International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 13 (3), pp. 001-008, March, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Effect of oligosaccharides and isoflavones aglycones in defatted soy meal fermented by *Lactobacillus paracasei* and *Bifidobacterium longum*

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Accepted 17 February, 2019

The study determined bacterial population, pH value, oligosaccharides and isoflavone in defatted soy meal fermented by *Lactobacillus paracasei* and *Bifidobacterium longum* at 37°C for 48 h. *B. longum* and *L. paracasei* fermented in defatted soy meal has significantly increased the maximum bacterial population over 9.6 log CFU/h and reduced pH to 4.9. Furthermore, content of oligosaccharides, including sucrose, raffinose and stachyose, in FDSM were significantly reduced by *L. paracasei* (97.7, 98.3 and 98.5%) and *B. longum* (98.0, 97.5 and 86.3%, P<0.05). Isoflavone aglycones concentration was significantly increased to 60% for *B. longum* and was higher than 52% for *L. paracasei* (P<0.05). Daidzein and genistein increased concentration to 28-folds and 14.4 to 15.6-folds, respectively, compared to unfermented defatted soy meal.

Key words: Oligosaccharides, isoflavones, defatted soy meal, *Lactobacillus, Bifidobacterium*.

INTRODUCTION

Soybeans (*Glycine max*. L. Merr) are a rich source of protein and economical protein food. Soybean- based food contains isoflavones phytoestrogens, which have been linked to reduced risk of most hormone-associated health disorders (Kurzer, 2000). Isoflavones have potentially beneficial effects, including reducing the risk of cardiovascular disease, lowering rates of breast, prostate and colon cancer, and improving bone health (Ishida et al., 1998; Ishimi et el., 1999). It occurs as the major proportion of conjugated glucosides (daidzin, genistin and glycitin), malonylglucosides, and acetylglucosides (Kudou et al., 1991) and less level of aglycones (daidzein, genistein and glycitein). Bioactive aglycones from isoflavones are absorbed faster and in greater proportion in the human intestine than the glucoside forms, which have higher molecular weight and greater hydrophilicity (Change and Nair, 1995; Izumi et al., 2000). Besides, soybean also contains various oligosaccharides including raffinose and stachyose that may cause a gastrointestinal discomfort to consumers (Scalabrini et al., 1998; Shin et al., 2000). Raffinose and stachyose are non-digestible in the gut due to the absence of -galactosidase in the human intestinal mucosa.

Consequently, intact oligosaccharides pass directly into the lower intestine where they are metabolized by bacteria that possess this enzyme, resulting in the production of gases (Tsangalis and Shah, 2004). Most researches reported that *Lactobacillus* and *Bifidobacteria* fermented with soymilk exhibit significant -glucosidase activity to hydrolyze the isoflavone glucoside forms to aglycones (Tsangalis et al., 2002; Otieno et al., 2006; Wei et al., 2007). -Glucosidase is the key enzyme for carbohydrate metabolism in gut microflora that is able to cleave - glucosidic linkages and other glucose conjugates (Esen, 1993). This enzyme is crucial for the

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conversion of the glucoside form of isoflavones to aglycone forms in fermented soybean products (Toda et al., 2000; Ribeiro et al., 2007). There has been an alternative approach that utilizes lactic acid bacteria processing -galactosidase activity which can hydrolyze the raffinose and stachyose during fermentation (Garro et al., 1999). This would provide simple sugar as substrate for the lactic acid bacteria, as well as reduce the level of raffinose and stachyose. Some of researchers have reported the reduction of raffinose and stachyose in soymilk fermented by *Lactobacillus* (Garro et al., 1994; Scalabrini et al., 1998; Garro et al., 1999; Mital et al., 2006) and *Bifidobacterium* (Scalabrini et al., 1998).

Defatted soy meal (DSM) is a commonly used source of protein and is commercially available to the food industry and in feed for farm animals. DSM is prepared by solvent extraction of whole soybeans to remove the oil in which there is no isoflavone, thus it has remained abundant of this beneficial compound (Eldridge and Kwolek, 1983; Wang and Murphy, 1996). However, there was a few reported simultaneously to investigate the oligosaccharides and isoflavones in DSM fermented by lactic acid bacteria. This study was to determine the effect on bacterial population and pH value, oligosaccharides content and isoflavones content in DSM fermented by *Lactobacillus* paracasei and *Bifidobacteria* longum.

