Appl Biochem Biotechnol (2008) 151:452–463
DOI 10.1007/s12010-008-8213-4

Biomolecules and Nutritional Quality of Soymilk Fermented with Probiotic Yeast and Bacteria

C. R. Rekha · G. Vijayalakshmi

Received: 17 December 2007 / Accepted: 4 March 2008 /
Published online: 8 July 2008

Abstract Soymilk was fermented with five isolates of probiotic lactic acid bacteria and in combination with probiotic yeast *Saccharomyces boulardii*. Nutritional profile like fat, protein, ash, pH, acidity, polyphenol, and protein hydrolysis were analyzed. Polyphenol content decreased from 265.88 to 119 μg/ml with different cultures. Protein hydrolysis ranged from 2.46 to 2.83 mmol l^{-1} with different cultures. The antioxidant activity was assessed using different methods like 1, 1-diphenyl-2-picrylhydrazyl free radical-scavenging assay, inhibition of ascorbate autoxidation, and measurement of reducing activity. The activities varied with the starters used but, nevertheless, were significantly higher than those found in unfermented soymilk. Bioconversion of the isoflavone glucosides (daidzin+genistin) into their corresponding bioactive aglycones (daidzein+genistein) was observed during soymilk fermentation. Total glucosides in soyamilk were 26.35 mg/100 ml. In contrast, aglycones genistein and daidzein were quantitatively lesser accounting 2.91 mg/100 ml (genistein 1.17 mg/100 ml and daidzein 1.19 mg/100 ml). Soymilk fermented with probiotic cultures resulted in the reduction of glycosides ranging from 0.40 mg to 1.36 mg/100 ml and increase in aglycones ranging from 6.32 mg to 13.66 mg/100 ml.

Keywords Antioxidant activity · Biomolecule · Isoflavones · Polyphenol · Probiotic · *S. boulardii*

Abbreviations

LAB	lactic acid bacteria
Sb	*Saccharomyces boulardii*
La	*Lactobacillus acidophilus*
Lb	*Lactobacillus bulgaricus*
Lc	*Lactobacillus casei*
Lp	*Lactobacillus plantarum*
Lh	*Lactobacillus helviticus*

C. R. Rekha · G. Vijayalakshmi (✉)
Department of Food Microbiology, Central Food Technological Research Institute, Mysore 570020, India
e-mail: vij19_99@yahoo.com

Introduction

Soymilk is the aqueous extract of whole soybeans (*Glycine max*). Soymilk is considered as a suitable economical substitute for cow's milk and an ideal nutritional supplement for lactose-intolerant population [1]. Soybean is a rich source of isoflavone, which are reported to have beneficial estrogenic effects [2–4] with potential bioactive antioxidant properties. Soy-based foods are promising supplements to overcome existing protein–calorie–malnutrition problems [5]. However, its consumption may lead to digestive problems associated with the presence of raffinose and stachyose. One method of overcoming such problem and also to improve the acceptability is by fermentation. Fermentation improves the bioavailability of isoflavones, assists in digestion of protein, provides more soluble calcium, enhances intestinal health, and supports immune system.

Flavonoids consumption has been associated with a reduced risk of most hormone-associated health disorders prevalent in current Western civilizations [6]. Isoflavones occur in two forms, glucosides and aglycones. Aglycone isomers are able to bind to estrogen receptor sites and hence mimic the functions of estradiol in the human body [7, 8]. Asian populations, with their high intake (50–70 mg/day) of soy-derived isoflavones [9], are known to have the lowest incidence of osteoporosis [10, 11], menopausal symptoms [11], mortality from cardiovascular disease [12], and cancer. Both in soybean and nonfermented soy foods, isoflavones are found in concentrations ranging from 0.1 to 5 mg/g [13] as biologically inactive glucoside conjugates, which comprise 80% to 95% of the isoflavone concentration. Glucoside isoflavones are very poorly absorbed in the small intestine compared with their aglycones because of their greater molecular weight and higher hydrophilicity [14]. Furthermore, the glucosides are known to be less bioactive than their respective aglycones [15].

Probiotic lactic acid bacteria and probiotic yeast *Saccharomyces boulardii*, when grown in milk, have the ability to bioconvert the glucoside isoflavones into their respective aglycones without the supplementation of any nutrition.

Growth of probiotic yeast, *S. boulardii*, in association with lactic acid bacteria, has been suggested to stimulate the growth of the probiotic lactic acid organisms and to assure their survival during shelf-life. The low pH of yogurt and the ability of yeasts to utilize organic acids create a selective environment for yeast growth [16]. The present study describes the bioconversion of glucosidic isoflavones to aglyconic form of isoflavones and improvement in nutritional quality of soymilk when fermented with probiotic yeast and lactic acid bacteria.

