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Hydrolysis of Isoflavone Glucosides in Soymilk Fermented with Single or Mixed Cultures of *Lactobacillus paraplantarum* KM, *Weissella* sp. 33, and *Enterococcus faecium* 35 Isolated from Humans

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Lactobacillus paraplantarum **KM (*Lp*), *Weissella* sp. 33 (*Ws*), and *Enterococcus faecium* 35 (*Ef*) were used in single (*Lp*, *Ws*, *Ef*) or mixed cultures (*Lp*+*Ws*, *Lp*+*Ef*, *Ws*+*Ef*) for soymilk fermentation (37°C, 12 h). After 12 h, the cell numbers, pH, and TA of soymilk were 7.4×10^8–6.0×10^9 CFU/ml, 3.8–4.5, and 0.59–0.70%, respectively. Changes in the contents of glycitin and genistin in soymilk fermented with *Ef* were not significant. The contents of isoflavone glucosides in soymilk fermented with the other cultures decreased significantly with an increase of aglycone contents ($p<0.05$). It corresponded well with a sharp increase in β-glucosidase activity during fermentation. About 92–100% of the daidzin and 98–100% of the genistin in soymilk were converted to corresponding aglycones by *Lp*, *Ws*, or *Lp*+*Ef* within 12 h.**

Keywords: Fermentation, isoflavones, lactic acid bacteria, soymilk

Isoflavones are phytochemicals present in leguminous plants, especially in soybeans. Soy isoflavones have been implicated in health benefits, including the potential to reduce the risk of age-related and hormone-related diseases including cancer, menopausal symptoms, cardiovascular disease, and osteoporosis [9, 16]. Researches indicate that differences in the chemical structure of isoflavones may result in variable bioavailabilities in biological systems [2, 26]. In general, isoflavones in soybeans exist mainly as glucoside forms and rarely as aglycone forms unless they have been fermented [23]. It has been reported that certain intestinal bacteria play major roles in the hydrolysis of isoflavone glucosides and promote their absorption in the intestine [22]. Biotransformation and the production of metabolites of isoflavones in the intestinal tract are highly dependent on the nature of intestinal microflora [16, 27]. However, little information is available regarding the specific bacteria responsible for the conversion of isoflavone glucosides into aglycones in humans.

Controversy exists regarding the extent of bioavailability of isoflavones, and the mechanism of intestinal absorption of isoflavones is unclear. Hutchin *et al.* [7] showed that fermentation of soybeans enhanced the bioavailability of isoflavones. Izumi *et al.* [8] stated that soy isoflavone aglycones were absorbed faster and more than their glucosidic forms in humans. Furthermore, Setchell *et al.* [22] reported that isoflavone glucosides were not absorbed intact across the intestinal epithelium in human adults, and the absorption of these glucosides required initial removal of the sugar moiety by intestinal β-glu (β-glucosidase). However, a study by Richelle *et al.* [20] showed that hydrolysis of isoflavone glycosides to aglycones by β-glu did not alter plasma and urine isoflavone pharmacokinetics in women. Despite of these contradictory findings, several studies on the fermentation of soymilk with probiotic bacteria have been conducted, attempting the enrichment of isoflavone aglycones [3, 17, 19]. These studies proceeded on the premise that both the enhancement of isoflavone aglycone contents before consumption of soy foods and the modulation of intestinal microflora through the ingestion of viable bacteria could improve the bioavailability of isoflavones from soy foods. Besides this, soymilk fermented with probiotic bacteria has some advantages: a reduced

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content of oligosaccharides, enhanced antioxidant activities, and improved flavor and sensory characteristics [6, 24, 25].

It has been reported that lactic acid bacteria (LAB) have effective cancer chemopreventive effects, which act through diverse mechanisms including alteration of the intestinal microflora, enhancement of the host's immune response, and antioxidative and antiproliferative activities [10, 11]. Recently, we isolated LAB with β-glu activity from human feces and investigated the hydrolysis of isoflavone glucosides in soymilk to corresponding aglycones [5]. In the present study, we selected other LAB (*Weissella* sp. 33 and *Enterococcus faecium* 35) among our LAB collections and tested for conversion of isoflavone glucosides in soymilk. All values were given as mean±standard deviation from three independent experiments. The analyses of variance were performed by using ANOVA and differences between the means of samples were analyzed by Duncan's test at α=0.05 [21].