MATERIALS AND METHODS

Chemicals and reagents

DSM was obtained from TTET Union Corp. (Chia-Yi, Taiwan). Daidzin, genistin, daidzein, genistein, sucrose, raffinose and stachyose were purchased from Sigma Chemical (St. Louis, MO). Malonyldaidzin, malonylglycitin, malonylgenistin, acetyldaidzin, acetylglycitin, acetylgenistin, glycitin and glycitein standards were purchased from LC Laboratories (Woburn, MA). Trifluoroacetic acid and liquid chromatography–grade acetonitrile, methanol, and n-hexane were obtained from Merck (Darmstadt, Germany).

Microorganisms

L. paracasei BCRC 14023 and *B. longum* BCRC 14661 were obtained from the BioResources Collection and Research Institute (Hsinchu, Taiwan). Pure cultures were stored at –80°C in 40% (v/v) glycerol. After two successive transfers of the test organisms in MRS medium (Difco, Detroit, MI) at 37°C for 24 h, the activated cultures were again inoculated into broth under the same conditions. The bacteria culture served as the inoculum to prepare fermented defatted soy meal (FDSM).

Preparation of FDSM

DSM was autoclaved at 121°C for 15 min, cooled to room temperature, then its surface was inoculated with 1 to 5 $\times$ 10^3 CFU/g of *L. paracasei* and *B. longum*. Following fermentation at 37°C for 48 h, samples were analyzed for bacterial population, pH, and stored at –20°C for further oligosaccharides and isoflavone analysis.

Bacterial population and pH measurement

The pour plate method was used to measure bacterial growth. MRS agar (Difco) was used during the measurement of colony formation. The plates were aerobic incubated at 37°C for *L. paracasei* and anaerobic incubated at 37°C for *B. longum*. The pH of each sample was determined using a pH meter (S20K, Mettler-Toledo, Switzerland).

Isoflavone analysis

Isoflavones were extracted from defatted soy meal samples as reported (Wei et al., 2004) . Samples were extracted with 20 ml of 80% methanol and stirred at 60°C for 1 h. The insoluble residue was separated by centrifugation (5,000 $\times$ *g* for 10 min), and the insoluble pellets were dried under vacuum. The dried pellet was dissolved in 5 ml of 50% methanol and then extracted 4 times with 20 ml of n-hexane to remove residual fat. After the n-hexane was removed with a pipette, the pellet was again dried. The insoluble residue was redissolved in 10 ml of 80% (v/v) methanol. The samples were filtered through a 0.45 μm millipore PVDF filter membrane (Schleicher and Schuell, GmbH, Dassel, Germany) prior to HPLC analysis.

HPLC analysis was performed according to Wang and Murphy (1994) with modifications, using a Hitachi Model L- 6200 equipped with an ODS-AM-303 column (250 $\times$ 4.6 mm i.d., 5 μm; YMC Inc., Kyoto, Japan) and an ultraviolet spectrophotometer L- 2000 (Hitachi Ltd.). The mobile phase consisted of 0.1% (v/v) glacial acetic acid in water (A) and 0.1% (v/ v) glacial acetic acid in acetonitrile (B). The gradient was as follows: 85% A was at 20 min, followed by a decrease to 76% A over 10 min, maintained for 10 min, followed by a decrease to 65% A over 4 min, steady for 8 min, and then increased to 85% A over 5 min prior to the next injection. The flow rate was 1.0 ml/min. The isoflavone components were detected at 254 nm. Quantitative data for each isoflavone was obtained by comparison to known standards.

Determination of oligosaccharides

The extraction of sugars from fermented and unfermented soymilk samples was modified as previously described by Kuo et al. (1988). 0.5 g of samples were extracted, the 15 ml of 80% ethanol and heating was at 70°C in water bath, then centrifuged at 10,000 x g for 15 min. Followed by filtration using a 0.45 m membrane filter. The concentration of oligosaccharides was determined with a Hitachi Model L-6200 equipped with an ASP-2 Hyperil column (250 $\times$ 4.6 mm i.d., 5 μm; Thermo electron Co., USA) and an evaporative light- scattering detector Sedex 75 (Sedere, France). The mobile phase consisted of acetonitrile (A) and distilled water (B), and the gradient was as follows: 80% A was decreased to 50% A over 15 min, maintained for 5 min and then increased to 80% A over 5 min prior to the next injection. The flow rate was 1.0 ml/min. Quantitative data for each oligosaccharide was obtained by comparison to known standards.

Statistical analysis

Results are presented as the mean ± standard deviation calculated from triplicate experiments. Data were analyzed with ANOVA using Statistical Analysis System software (version 6.03, SAS Institute Inc., Gary, NC). Duncan's multiple range tests were used to compare differences among isoflavone isomers at different time points. Differences were considered to be significant at $P<0.05$.

