Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2017.09.007

Lactic acid bacteria mediated fermented soybean as a potent nutraceutical candidate

Sasithorn Sirilun[1], Bhagavathi Sundaram Sivamaruthi[1], Periyanaina Kesika[1], Sartjin Peerajan[2], Chaiyavat Chaiyasut[1*]

[1]Innovation Center for Holistic Health, Nutraceuticals, and Cosmeceuticals, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand

[2]Health Innovation Institute, Chiang Mai 50230, Thailand

ARTICLE INFO

Article history:
Received 7 Aug 2017
Received in revised form 21 Aug 2017
Accepted 8 Sep 2017
Available online 11 Sep 2017

Keywords:
Lactic acid bacteria
Fermentation
Lactobacillus paracasei HII02
Soybean
Northern Thailand

ABSTRACT

Objective: To study some soybean cultivars commonly used in Northern Thailand that exhibit high nutritional profile and to investigate the changes in bioactive principles and antioxidant capacity of the fermented soy broth that was prepared using the selected soybean cultivar and *Lactobacillus paracasei* HII02 mediated fermentation process.

Methods: The best soybean cultivar was subjected to fermentation, and then analyzed the phytochemical, antioxidant and nutritional changes by high performance liquid chromatography and spectrophotometric analysis.

Results: Sor Jor 2 soybean cultivar showed rich nutritional profile and was subjected to fermentation process. *Lactobacillus paracasei* HII02 mediated fermentation of Sor Jor 2 soybean exhibited stable physical and chemical characteristics. Lactic acid bacteria mediated fermentation also increased the aglycone forms of isoflavone content, exhibited antioxidant capacity and thereby enhanced the quality of the fermented soy broth. It also prevented the growth of coliforms in fermented soybean.

Conclusions: The study results suggest that fermented soybean is rich in nutrition and considered to be safe for consumption for the improvement of health and to treat the malnutrition.

1. Introduction

The fermented soybean products are one of the ancient and commonly used plant-based health supplement with the history of thousands of years [1]. Soybean-based products, like tofu, has taken an increasing share in market because of low cost and nutritional richness (includes protein, oligosaccharides, vitamins, and minerals), especially in Asian countries [2–4].

First author: Sasithorn Sirilun, Innovation Center for Holistic Health, Nutraceuticals, and Cosmeceuticals, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand.

*Corresponding author: Chaiyavat Chaiyasut, Ph.D, Innovation Center for Holistic Health, Nutraceuticals, and Cosmeceuticals, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand.

Tel: +66 53944340

Fax: +66 53894163

E-mail: chaiyavat@gmail.com (C. Chaiyasut).

Foundation project: Chiang Mai University provided the financial supports for Innovation Center for Holistic Health, Nutraceuticals, and Cosmeceuticals (CMU-grant sanction, Dated: 1 October 2016).

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

With the advancement of food science and technology, government regulatory bodies are controlling each and every step in food processing, production and distribution. The production of fermented functional soybean products is majorly attributed to the starter culture. The most acceptable and commonly used starter strains are lactic acid bacteria (LAB), a well-known probiotic bacteria with several desired bioactivities. LAB are frequently related to dairy products, but they also play a part in other food forms and processes such as sausages, drinks, food preservation, *etc*.

The LAB fermented soybean products are reported for antithrombotic [5], anticancer properties [6], and are rich in antioxidants and isoflavones (reported to have tyrosine kinase inhibition, anti-angiogenic effects and activation of natural killer cells). Though the soy milk itself is an excellent functional food, fermentation process may add extra nourishment in terms of bioactive compounds by the metabolic reaction of the microbes. The nutritional value of the fermented soy milk is varied based on the type of probiotic strain used for the fermentation. The versatile nature of LAB strains aid the food industries to develop innovative and functional food products.

The probiotic LAB strains like *Lactobacillus paracasei (L. paracasei)*, *Lactobacillus casei* (*L. casei*), *Lactobacillus mali*, and *Bifidobacterium breve* are reported as a potent starter for the fermentation of soy milk to produce improved fermented soy milk products with pleasant flavor and bioactivities [7]. The glycoside form of isoflavones of soybean can be changed to aglycones by fermentation, and it also depends on the bacterial strain which facilitates the natural absorption of isoflavone [8,9]. The efficiency of isoflavone absorption has been reported and proved that the fermented soy milk is superior to raw soy milk in terms of improvement of the biological efficiency of isoflavones [10].