Growth and Acid Production of LAB During Fermentation of Soymilk

Soymilk was prepared from Tae-Kwang variety soybean grown in Kyungpook Province of Korea (2004 crop year) according to Chun *et al.* [5]. Three LAB, *Lactobacillus paraplantarum* KM, *Weissella* sp. 33, and *Enterococcus faecium* 35, were from our LAB collections isolated according to Chun *et al.* [5]. They were briefly named *Lp*, *Ws*, and *Ef*, respectively. The three single strains (*Lp*, *Ws*, and *Ef*) and three mixed combinations (*Lp*+*Ws*, *Lp*+*Ef*, and *Ws*+*Ef*, 1:1, v/v) were used as starters. Soymilk (150 ml) was transferred into a flask and inoculated with 3 ml (2% of soymilk, v/v) of either a single or a mixed culture to give an initial population of 3–6×10^7 CFU/ml. Inoculated soymilks (150 ml) were aliquoted into sterile 15-ml centrifuge tubes and put into an incubator at 37°C. Soymilk sample tubes were taken out at 3-h intervals up to 12 h. Total viable cells of the samples were measured in triplicates by using the pour-plate method with lactobacilli MRS media (Difco). The pH was measured by using a pH meter (DMS, Seoul, Korea). Titratable acidity (TA) was determined by titration with a 0.1 N NaOH solution and expressed as % lactic acid [1].

Fig. 1 shows the changes in viable cell counts during soymilk fermentation. The growth patterns of single cultures varied depending on the species. Cell numbers of soymilk inoculated with *Lp* gradually increased from 10^7 to 10^9 CFU/ml during 12 h of fermentation. Cell numbers of soymilk fermented with either *Ws* or *Ef* rapidly increased to higher numbers than *Lp* during the initial 6 h. However, during the next 6 h, the cell number of *Ws* decreased to lower numbers than *Lp*, while that of *Ef* remained unchanged, resulting in the order of cell numbers by *Ef*>*Lp*>*Ws* at 12 h. On the other hand, the growth patterns of individual cultures were not observed in mixed cultures. Cell numbers of soymilk fermented with either *Lp*+*Ws*, *Lp*+*Ef*, or *Ws*+*Ef* significantly increased from 10^7 to 10^9 CFU/ml during the initial 6 h of fermentation and then remained without remarkable changes. At 6 h of fermentation, the cell numbers of all three mixed cultures were higher than that of *Lp* but less than those of *Ws* and *Ef*. These results indicated that growth of *Lp*, *Ws*, and *Ef* may not be stimulated by the presence of other LAB in soymilk.

Changes in TA and pH of soymilk during fermentation are shown in Figs. 2 and 3, respectively. The TA of soymilk increased from 0.15% at the beginning to the range of 0.59–0.70% at the end of fermentation. Accordingly, the initial pH of 6.3 rapidly decreased to 4.1–4.7 during the

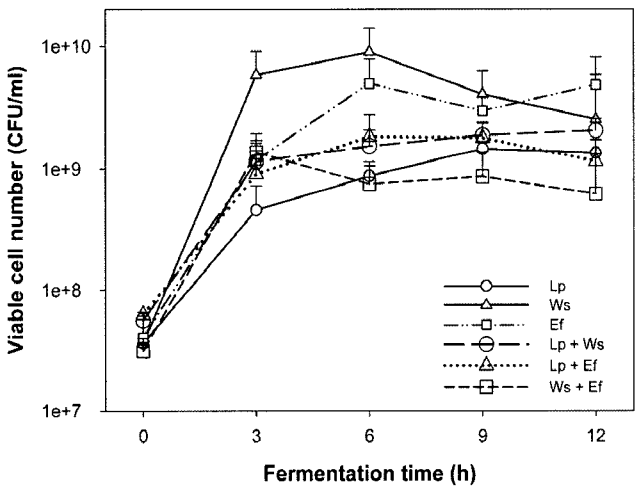

Fig. 1. Changes in viable cell counts of soymilk fermented with lactic acid bacteria at 37°C.
Lp, *Ws*, and *Ef* indicate *L. paraplantarum* KM, *W.* spp 33, and *E. faecium* 35, respectively.

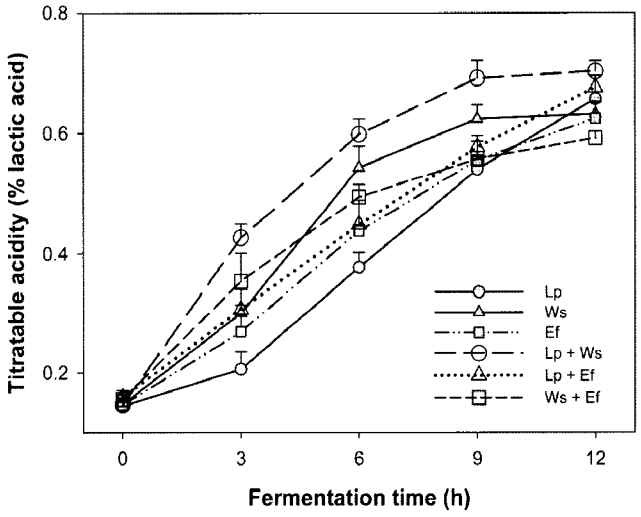

Fig. 2. Changes in titratable acidity of soymilk fermented with lactic acid bacteria at 37°C.
Lp, *Ws*, and *Ef* indicate *L. paraplantarum* KM, *W.* spp 33, and *E. faecium* 35, respectively.