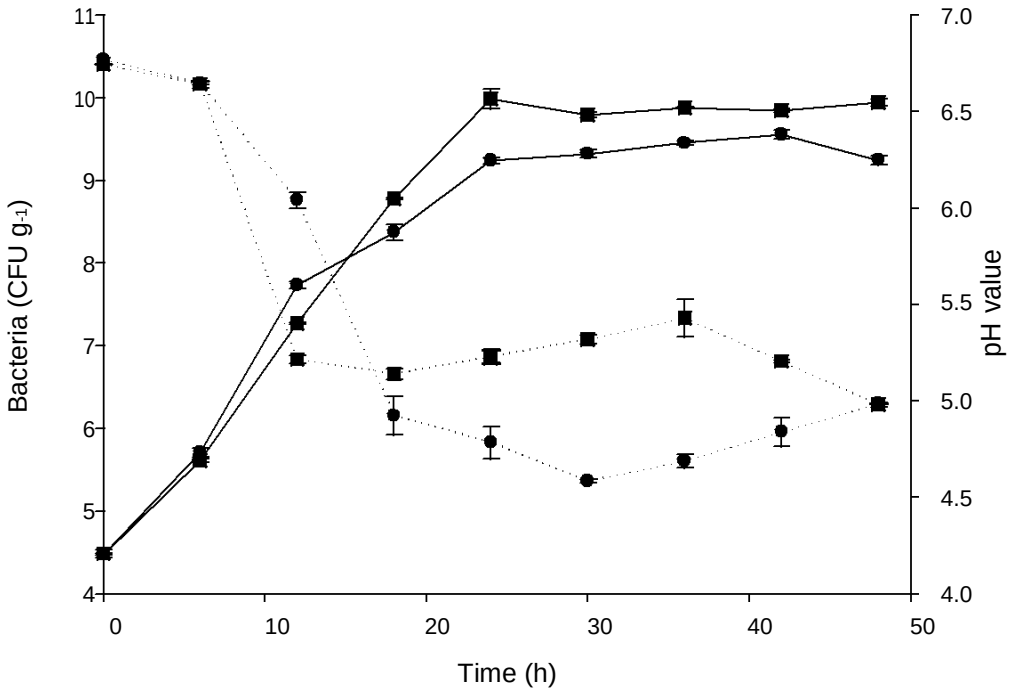

Figure 1. Change of bacterial population and pH in defatted soy meal fermented with *Lactobacillus paracasei* and *Bifidobacterium longum* at 37°C for 48 h. : *Bifidobacteriaum longum;* : *Lactobacillus paracasei*; Bacteria; ….. : pH value.

RESULTS

Change of bacterial population and pH value in FDSM

Bacterial growth in DSM fermented with either *L. paracasei* or *B. longum* at 37°C for 48 h are shown in Figure 1. The bacterial population of *B. longum* and *L. paracasei* exhibited typical growth curves in DSM. At 18 h after inoculation, these bacteria entered the stationary phase from the exponential phase. After 24 h of incubation, maximum bacterial population of *L. paracasei* and *B. longum* reached 9.69 and 9.66 CFU/g, respectively (Figure 1). The pH value in the FDSM with *L. paracasei* and *B. longum* decreased substantially from 6.77 to 4.99 and 4.98, respectively (Figure 1).

Change of oligosacchrides content in FDSM

Change of oligosaccharides including sucrose, raffinose and stachyose in DSM fermented by *L. paracasei* and *B. longum* at 37°C for 48 h is shown in Figure 2. The concentration of sucrose, raffinose and stachyose in FDSM were significantly reduced (P<0.05) from 6.51, 1.22 and 4.04 to 0.15, 0.02 and 0.18, respectively after being fermented by *L. paracasei* (Figure 2A). There were also exhibited, similar results in DSM fermented by *B. longum* decreased to 0.13, 0.03 and 0.55, respectively (Figure 2B).

