.4 ± 0.4a	3.0 ± 0.5a	2.7 ± 0.2a	3.3 ± 0.4b	2.2 ± 0.3a	3.1 ± 0.2b
appearance	3.4 ± 0.4a	4.0 ± 0.5b	3.1 ± 0.4a	3.8 ± 0.2b	2.8 ± 0.2a	3.8 ± 0.4b
acceptability	2.8 ± 0.3a	3.4 ± 0.2b	2.9 ± 0.1a	3.2 ± 0.3a	2.5 ± 0.2a	3.0 ± 0.3b

[a] All data are expressed as mean ($n = 3$). Different letters between two sogurts represent significant differences ($p < 0.05$).

sides. An overall significant increase ($p < 0.05$) in aglycones was observed even though there was a substantial loss of total isoflavone content during the fermentation. Total isoflavone contents in MS decreased about 14.3% (from 168.9 ± 11.7 to 144.7 ± 2.6 mg/100 g) after 30 days of storage (**Tables 1** and **2**). On the other hand, the content of aglycones in MS increased from 10.2 to 68.9 mg/100 g (about 6.8-fold) after 48 h of fermentation (**Table 2**). Thus, the remarkable increase of aglycones content noted in the medicinal sogurt may be based on the hydrolytic reaction catalyzed by β-glucosidase produced by LAB strains used in this study (*5*, *6*). Most interesting was that the glucosides have less estrogenic acitivity when compared to their respective aglycone forms, because of hydrophilic capacity and lower molecular weight, with better absorption (*4*, *29*). Generally, the bioavailability of soybean isoflavones in humans depends on their metabolism capacity, which is related to ethnic backgrounds, dietary habits, and intestinal microflora that cause variations in the amount as well as activity of the gut β-glucosidases (*29*). Therefore, dose−effect relationships for isoflavones have not been established in animal and human studies. Also, at the present time, the 50% effective dose, the 50% lethal dose, or equivalent values cannot be determined because neither the effects nor the risk has been well-defined (*30*).

Sensory Evaluation. Sensory properties of medicinal sogurt were evaluated by a consumer panel consisting of 10 trained assessors with a mean age of 28 ± 4 years, and the results are summarized in **Table 3**.

Flavor. The flavor scores of medicinal sogurt were slightly higher than those of control sogurt over storage. This may be partly due to the objectionable beany flavor or taste of soy milk, which remained in the control sogurts. Interestingly, trained panel evaluations showed that MFSE-added sogurts had the lowest beany flavor. Similar observations were reported that a legume-milk yogurt with little or no beany flavor has good possibilities of being sensorially accepted (*22*, *23*). The flavor score of medicinal sogurt was the highest by 3.8 ± 0.1 on a five-point scale at day 1. After that, the storage time negatively affected the flavor of medicinal sogurts (**Table 3**) At day 30 the medicinal sogurts had reached their shelf life, and the main criticism was high acid due to their lower pH. A pH drop was probably the reason for increasing the intensity of sour taste over storage. This is in agreement with Fernandez et al. (*31*); they evaluated organic acids in oat fiber-fortified yogurt during refrigerated storage for 4 weeks and reported significantly higher amounts of acetic and propionic acids in fortified yogurts.

Appearance. The control sogurts being shrunken and/or lumpy also influence appearance after 15 days of cold storage, but none of the medicinal sogurts were shrunken or lumpy. As presented in **Table 3**, the appearance values in medicinal sogurts were above 3.8 ± 0.2 on a scale of 1−5. Therefore, neither MFSE addition nor storage time significantly affected the appearance of soy yogurt.

Texture. The storage time significantly ($p < 0.05$) affected the texture of sogurts. Texture scores (from 3.0 ± 0.5 to 3.3 ± 0.4) for medicinal sogurts aged for 30 days were significantly higher than those of the control sogurts (from 2.2 ± 0.3 to 2.7 ± 0.2) at 15 and 30 days. At the beginning of storage, the control sogurts were superior to medicinal sogurts, mainly because of softer gel textures. However, after 15 days, the control sogurt appeared to be a less thick and homogeneous fluid, so it received lower scores than the medicinal sogurts. This could be related to compositional differences and/or acidity between the samples. During storage of medicinal sogurt, whey separation and pH decreased, whereas TA increased in comparison to the control sogurt.