Materials and Methods

Materials

Soybean (*Glycine max*) was purchased from the local market. Lactic cultures were obtained from culture collection of Food Microbiology Department, CFTRI.

S. boulardii was isolated from the dietary supplement sachet 'Darolac' obtained from local drug shop. Isoflavones Genistin, Daidzin, Genistein, and Daidzein were obtained from Sigma USA. All other chemicals used were of analytical grade.

Microorganisms and Culture Conditions

Five strains of lactic acid bacteria namely *Lactobacillus acidophilus* B4496, *Lactobacillus bulgaricus* CFR 2028, *Lactobacillus casei* B1922, *Lactobacillus plantarum* B4495, and

Lactobacillus helviticus B4526 were maintained in MRS Agar stabs. After two successive transfers of the test organisms in MRS broth at 37 °C for 12–15 h, the activated culture was again inoculated into MRS broth incubated at 37°C for 16 h. This was used as the inoculum and was maintained in MRS agar stab stored at 4°C. *S. boulardii* was maintained on potato dextrose agar slants and stored at 4 °C.

Preparation and Fermentation of Soymilk

Whole soybeans were first washed and soaked overnight in distilled water. After decanting the water, the soaked soybeans were comminuted in a blender for 3 min using distilled water (1:6 *w*/*v*). The resultant slurry was then filtered through double-layered cheesecloth to yield soymilk. Fifty milliliters of soymilk was dispensed into screw cap containers and autoclaved for 15 min at 121 °C. Sixteen-hour-old LAB suspension and yeast suspension with the OD_{600} of 1.0 (approximately 7–8log CFU/ml) in the ratio 1:1 was used as inoculum described above and incubated at 37 °C for a period of 24 h.

Determination of Fat, Protein, and Ash

Total nitrogen was determined by Kjeldahal method (AOAC) [17]. Nitrogen-to-protein conversion factor of 6.25 was used. Fat and ash were determined by AOAC [17] procedures.

pH and Titrable Acidity

The pH of the samples was measured using a pH meter (cyberscan-Eutech Instruments). Titrable acidity (TA) was determined by titration with 0.1 N NaoH solution and expressed as percent lactic acid AOAC [18].

Determination of Polyphenols

Polyphenols was determined using Folin–Ciocalteu reagent [19]. The sample (0.1 ml) was mixed with 0.9 ml of distilled water and was extracted for 2 h at room temperature on a mechanical shaker. To this, 1 ml of Folin–Ciocalteu reagent (1:2 dilution) and 2 ml of 10% Na_2Co_3 was added. The mixture was centrifuged at 20,000×*g* for 20 min, and the supernatant was decanted and filtered through Whatman No. 1 filter paper. The absorbance of the clear supernatant solution was measured at 765 nm (Shimadzu 160 uv A). Gallic acid was used as a standard. Each sample was analyzed twice with duplicates. Results were expressed as milligram gallic acid equivalent per 100 g dry weight.

Protein Hydrolysis

The degree of protein hydrolysis in soymilk during fermentation, expressed as contents of leucine amino equivalent, was determined according to the method described by Adler-Nissen [20]. Samples (225 μl) were mixed with 2.0 ml of 0.10% trinitrobenzensulfonic acid, followed by incubation in the dark for 60 min at 50 °C. The reaction was quenched by adding 4.0 ml of 0.1 N HCL solution, and the absorbance at 340 nm was measured with a spectrophotometer (Model 7800, Jasco, and Tokyo, Japan). L-Leucine (Sigma, St. Louis, USA) was used as the standard to prepare a standard curve.

Determination of Antioxidant Activity

DPPH Free Radical-Scavenging Assay

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was assessed according to Moon and Terao [21]. To 1.0 ml DPPH (500 μM in ethanol), 200 μl aliquot was added, and the reaction mixture was made to 2.0 ml with Tris–HCL buffer (100 mM, pH 7.4). The mixture was shaken vigorously and incubated at room temperature for 30 min. The absorbance of the resulting solution was measured at 517 nm. Reaction mixture without DPPH was used as control.

Measurement of Inhibition of Ascorbate Autoxidation

The method described by Mishra and Kovachich [22] was used to determine the inhibition of ascorbate autoxidation. The sample (0.1 ml) and distilled water, which served as control, was mixed with 0.1 ml ascorbate solution (5.0 mM, Sigma) and 9.8 ml phosphate buffer (0.2 M, pH 7.0). After being placed at 37 °C for 10 min, the absorbance of this mixture at 265 nm was measured. The ascorbate autoxidation inhibition rate of the sample was then calculated.