Even though soy milk and fermented soybean products are enriched with vital nutrition, the acceptance rate of such foods is limited because of the discrete odor of soybeans, mainly in Western countries. The fermentation process improves the smell of the soy milk. LAB strains which are capable of reducing *n*-hexanal, *n*-pentanal, and group A saponin glycosides are used to improve the odor and taste of the fermented soy milk [11]. Thus, the selection of appropriate probiotic starter strain could be the critical step in the production of fermented soybean products.

The nutritional profile of commonly used soybean cultivars of Northern Thailand was assessed in the present study. Subsequently, one soybean cultivar was selected for *L. paracasei* HII02 mediated fermentation process, and the changes in bioactive principles and antioxidant ability were estimated.

2. Materials and methods

2.1. Sample collection and microorganism

The fresh soybean samples, five different cultivars (Chiangmai 1, Chiangmai 60, Rajamangala, Sor Jor 2, Sor Jor 4 and Sor Jor 5) were collected from local market of Chiang Mai, Thailand. The lactic acid bacterial strain *L. paracasei* HII02 was obtained from Health Innovation Institute and the strain was maintained in MRS (de Man, Rogosa and Sharpe) medium until use.

2.2. Quantification of total protein, fat, carbohydrates, fiber, and minerals

The soybean samples were mechanically crushed and the total protein content in soybean was measured by Bradford's method [12] and Lowry's method [13]. The fat content was measured by following the method of Association of Analytical Communities [14]. The percentage of fat in the sample was calculated as per the following formula:

$$\text{Fat content }(\%) = \frac{(\text{Weight of fat extracted})}{(\text{Weight of samples})} \times 100$$

Carbohydrate and fiber content was measured by following the standard methods of Association of Official Analytical Chemist (AOAC) [14]. Mineral content was measured by following the AOAC Official Method 975.03 [15] as described in the previous publication [16].

2.3. Quantification of isoflavones

The soybean samples were subjected to isoflavones extraction using 80% methanol as described by Achouri *et al* [17]. The concentration of genistein, daidzein and their glucoside forms (genistin and daidzin) were assessed by high performance liquid chromatography (HPLC) method with respective HPLC grade standards. To detect the isoflavones in fermented soybean (FSB) broth, samples were collected at various time points and filtered through whatman no. 1 filter, and then the filtrate was subjected to analysis. HPLC was performed as detailed in previous reports with slight modifications [18,19]. Briefly, reversed-phase HPLC system (Waters Inc., Ireland; Model No.: 2995) was equipped with UV detector. An ACE® C18 column (250 mm × 4.6 mm; 5 μm) was used. The mobile phase A (water:methanol:acetic acid at the ratio of 88:10:2) and mobile phase B (methanol:acetic acid at the ratio of 98:2) were used at the flow rate of 1.5 mL/min. All the samples were assessed in triplicate.

2.4. Fermentation process

The soybeans were submerged in drinking water for 12 h and subjected to steam sterilization (cooking) by autoclave at 121 °C for 15 min. The cooked soybean was minced and mixed with brown sugar and water at the ratio of 3:1:10 (soybean:brown sugar:water), and filtered through cheesecloth to obtain soybean milk. Sugar (10%) and *L. paracasei* HII02 starter (10%) were added to the soybean milk, which was incubated at 30 °C for 3 weeks. The samples were collected aseptically at regular intervals to study the various parameters. The soybean milk with all the ingredients at the same ratio without LAB starter was considered as a control.

2.5. Physical and chemical parameters

The changes in the color, odor, taste, turbidity and gas formation were monitored and noted at regular intervals by manual observation. The total acidities of FSB at different time points were measured by titration technique as described in previous report [16]. The moisture content of the sample was calculated by measuring the total solid by AOAC method [20]. Briefly, 2.5–3.0 g of sample was placed in a moisture can and incubated in steam bath for 10–15 min, followed by baking at 90–100 °C for 3 h. Then the total solid and moisture content (%) were calculated by the following formula.

Total solid content (%) = Weight of can with sample after baking−Weight of empty can/Weight of sample × 100

Moisture content (%) = Weight of can with wet sample−Weight of can with sample after baking/ Weight of can with wet sample × 100

2.6. Antioxidant assays

The antioxidant capacity of FSB broth and control samples were evaluated by 2, 2′-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) assay [21], nitric oxide scavenging, superoxide anion scavenging assays [18] and ferric reducing antioxidant power (FRAP) [22] as detailed earlier.

