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Protein bioaccessibility of soymilk and soymilk curd prepared with two *Lactobacillus plantarum* strains as assessed by *in vitro* gastrointestinal digestion

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ABSTRACT

The current study was conducted to compare the protein bioaccessibility of soymilk and two soymilk curds generated by fermentation with *Lactobacillus plantarum* B1-6 and *Lactobacillus plantarum* 70810. Soluble protein content, degree of hydrolysis, and electrophoretic patterns were monitored during *in vitro* gastrointestinal digestion of the soymilk and curds. Soluble protein content of digested soymilk was 2.0–2.6 times higher than that of digested curds as measured by the Bradford assay. The degree of hydrolysis was 1.6 times higher in digested soymilk than in digested soymilk curds as determined by the *ortho*-phthalaldehyde (OPA) method. Electrophoretic data showed that soymilk curds had slower protein digestion rates during simulated gastric and intestinal digestion. The soy protein 7S α′, 7S α and 11S acidic subunits and a peptide of 28 kDa were observed even after simulated intestinal digestion. The different degradation profiles suggest that the soymilk curd hinders enzymatic hydrolysis during simulated gastrointestinal digestion.

Industrial relevance: Soymilk curd is a popular food in East and Southeast Asian countries. Lactic acid fermentation is an emerging technology to form soymilk curds that creates a product with improved functional and probiotic properties. The purpose of the present study was to compare protein degradation profiles of soymilk curds produced by fermentation with *Lactobacillus plantarum* B1-6 and 70810, and of soymilk using an *in vitro* gastrointestinal digestion system. Results of the study will expand the knowledge base of the effects of this novel coagulation technology on the nutritional characteristics, especially protein bioaccessibility of soymilk curds and thus would be expected to provide fundamental information in the future application of the emerging coagulation technology using lactic acid bacteria.

1. Introduction

Soymilk, a water extract from crushed soybeans, is a nutritious drink that contains appreciable amounts of protein, and is free of cholesterol and lactose. Soymilk curd, a gel-like food made through the addition of coagulants into heated soymilk is widely consumed in East and Southeast Asian countries (Poysa, Woodrow, and Yu, 2006). Recently, lactic acid bacteria (LAB) were introduced into soymilk as a means of inducing soymilk curd formation. This technology has allowed the production of soymilk curd with improved functional and probiotic properties (Ghosh, Chattoraj, and Chattopadhyay, 2013; Serrazanetti, Ndagijimana, Miserocchi, Perillo, and Guerzoni, 2013). The gelation process induced by LAB has already been studied (Grygorczyk and Corredig, 2013; Ringgenberg, Alexander, and Corredig, 2013); however, little is known about the potential changes in nutritional characteristics due the fermentation process.

In vitro gastrointestinal digestion is regarded as an effective and valid strategy to simulate some of the digestive activities of the human gastrointestinal tract (Guerra et al., 2012). The human digestive tract carries out mechanical, chemical and enzymatic activities that result in the disintegration of the food matrix. The nutrients released by this process become "bioaccessible" to the host organism (Rinaldi, Rioux, Britten, and Turgeon, 2015). Several studies have indicated that food with the same nutrient composition but different molecular structures can have different levels of bioaccessibility and thus different nutritional values as can be observed with milk *versus* yogurt (Rinaldi, Gauthier, Britten, and Turgeon, 2014), or with meat treated with different cooking temperatures (Wen et al., 2015).

Therefore, the current study was conducted to compare the protein bioaccessibility of soymilk and two soymilk curds induced by *Lactobacillus plantarum* B1-6 and *L. plantarum* 70810 isolated from a traditional fermented cereal-based drink, namely, *Kirgiz boza* and Chinese fermented cabbage, respectively. An *in-vitro* gastrointestional digestion

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model was applied and soluble protein content, degree of hydrolysis, and protein degradation profiles were investigated. Results of the study will be helpful to understand potential bioaccessible proteins from consumption of LAB-induced soymilk curds and might encourage further studies in optimization of fermentation parameters targeting the optimum nutrient releasing profiles using this novel coagulation technology.

2. Materials and methods

2.1. Inoculum preparation

The *Lactobacillus plantarum* strains selected for the current study were isolated in our laboratory. The strains were identified based on their 16s rDNA sequences. *L. plantarum* 70810 was isolated from Chinese fermented cabbage, whereas *L. plantarum* B1-6 was from *Kirgiz boza*, a traditional fermented cereal-based drink from the Xinjiang province of China. *L. plantarum* B1-6 and *L. plantarum* 70810 were grown at 37 °C and 31 °C, respectively, based on their optimum incubation temperature for two successive transfers in de Man Rogosa and Sharp broth (MRS, pH 6.2) for 24 h. The activated cultures were inoculated into MRS broth and cultured for another 16 h. The cells were harvest by centrifugation at 7500 ×*g*, 4 °C for 10 min, and washed twice with sterilized physiological saline.

2.2. Soymilk and soymilk curd preparation

The soybeans were rinsed and soaked in distilled water at room temperature for about 12 h, drained and homogenized in six volumes of distilled water with a homogenizer (BE601AB, Midea, China). The insoluble soybean residue, okara (soy pulp), was removed by filtration with a 200-mesh screen cloth. The resulting water extract (soymilk) was then autoclaved at 108 °C for 15 min. Soymilk curds were prepared by inoculation of the soymilk with 10^9 cfu/ml of *L. plantarum* B1-6 or *L. plantarum* 70810 and incubation at the optimum growth temperatures of 37 °C and 31 °C for *L. plantarum* B1-6 and *L. plantarum* 70810, respectively, until curd formation. Duration of the fermentation was around 4 h for both strains and two replicates were conducted. After fermentation, curds were stored at 4 °C before *in vitro* gastrointestinal digestion.