Inhibition effect (%):

$$\left[\frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} - 1\right] \times 100\%$$

Measurement of Reducing Activity

Reducing activity was determined according to the method of Oyaizu [23]. Sample (0.5 ml) and distilled water (0.5 ml), which served as control, was mixed with 0.5 ml potassium ferricyanide (1.0%) and 0.5 ml of sodium phosphate buffer (0.02 M, pH 7). This was incubated at 50 °C for 20 min, and then 0.5 ml of trichloroacetic acid (10%) was added. The mixture was centrifuged at 780×*g* for 5 min. Of the supernatant, 1.5 ml (upper layer) was mixed with 0.1% ferrichloride (0.2 ml), and the absorbance was measured at 700 nm.

HPLC Analysis of Isoflavones

One milligram of each of standard compounds of Daidzein, Genistein, Daidzin, and Genistin was dissolved individually in 80% methanol in water as stock solutions. The stock solutions were stored in the deep freezer (−20 °C). Each isoflavone standard solution was injected into the high-performance liquid chromatography (HPLC), and the peak areas were determined.

The procedure of Chiou and Cheng [24] was followed. One milliliter of fermented milk was taken in 10-ml centrifuge tube; 4 ml of methanol (100%) was added and the tube was screw capped. After vortexing, the tube was heated at 70 °C for 30 min. During heating, the tubes were inverted by hand for agitation at 5-min intervals. The tubes were centrifuged at 20 °C at 18,000×*g* for 30 min. One milliliter of the sample withdrawn from the middle layer was membrane-filtered (0.45 μm), and 20 μl of the solution was injected into the HPLC system (Shimadzu, Japan Lc 10A UV-Vis det 265 nm) for analysis. A reversed-phase water C18 Column (Spherisorb ODS 2, 4.6×250 mm) was used. A gradient solvent system started with 20% solvent A (methanol) and 80% solvent B (water) and progressed to 80% A

and 20% B within 16 min followed by holding for an additional 2 min. The flow rate was 1.0 ml/min.

Results and Discussion

Fat, Protein, and Ash

The nutritional profile (on dry weight basis in percent) of fermented soymilk is represented in Fig. 1. Fat content was more in soymilk fermented with *S. boulardii* + *L. acidophilus* (Sb+La) curd (23.17%) followed by *S. boulardii* + *L. helviticus* (Sb+Lh) (22.50%), *S. boulardii* + *L. bulgaricus* (Sb+Lb) (21.56%), and *S. boulardii* + *L. casei* (Sb+Lc) (20.47%). The least was in *S. boulardii* + *L. plantarum* (Sb+Lp) (10.81). Protein content was more in soymilk fermented with Sb+Lh curd (58.67%) followed by Sb+Lp (45.48%), Sb+La (45.12%), Sb+Lc (44.42%), and in Sb+Lb (42.31%). The ash content was high in Sb+Lc (5.3%), and the least was in Sb+Lb curd (4.3%).

There was no significant difference in protein content of test curds when compared to control. The protein originating from *L. helviticus* may be contributed to the increased protein content in fermented milk. Similar finding was reported by Hou et al [25] in Bifidobacteria. The increased protein in milk fermented with Bifidobacteria was due to the protein present in cells itself. The difference in fat content was more significant in some of the combinations. Sb+Lp curd, though had high protein content, had very less fat content (10.81%).

pH and Acidity of Fermented Soymilk

The change in pH and % TA in fermented soymilk is represented in Fig. 2. The pH of soymilk fermented with Sb+La culture was highest (6.13) followed by Sb+Lp (5.87), while the others ranges from 5.67 to 5.16 at the end of 24 h of fermentation at 37 °C. An increase in TA of soymilk from 3.60% to 8.55% was observed after 24 h of fermentation with Sb+La combination. Marginal increase was observed with Sb+Lc and Sb+Lp (4.50%) and Sb+Lh (5.80%). On the other hand, relatively lower TA of 3.69% was seen in curd fermented with Sb+Lb combination. The texture, physical stability, flavor, and aroma of the soy yogurt were related to pH [26]. In general, coagulation of sterilized soymilk occurs at pH 5.7 [27]. Previous research has shown that a common problem associated with soymilk

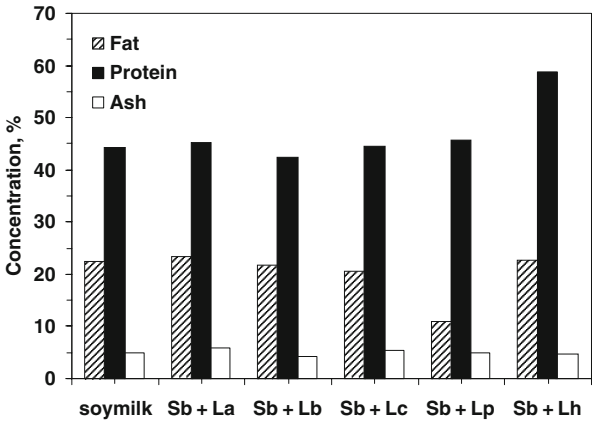

Fig. 1 Nutritional profile of soymilk and fermented soymilk with different LAB and *S. boulardii* for 24 h at 37 °C