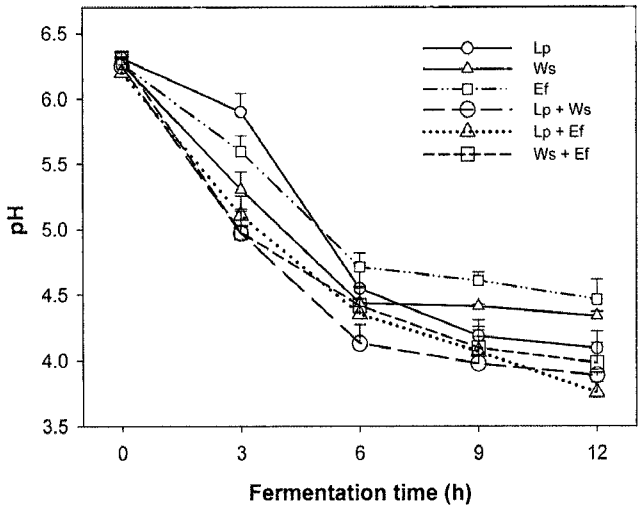

Fig. 3. Changes in pH of soymilk fermented with lactic acid bacteria at 37°C.
Lp, *Ws*, and *Ef* indicate *L. paraplantarum* KM, *W.* spp 33, and *E. faecium* 35, respectively.

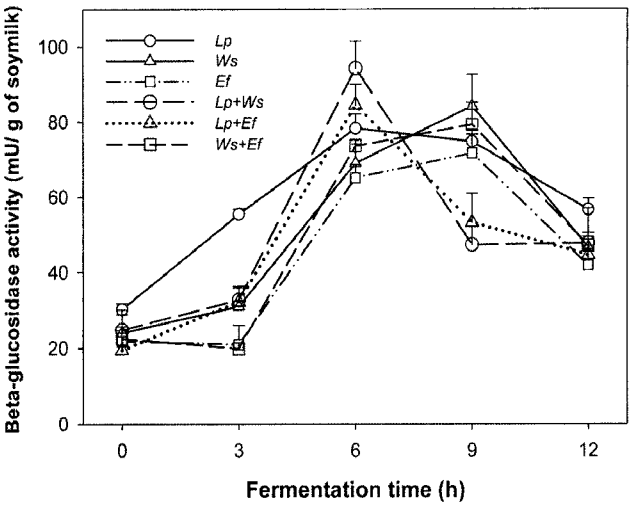

Fig. 4. Changes in β-glucosidase activity of soymilk fermented with lactic acid bacteria at 37°C.
Lp, *Ws*, and *Ef* indicate *L. paraplantarum* KM, *W.* spp 33, and *E. faecium* 35, respectively.

initial 6 h of fermentation and then decreased slowly during the rest of fermentation, resulting in the final pH of 3.8–4.5. During the overall 12 h of fermentation, the lowest pH and highest TA were observed in soymilk fermented with *Lp+Ws*, indicating that *Lp+Ws* produced the most acid. The pH ranges of mixed cultures were lower than those of single cultures at all stages of fermentation, indicating more acid production by using mixed cultures. The observed pH range after 12 h was 4.1–4.5 for single cultures and 3.8–4.0 for mixed cultures. Appropriate concentration of the acid is one of the important factors ensuring the quality of fermented soymilk [6]. Commercial yoghurts have the pH range of 4.2 to 4.4 [18]. In the present study, the commercial pH range was achieved when soymilk was fermented with *Ws*, *Lp+Ws*, *Lp+Ef*, or *Ws+Ef* for 6 h. The corresponding pH value was obtained by fermentation of soymilk with *Lp* and *Ef* for 9 h and 12 h, respectively.

