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Lactic acid bacteria mediated fermented soybean as a potent nutraceutical candidate

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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].

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.

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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