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Improvement of bioactivity of soybean meal by solid-state fermentation with *Bacillus amyloliquefaciens* versus *Lactobacillus* spp. and *Saccharomyces cerevisiae*

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ABSTRACT

To evaluate the impact of fermentation with *Bacillus amyloliquefaciens* U304 on nutritional quality and bioactivity of soybean meal (SBM), we analyzed the solid-state fermentation process for *Bacillus amyloliquefaciens, Lactobacillus* spp., and *Saccharomyces cerevisiae*. *B. amyloliquefaciens* showed significant improvement in nutritional quality and bioactivity by removing the protein- and carbohydrate-based anti-nutritional factors (ANFs), as well as allergens. The total phenolic content, reducing power, free radical scavenging ability, and Ca^{2+} chelating ability of SBM, as indicators of the antioxidant activity, increased to 195.8, 201.7% (at 10 mg/mL), 136.6% (at 10 mg/mL), and 122.3%, respectively, after *Bacillus* fermentation. *S. cerevisiae* decomposed carbohydrate-based but not protein-based ANFs, and fermentation with this organism produced similar values of the antioxidant markers of unfermented soybean meal, except for the reducing power (160.0% at 10 mg/mL). *Lactobacillus* spp. was only effective for decreasing the activity of trypsin inhibitors, but not other ANFs, resulting in lower bioactivity of fermented soybean meal. *B. amyloliquefaciens* U304 can substantially improve both the nutritional quality and bioactivity of SBM.

1. Introduction

For generations, the soybean has been an important crop in Asia. In addition to being a component of such foods as tofu, natto, and tempeh, this crop is used for production of soy vegetable oil. Furthermore, the part remaining from processing of soybean-based food, defatted soybean meal (SBM), is an important and cheap protein source for food and animal feed. SBM is known for its high protein content, balanced amino acid composition, and high level of lysine in comparison with other vegetable protein sources. However, other components of SBM, namely anti-nutritional factors (ANFs) and allergens, cause allergy reactions in children with atopic dermatitis (Kleine-Tebbe et al., 2002), and decrease digestibility and absorption based on young animal studies (Goebel & Stein, 2011; Gu, Pan, Sun, & Qin, 2010; Schneeman & Gallaher, 1986), thus limiting its nutrition utilization.

One of prevalent ANFs is trypsin inhibitor (TI). It is a protein-based ANF, which inhibits pancreatic protease, proteolysis, and the absorption of dietary proteins (Liener et al., 1988; Perez-Maldonado, Mannion, & Farrell, 2003). Increased secretion of trypsin is caused by activity reduction and results in endogenous nitrogen loss, especially in sulfur-containing amino acid content (Schneeman & Gallaher, 1986). In addition, carbohydrate-based ANFs such as the non-digestible oligosaccharides (raffinose and stachyose) can induce increased gas production in humans (Sumarna, 2008) and diarrhea in poultry due to the absence of endogenous α-(1,6)-galactosidase enzyme in these species (Gitzelmann & Auricchio, 1965; Sun, Li, Dong, Qiao, & Ma, 2008).

The soybean is one of the “Big 8” food allergens. The allergen proteins account for 65–80% of total protein content in the soybean and approximately 30% in SBM. The major allergen proteins are β-conglycinin (α, α′ subunit, β subunit), the 30-kDa allergen (Gly m Bd 30), and glycinin. In human subjects, these allergens can induce symptoms ranging from skin, gastrointestinal, or respiratory reactions to anaphylaxis (Holzhauser et al., 2009). They also cause hypersensitivity in weaned piglets, with the primary adverse effect being diarrhea (Hotz & Gibson, 2007).

Different approaches, including over-heating, chemical treatment, and alcohol extraction, were used in order to solve these

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problems. However, all of these methods were able only to denature ANFs and allergens, but not to eliminate them completely. In addition to being the most efficient method to remove the ANFs and allergens, fermentation provides other benefits. It has been shown that fermentation can help to reduce the immunoreactivity and allergic reactions caused by soy products (Frias, Young, Martínez-Villaluenga, De Mejia, & Vidal-Valverde, 2008). Fermented soybean meal (FSBM) enhanced the bioavailability of nutritious components and decreased the incidence of diarrhea in weaned pigs (Egounlety & Aworh, 2003; Teng et al., 2012), due to the degradation of allergens into peptides. The peptides can be easily absorbed by an animal and transported within an organism (Gilbert, Wong, & Webb, 2008). They also exhibit high specific bioactivities, such as anti-oxidative activity and metal-chelating activity. Furthermore, soybean protein hydrolysate has lipid peroxidation inhibitory activity attributed mainly to the low-molecular weight (3 kDa) peptide (Karki, Maurer, & Jung, 2011; Park, Lee, Baek, & Lee, 2010), and peptide fragments with molecular weights of either 14.4 or 8–9 kDa exhibit high Ca^{2+}-binding capacity (Bao, Song, Zhang, Chen, & Guo, 2007). Soybean hydrolysate shows high peptide content and antioxidant activity (Sefatie, Fatoumata, & Eric, 2013). However, the overall benefits of soybean hydrolysate in comparison with FSBM remain unclear. Although soybean hydrolysate can decompose the protein-based ANFs, the carbohydrate-based ANFs do persist. Considering the commercial value improvement, solid-state fermentation remains much more economical and beneficial than other methods. It has been reported that *Bacillus* sp. *Lactobacillus* spp., and *Saccharomyces cerevisiae* could reduce the level of ANFs and improve the nutritional value of soybean products (Frias et al., 2008; Hansen, 2012; Murashita et al., 2013; Seo et al., 2011; Zhao, Huang, Cai, Hong, & Wang, 2014). However, there are needs of more information on the nutritional effects of using solid-state fermentation on SBM.