β-Glu Activity of LAB in Soymilk

β-Glu activity in soymilk during fermentation was determined by a modified McCue and Shetty method [15] using *p*-nitrophenyl-β-D-glucopyranoside (*p*-NPG) as a substrate. Two g of fermented soymilk samples was homogenized with 20 ml of 0.2 M sodium acetate buffer (pH 5.5) at 4°C. Aliquots of the homogenate were placed in a 1.8-ml Eppendorf tube, followed by centrifugation (14,000 ×*g*, 15 min, 4°C). The sediment was suspended in 1 ml of 0.2 M acetate buffer (pH 5.5) and used for enzyme assay. One unit of β-glu activity (U) was defined as the amount of enzyme that released 1 μmole of *p*-nitrophenol from the substrate (*p*-NPG) per minute under the assay conditions. Enzyme activity was expressed as U/g of soymilk sample.

Changes in β-glu activity of the 3 single and 3 mixed cultures in soymilk during fermentation at 37°C for 12 h are shown in Fig. 4. β-Glu activity of all cultures rapidly increased during the first 6 h, reaching to the range of 65.0±3.5 to 94.2±7.1 mU/g of soymilk. At 6 h, *Lp+Ws* showed the highest activity of 94.2±7.1 mU/g of soymilk followed by *Lp+Ef* with 84.4±5.5 mU/g of soymilk. After 6 h, β-glu activities of *Lp+Ws* and *Lp+Ef* rapidly decreased while those of *Ws+Ef*, *Ws*, and *Ef* increased up to 9 h and then decreased during the rest of the fermentation period. β-Glu activity of *Ws* at 9 h was the highest by 83.1± 8.3 mU/g of soymilk, whereas that of *Lp+Ws* was the lowest by 47.1± 4.3 mU/g of soymilk. An increase and a decrease in β-glu activity during fermentation corresponded well with the exponential and stationary growth phases, respectively (Fig. 1). This phenomenon is in agreement with the reports of Pyo *et al.* [19] and Otieno *et al.* [17].

Changes in Isoflavone Glucosides and Aglycones in Soymilk Fermented with LAB

Isoflavones were extracted from freeze-dried soymilk samples with 80% methanol at 60°C for 2 h, as described by Chun *et al.* [5]. Recovery of the apigenin internal standard was 97.5± 3.2% on average. Isoflavone separation and quantification by HPLC were the same as described by Chun *et al.* [5]. The respective isoflavone contents in nonfermented soymilk were 1,423.7±19.4 for daidzin, 307.6±17.8 for glycitin, 1,580.2±85.1 for genistin, 319.2±13.0 for daidzein, 138.8± 23.7 for glycitein, and 405.3±21.6 for genistein in μg/100 g of dry soymilk (Tables 1 and 2). Changes in the contents of isoflavone glucosides and aglycones in soymilk fermented with 1 of the 6 cultures (*Lp*, *Ws*, *Ef*, *Lp+Ws*, *Lp+Ef*, and *Ws+Ef*) were summarized in Tables 1 and 2, respectively.

Table 1. The content of isoflavone glucosides (μg/100 g of dry sample) in soymilk fermented with lactic acid bacteria at 37°C.

