Food Control 41 (2014) 1–6

Contents lists available at ScienceDirect

Food Control

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

Whole soybean as probiotic lactic acid bacteria carrier food in solid-state fermentation

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ARTICLE INFO

Article history:
Received 26 October 2013
Received in revised form
18 December 2013
Accepted 20 December 2013

Keywords:
Whole soybean
Solid-state fermentation
Lactic acid bacteria
Bacillus subtilis natto

ABSTRACT

For the purpose of preparing lactic acid bacteria (LAB) carrier food, the solid-state fermentation of whole soybean was performed using *Bifidobacterium animalis* 937, *Lactobacillus casei* Zhang and *Lactobacillus plantarum* P-8 mixed with *Bacillus subtilis* natto, respectively. The physicochemical properties, the amino nitrogen content and peptide molecular weight distribution of the fermented whole soybean products were examined during this process. After 48 h of fermentation, the viable counts of the three samples were 1.41×10^8 CFU/g (*B. animalis* 937), 1.74×10^{10} CFU/g (*L. casei* Zhang) and 2.19×10^{10} CFU/g (*L. plantarum* P-8), with the pH declined rapidly from 6.32 to 5.78, 5.60 and 5.44 at the early stage of the fermentation and increased to 6.71, 6.47 and 6.60 at the later stage of the fermentation. The fermentation caused a sharply increase in the content of the free amino nitrogen from 99.7 μmol/g to 301.9 μmol/g, 390.1 μmol/g and 529.1 μmol/g in the solid fermented soybean products, due to the multiplication of microorganism and the effect of enzyme system. Furthermore, the levels of soybean peptide with molecular weight less than 1000 Da increased 30.7%, 71.2% and 81.3% relative to that of the control group at 48 h. The result of the present work implied that whole soybean fermented by LAB can provide the different probiotics for the host, and there is potential to develope nutritious fermented soybean products.

1. Introduction

Soybean is an excellent source for nutrition that include plant protein, oligosaccharides, V_B, V_E and mineral substance (Dajanta, Chukeatirote, & Apichartsrangkoon, 2012). Acceptance of soybean protein products has increased because of the low cost and high nutritional quality for human consumption and also as protein source for animal meals (Frias, Song, Martinez-Villaluenga, Gonzalez De Mejia, & Vidal-Valverde, 2008). Soybean is also a crucial protein source for Asian people (Ojokoh & Wei, 2011). For example, soybean curd and soymilk are their favorite food (Amadou, Le, Shi, & Jin, 2011; Feng, Eriksson, & Schnurer, 2005). It has been shown that the production of soybean have some functional compound which can reduce the risk of cardiovascular diseases and cancers (Amadou, 2011). In addition, fermented soybean products such as black bean sauce, natto and tempeh are people's daily diet (Juan & Chou, 2010; Lv, Guo, & Yang, 2009). After fermentation, the nutrition inhibitory factor in soybean was eliminated. Under the action of the microbial enzyme, insoluble macromolecular substances such as protein, fat and carbohydrate were degraded into polypeptides, fatty acids and oligopeptides, which can improve the nutrition utilization of soybean.

Lactic acid bacteria (LAB) and *Bacillus subtilis* natto as probiotics can exert different health effects on the consumers, which leads to the developing of certain functional foods (Molina, Médici, Font de Valdez, & Taranto, 2012). LAB are well known to be major beneficial microflora in human intestine, and they have been widely utilized to manufacture fermented soymilk products and other types of foods (Kim, Lee, & Yoo, 2012). For example, it can reduce the soy flour immunoreactivity by fermenting with *Lactobacillus plantarum* by decreasing the IgE immunoreactivity (Frias, Song, Martinez-Villaluenga, Gonzalez De Mejia, & Vidal-Valverde, 2008; Nguyen, Guyot, Icard-Vernière, Rochette, & Loiseau, 2007). *Lactobacillus casei* as a significant member of LAB can survive naturally in the intestinal tract to regulate the gut microorganism and reduce the risk of cancer, and also be used for fermenting soymilk (John, Nampoothiri, & Pandey, 2007). In addition, soybean also has natural oligosaccharide, such as raffinose, stachyosea, and sugar, and they have some unique characteristics to make *Bifidobacterium* proliferating, to improve the *Bifidobacterium* viable count, and to reduce harmful