2.7. Microbiological examination

The microbial load in FSB broth was assessed at the beginning and the end of the fermentation process by plating the serially diluted samples in specific media. The samples were plated on plate count agar (total bacterial load), potato dextrose agar (yeast and mold), phenol red egg-yolk kanamycin agar [*Bacillus cereus* (*B. cereus*)], mannitol salt phenol red egg yolk agar [*Staphylococcus aureus* (*S. aureus*)], Salmonella-Shigella agar (*Salmonella* spp.) and cooked meat medium [*Clostridium perfringens* (*C. perfringens*)], and were incubated at the optimum temperatures of the respective microbes for 24–48 h. Then, the colony forming unit (CFU) was calculated [23].

2.8. Statistical analysis

The analysis was performed in triplicates, and values are represented as mean ± SD. The significant difference among the samples were assessed by analysis of variance using statistical SPSS software version 16 (Chicago, SPSS Inc, USA) at the 95% confidential level ($P < 0.05$).

3. Results

3.1. Nutritional value of soybean

Five different cultivars of soybean samples were collected, and their protein, fat, carbohydrate, fiber and mineral contents were assessed. The range of (37.59 ± 0.30)% to (39.49 ± 0.33)%, (14.71 ± 0.10)% to (20.82 ± 0.28)%, (35.79 ± 0.41)% to (42.24 ± 0.17)%, (4.60 ± 0.21)% to (8.02 ± 0.44)%, and (5.31 ± 0.01)% to (5.49 ± 0.12)% of protein, fat, carbohydrate, fiber and mineral were found in tested soybean samples, as shown in Table 1.

3.2. Isoflavones content of different soybean cultivars

The glucone and aglucone forms of isoflavones (daidzin, genistin, daidzein and genistein) were quantified in soybean samples. The daidzin content was found to be ranging from (303.2 ± 18.0) mg/kg to (948.6 ± 19.0) mg/kg among the tested soybean samples. The genistin content was found to be ranging from (281.1 ± 11.0) mg/kg to (949.2 ± 31.5) mg/kg among the tested soybean cultivars. Whereas, the daidzein and genistein content was found to be ranging from (2.8 ± 0.6) mg/kg to (5.2 ± 1.2) mg/kg and (7.1 ± 1.0) mg/kg to (27.5 ± 4.5) mg/kg, respectively among the tested soybean samples (Table 2).

3.3. Physical and chemical characteristics of unfermented and fermented Sor Jor 2 soybean broth

The physical properties such as color, odor, taste, turbidity and gas of soybean broth (SB) and FSB broth were observed manually. The color of both SB and FSB was observed to be light brown. The fragrant of SB was found to be changed into sour smell after 7 days, and FSB was found to exhibit sour odor after 4 days. The turbidity of SB was observed to be slightly opaque and started sediment after 23 h, whereas the turbidity of FSB remained slightly opaque until the monitoring duration of this study. The SB samples were found to exhibit bubbles on the surface throughout the study and produced gas after 7 days. While the FSB samples exhibited bubbles on the surface until 18 h and exhibited no bubbles and gas after 23 h (Table 3).

The chemical characteristics that include pH, acidity, moisture, soluble protein and fat content of SB and FSB were assessed as described in the methods section. The pH of the SB and FSB were found to be reduced at all the time points compared to that of the initial time point. The slight gradual increases in acidity of the SB and FSB were observed throughout the experiment. The moisture content and fat content of SB and FSB were found to be varied until the final day of the experiment. About 4.58% and 5.76% soluble protein content of SB were detected on the initial day and 21st day of the experiment, respectively, whereas, about 4.79% soluble protein content of FSB was detected on the initial day and finally reached about 14.06% on 21st day of the experiment (Table 4).

3.4. Isoflavones content of fermented Sor Jor 2 soybean broth during fermentation

Daidzin, genistin, daidzein and genistein content of FSB during fermentation were assessed. Both forms of tested isoflavones content were found to be varied at different time durations during fermentation. On the initial day (day zero) of FSB possessed about 55.31, 36.17, 14.48, and 6.36 mg of daidzin, genistin, daidzein, and genistein per kg of FSB, respectively. FSB possessed about 55.38, 10.77, 75.42, and 56.04 mg of daidzin, genistin, daidzein, and genistein per kg of FSB, respectively on the 21st day of the experiment (Table 5).

Table 2
Quantity of isoflavones content of selected soybean cultivars (Mean ± SD, $n = 4$) (mg/kg).

Cultivars	Daidzin	Genistin	Daidzein	Genistein
Chiangmai 1	446.3 ± 104.7	360.2 ± 25.7	2.8 ± 0.6	10.0 ± 2.8
Chiangmai 60	588.5 ± 68.4	825.6 ± 8.9	3.8 ± 0.7	26.0 ± 2.1
Rajamangala	927.1 ± 63.5	949.2 ± 31.5	3.1 ± 0.9	7.1 ± 1.0
Sor Jor 2	948.6 ± 19.0	834.5 ± 14.0	5.2 ± 1.2	27.5 ± 4.5
Sor Jor 4	408.4 ± 65.3	610.3 ± 95.0	3.9 ± 0.6	18.9 ± 2.2
Sor Jor 5	303.2 ± 18.0	281.1 ± 11.0	2.9 ± 0.9	9.4 ± 3.5

Table 1
Nutritional profile of various cultivars of soybean (Mean ± SD) (% per dry weight).