Glucosides	Time	Isoflavone glucoside content in soymilk fermented with[I]					
		Lp	*Ws*	*Ef*	*Lp+Ws*	*Lp+Ef*	*Ws+Ef*
Daidzin	0 h	1,423.7±19.4^{aA} (100)[II]	1,423.7±19.4^{A} (100)	1,423.7±19.4^{aA} (100)	1,423.7±19.4^{aA} (100)	1,423.7±19.4^{aA} (100)	1,423.7±19.4^{aA} (100)
	3 h	1,243.4±19.0^{bA} (87.3)	311.3±16.0^{bD} (21.9)	1,325.0±35.4^{abA} (93.1)	819.0±97.6^{bC} (57.5)	1,278.1±11.4^{bA} (89.8)	1,084.8±22.9^{bB} (76.2)
	6 h	166.9±18.5^{cD} (11.7)	22.5±10.6^{cE} (1.6)	1,267.4±16.1^{bA} (89.0)	384.0±48.1^{cB} (27.0)	277.1±32.4^{cC} (19.5)	354.5±12.9^{cB} (24.9)
	9 h	123.2±9.6^{dC} (8.7)	0.0±0.0^{cD} (0.0)	1,270.5±71.4^{bA} (89.2)	268.0±59.4^{cdB} (18.8)	193.4±24.9^{dBC} (13.6)	186.6±20.4^{dBC} (13.1)
	12 h	90.0±14.1^{dC} (6.3)	0.0±0.0^{cD} (0.0)	1,263.2±84.5^{bA} (88.7)	188.6±16.1^{dB} (13.2)	116.9±10.7^{cBC} (8.2)	115.5±6.3^{eBC} (8.1)
Glycitin	0 h	307.6±17.8^{aA} (100)	307.6±17.8^{aA} (100)	307.6±17.8^{aA} (100)	307.6±17.8^{aA} (100)	307.6±17.8^{aA} (100)	307.6±17.8^{aA} (100)
	3 h	276.5±37.4^{aA} (89.9)	255.9±42.3^{abA} (83.2)	300.0±28.2^{aA} (97.5)	327.1±21.4^{aA} (106.3)	301.5±74.3^{aA} (98.0)	324.6±64.5^{aA} (105.5)
	6 h	148.7±57.0^{bA} (48.3)	188.4±17.0^{bcBC} (61.2)	286.2±17.2^{aAB} (93.0)	283.3±14.6^{aAB} (92.1)	197.4±8.0^{bBC} (64.2)	314.8±71.8^{aA} (102.3)
	9 h	132.7±29.3^{bB} (43.2)	130.2±28.1^{cdB} (42.3)	307.3±3.8^{aA} (99.9)	190.1±22.4^{bB} (61.8)	160.8±39.3^{bB} (52.3)	286.4±55.7^{aA} (93.1)
	12 h	69.7±24.4^{bD} (22.7)	71.5±24.7^{dD} (23.2)	299.5±14.8^{aA} (97.3)	177.5±43.1^{bBC} (57.7)	118.8±15.8^{bcD} (38.6)	243.9±33.8^{aAB} (79.3)
Genistin	0 h	1580.2±85.1^{aA} (100)	1,580.2±85.1^{aA} (100)	1,580.2±85.1^{aA} (100)	1,580.2±85.1^{aA} (100)	1,580.2±85.1^{aA} (100)	1,580.2±85.1^{aA} (100)
	3 h	1,559.9±59.2^{aA} (98.7)	503.3±23.6^{bD} (31.9)	1,576.7±51.9^{aA} (99.8)	1,177.0±101.9^{bC} (74.5)	1,454.3±45.7^{aB} (92.0)	1,452.8±70.4^{aB} (91.1)
	6 h	51.5±40.3^{bC} (3.3)	68.2±47.8^{cC} (4.3)	1,554.2±92.1^{aA} (98.4)	906.6±53.2^{cB} (57.4)	117.8±72.8^{bC} (7.5)	797.4±10.4^{bB} (50.5)
	9 h	12.9±3.0^{bC} (0.8)	31.6±30.3^{cC} (2.0)	1,554.0±87.7^{aA} (98.4)	47.6±14.7^{dC} (3.0)	46.2±34.1^{bC} (2.9)	495.5±62.9^{cB} (31.4)
	12 h	4.0±1.4^{bC} (0.3)	17.9±10.1^{cC} (1.1)	1,569.7±61.8^{aA} (99.3)	28.5±7.8^{dC} (1.8)	26.0±8.6^{bC} (1.6)	244.4±61.3^{dB} (15.5)

[I]Mean±standard deviation. Values at 0 h indicate the isoflavone contents of nonfermented soymilk before inoculation of lactic acid bacteria. Values in the same column of each isoflavone with different superscript small letters are statistically different ($p<0.05$). a>b>c>d. Values in the same row with different superscript capital letters are statistically different ($p<0.05$). A>B>C>D>E. *Lp*, *Ws*, and *Ef* indicate *L. paraplantarum* KM, *W.* spp. 33, and *E. faecium* 35, respectively.

[II]Numbers in parentheses indicate % remaining content of isoflavone glucosides in soymilk after the corresponding fermentation period. These values were calculated by 100×(the isoflavone content in soymilk fermented for the corresponding period/the isoflavone content in non-fermented soymilk).

Significant decreases in the content of isoflavone glucosides in soymilk fermented with a single (except for *Ef*) or a mixed culture during 12 h of fermentation were observed ($p<0.05$) (Table 1). Consequently, the content of corresponding aglycones significantly increased with fermentation time ($p<0.05$) (Table 2), resulting in that the contents of daidzein and genistein ranged from 1,115.6± 34.5 to 1,255.0±18.4 μg/100 g of dry soymilk and from 1,711.4±116.5 to 2,220.4±58.6 μg/100 g of dry soymilk, respectively, after 12 h (data from *Ef* was excluded). The amounts of aglycones were 3.5 to 3.9 and 4.2 to 5.5 times higher than the initial levels of daidzein and genistein in soymilk, respectively. Among the single cultures, *Ws* showed the highest hydrolysis rate, especially during the initial 3 h. Compared with single cultures, the rates of hydrolysis of mixed cultures were relatively lower. More than 25% and 50% of the initial daidzin and genistin, respectively, remained in soymilk fermented with either *Lp+Ws* or *Ws+Ef* for 6 h. Interestingly, *Lp+Ws* showed lower hydrolysis rates of glucosides than the other mixed cultures, although *Lp* and *Ws* as a single culture showed high hydrolysis rates of genistin and daidzin. It suggests that the presence of *Lp* or *Ws* might adversely affect the hydrolyzing activity of other organisms.