Overall, on the basis of the acceptability mean scores, the medicinal sogurt appeared to be more acceptable by the trained panel (from 3.0 ± 0.3 to 3.4 ± 0.2) than the control sogurt (from 2.5 ± 0.2 to 2.9 ± 0.1) (**Table 3**). The overall acceptability score of medicinal sogurt was the highest at 3.4 ± 0.2 on a five-point scale at day 1. The result indicates that the addition of MFSE improved the sensory characteristics of the soy yogurts.

In conclusion, the effects of MFSE addition on some physicochemical and sensory properties of sogurt were investigated. Medicinal sogurt with MFSE added (1.5%, w/v) in soy milk was fermented with a mixture of strains (1:1) of *L. delbrueckii* subsp. *latis* and *L. plantarum* at 35 °C for 48 h and stored at 4 °C for 30 days. The contents of GABA, FAAs, and isoflavone aglycones except for statins in medicinal sogurts were increased with the time of fermentation and storage (**Tables 1** and **2** and **Figure 4**). Also, medicinal sogurt displayed higher % WHC and % TA and total bacterial cells and lower pH than the control sogurt during cold storage. On the basis of sensory evaluation, overall acceptance of medicinal sogurt supplemented with MFSE was higher than that of the control sogurt prepared without MFSE. Thus, addition of the appropriate MFSE concentrations improved the physicochemical properties as well as sensory characteristics of soy yogurt. The results indicate that the addition of MFSE (1.5%, w/v) in sogurt can be used satisfactorily for the production of medicinal sogurt enriched with GABA, FAAs, isoflavone aglycones, and natural statins.

LITERATURE CITED

(1) Sarkar, S. Potential of soyoghurt as a dietetic food. *Nutr. Food Sci.* **2006**, *36*, 43–49.

(2) Hwang, C. S.; Kwak, H. S.; Lim, H. J.; Lee, S. H.; Kang, Y. S.; Choe, T. B.; Hur, H. G.; Han, K. O. Isoflavone metabolites and their *in vitro* dual functions: they can act as an estrogenic agonist or antagonist depending on the estrogen concentration. *J. Steroid Biochem.* **2006**, *101*, 246–253.

(3) Faraj, A.; vasanthan, T. Soybean isoflavones: effects of processing and health benefits. *Food Rev. Int.* **2004**, *20*, 51–75.

(4) Isumi, T.; Piskula, M. K.; Osawa, S.; Obata, A.; Saito, M.; Kikuchi, M. Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *J. Nutr.* **2000**, *130*, 1695–1699.