Cultivars	Protein	Fat	Carbohydrate	Fiber	Minerals
Chiangmai 1	37.59 ± 0.30[c]	20.82 ± 0.28[a]	36.28 ± 0.56[c]	4.60 ± 0.21[c]	5.31 ± 0.01
Chiangmai 60	37.62 ± 0.04[c]	18.00 ± 0.62[d]	38.90 ± 0.77[b]	8.02 ± 0.44[a]	5.49 ± 0.09
Rajamangala	37.61 ± 0.35[c]	14.71 ± 0.10[e]	42.24 ± 0.17[a]	6.11 ± 0.40[b]	5.44 ± 0.10
Sor Jor 2	39.45 ± 0.18[a]	19.19 ± 0.19[c]	35.91 ± 0.25[c]	5.09 ± 0.50[c]	5.46 ± 0.01
Sor Jor 4	38.54 ± 0.35[b]	20.34 ± 0.06[b]	35.79 ± 0.41[c]	5.91 ± 0.61[b]	5.34 ± 0.09
Sor Jor 5	39.49 ± 0.33[a]	18.54 ± 0.12[d]	36.48 ± 0.46[c]	5.32 ± 0.81[c]	5.49 ± 0.12

[a–d] indicates the statistically significant differences among the samples ($P < 0.05$).

Table 3
pH, total acid, moisture, soluble protein and fat content of soybean broth and fermented soybean broth.

Time (Day)	pH		Total acid (%)		Moisture (%)		Soluble protein (%)		Fat (%)	
	SB	FSB	SB	FSB	SB	FSB	SB	FSB	SB	FSB
0	4.76	4.59	0.06	0.06	95.14	95.09	4.58	4.79	0.22	0.19
1	4.49	3.68	0.07	0.14	94.82	95.17	4.21	17.88	0.23	0.56
3	4.41	3.46	0.08	0.21	95.12	94.94	3.40	19.97	0.22	0.57
7	4.17	3.12	0.09	0.31	95.88	97.81	4.53	18.43	0.18	0.55
10	4.12	3.03	0.21	0.43	96.63	97.78	5.56	16.23	0.20	0.55
14	4.10	3.03	0.26	0.44	96.87	98.20	5.81	18.13	0.20	0.61
17	4.12	3.11	0.21	0.44	97.45	98.24	5.86	13.64	0.21	0.67
21	4.13	3.05	0.21	0.47	96.92	98.31	5.76	14.06	0.24	0.64

Table 4
Quantity of isoflavones in fermented soybean broth at various time points (mg/kg).

Time (Day)	Daidzin	Genistin	Daidzein	Genistein
0	55.31	36.17	14.48	6.36
1	71.94	33.54	21.10	19.45
3	37.46	11.52	15.64	16.82
7	55.58	13.31	45.01	42.36
10	84.38	16.20	89.23	79.17
14	56.39	7.57	67.92	56.06
17	80.72	32.58	91.12	54.62
21	55.38	10.77	75.42	56.04

3.5. Microbial load in unfermented and fermented Sor Jor 2 soybean broth

The microbial load in SB and FSB on both initial day and the end of the experiment were assessed. Total microbial count of SB on zero hours was found to be nil, whereas, on day 21, the total microbial count of SB was (4.9×10^4) CFU/mL. About (1.7×10^8) and (2.2×10^6) CFU per mL of FSB was the total microbial count on zero hour and 21st day of the experiment, respectively. Total coliforms (except SB on day 21), total yeast and mold count, *B. cereus*, *C. perfringens*, *Salmonella* spp., and *S. aureus* were nil in the SB and FSB on both zero and 21st day of the experiment (Table 6).

Table 5
The quantity of isoflavones in fermented soybean broth at various time points (mg/kg).