2.3. Viable cell counts and pH analysis

Lactobacillus spp. was enumerated on MRS agar (pH 6.2 ± 0.2, Oxoid-CM0361, Unipath, Basingstoke, UK) after anaerobic incubation under 80–85% nitrogen and 5% carbon dioxide at 37 °C for 48 h using a controlled atmosphere chamber (Plaslabs, Lansing, MI, USA). Cell counts were expressed as log cfu/ml. pH values were analyzed by a pH meter (Schoot Lab 850, Mainz, Germany).

2.4. In vitro gastrointestinal digestion

In vitro gastrointestinal digestion was conducted according a previously published protocol to simulate human buccal, gastric and duodenal digestion (Shim et al., 2010). The ratio of food to digestive juices was set at 2.5:1:1:1:1 (food: saliva: gastric juice: bile: pancreatic juice) to mimic human physiology conditions (Guyton, 1991). The pH was adjusted with HCl or NaOH (4 M) during digestion. 25 ml of soymilk or 25 g of soymilk curd was added to 10-ml of α-amylase solution (w/v, 0.2 mg/20 mM phosphate buffer, pH 7.0) and homogenized to simulate buccal digestion. The digestion was conducted at 37 °C for 3 min in a shaking water bath at 55 rpm (SWB series, Biobase, Shandong, China). Subsequently, the pH of the slurry was adjusted to 2.0, and 15 ml of gastric juice prepared with pepsin in 0.1 M HCl (w/v, 3.2 mg/0.1 M HCl) was added. Gastric digestion was conducted at 37 °C for 1 h at 55 rpm. The pH of samples was adjusted to 7.0 and 10 ml bile acids and pancreatic juice (0.4 mg/10 mM phosphate buffer, pH 7.0) were added to simulate small intestinal digestion. The digestion was performed at 37 °C for 2 h at 150 rpm. Aliquots were taken before digestion (P0), after buccal digestion (P1), at 1 min, 5 min, 20 min and 60 min of gastric digestion (P2), and at 1 min, 5 min, 30 min and 120 min of intestinal digestion (P3). All collected samples were boiled for 5 min to terminate the enzymatic hydrolysis. Samples were centrifuged at 9800 ×*g* at 4 °C for 20 min and the supernatants were collected. All samples were immediately frozen at −20 °C.

2.5. Soluble protein content and degree of hydrolysis

The total soluble protein content was determined according to the Bradford assay with bovine serum albumin as a standard (Bera and Mukherjee, 1989). The degree of hydrolysis was determined using the OPA method (Church, Swaisgood, Porter, and Catignani, 1983). Serine was used as a standard.

2.6. Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out under reducing conditions (5% β-mercaptoethanol) in a 12% polyacrylamide gel using a Bio-Rad Miniprotein 3 unit (Hercules, CA, USA). The voltage was set at 60 V during sample migration through the stacking gel, and then increased to 120 V for peptide separation in the separating gel. A pre-stained molecular mass standard (15–150 kDa, Sangon Biotech Co., Shanghai, China) was used. Gels were scanned with Image Scanner III (GE Healthcare Biosciences, Uppsala, Sweden) and then analyzed using Quantity One software, version 4.6.2 (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

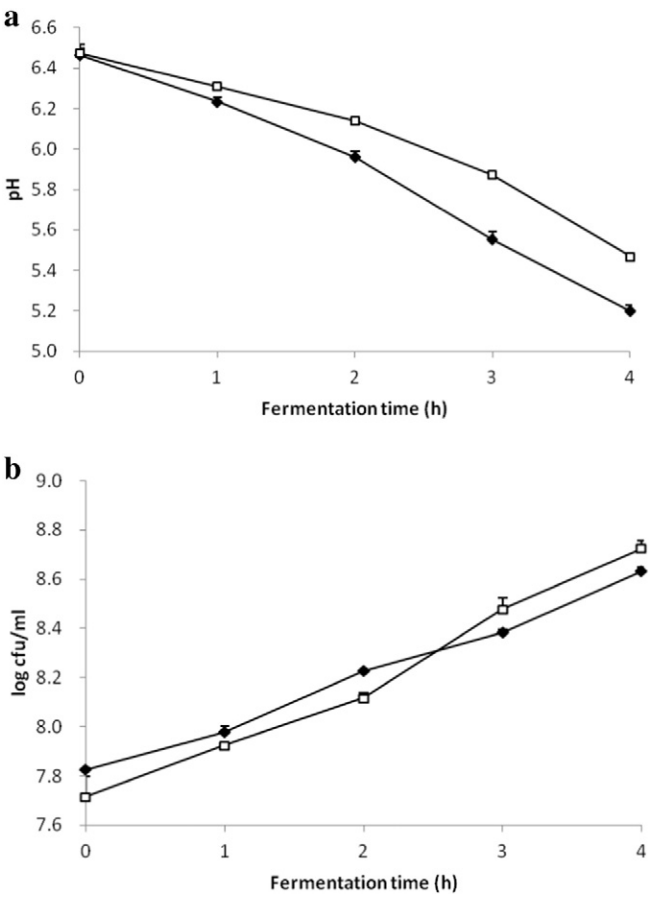

Fig. 1. Decline of pH (a) and change in viable counts (b) during growth of *L. plantarum* 70810 (◆), *L. plantarum* B1-6(□) in soymilk.