Overall, a rapid decrease in the content of isoflavone glucosides and a subsequent increase in the content of corresponding aglycones corresponded well with a sharp increase in β-glu activity of soymilk. The same phenomenon was observed in the previous studies by Matsuda *et al.* [13], Pyo *et al.* [19], and Otieno *et al.* [17]. Exceptionally,

Table 2. The content of isoflavone aglycones in soymilk fermented with lactic acid bacteria at 37°C (μg/100 g of dry sample).

Aglycones	Time	Isoflavone aglycone content in soymilk fermented with [1]					
		Lp	*Ws*	*Ef*	*Lp+Ws*	*Lp+Ef*	*Ws+Ef*
Daidzein	0 h	319.2±13.0^{bA}	319.2±13.0^{cA}	319.2±13.0^{cA}	319.2±13.0^{dA}	319.2±13.0^{cA}	319.2±13.0^{dA}
	3 h	351.4±44.4^{bC}	910.9±57.8^{bA}	314.8±6.8^{eC}	666.5±13.4^{cB}	408.7±58.5^{eC}	415.8±28.0^{eC}
	6 h	1,165.7±50.5^{aA}	1,149.0±41.0^{aAB}	330.4±14.7^{bcD}	1,009.8±116.2^{bBC}	990.6±43.3^{bC}	996.1±22.8^{bC}
	9 h	1260.8±56.9^{aA}	1,155.9±19.9^{aBC}	366.2±7.4^{aD}	1,197.2±40.7^{aAB}	1,056.9±52.2^{abC}	1,110.3±42.9^{aBC}
	12 h	1,242.5±13.5^{aA}	1,220.0±14.1^{aA}	360.9±15.4^{abC}	1,255.0±18.4^{aA}	1,175.2±70.4^{aAB}	1,115.6±34.5^{aB}
Glycitein	0 h	138.8±23.7^{bA}	138.8±23.7^{cA}	138.8±23.7^{aA}	138.8±23.7^{bA}	138.8±23.7^{cA}	138.8±23.7^{aA}
	3 h	176.6±17.6^{bA}	180.7±13.2^{cA}	125.4±14.7^{aB}	121.1±20.8^{bB}	137.8±12.7^{cB}	126.0±14.1^{bB}
	6 h	324.6±33.3^{aA}	291.3±26.5^{bA}	133.0±24.0^{aCD}	199.2±33.7^{bB}	179.2±1.7^{bcBC}	129.3±14.6^{abD}
	9 h	353.0±46.7^{aA}	306.7±40.6^{abAB}	153.7±12.3^{aC}	303.5±13.4^{aAB}	232.8±37.8^{abB}	136.7±8.9^{abC}
	12 h	397.7±16.5^{aA}	369.7±22.1^{aAB}	147.7±19.4^{aD}	328.7±19.4^{aB}	280.3±14.6^{aC}	134.7±20.8^{abD}
Genistein	0 h	405.3±21.6^{cA}	405.3±21.6^{dA}	405.3±21.6^{bA}	405.3±21.6^{dA}	405.3±21.6^{cA}	405.3±21.6^{cA}
	3 h	612.0±79.2^{bC}	1,206.5±67.1^{cA}	401.0±59.4^{bD}	841.4±74.0^{cB}	436.2±36.2^{cD}	437.0±49.4^{cD}
	6 h	2,139.4±51.4^{aA}	1,975.2±35.6^{bB}	449.5±33.2^{abF}	1,084.2±86.5^{bE}	1,801.3±68.4^{bC}	1,293.7±64.6^{bD}
	9 h	2,159.9±57.9^{aA}	2,158.2±73.3^{aA}	489.9±23.9^{abD}	2,048.4±69.9^{aAB}	1,907.7±78.8^{abB}	1,678.7±80.2^{aC}
	12 h	2,220.4±58.6^{aA}	2,138.4±98.1^{abAB}	526.9±4.4^{aD}	2,157.0±99.4^{aAB}	2,000.8±20.9^{aB}	1,711.4±116.5^{aC}