Change of isoflavone concentration in FDSM

Isoflavone concentration in DSM fermented with *L. paracasei* and *B. longum* are shown in Tables 1 and 2, respectively. Figure 3A is the HPLC profile of isoflavones of standards. The major components in unfermented DSM were -glucoside forms 1.92 mg/g (61%) higher than acetylglucosides 0.13 mg/g (4%), malonylglucosides 1.07 mg/g (33%) or aglycones forms 0.08 mg/g (2%). Incubation of *B. longum* or *L. paracasei* with defatted soy meal at 37°C for 48 h, exhibited a significant decrease in -glucoside isoflavone concentrations, with less drastic changes in acetylglucosides and malonylglucosides during the fermentation period (P<0.05). Conversely, isoflavone aglycone concentrations increased signifi-cantly in fermented defatted soy meal (P<0.05). Total glucoside isoflavones, which constituted 98% of total isoflavones prior to fermentation (0 h), was progressively decreased to 89% for *L. paracasei* (Table 1) and 87% for *B. longum* after 24 h of fermentation (Table 2). After prolonged incubation for 48 h, *B. longum* and *L. paracasei* isoflavone glucosides were significantly reduced (P<0.05) to 40 and 48%, respectively.

In contrast, aglycone isoflavones which constituted 2% of total isoflavones prior to fermentation (0 h) was significantly increased (P<0.05) to 52% for *L. paracasei* (Table 1) and 60% for *B. longum* (Table 2) after 48 h of

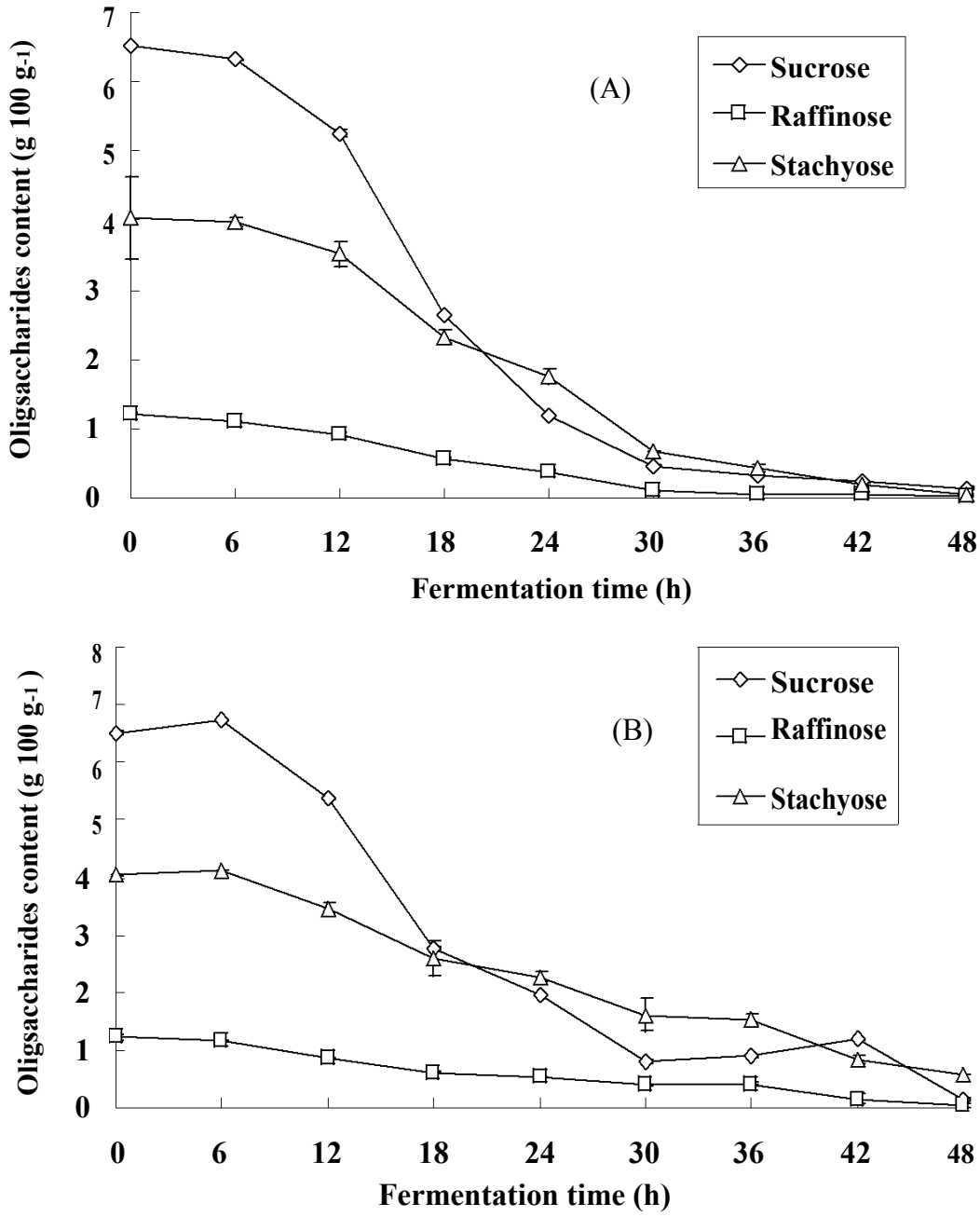

Figure 2. Effect of oligosaccharides content in defatted soy meal fermented by *Lactobacillus paracasei* BCRC 14023 (A) and *Bifidobacterium longum* BCRC 14661 (B) at 37°C for 48 h.