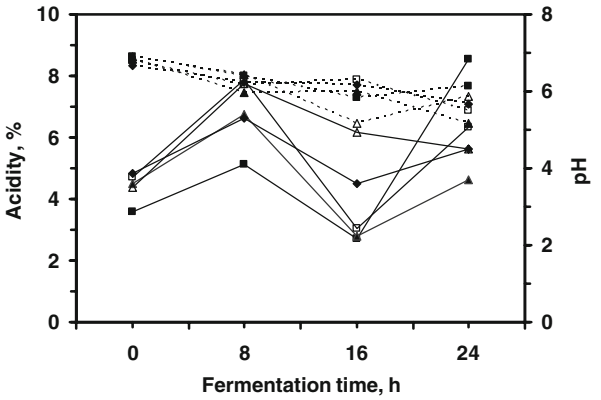

Fig. 2 pH and TA of soymilk fermented with different combination of LAB and yeast *S. boulardii* for 24 h at 37 °C

yogurt is low acidity and flavor intensity [28]. The reported optimum pH of soymilk yogurt is 4.2 to 4.3 [29]. The increase in TA of fermented soymilk is due to acid production during fermentation. Sb+Lc and Sb+Lp curd can be preferred in terms of acidity.

Polyphenols

The changes in polyphenol content are shown in Fig. 3. Polyphenols are present in considerable amount in soymilk. This content decreased from 26.58 mg/100 ml to 11.9 mg/100 ml in various fermented milk, which was incubated for 24 h. In Sb+La curd, not much reduction was observed. Around 55% reduction in polyphenol content was observed with Sb+Lb curd, followed by Sb+Lh (52.68%, 52.04%) and Sb+Lc (51.92%). Similar observation was reported by Sindhu and Khetarpaul [30] in indigenous mixture fermented with *S. boulardii* + *L. plantarum* wherein 24% reduction in polyphenol content was observed. These antinutrients interfere with mineral bioavilability and digestibility of proteins [31] and carbohydrates. Reduction in polyphenol content through fermentation may imply improved digestibility of proteins and carbohydrates and also enhance bioavailability of minerals in the fermented product, thereby improving the nutritive value. Besides nutritional benefits, probiotic organisms have a role in improving metabolism, reducing constipation, lowering cholesterol levels in the blood, and increasing phenol tolerance [30]. Fermentation with different probiotics resulted in reduction of polyphenols. The diminishing effect of fermentation on polyphenols may be due to the activity of

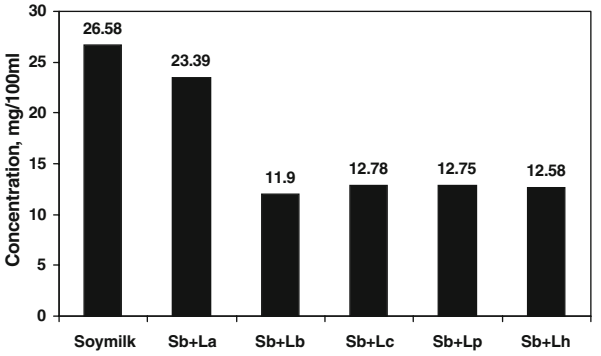

Fig. 3 Polyphenol content of soymilk and soymilk fermented with different LAB and yeast *S. boulardii* for 24 h at 37 °C

polyphenol oxidase present in the food grain or microflora. This reduction in polyphenolic content by fermentation results in less astringency.

Protein Hydrolysis

The proteolytic activity of soymilk fermentation is shown in Fig. 4. Probiotic organisms are rich in proteolytic activity. The rate of protein hydrolysis ranged from 2.46 to 2.83 mmol l^{-1} with different strains of LAB and yeast *S. boulardii* combinations. The highest was seen in Sb+Lb (2.83) followed by Sb+La (2.69), Sb+Lp (2.66), and Sb+Lc (2.52); the least was in Sb+Lh (2.46) combination. The addition of probiotic organisms to soymilk results in increased free amino acid content. The degree of protein hydrolysis is expressed as content of leucine amino equivalent in soymilk after 24 h of fermentation. Similar observation was reported by Kurmann and Rasic [32] in yogurt fermented with *Bifidobacterium fifidum* increased free amino acid content of 7.99 mmol l^{-1} in *Bifidobacterium infantis* at 24 h and 8.36 mmol l^{-1} in *Bifidobacterium longum* was observed (on dry weight basis).