Time (Day)	Daidzin	Genistin	Daidzein	Genistein
0	55.31	36.17	14.48	6.36
1	71.94	33.54	21.10	19.45
3	37.46	11.52	15.64	16.82
7	55.58	13.31	45.01	42.36
10	84.38	16.20	89.23	79.17
14	56.39	7.57	67.92	56.06
17	80.72	32.58	91.12	54.62
21	55.38	10.77	75.42	56.04

3.6. Antioxidant property of unfermented and fermented Sor Jor 2 soybean broth

Free radical scavenging property of SB and FSB was assessed using FRAP assay, ABTS, superoxide, and nitric oxide radical scavenging assay. Trolox equivalent antioxidant capacity of both SB and FSB were found to be similar in ABTS assay (Figure 1A). FRAP of SB at different time points were found to be slightly varied when compared with that of the FSB (Figure 1B). Both nitric oxide and superoxide radical scavenging property of SB were almost similar to those of the FSB throughout the experiment (Figure 1C, D).

4. Discussion

Messina *et al* [24] have reported the content of protein, fat, dietary fiber, Ca, Fe, Zn, riboflavin, and folate in cooked soybeans. Giri and Mangaraj [25] have reported the effect of processing on the composition of soymilk. Zhang group have reviewed the composition of soybean curd reside, which is obtained while processing the soybean to prepare soybean products like tofu, soymilk, *etc.* [26]. In the present study, all the tested cultivars contain a relatively equal distribution of protein, fat, carbohydrate, fiber and mineral content. The nutritional composition of soybean varied among the cultivars. Sor Jor 2 cultivar appeared to be superior in case of protein content when compared to that of the other tested cultivars. The content and the form of isoflavones were found to be varied among the cultivars. The almost high content of both forms of tested isoflavones was found in Sor Jor 2 samples compared to that of the other soybean cultivars used in this study. Achouri *et al* [17] have reported the appropriate conditions for the extraction of isoflavones from two soybean products. Mujic *et al* [27] have determined the total isoflavone content present in the soybean seeds and also reported that soybean seeds were found to be rich in genistein. Kuo *et al* [28] reported that media milled soymilk contains more

Table 6
The microbial load in fermented soybean broth.

Sample	Total microbes (CFU/mL)	Yeast and mold (CFU/mL)	Coliforms (MPN/100 mL)	*Salmonella* spp., *S. aureus*, *C. perfringens*, *B. cereus* (CFU/mL)
SB (0 h)	Nil	Nil	Nil	Nil
SB (Day 21)	4.9×10^4	Nil	2.0	Nil
FBS (0 h)	1.7×10^8	Nil	Nil	Nil
FSB (Day 21)	2.2×10^6	Nil	Nil	Nil