fermentation. Among glucosides from isoflavones of daidzin and genistin had a significantly decreased concentration ($P<0.05$) from both bacteria (Tables 1 and 2) (Figure 3B). Malonyl form isoflavones of daidzin and genistin showed a little of reduce concentration, then acetyl forms isoflavones of daidzin and genistin was not obviously change in FDSM. Contrary isoflavones aglycones of daidzein and genistein exhibited a significant increase ($P<0.05$) after fermentation for 48 h (Tables 1 and 2). These components had great increase amounts approximately 28.0 folds for daidzein and 14.5 ~ 15.6 folds for genistein, compared to unfermented defatted soy meal. However, all of glucosides form glycitin and aglycones forms of glycitein were a less change in FDSM.

DISCUSSION

L. paracasei and *B. longum* had shown growth, well after

Table 1. Changes of isoflavone contents in defatted soy meal fermented with *B. longum* BCRC 14661 at 37°C for 48 h.

Isoflavones (mg/g)	Time (h)								
	0	6	12	18	24	30	36	42	48
Daidzin	0.95±0.01^{ab}	0.97±0.02^{b}	1.03±0.01^{a}	0.91±0.03^{c}	0.72±0.02^{d}	0.30±0.00^{e}	0.15±0.01^{f}	0.11±0.00^{fg}	0.10±0.02^{g}
Glycitin	0.19±0.01^{ab}	0.19±0.01^{b}	0.25±0.03^{a}	0.18±0.01^{b}	0.19±0.00^{b}	0.12±0.00^{c}	0.06±0.00^{d}	0.04±0.00^{d}	0.04±0.00^{d}
Genistin	0.78±0.00^{bc}	0.83±0.04b	0.84±0.00^{b}	0.79±0.03^{b}	0.60±0.00^{c}	0.26±0.01^{d}	0.14±0.01e	0.10±0.00^{e}	0.09±0.01^{e}
Acetyldaidzin	0.03±0.00^{de}	0.04±0.00^{def}	0.04±0.00^{cd}	0.03±0.00^{ef}	0.03±0.00^{f}	0.06±0.00^{a}	0.04±0.00^{bcd}	0.05±0.00^{b}	0.05±0.01^{bc}
Acetylglycitin	0.04±0.00^{b}	0.04±0.00^{d}	0.04±0.00^{d}	0.05±0.00^{c}	0.05±0.00^{c}	0.10±0.00^{a}	0.08±0.00^{b}	0.07±0.00^{b}	0.08±0.00^{b}
Acetylgenistin	0.06±0.00^{bc}	0.07±0.00^{bc}	0.07±0.00^{bc}	0.07±0.00^{ab}	0.06±0.00^{bc}	0.08±0.00^{a}	0.06±0.00^{d}	0.06±0.00^{cd}	0.02±0.00^{e}
Malonyldaidzin	0.54±0.01^{b}	0.54±0.03^{b}	0.54±0.02^{b}	0.54±0.00^{b}	0.48±0.01^{c}	0.36±0.01^{d}	0.26±0.01^{e}	0.19±0.00^{f}	0.20±0.00^{f}
Malonylglycitin	0.10±0.01^{ab}	0.09±0.01^{b}	0.10±0.00^{b}	0.10±0.00^{b}	0.09±0.00^{b}	0.08±0.00^{c}	0.06±0.00^{d}	0.05±0.00^{e}	0.05±0.00^{e}
Malonylgenistin	0.43±0.00^{bc}	0.43±0.02^{a}	0.44±0.02^{a}	0.43±0.00^{a}	0.39±0.01^{b}	0.30±0.00^{c}	0.25±0.01^{d}	0.20±0.01^{e}	0.19±0.00^{e}
Daidzein	0.02±0.00^{f}	0.02±0.00^{f}	0.02±0.00^{f}	0.04±0.00^{f}	0.16±0.01^{e}	0.40±0.00^{d}	0.50±0.01^{c}	0.53±0.01^{b}	0.56±0.01^{a}
Glycitein	0.02±0.00^{d}	0.02±0.00^{e}	0.01±0.00^{e}	0.02±0.00^{e}	0.03±0.00^{d}	0.06±0.00^{c}	0.08±0.00^{b}	0.08±0.00^{b}	0.09±0.00^{a}
Genistein	0.04±0.00^{e}	0.04±0.00^{e}	0.04±0.00^{e}	0.06±0.00^{e}	0.21±0.01^{d}	0.43±0.01^{c}	0.54±0.02^{b}	0.58±0.02^{a}	0.60±0.01^{a}
Total isoflavones	3.20±0.01^{ab}	3.28±0.10^{bc}	3.42±0.00^{ab}	3.22±0.05^{c}	3.02±0.04^{d}	2.56±0.03^{e}	2.22±0.03^{f}	2.06±0.06^{f}	2.07±0.02^{f}