(5) Pyo, Y. H.; Lee, T. C.; Lee, Y. C. Enrichment of bioactive isoflavones in soymilk fermented with β-glucosidase-producing lactic acid bacteria. *Food Res. Int.* **2005**, *38*, 551–559.
(6) Tsangalis, D.; Ashton, J. F.; McGill, A. E. J.; Shah, N. P. Enzymatic transformation of isoflavone phytoestrogens in soymilk by β-glucosidase-producing bifidobacteria. *J. Food Sci.* **2002**, *67*, 3104–3113.
(7) Drake, M. A.; Gerard, P. D. Consumer attitudes and acceptability of soy-fortified yogurts. *J. Food Sci.* **2003**, *68*, 1118–112.
(8) Donkor, O. N.; Henriksson, A.; Vasiljevic, T.; Shah, N. P. Probiotic strains as starter cultures improve angiotensin-converting enzyme inhibitory activity in soy yogurt. *J. Food Sci.* **2005**, *70*, M375−M381.
(9) Donkor, O. N.; Nilmini, S. L.; Stolic, P.; Vasiljevic, T.; Shah, N. P. Survival and activity of selected probiotic organisms in set-type yogurt during cold storage. *Int. Dairy J.* **2007**, *17*, 657–665.
(10) Fuller, R.; Gibson, G. R. Modification of the intestinal microflora using probiotics and prebiotics. *Scand. J. Gastroenterol.* **1997**, *32*, 28–31.
(11) Brown, A. W.; Shelp, B. J. The metabolism and functions of γ-aminobutyric acid (GABA). *Plant Physiol.* **1997**, *115*, 1–5.
(12) Tsai, J. S.; Lin, Y. S.; Pan, B. S.; Chen, T. J. Antihypertensive peptides and γ-aminobutyric acid from Prozyme 6 facilitated lactic acid bacteria fermentation of soymilk. *Process Biochem.* **2006**, *41*, 1282–1288.
(13) Abe, Y.; Umemura, S.; Sugimoto, K.; Hirawa, N.; Kato, Y.; Yokoyama, N. Effect of green tea rich in γ-aminobutyric acid on blood pressure of salt-sensitive rats. *Am. J. Hypertens.* **1995**, *8*, 74–79.
(14) Endo, A.; Hasumi, K.; Negishi, S. Monacolins J and L new inhibitors of cholesterol biosynthesis produced by *Monascus ruber*. *J. Antibiotechnol.* **1985**, *38*, 420–422.
(15) Armitage, J. The safety of statins in clinical practice. *Lancet* **2007**, *370*, 1781–1790.
(16) Thavendiranathan, P.; Bagai, A.; Brookhart, M. A.; Choudhry, N. K. Primary prevention of cardiovascular diseases with statin therapy: a meta-analysis of randomized controlled trials. *Arch. Intern. Med.* **2006**, *166*, 2307–2313.
(17) Pyo, Y. H. Production of a high value-added soybean containing bioactive mevinolins and isoflavones. *J. Food Sci. Nutr.* **2007**, *12*, 29–34.
(18) Pyo, Y. H.; Lee, T. C. The potential antioxidant capacity and angiotensin I- converting enzyme inhibitory activity of *Monascus*-fermented soybean extracts: Evaluation of *Monascus*-fermented soybean extracts as multifunctional food additives. *J. Food Sci.* **2007**, *72*, S708−S713.
(19) Lucey, J. A. The relationship between rheological parameters and whey separation in milk gels. *Food Hydrocolloids* **2001**, *15*, 603–608.
(20) Barnes, S.; Coward, L.; Kirk, M.; Sfakianos, J. HPLC-mass spectrometry analysis of isoflavones. *Proc. Soc. Exp. Biol. Med.* **1998**, *217*, 254–262.
(21) Yang, D. J.; Hwang, L. S. Study on the conversion of three natural statins from lactone forms to their corresponding hydroxyl acid forms and their determination in Pu-Erh tea. *J. Chromatogr., A.* **2006**, *1119*, 277–284.
(22) Buono, M. A.; Setser, C.; Erickson, L. E.; Fung, D. Y. C. Soymilk yoghurt: sensory evaluation and chemical measurement. *J. Food Sci.* **1999**, *55*, 528–531.
(23) Donkor, O. N.; Henriksson, A.; Vasiljevic, T.; Shah, N. P. Rheological properties and sensory characteristics of set-type soy yogurt. *J. Agric. Food Chem.* **2007**, *55*, 9868–9876.
(24) Ouwehand, A. C.; Salminen, S. J. The health effects of cultured milk products with viable and non-viable bacteria. *Int. Dairy J.* **1998**, *8*, 749–758.
(25) Mital, B. K.; Steinkraus, K. H. Growth of lactic acid bacteria in soy milk. *J. Food Sci.* **1974**, *39*, 1018–1022.
(26) Kamiya, T. Biological functions and health benefits of amino acids. *Food Inaredients J. Jpn.* **2002**, *206*, 33–44 (in Japanese).
(27) Hoppe, M. B.; Jha, H. C.; Egge, H. Structure of antioxidant from fermented soybeans (tempeh). *J. Am. Oil Chem. Soc.* **1997**, *74*, 477–479.
(28) Pyo, Y. H.; Lee, Y. C. Mevinolin production by *Monascus pilosus* KFRI-1140 in solid state fermentation using soymeal. *Food Sci. Biotechnol.* **2006**, *15*, 647–649.
(29) Xu, X.; Keecha, S. H.; Wang, H. J.; Murphy, P. A.; Hendrich, S. Bioavailability of soybean isoflavones depends upon gut microflora in women. *J. Nutr.* **1995**, *125*, 2307–2315.
(30) Choi, M. S.; Rhee, K. C. Production and processing of soybeans and nutrition and safety of isoflavone and other soy products for human health. *J. Med. Food* **2006**, *9*, 1–10.
(31) Fernandez, G.; McGregor, J. U.; Traylor, S. The addition of oat fiber and natural alternative sweeteners in the manufacture of plain yogurt. *J. Dairy Sci.* **1998**, *81*, 655–663.

Received for review September 1, 2008. Revised manuscript received November 14, 2008. Accepted November 14, 2008. This work was supported by the Sungshin Women's University Research Grant.

JF8026952