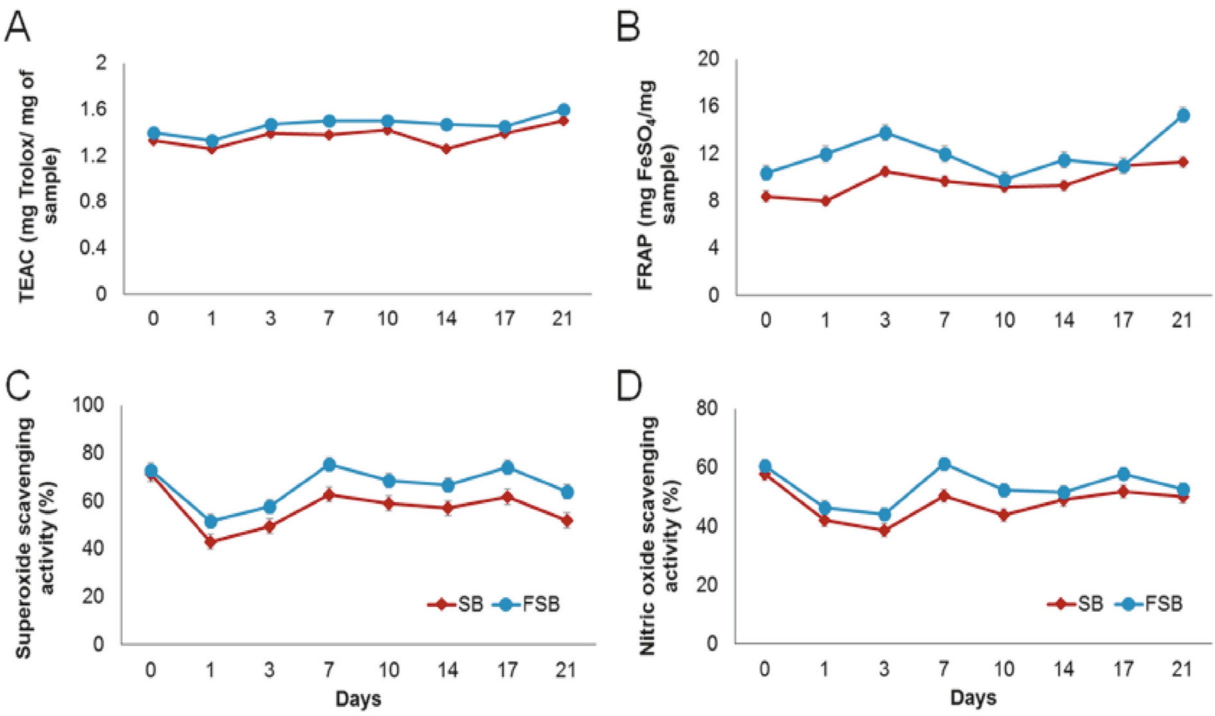

Figure 1. Free radical scavenging property of SB and FSB.
(A) ABTS scavenging activity of SB and FSB was represented as Trolox equivalent antioxidant capacity. (B) Ferric reducing antioxidant power of SB and FSB. (C) Superoxide radical scavenging activity of SB and FSB. (D) Nitric oxide radical scavenging activity of SB and FSB.

aglycone forms of isoflavones compared to that of the filtered soymilk. Recent studies have reported about the isoflavone content of different soybean cultivars [29,30]. In the present study, among the tested cultivars, Sor Jor 2 samples were rich in daidzin, genistin, daidzein and genistein content.

Liu *et al* [31] investigated the physical and chemical characteristics of about 39 commercially available soymilk products and revealed that both physical and chemical properties of tested soymilk products widely differed from each other. A study has been reported investigating the physical and chemical properties of commercially available six soymilk plain beverages and suggested that consumers prefer the soymilk products that are rich in protein content, dark color and high viscosity [32]. Kuo *et al* [28] compared the physical and chemical properties of blended soymilk, filtered soymilk and media milled soymilk and suggested that media milled soymilk was more stable when compared to that of the other tested soymilk. In the present study, FSB exhibited a stable physiochemical property compared to that of the SB.

Aglycone forms of soy isoflavone (daidzein and genistein) are the most active forms and gets easily absorbed in higher quantity in humans compared to that of the glucoside forms of soy isoflavones (daidzin and genistin) [33]. Gardner *et al* [34] investigated the effect of isoflavone content in healthy adults administered (three doses per day) with soy isoflavone tablets (total dose per day: 144 or 288 mg of isoflavone) or soy foods (total dose per day: 96 mg of isoflavone) and revealed that genistein content was higher in the plasma of healthy adults who consumed soy foods when compared to that of the healthy adults supplemented with tablets. Therefore, intake of isoflavones in the form of soy food source is more effective than that of the tablet form. Marazza *et al* [35] have reported that *Lactobacillus rhamnosus* CRL981 mediated fermentation of soy milk increased the aglycone forms of isoflavone content of FSB. In the present study, the daidzein and genistein content of FSB were found to be increased after fermentation. Increased concentration of aglycone forms of soy isoflavone in FSB suggests that consumption of FSB might provide beneficial effects of daidzein and genistein in humans.

Agboke group investigated the microbial quality of some commercially available soymilk products that are consumed in Nigeria and revealed that most of the soymilk products might cause health risk due to the high bacterial count and presence of either pathogenic microorganisms like *Escherichia coli*, *S. aureus*, and *Candida* species [36]. A study has been reported about the bactericidal activity of fermented soybean broth using *in vitro* assays, rat, and mouse models [37]. In the current study, the coliforms count of SB was higher on day 21 compared to that of the FSB. Yeast, mold, coliforms, and pathogenic microorganisms like *Salmonella* spp., *S. aureus*, *C. perfringens*, *B. cereus* were not found in FSB, which proved that consumption of FSB is safe for human.

Marazza *et al* [35] have reported that fermentation of soy milk with *Lactobacillus rhamnosus* CRL981 increased the antioxidant capacity of the fermented soymilk. Similarly, *Lactobacillus* species-mediated fermentation of soymilk increased the antioxidant capacity of the fermented soymilk [38]. A recent study reported the antioxidant capacity of *L. paracasei* KUMBB005 mediated fermented soymilk using diphenyl picryl hydrazinyl radical and FRAP assays [39]. In this study, both SB and FSB showed antioxidant property, and even though the antioxidant capacity of the FSB was slightly higher than that of the SB, no significant changes were observed between the antioxidant capacity of SB and FSB at the different time points during the experiment.

Among the tested soybean samples, was rich in protein and isoflavone content. This study concludes that fermentation of Sor Jor 2 cultivar soybean with *L. paracasei* HII02 increases the quality of the soy broth by exhibiting stable physiochemical properties, increasing the aglycone forms of soy isoflavone content, preventing the growth of coliforms and slightly increasing the antioxidant capacity. Therefore, FSB was considered safe for consumption and healthy for humans. Further study is required for evaluating the customer satisfaction on consuming FSB.