Means ± SD of three replicate analyses (n=3). Means in the same row with different letters are significantly different by Duncan's multiple range test (P<0.05).

Table 2. Changes of isoflavone contents in defatted soy meal fermented with *L. paracasei* BCRC 14023 at 37°C for 48 h.

Isoflavones (mg/g)	Time (h)								
	0	6	12	18	24	30	36	42	48
Daidzin	0.95±0.01^{ab}	1.02±0.01^{a}	0.97±0.00^{ab}	0.90±0.01^{b}	0.69±0.07^{c}	0.33±0.01^{d}	0.20±0.04^{e}	0.21±0.04^{e}	0.10±0.02^{f}
Glycitin	0.19±0.01^{ab}	0.21±0.00^{a}	0.21±0.00^{a}	0.21±0.00^{a}	0.21±0.01^{a}	0.16±0.00^{b}	0.18±0.03^{ab}	0.11±0.02^{c}	0.08±0.00^{c}
Genistin	0.78±0.00^{bc}	0.88±0.01^{a}	0.84±0.01^{ab}	0.80±0.01^{abc}	0.74±0.04^{c}	0.38±0.01^{d}	0.22±0.06^{e}	0.25±0.04^{e}	0.08±0.01^{f}
Malonyldaidzin	0.03±0.00^{de}	0.03±0.00^{de}	0.04±0.00^{cd}	0.04±0.00^{cd}	0.03±0.00^{e}	0.06±0.00^{a}	0.05±0.00^{b}	0.04±0.00^{bc}	0.08±0.01^{a}
Malonylglycitin	0.04±0.00^{b}	0.03±0.00^{b}	0.05±0.00^{b}	0.05±0.00^{b}	0.04±0.00^{b}	0.09±0.01^{a}	0.08±0.00^{a}	0.08±0.01^{a}	0.05±0.01^{a}
Malonylgenistin	0.06±0.00^{bc}	0.07±0.00^{ab}	0.07±0.01^{abc}	0.07±0.00^{abc}	0.05±0.00^{c}	0.08±0.00^{a}	0.06±0.01^{bc}	0.05±0.00^{c}	0.09±0.01^{c}
Acetyldaidzin	0.54±0.01^{b}	0.55±0.01^{b}	0.59±0.00^{a}	0.56±0.00^{ab}	0.49±0.00^{c}	0.30±0.01^{d}	0.25±0.01de	0.22±0.01^{e}	0.25±0.01^{f}
Acetylglycitin	0.10±0.01^{ab}	0.10±0.00^{b}	0.11±0.00^{a}	0.11±0.00^{a}	0.10±0.00^{b}	0.07±0.00^{d}	0.08±0.00^{de}	0.06±0.00^{e}	0.16±0.03^{f}
Acetylgenistin	0.43±0.00^{bc}	0.42±0.01^{bc}	0.46±0.01^{a}	0.44±0.00^{ab}	0.41±0.00^{b}	0.30±0.00^{d}	0.27±0.01^{c}	0.25±0.01^{e}	0.25±0.00^{f}
Daidzein	0.02±0.00^{f}	0.03±0.00^{f}	0.03±0.00^{f}	0.06±0.00^{f}	0.17±0.03^{e}	0.35±0.00^{d}	0.40±0.00^{c}	0.46±0.02^{b}	0.56±0.00^{a}
Glycitein	0.02±0.00^{d}	0.02±0.00^{d}	0.02±0.00^{d}	0.02±0.00^{d}	0.02±0.00^{d}	0.05±0.00^{c}	0.06±0.01^{b}	0.05±0.00^{bc}	0.09±0.00^{a}
Genistein	0.04±0.00^{e}	0.04±0.00^{e}	0.05±0.00^{e}	0.06±0.00^{e}	0.14±0.02^{d}	0.38±0.01^{c}	0.41±0.00^{c}	0.51±0.02^{b}	0.58±0.00^{a}
Total isoflavones	3.20±0.01^{ab}	3.40±0.03^{a}	3.43±0.01^{a}	3.31±0.01^{a}	3.09±0.09^{ab}	2.55±0.02^{c}	2.27±0.07^{de}	2.32±0.09^{d}	2.07±0.01^{e}

Means ± SD of three replicate analyses (n=3). Means in the same row with different letters are significantly different by Duncan's multiple range test (P<0.05).