Determination of Antioxidative Activity

DPPH Scavenging Activity

The DPPH scavenging activity of fermented soymilk is shown in Table 1. There was a significant antioxidative activity increase in fermented milk when compared to unfermented soymilk under similar condition. The milk fermented with Sb+Lh strain expressed highest radical scavenging activity (28.53%) compared to others with an increase by 21% compared to control. The least antioxidative activity of 8.77% was observed in Sb+La combination. These results suggested that each extract might react as free radical scavengers by contributing hydrogen from their phenolic hydroxyl groups thereby forming stable free radicals that do not initiate or propagate further oxidation of lipids. The isoflavones and tocopherols are the main phenols responsible for the antioxidant properties [33].

Similar results were published by Pyo et al. [33] in lactic acid bacteria and Bifidobacteria wherein an increase in antioxidative activity of 47% and 38% in lactic acid bacteria and Bifidobacteria was seen compared to control.

Measurement of Inhibition of Ascorbate Autoxidation

Results showed that unfermented soymilk exhibited ability to inhibit ascorbate autoxidation, which is consistent with the finding of previous investigators [34]. Fermentation of

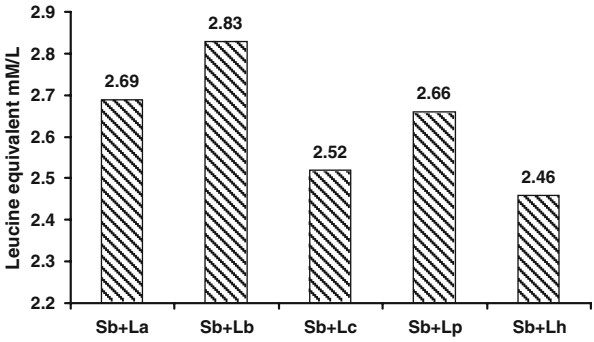

Fig. 4 Protein hydrolysis of Soymilk fermented with different LAB and yeast *S. boulardii* for 24 h at 37 °C

Table 1 Antioxidative activities of soymilk fermented with different lactic acid bacteria with yeast *S. boulardii*.

Fermented Samples	Antioxidative activity		
	DPPH scavenging (%)	Inhibition of ascorbate autoxidation (%)	Reducing (equivalent cysteine, μM)
Soymilk	7.21	4.26	0.727
Sb+La	15.98	8.23	0.747
Sb+Lb	26.22	6.12	0.762
Sb+Lc	26.68	6.34	0.776
Sb+Lp	16.40	8.80	0.808
Sb+Lh	28.53	9.16	0.751

soymilk with yeast and lactic acid bacteria significantly increased the inhibition rate of ascorbate autoxidation. Liberation of aglyconic form of genistein and daidzein through the catalytic action of β-glucosidase during fermentation [35] and the presence of intracellular antioxidants of starter organism may account for the increased inhibition of ascorbate autoxidation found in the fermented soymilk.

As shown in Table 1, the inhibition rate of fermented soymilk to inhibit ascorbate autoxidation ranged from 6.12–9.16% depending on the starter organisms used. Soymilk fermented with Sb+Lh exhibited a significantly higher inhibition rate of ascorbate autoxidation (9.16%) after 24 h of fermentation.

Reducing Activity of Fermented Soymilk

The reducing activity of soymilk is expressed as an equivalent amount of cysteine (μM). Soymilk fermented with Sb+Lp exhibited the highest reducing activity (0.808) among the various fermented soymilk. One milliliter of this fermented soymilk showed the reducing activity equivalent to 0.808 μM cysteine.

The intracellular antioxidants, peptides of starter organism [34], and their hydrogen-donating ability [36] may contribute to the increased reducing activity of soymilk after fermentation.

HPLC Analysis of Isoflavones

The isoflavone content of fermented and unfermented soymilk is shown in Table 2 and Fig. 5. The HPLC chromatogram of isoflavones is shown in Fig. 6. The isoflavones

Table 2 Isoflavone content of fermented and unfermented soymilk with different lactic acid bacteria with yeast *S. boulardii* (on wet weight basis) incubated for 24 h at 37 °C.

	Glucosides (mg/100 ml)			Aglycones (mg/100 ml)			Total (mg/100 ml)
	Daidzin	Genistin	Sub-Total	Daidzein	Genistein	Sub-Total	
Soymilk	6.65	19.7	26.35	1.19	1.71	2.91	29.26
Sb+La	0.23	0.16	0.40	3.60	9.55	13.16	13.56
Sb+Lb	0.16	1.02	1.18	3.33	8.09	11.43	12.61
Sb+Lc	0.12	0.66	0.78	1.84	4.48	6.32	7.10
Sb+Lp	0.17	1.05	1.23	3.71	8.95	12.67	14.00
Sb+Lh	0.19	1.17	1.36	3.98	9.68	13.66	15.02