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http://dx.doi.org/10.1016/j.foodcont.2013.12.026

bacteria, so as to improve the quality of soybean fermentation products (Han, Ebert, Zhao, Li, Zhang, & Tian, 2005). *Bacillus subtilis* is the most broadly used strain for soybean fermentation, which can increase antioxidant activity, anti-allergic activity and fibrinolytic function of the soybean (John, Nampoothiri, & Pandey, 2007; Juan, Wu, & Chou, 2010; Kwon, Lee, Lee, Chang, & Chang, 2000).

Many studies suggested that LAB was widely used in the fermentation of soybean derived products, such as sufu (a Chinese fermented soybean food), soybean flour and soymilk (Georgetti et al., 2009; Han, Cao, Rombouts, & Nout, 2004; Marazza, Nazareno, de Giori, & Garro, 2012). However, to our knowledge, the whole soybean fermented by LAB mixed with *B. subtilis* natto as probiotics carrier food was not investigated. Besides, the solid-state fermentation possesses several biotechnological advantages, such as higher fermentation productivity, higher end-concentration of products, higher product stability, lower catabolic repression, cultivation of microorganisms specialized for water-insoluble substrates and lower demand on sterility (Holker, Hofer, & Lenz, 2004). After the solid-state fermentation of the whole soybean, the products can be lyophilized directly without centrifugation. As a result, the solid-state fermentation of the whole soybean is a more economical and simple fermentation technology in order to produce probiotics carrier food.

In our research, the solid-state fermentation of whole soybean was performed using *Bifidobacterium animalis* 937, *L. casei* Zhang and *L. plantarum* P-8 mixed with *Bacillus subtilis* natto, respectively. The physicochemical properties, the amino nitrogen content and peptide molecular weight distribution of the fermented whole soybean products were examined during this process.

2. Material and methods

2.1. Microorganisms

The microorganism, *L. casei* Zhang and *L. plantarum* P-8 were obtained from Key Laboratory of Dairy Biotechnology and Engineering, Ministry of Education, Inner Mongolia Agricultural University (Inner Mongolia, PR China), which had been considered as new probiotic strains (Bao, Wang, & Zhang, 2012; Bao, Zhang, & Li, 2012; Wang, Zhang, Zhang, Wei, Bao, Zhang, Sun, Postnikoff, Meng, & Zhang, 2012; Wu, Zhang, Sun, Wu, Yue, Meng, & Zhang, 2011) isolated form traditional fermented foods in Inner Mongolia of China. *B. animalis* 937 was isolated from the feces of a healthy child in our laboratory and *B. subtilis* natto was isolated in our laboratory from a traditional fermented food (natto) collected from Heilongjiang, China (Wang, Zhang, Sun, & Dai, 2013).

2.2. Inoculum preparation

For inoculum preparation, *L. casei* Zhang and *L. plantarum* P-8 were grown in MRS broth (peptone 10 g/L, yeast extract 5 g/L, beef extract 10 g/L, sodium acetate anhydrous 5 g/L, ammonium citrate tribasic 2 g/L, K_2HPO_4 2 g/L, $MgSO_4 \cdot 7H_2O$ 0.1 g/L, $MnSO_4 \cdot H_2O$ 0.05 g/L, tween-80 1 g/L, pH 6.8) at 37 °C for 20 h. *B. animalis* 937 was grown in MRS broth at 37 °C for 20 h under anaerobic conditions. *B. subtilis* natto was grown in the broth containing 1% soybean meal and 2% glucose at 37 °C , 150 rpm for 20 h. The cells were then harvested and resuspended in sterilized physiological saline and adjusted to 10^6 CFU/mL. This cells suspension was ready to serve as inoculum for soybean fermentation.