[1]Mean±standard deviation. Values at 0 h indicate the isoflavone content of nonfermented soymilk before inoculation of lactic acid bacteria. Values in the same column of each isoflavone with different superscript small letters are statistically different ($p<0.05$). a>b>c>d. Values in the same row with different superscript capital letters are statistically different ($p<0.05$). A>B>C>D>E> F. *Lp*, *Ws*, and *Ef* indicate *L. paraplantarum* KM, *W.* spp. 33, and *E. faecium* 35, respectively.

in the present study, there was no significant decrease in the content of glycitin and genistin in soymilk fermented with *Ef*, whereas the β-glu activity of *Ef* increased remarkably from 21.8±3.9 to 71.6±3.9 during 9 h of fermentation. These results suggest that *Ef* may produce β-glu with a very low hydrolyzing activity for soybean isoflavone glucosides but with a high activity for *p*-NPG or other glucosides present in soymilk. The hydrolyzing capacity of β-glu for isoflavone glucosides could be different depending on the sources of microorganisms, growth medium, substrate specificity, and presence of isozymes [4, 12–14, 19]. Matsuura and Obata [14], who isolated 3 isoforms of β-glu (A, B, and C) from soybeans, observed that the relative activity for daidzin, genistin, and *p*-NPG was 100, 103, and 126, respectively, for β-glu B, and 100, 136, and 213, respectively, for β-glu C. β-Glu produced by organisms tested in the present study may prefer to hydrolyze daidzin and genistin rather than glycitin. In addition, *Lp*, *Lp+Ws*, and *Lp+Ef* showed a higher rate of hydrolysis for genistin than daidzin, resulting in that less than 2% of the initial genistin was retained in soymilk fermented with those cultures, whereas the % remaining content of daidzin was 6.3% for *Lp*, 13.2% for *Lp+Ws*, and 8.2% for *Lp+Ef* after 12 h of fermentation.

Choi *et al.* [4] reported that approximately 87% and 92% of the initial daidzin and genistin, respectively, in soymilk was hydrolyzed by *L. delbrueckii* subsp. *delbrueckii* KCTC 1047 during 6 h. Otieno *et al.* [17] showed that % conversion of β-glucosides in soymilk fermented with *L. acidophilus* was less than 50% after 6 h and 80% after 12 h. In our study, about 92–100% of the daidzin and 98–100% of the genistin were converted to corresponding aglycones by *Lp*, *Ws*, or *Lp+Ef* within 12 h. In particular, a 100% conversion of daidzin to daidzein by *Ws* was completed within 9 h. In the present study, we tried to improve the hydrolysis rate of isoflavones by mixing *Lp* with new organisms, but it was not efficient. However, acid production could be improved by using the mixed cultures. In conclusion, cultures including *Lp*, *Ws*, and *Lp+Ef* tested in the present study seem to be potential starters for the development of bioactive fermented soymilk with higher estrogenicity and appropriate acidity within a short time.

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References

1. AOAC. 1984. *Official Methods of Analysis*, 14th Ed. Association of Official Analytical Chemists, Washington, D.C. U.S.A.