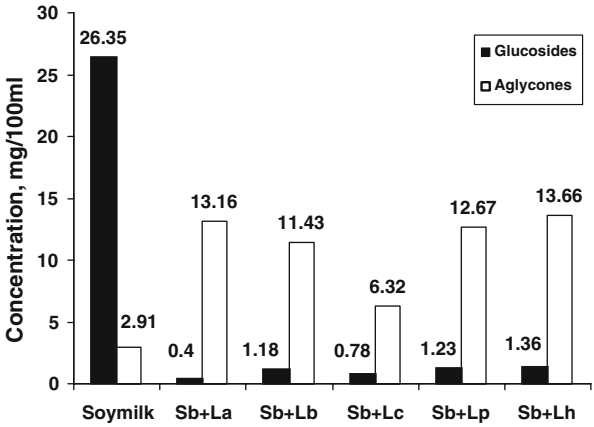

Fig. 5 Isoflavone content of Soymilk fermented with different LAB and yeast *S. boulardii* for 24 h at 37°C

genistin, daidzin, genistein, and daidzein were successfully separated and identified. The most abundant form of isoflavones in soybean and nonfermented soy foods are glycosides (genistin and daidzin), and in fermented foods, they are in the form of aglycones (genistein and daidzein). Unfermented soymilk is rich in glycosides, and total glucoside in unfermented soyamilk was 26.35 mg/100 ml (genistin 19.7 mg/100 ml and daidzin 6.65 mg/100 ml). In contrast, aglyconic form was quantitatively lesser in soymilk accounting of 2.91 mg/100 ml (genistein1.17 mg and daidzein 1.19 mg/100 ml).

Soymilk fermented with different cultures show decrease in glucosidic content ranging from 0.40 to 1.36 mg/100 ml and increase in aglyconic content ranging from 6.32 to 13.66 mg/100 ml.

The concentration of isoflavone isomers (genistin, daidzin, ginistein, and daidzein) in soymilk was 29.26 mg/100 ml after 24 h. The non-bioavailable, biologically inactive glucoside forms (genistin and daidzin) contributed the greatest concentration of isomers (90%) (Fig. 7). But soymilk fermented with five combinations contained a total isoflavone content of 7.10–15.02 mg/100 ml, of bioactive aglycones (genistein and daidzein) after 24 h of fermentation (Sb+La—97.05%, Sb+Lb—90.64%, Sb+Lc—89.01%, Sb+Lp—80.5%, and Sb+Lh—90.94%) of the total isoflavones (Table 2). After 24 h of incubation, the concentration of aglycones in soymilk fermented with Sb+Lh was higher than that of other combinations (13.66 mg/100 ml) followed by Sb+La—13.16 mg/100 ml, Sb+Lp—12.67 mg/100 ml, Sb+Lb—11.43 mg/100 ml, and Sb+Lc—6.32 mg/100 ml.

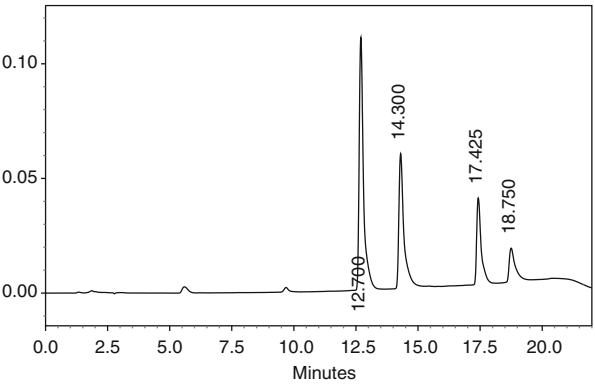

Fig. 6 HPLC chromatogram showing the retention time of standard isoflavones -Daidzin (12.70 min), Genistin (14.30 min), Daidzein (17.42 min) and Genistein (18.75 min)

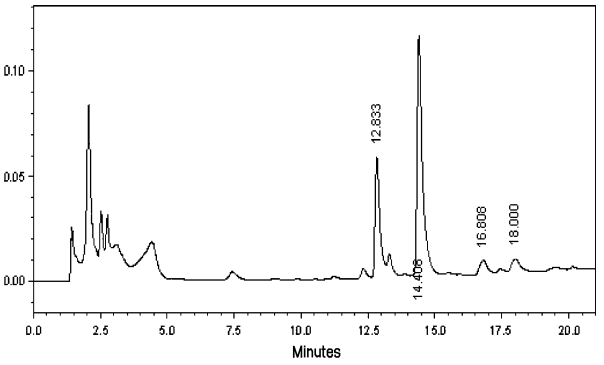

Fig. 7 HPLC chromatogram showing the retention time of isoflavone in soymilk (control): Daidzin (12.83 min), Genistin (14.40 min), Daidzein (16.80 min) and Genistein (18.00 min)