2.3. Soybean fermentation

The soybeans (Northeast soybean, Heilongjiang, PR China) were cleaned and soaked in the water (pH 6.5) overnight at 4 °C and then the soaking water was discarded. The soybeans were sterilized at 115 °C for 25 min and cooled to 24 °C and then were inoculated with 10^6 CFU/g of *L. plantarum* P-8, *L. casei* Zhang and *B. animalis* 937 mixed with 10^6 CFU/g of *B. subtilis* natto, respectively. The fermentation process was carried out at 37 °C for 48 h and the fermentation of *B. animalis* 937 mixed with *B. subtilis* natto was cultured with anaerobic conditions. The samples were taken for analysis after 0, 24, 28, 32, 36 and 48 h of the fermentation.

2.4. Microbiological analysis (total viable bacterial count)

Total viable counts were determined during the fermentation. The fermented soybeans (10 g) were homogenized with 90 ml of the sterilized physiological saline (0.85%). Serial dilutions were prepared in sterilized physiological saline and 1 ml of appropriate dilutions was poured in triplicate plates for total viable count. Total viable counts of *L. plantarum* P-8, *L. casei* Zhang and *B. animalis* 937 were made using a pour plate method and MRS agar after serial dilution in maximum recovery diluents. A pre-prepared test sample (1 ml) of 10^{-7}, 10^{-8}, and/or 10^{-9} dilution was transferred into a sterile petri dish, in triplicate, and warm (45 ± 2 °C) sterile plate count MRS agar (15 ml) was mixed with the inoculums. Cultures were incubated anaerobically at 37 °C for 48 ± 2 h. Total Viable counts of *B. subtilis* natto was made using spread plate technique. About 15 ml nutrient agar medium was poured into plates prior to use. A pre-prepared test sample (0.1 ml) of 10^{-6}, 10^{-7}, and/or 10^{-8} dilution was transferred to the surface of nutrient agar medium, the inoculums was spread evenly over the entire surface of the agar by a rotary twirling motion of the plate under the rod. Cultures were incubated at 37 °C for 24 ± 2 h. The colonies were then counted and expressed as logarithmic colony forming units per gram (lg CFU/g) of the sample.

2.5. Physicochemical analysis

2.5.1. The determination of pH

10 g of the fermented soybeans (wet weight) were homogenized in a blender with 90 ml of distilled water for 30 s and the pH value of the suspension was measured with an FE20 pH meter (Mettler-Toledo, Shang Hai, PR China).

2.5.2. The determination of free amino nitrogen

10 g of the fermented soybeans (wet weight) were homogenized in a blender with 90 ml of distilled water for 30 s. The free amino nitrogen of the homogenate was extracted at 4 °C for 24 h, centrifuged at 7000 rpm for 15 min and the supernatant was reserved. The free amino nitrogen was determined according to the ninhydrin method (Abernathy, Spedding, & Starcher, 2009).

2.5.3. The determination of molecular weight distribution

Fermented soybeans (10 g) were homogenized with 50 ml of distilled water and incubated at 4 °C for 4 h, centrifuged at 4500 rpm for 10 min at 4 °C and then the supernatant was passed through 0.45 μm Millipore filters (Minisart, Sartorious, Germany). The filtered supernatant was collected, lyophilized and stored at −20 °C until further used.

1.0 g freeze-dried samples was dissolved in 99 ml mobile phase (50 mM phosphate buffer containing 0.15 M NaCl, pH = 7.2). The sample was loaded onto GE Superdex™ Peptide 10/300 GL column (i.d. 10 × 300 nm), eluted with mobile phase at a flow rate of 0.5 ml/min and monitored at 220 nm. A molecular weight calibration curve was obtained from the following standards: Cytochrome C (12,500 Da), Trasylol (6512 Da), Glutathione Disulfide (615 Da), Reduced Glutathione (310 Da) and Glycine (75 Da).