Conflict of interest statement

The authors declare there is no conflict of interest.

Acknowledgments

Authors gratefully acknowledge the Chiang Mai University grant for Innovation Center for Holistic Health, Nutraceuticals, and Cosmeceuticals (CMU-grant sanction, Dated: 1 October 2016) for the financial supports. Authors also gratefully acknowledge the Faculty of Pharmacy and Chiang Mai University, Thailand for the support and instrumentation facilities.

References

[1] Shurtleff W, Aoyagi A. *History of fermented black soybeans (165 B.C. to 2011).* 1st ed. California: Soyinfo Center; 2011.

[2] Frias J, Song YS, Martínez-Villaluenga C, González de Mejia E, Vidal-Valverde C. Immunoreactivity and amino acid content of fermented soybean products. *J Agric Food Chem* 2008; **56**(1): 99-105.

[3] Ojokoh AO, Yimin W. Effect of fermentation on chemical composition and nutritional quality of extruded and fermented soya products. *Int J Food Eng* 2011; **7**(4): 6.

[4] Dajanta K, Chukeatirote E, Apichartsrangkoon A. Improvement of *thua nao* production using protein-rich soybean and *Bacillus subtilis* TN_{51} starter culture. *Ann Microbiol* 2012; **62**(2): 785-95.

[5] Kamiya S, Ogasawara M, Arakawa M, Hagimori M. The effect of lactic acid bacteria-fermented soybean milk products on carrageenan-induced tail thrombosis in rats. *Biosci Microbiota Food Health* 2013; **32**(3): 101-5.

[6] Takagi A, Kano M, Kaga C. Possibility of breast cancer prevention: use of soy isoflavones and fermented soy beverage produced using probiotics. *Int J Mol Sci* 2015; **16**(5): 10907-20.

[7] Shimakawa Y, Matsubara S, Yuki N, Ikeda M, Ishikawa F. Evaluation of *Bifidobacterium breve* strain Yakult-fermented soymilk as a probiotic food. *Int J Food Microbiol* 2003; **81**(2): 131-6.

[8] Setchell KD, Brown NM, Zimmer-Nechemias L, Brashear WT, Wolfe BE, Kirschner AS, et al. Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. *Am J Clin Nutr* 2002; **76**(2): 447-53.

[9] Chien HL, Huang HY, Chou CC. Transformation of isoflavone phytoestrogens during the fermentation of soymilk with lactic acid bacteria and Bifidobacteria. *Food Microbiol* 2006; **23**(8): 772-8.

[10] Kano M, Takayanagi T, Harada K, Sawada S, Ishikawa F. Bioavailability of isoflavones after ingestion of soy beverages in healthy adults. *J Nutr* 2006; **136**(9): 2291-6.

[11] Murti TW, Bouillanne C, Landon M, De Smazeaud MJ. Bacterial growth and volatile compounds in yoghurt-type products from soymilk containing *Bifidobacterium* spp. *J Food Sci* 1993; **58**(1): 153-7.

[12] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72**: 248-54.

[13] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**(1): 265-75.

[14] Association of Official Analytical Chemist. *Official methods of analysis*. 18th ed. Washington DC, USA: AOAC International; 2005.

[15] Horwitz W. *Official methods of analysis of AOAC international*, vol. I. Gaithersburg, MD: AOAC International; 2000.

[16] Chaiyasut C, Sivamaruthi BS, Peerajan S, Sirilun S, Chaiyasut K, Kesika P. Assessment of heavy metals, minerals, alcohol, and fusel oil content of selected fermented plant beverages of Thailand. *Int Food Res J* 2017; **24**(1): 126-33.

[17] Achouri A, Boye JI, Belanger D. Soybean isoflavones: efficacy of extraction conditions and effect of food type on extractability. *Food Res Int* 2005; **38**(10): 1199-204.

[18] Pengkumsri N, Chaiyasut C, Sivamaruthi BS, Saenjum C, Sirilun S, Peerajan S, et al. The influence of extraction methods on composition and antioxidant properties of rice bran oil. *Food Sci Technol (Campinas)* 2015; **35**(3): 493-501.

[19] Woraharn S, Lailerd N, Sivamaruthi BS, Wangcharoen W, Sirisattha S, Peerajan S, et al. Evaluation of factors that influence the *L*-glutamic and γ-aminobutyric acid production during *Hericium erinaceus* fermentation by lactic acid bacteria. *CyTA-J Food* 2016; **14**(1): 47-54.