In this study, we evaluated the changes in nutritional quality, anti-oxidative activity and metal-chelating ability of FSBM depending on the strain chosen for solid fermentation and made a comparison.

2. Materials and methods

2.1. Strains

Bacillus amyloliquefaciens U304 was isolated from the Korean traditional soybean paste, Cheonggukjang and grown in GYP medium (1% glucose, 0.8% yeast extract, 0.2% soy peptone) at 37 °C with shaking. *Lactobacillus acidophilus* and *Lactobacillus plantarum* were isolated from the Korean traditional fermented vegetable, Kimchi and grown in MRS medium (Difco) at 37 °C anaerobically. *Saccharomyces cerevisiae* CJ1697 was isolated from the Korean rice wine, Makgeolli and cultured in GYP medium 30 °C with shaking. When the optical density at 660 nm for the growing cultures exceeded 5, the strains were used in solid-state fermentation.

2.2. Fermentations

SBM with moisture content 45% was steamed at 100 °C for 30 min in an autoclave. After inoculation with a 10% ratio of *B. amyloliquefaciens U304* to *L. acidophilus, L. plantarum*, or *S. cerevisiae* CJ1697, it was incubated at 37 °C for 24 h, 37 °C for 36 h anaerobically, or 30 °C for 48 h, in a constant temperature and humidity test chamber, respectively. FSBM was then dried at 60 °C for 12 h and ground and then used for analysis.

2.3. Proximate analysis

Moisture, protein, ash and fat content of FSBM were determined by the official methods and recommended practices of the American Oil Chemists' Society (AOCS, 2006). Crude protein was measured by the Kjeldahl method using the automatic Kjeltec TM8400 system (Foss, Denmark) by AOCS Ba 4d-90. Ash was determined by AOCS Ba 5a-49 and fat was measured by AOCS Ba 3-38. The nitrogen solubility index (NSI) was measured by AOCS Ba 11-65. Shortly, sample was mixed with 40 volumes of distilled water and incubated at 30 °C for 120 min with agitation, then centrifuged at 1500 g for 10 min. The nitrogen content of supernatant and sample was measured by Kjeldahl method. The NSI was calculated as follows:

$$\mathrm{NSI}(\%) = \frac{\text{nitrogen content of supernatant}}{\text{total nitogen content of sample}} \times 100$$

Methionine and lysine were determined after acid hydrolysis with 6 N HCl for 24 h at 110 °C by high-performance liquid chromatography (Rayner, 1985). Samples were assessed in triplicate.

2.4. Antinutritional factors

Trypsin inhibitor (TI) was determined by AOCS Ba 12-75 using BAPNA (Benzoyl-DL-arginine-p-nitroanilide) as substrate (AOCS, 2006). The level of raffinose and stachyose was analyzed by thin layer chromatography (TLC). The samples were extracted in 20 volumes of water and centrifuged at 10,000 g for 5 min following filtration using a 0.22-μm filter. The mobile phase consisted of 50% ethyl acetate and 35% acetic acid.

2.5. Protein molecular weight distribution

2.5.1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

We used 8 M urea as a solvent to extract proteins with a sonicator (Bandelin, GM 2070, 70 W power, BR, Germany) at 35% power. After centrifugation at 6000 g for 10 min, protein concentration in the supernatant was measured by the BCA assay (Sapan, Lundblad, & Price, 1999). The samples were denatured with 5 × Tris-glycine SDS loading buffer, and 30 μg of proteins were loaded and separated using a 10% gel for SDS-PAGE (BIO-RAD, Hercules, CA, USA) at 90 V. Protein bands were visualized by staining with Coomassie Brilliant Blue R250 (Sigma, St. Louis, MO, USA).

2.5.2. Peptide content assay

To calculate the peptide contents, we performed gel permeation chromatography (GPC). The protein were extracted as described for SDS-PAGE, and centrifuged at 10,000 g for 5 min following filtration with a 0.22-μm polyvinylidene fluoride filter. The analysis was performed using an Agilent HPLC system with a Superdex 75 10/300 GL column (GE Healthcare, BKM, UK), and the protein was detected at 214 nm.

2.6. Anti-oxidative activity assay

2.6.1. Preparation of solvent extracts

For the anti-oxidative activity assay, SBM and FSBMs were extracted twice by shaking at 30 °C for 3 h with 70% (v/v) ethanol (1:10, w/v) and filtered through Whatman No.1 filter paper (Whatman, Maidstone, UK) and freeze-dried.

2.6.2. Total phenolic content

The total phenolic content of the samples extracted with ethanol