It has been found that the isoflavone glucosides are the predominant isomeric forms in nonfermented soymilk. Soymilk requires bacterial-induced hydrolytic deconjugation for transformation into a bioavailable aglycone form [13]. In this study, the significant bioconversion of the glucoside isoflavones into their corresponding aglycones during soymilk fermentation was because of cleavage of glycosyl bond of by microbial fermentation. Thus, there was an average 14.2-fold increase in the concentration of aglycones in soymilk fermented with five different combinations of LAB and yeast *S. boulardii* with an average of 90% of the original glucosides (genistin and daidzin) bioconverted into aglycones (genistein + daidzein) (Fig. 8).

Interesting feature was that the genistein contributed to the greatest concentration of aglyconic form (3.13–9.55 mg/100 ml wet weight) than daidzein (1.84–3.98 mg/100 ml). Similar observation was reported by Tsangalis et al. [37]. This was possibly due to the higher content of genistin in the original soymilk compared with the other isomers. The reduction in the contents of glucosides (daidzin and genistin) and the increase in the contents of their respective aglycones (daidzein and geinistein) may be based on the hydrolytic reaction catalyzed by β-glucosidase produced by the bacterial and yeast strain. The isoflavone aglycones were absorbed faster and in greater amounts than their glucosides in humans [38]. Isoflavone aglycone-rich products may be more effective than glucoside-rich products in preventing chronic disease such as coronary heart disease and cancer [39].

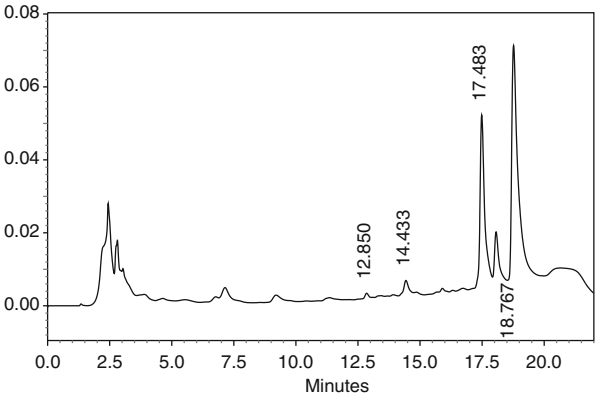

Fig. 8 HPLC chromatogram showing the retention time of isoflavone in soymilk fermented with Sb+La combination: Daidzin (12.85 min), Genistin (14.43 min), Daidzein (17.48 min) and Genistein (18.70 min)

Conclusions

The conclusion drawn from the present study is the increase in antioxidant activity of fermented soymilk is due to the significant bioconversion of the glucosidic form of isoflavones (genistin and daidzin) into their bioactive aglyconic form of isoflavones (genistein and daidzein) [40]. The novelty of this work is fermentation of soymilk with lactic probiotic bacteria with non-lactic probiotic yeast in combination. Yeast sps *S. boulardii* is capable of utilizing the yogurt constituents as growth substrates, and its application as a probiotic microorganism seems promising, as no gas and alcohol are produced [41]. Fermentation with *S. boularadii* + lactics reduced antinutrients like phytic acid, polyphenols, and trypsin inhibitor [32]. According to the literature, the minimum dosage of probiotic cells/day for any beneficial effect is considered to be 10^8–10^9 probiotic cfu/ml [30], but lactics present in yogurt are unstable. Their poor survival is attributed to low pH and low acid tolerance. Yeasts have the ability to utilize organic acids, thereby increasing the pH of the environment. Thus, growth of probiotic yeast in association with probiotic bacteria has been suggested for enhancing the viability of lactic acid bacteria.