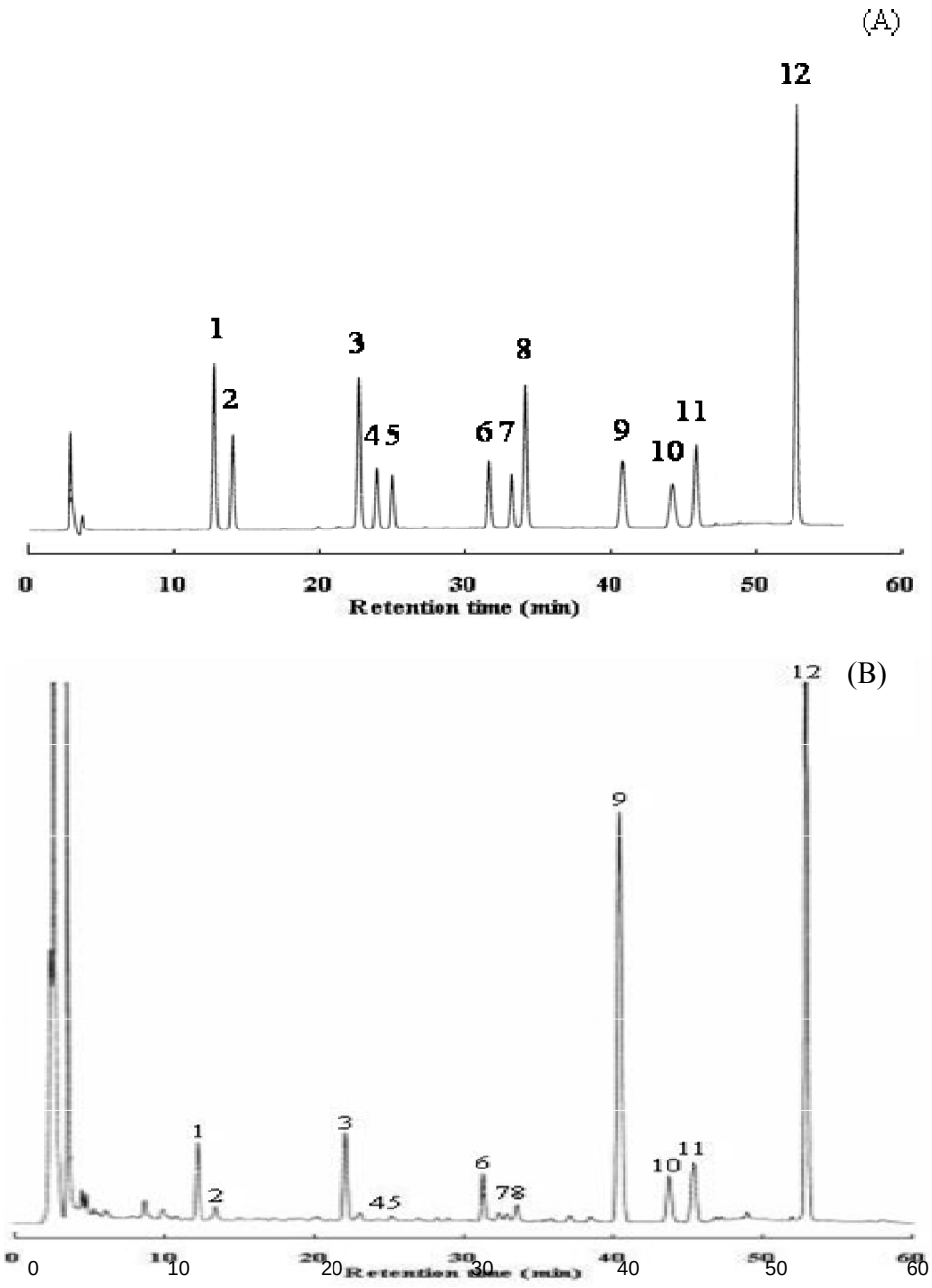

Figure 3. HPLC profile of isoflavone of standard (A) and defatted soybean fermented by *Bifidobacterium longum* BCRC 14661 at 37°C for 48 h (B). 1, Daidzin (12.80 min); 2, Glystin (14.07 min); 3, Genistin (22.86 min); 4, Malonyldaidzin (24.08 min); 5, Malonylglycitin (25.15 min); 6, Acetyldaidzin (31.84 min); 7, Acetylglystin (33.39 min); 8, Malonylgenistin (34.32 min); 9, Daidzein (41.10 min); 10, Glyctein (44.51 min); 11, Acetylgenistin (46.11 min); 12, Genistein (53.10 min).