2. Brown, J. P. 1988. Hydrolysis of glycosides and esters, pp. 109–144. *In* R. Rowland (ed.). *Role of the Gut Flora in Toxicity and Cancer.* Academic Press, San Diego, CA.
3. Chien, H. L., H. Y. Huang, and C. C. Chou. 2006. Transformation of isoflavone phytoestrogens during the fermentation of soymilk with lactic acid bacteria and bifidobacteria. *Food Microbiol.* **23:** 772–778.
4. Choi, Y. B., J. G. Woo, and W. S. Noh. 1999. Hydrolysis of β-glucosidic bonds of isoflavone conjugates in the lactic acid fermentation of soy milk. *Kor. J. Food Sci. Technol.* **31:** 189–195.
5. Chun, J. Y., G. M. Kim, G. H. Kwon, J. Y. Park, S. J. Jeong, K. W. Lee, I. D. Choi, J. S. Kim, and J. H. Kim. 2007. Conversion of isoflavone glucosides to aglycones in soymilk by fermentation with lactic acid bacteria. *J. Food Sci.* **72:** M39–M44.
6. Granata, L. A. and C. V. Morr. 1996. Improved acid, flavor and volatile compound production in a high-protein and fiber soymilk yogurt-like product. *J. Food Sci.* **61:** 331–336.
7. Hutchins, A. M., J. L. Slavin, and J. W. Lampe. 1995. Urinary isoflavonoid phytoestrogen and lignan excretion after consumption of fermented and unfermented soy products. *J. Am. Diet. Assoc.* **95:** 545–551.
8. Izumi, T., M. K. Piskula, S. Osawa, A. Obata, K. Tobe, M. Saito, S. Kataoka, Y. Kubota, and M. Kikuchi. 2000. Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *J. Nutr.* **130:** 1695–1699.
9. Jacobsen, B. K., S. F. Knutsen, and G. E. Fraser. 1998. Does high soy milk intake reduce prostate cancer incidence ? The Adventist Health Study (United States). *Cancer Causes Control* **9:** 553–557.
10. Kim, J.-E., J. Y. Kim, K. W. Lee, and H. J. Lee. 2007. Cancer chemopreventive effects of lactic acid bacteria. *J. Microbiol. Biotechnol.* **17:** 1227–1235.
11. Kim, J.-E., H. J. Hur, K. W. Lee, and H. J. Lee. 2007. Anti-inflammatory effects of recombinant arginine deiminase originating from *Lactococcus lactis* ssp. *lactis* ATCC 7962. *J. Microbiol. Biotechnol.* **17:** 1491–1497.
12. Kim, S.-J., C.-M. Lee, M.-Y. Kim, Y.-S. Yeo, S.-H. Yoon, H.-C. Kang, and B.-S. Koo. 2007. Screening and characterization of an enzyme with β-glucosidase activity from environmental DNA. *J. Microbiol. Biotechnol.* **17:** 905–912.
13. Matsuda, S., F. Norimoto, Y. Matsumoto, R. Ohba, Y. Teramoto, N. Ohta, and S. Ueda. 1994. Solubilization of a novel isoflavone glycoside-hydrolyzing β-glucosidase from *Lactobacillus casei* subsp. *rhamnosus*. *J. Ferment. Bioeng.* **77:** 439–441.
14. Matsuura, M. and A. Obata. 1993. β-Glucosidase from soybeans hydrolyze daidzin and genistin. *J. Food Sci.* **58:** 144–147.
15. McCue, P. P. and K. Shetty. 2005. Phenolic antioxidant mobilization during yogurt production from soymilk using Kefir cultures. *Process Biochem.* **40:** 1791–1797.
16. Omoni, A. O. and R. E. Aluko. 2005. Soybean foods and their benefits: Potential mechanisms of action. *Nutr. Rev.* **63:** 272–283.
17. Otieno, D. O., J. F. Ashton, and N. P. Shah. 2006. Evaluation of enzymic potential for biotransformation of isoflavone phytoestrogen in soymilk by *Bifidobacterium animalis*, *Lactobacillus acidophilus*, and *Lactobacillus casei*. *Food Res. Int.* **39:** 394–407.
18. Pinthong, R., R. Macrae, and J. Dick. 1980. The development of a soya-based yoghurt. *J. Food Technol.* **15:** 647–667.
19. Pyo, Y. H., T. C. Lee, and Y. C. Lee. 2005. Enrichment of bioactive isoflavones in soymilk fermented with β-glucosidase-producing lactic acid bacteria. *Food Res. Int.* **38:** 551–559.
20. Richelle, M., S. Pridmore-Merten, S. Bodenstab, M. Enslen, and E. A. Offord. 2002. Hydrolysis of isoflavone glycosides to aglycones by beta-glycosidase does not alter plasma and urine isoflavone pharmacokinetics in postmenopausal women. *J. Nutr.* **132:** 2587–2592.
21. SAS Institute, Inc. 1990. *SAS User's Guide.* SAS Institute, Inc., Cary, N.C.
22. Setchell, K. D. R., N. M. Brown, L. Zimmer-Nechemias, W. T. Brashear, B. E. Wolfe, A. S. Kirschner, and J. E. Heubi. 2002. Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. *Am. J. Clin. Nutr.* **76:** 447–453.
23. Wang, H. and P. A. Murphy. 1994. Isoflavone content in commercial soybean foods. *J. Agric. Food Chem.* **42:** 1666–1673.
24. Wang, Y. C., R. C. Yu, H. Y. Yang, and C. C. Chou. 2003. Sugar and acid contents in soymilk fermented with lactic acid bacteria alone or simultaneously with bifidobacteria. *Food Microbiol.* **20:** 333–338.
25. Wang, Y. C., R. C. Yu, and C. C. Chou. 2006. Antioxidative activities of soymilk fermented with lactic acid bacteria and bifidobacteria. *Food Microbiol.* **23:** 128–135.
26. Xu, X., H. J. Wang, and P. A. Murphy. 1994. Daidzein is a more bioavailable soymilk isoflavone than genistein in adult woman. *J. Nutr.* **124:** 825–832.
27. Xu, X., S. H. Keecha, H. J. Wang, P. A. Murphy, and S. Hendrich. 1995. Bioavailability of soybean isoflavones depends upon gut microflora in women. *J. Nutr.* **125:** 2307–2315.