References

1. Dhananjay, S., Kulkarni, S. S., Kapanoor, K. G., Naganagouda, V. K., & Veerappa, H. M. (2006). *Biotechnology and Applied Biochemistry*, *45*, 51–57.
2. Adlercreutz, H. (2002). *Lancer Oncology*, *3*, 364–373.
3. Brouns, F. (2002). *Food Research International*, *35*, 187–193.
4. Corn well, T., Cohick, W., & Raskin, I. (2004). *Phytochemistry*, *65*, 995–1016.
5. Khare, S. K., Krishna, J., & Manishwara, M. N. (1994). *Biotechnology and Applied Biochemistry*, *19*, 193–198.
6. Beck, V., Unterrieder, E., Krenn, L., Kubelka, W., & Jungbauer, A. (2003). *Journal of Steroid Biochemistry and Molecular Biology*, *84*, 259–268.
7. Setchell, K. D. R. (1998). *American Journal of Clinical Nutrition*, *68*(suppl), 1333S–1346S.
8. Setchell, K. D. R., & Cassidy, A. (1999). *Journal of Nutrition*, *129*(suppl), 758S–767S.
9. Fukutake, M., Takahashi, M., Ishida, K., Kawamaru, H., Sugimura, T., & Wakabayashi, K. (1996). *Food&chemical Toxicology*, *34*, 457–461.
10. Arjmandi, B. H., Birnbaum, R., Goyal, N. V., Getlinger, M. J., Juma, S., & Alekel, L. (1998). *American Journal of Clinical Nutrition*, *68*, 1364S–1368S.
11. Potter, S. M., Baum, J. A., Teng, H., Stillman, R. J., Shay, N. F., & Erdman, J. R. (1998). *American Journal of Clinical Nutrition*, *68*, 1375S–1379S.
12. Wagner, J. D., Cefalu, W. T., Anthony, M. S., Litwak, K. N., Zhang, L., & Clarkson, T. B. (1997). *Metabolism*, *46*, 698–705.
13. King, R. A., & Bignell, C. M. (2000). *Aus Journal of Nutrition and Dietetics*, *57*(2), 70–78.
14. Chang, Y. C., & Nair, M. G. (1995). *Journal of Natural Products*, *58*, 1892–1896.
15. Xu, X., Harris, K. S., Wang, H., Murphy, P. A., & Hendrich, S. (1995). *Journal of Nutrition*, *125*, 2307–2315.
16. Fleet, G. H., & Mian, M. A. (1987). *International Journal of Food Microbiology*, *4*, 145–155.
17. AOAC. (1970). *Official methods of analysis* (11th ed.). Washington, DC: Association of Official Analytical Chemists.
18. AOAC. (1984). *Official methods of analyis* (14th ed.). Washington, DC: Association of Official Analytical Chemists.
19. Singleton, V. L., & Rossi, J. A. (1965). *American Journal of Enology and Viticulture*, *16*, 144–158.
20. Adler-Nissen, J. (1979). *Journal of Agricultural and Food Chemistry*, *27*, 1256–1262.
21. Moon, J. H., & Terao, J. (1998). *Journal of Agricultural and Food Chemistry*, *46*, 5062–5065.
22. Mishra, O. P., & Kovachich, G. B. (1984). *Life Sciences*, *35*, 849–854.
23. Oyaizu, M. (1986). *Japanese Journal of Nutrition*, *44*, 307–315.
24. Chiou, R. Y. Y., & Cheng, S. L. (2001). *Journal of Agricultural and Food Chemistry*, *49*, 3656–3660.
25. Hou, J-W., Yu, R-C., & Chou, C-C. (2000). *Food Research International*, *33*, 393–397.
26. Ankenman Granata, L., & Morr, C. V. (1996). *Journal of Food Science*, *61*(2), 331–336.

27. Chou, C-C., & Hou, J-W. (2000). *International Journal of Food Microbiology*, *56*, 113–121.
28. Karleskind, D., Laye, I., Halpin, E., & Morr, C. V. (1991). *Journal of Food Science*, *55*, 999–1001.
29. Oberman, H. (1985). *CH3*. London, England: Applied Science Publishers.
30. Sindhu, S. C., & Khetarpaul, . (2003). *Plants Foods for Human Nutrition*, *58*, 1–10.
31. Serraino, M. R., Thompton, L. U., Savoie, L., & Parent, G. (1985). *Journal of Food Science*, *50*, 1689–1692.
32. Kurmann, J. A., & Rasic, J. L. (1991). *Therapeutic properties of fermented milks* pp. 117–158. London: Elsevier Applied Sciences.
33. Pyo, Y-H., Lee, T-C., & Lee, Y-C. (2005). *Food Research International*, *38*, 551–559.
34. Wang, Y-C., Yu, R-C., & Chou, C-C. (2006). *Food Microbiology*, *23*, 128–135.
35. Chien, H. L. (2004). M.S.Thesis, National Taiwan University, Taipei, Taiwan.
36. Yang, J. H., Mau, J. L., Ko, P. T., & Huang, L. C. (2000). Antioxidant properties of fermented soybean broth. *Food Chemistry*, *71*, 249–254.
37. Tsangalis, D., Ashton, J. E., Magill, A. E. J., & Shah, N. P. (2002). *Journal of Food Science*, *68*, 623–631.
38. Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (1992). *Journal of Agricultural and Food Chemistry*, *40*, 945–948.
39. Izumi, T., Nasu, A., Kataoka, S., Obata, A., & Tobe, K. (2000). *Chem Pharma*, 1593–1595.
40. Pyo, Y-H., Lee, T-C., & Lee, Y-C. (2005). *Journal of Food Science*, *70*, S215–S220.
41. Laurens-Hatting, A., & Viljoen, B. C. (2001). *Food Research International*, *34*, 791–796.