fermentation in defatted soy meal at 37°C for 48 h. Mital et al. (1974) also reported that certain organisms such as strains of *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Lactobacillus cellobiosis* and *Lactobacillus plantarum*, which utilized sucrose, grew well and produced large amounts of acid in soymilk. During DSM fermentation, the metabolic end products include organic acids, which reduce the pH of the medium. Previous studies have reported similar pH findings (Angeles and Marth, 1971; Kamaly, 1997). Our results also showed a

significant decrease (P<0.05) in the content of sucrose, raffinose and stachyose for *L. paracasei* (97.7, 98.3 and 98.5%) and *B. longum* (98.0, 97.5 and 86.3%) fermented in DSM. Raffinose and stachyose are -galactosides of sucrose comprising three and four monomeric units respectively, which is not digestible in the gut due to the absence of -galactosidase in the human intestinal mucosa. Schrezenmeir and De Vrese (2001) reported that *Bifidobacterium* sp., *L. acidophilus*, and *Lactobacillus casei* have been associated with health-promoting effects and are classified as probiotic organisms since they are thought to improve the microbial balance in the human gastrointestinal tract. Lactic acid bacteria cultures possessing - galactosidase activity reduced raffinose and stachyose contents in fermented soymilk (Mital and Steinkraus, 1975). *Bifidobacterium* strains have been reported to produce varying levels of -D-galactosidase, which metabolized -D- galactosyl oligosaccharides in soymilk (Scalabrini et al., 1998). The removal of raffinose was due to the -galactosidase (-gal) activity of this bifidobacteria, which was highest at pH 6.0 to 5.5 (Garro et al., 2006). *L. plantarum* SMN, 25, *L. plantarum pentosus* SMN, 01 and *L. plantarum pentosus* FNCC, 235 reduced raffinose and stachyose by 81.5, 73.0, 67.0%, and 78.0, 72.5, 66.0% respectively in soymilk (Sumarna, 2008).

Isoflavone aglycones concentrations had a significant increase in FDSM after being fermented by *L. paracase* (52%) and *B. longum* (60%). Similar results reported by Tsangalis et al. (2002) that *Bifidobacterium animalis* Bb-12 was grown in soymilk increasing hydrolysis isoflavone glucosides into aglycones from 8 to 50% of total isoflavones. *Bifidobacterium breve*, *Bifidobacterium bifidum* and *L. casei subsp. rhamnosus* were also been shown to produce high content of isoflavone aglycone in fermented soybean milk (Bordignon et al., 2004). Isoflavone glucosides in soybeans are hydrolyzed by -glucosidase produced by microorganisms, thereby increasing isoflavone aglycones during fermentation. *Lactobacillus* and *Bifidobacteria* in the intestinal microflora can produce -glucosidase activity for enzyme-induced biotransformation of isoflavone glucosides to aglycones (Setchell, 2000; Esaki et al., 2004; Gheytanchi et al., 2010) . Soymilk fermented with *L. paraplantarum* KM were 6 and 7- folds of daidzein and genistein, respectively after 6 h of fermentation (Chun et al., 2007). Pyo et al. (2005) also reported that four lactic acid bacteria were increasing an average 7.1 folds of isoflavone aglycones concentration in fermented soymilk. Pham and Shah (2007) reported *B. animalis* B hydrolyzed higher level of isoflavone glycosides to aglycones than *B. animals* A in skim milk powder.

Our results showed DSM fermented with *L. paracasei* and *B. longum* had a significant decrease in content of raffinose and stachyose and increase level of bioactive daidzein and genistein. Thus, applied lactic acid bacteria fermented with soy-containing products was not only provided to enrich isoflavones aglycone, but also to reduce non-digest of oligosaccharide for consumers. These results may be useful for future applications on food industry and in feed for farm animals.

ACKNOWLEDGEMENTS

This study was financially supported by the National Science Council of the Republic of China, Taiwan, under contract No. 94-2214-E-273-001.

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