[20] Association of Official Analytical Chemist. *Official methods of analysis*. 14th ed. Washington DC, USA: AOAC International; 1984.

[21] Sivamaruthi BS, Pengkumsri N, Saelee M, Kesika P, Sirilun S, Peerajan S, et al. Impact of physical treatments on stability and radical scavenging capacity of anthocyanidins. *Int J Pharm Pharm Sci* 2016; **8**(1): 162-7.

[22] Peerajan S, Chaiyasut C, Sirilun S, Chaiyasut K, Kesika P, Sivamaruthi BS. Enrichment of nutritional value of *Phyllanthus emblica* fruit juice using the probiotic bacterium, *Lactobacillus paracasei* HII01 mediated fermentation. *Food Sci Technol (Campinas)* 2016; **36**(1): 116-23.

[23] Pattananandecha T, Sirilun S, Duangjitcharoen Y, Sivamaruthi BS, Suwannalert P, Peerajan S, et al. Hydrolyzed inulin alleviates the azoxymethane-induced preneoplastic aberrant crypt foci by altering selected intestinal microbiota in Sprague–Dawley rats. *Pharm Biol* 2016; **54**(9): 1596-605.

[24] Messina MJ. Legumes and soybeans: overview of their nutritional profiles and health effects. *Am J Clin Nutr* 1999; **70**(3 Suppl): 439S-50S.

[25] Giri SK, Mangaraj S. Processing influences on composition and quality attributes of soymilk and its powder. *Food Eng Rev* 2012; **4**: 149-64.

[26] Li S, Zhu D, Li K, Yang Y, Lei Z, Zhang Z. Soybean curd residue: composition, utilization, and related limiting factors. *ISRN Ind Eng* 2013; **2013**. 423590.

[27] Mujic I, Sertovic E, Jokic S, Saric Z, Alibabic V, Vidovic S, et al. Isoflavone content and antioxidant properties of soybean seeds. *Croat J Food Sci Technol* 2011; **3**(1): 16-20.

[28] Kuo HY, Chen SH, Yeh AI. Preparation and physicochemical properties of whole-bean soymilk. *J Agric Food Chem* 2014; **62**(3): 742-9.

[29] Zhang J, Ge Y, Han F, Li B, Yan S, Sun J, et al. Isoflavone content of soybean cultivars from maturity group 0 to VI grown in Northern and Southern China. *J Am Oil Chem Soc* 2014; **91**(6): 1019-28.

[30] Hasanah Y, Nisa TC, Armidin H, Hanum H. Isoflavone content of soybean [*Glycine max* (L). Merr.] cultivars with different nitrogen sources and growing season under dry land conditions. *JAEID* 2015; **109**(1): 5-17.

[31] Liu Z-S, Chang SKC. Nutritional profile and physicochemical properties of commercial soymilk. *J Food Proc Preserv* 2013; **37**: 651-61.

[32] Terhaag MM, Almeida MB, Benassi MdeT. Soymilk plain beverages: correlation between acceptability and physical and chemical characteristics. *Food Sci Technol (Campinas)* 2013; **33**(2): 387-94.

[33] Izumi T, Piskula MK, Osawa S, Obata A, Tobe K, Saito M, et al. Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *J Nutr* 2000; **130**(7): 1695-9.
[34] Gardner CD, Chatterjee LM, Franke AA. Effects of isoflavone supplements vs. soy foods on blood concentrations of genistein and daidzein in adults. *J Nutr Biochem* 2009; **20**(3): 227-34.
[35] Marazza JA, Nazareno MA, de Giori GS, Garro MS. Enhancement of the antioxidant capacity of soymilk by fermentation with *Lactobacillus rhamnosus*. *J Funct Foods* 2012; **4**(3): 594-601.
[36] Agboke AA, Osonwa UE, Opurum CC, Ibezim EC. Evaluation of microbiology quality of some soybean milk products consumed in Nigeria. *Prim Res Med* 2011; **1**(2): 025-30.
[37] Chin YP, Tsui KC, Chen MC, Wang CY, Yang CY, Lin YL. Bactericidal activity of soymilk fermentation broth by *in vitro* and animal models. *J Med Food* 2012; **15**(6): 520-6.
[38] Subrota H, Shilpa V, Brij S, Vandna K, Surajit M. Antioxidative activity and polyphenol content in fermented soy milk supplemented with WPC-70 by probiotic *Lactobacilli*. *Int Food Res J* 2013; **20**(5): 2125-31.
[39] Rani VU, Pradeep BV. Antioxidant properties of soy milk fermented with *Lactobacillus paracasei* KUMBB005. *Int J Pharm Sci Rev Res* 2015; **30**(1): 39-42.