World Journal of Dairy & Food Sciences 5 (2): 114-118, 2010
ISSN 1817-308X

Physicochemical and Nutritional Analysis of Fermented Soybean Protein Meal by *Lactobacillus plantarum* Lp6

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Abstract: This study investigated some physicochemical and nutritional analysis of solid state fermented soybean protein meal by *Lactobacillus plantarum* Lp6. The extracts were investigated for changes in amino acid composition, Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) assay and scanning electron microscopy analysis (SEM). The amino acid compositions and the estimated nutritional parameters showed significant variations among the samples data. The fermented soybean protein meal (FSPM) showed higher total free amino acid (8.86 g/100 g sample) compared to 0.33 g/100g sample obtained for unfermented soybean protein meal (Control). The results of SEM showed an empirical decrease in protein aggregates and the SDS-PAGE showed considerable decrease in the intensity of molecular size of polypeptide bands.

Key words: *Lactobacillus plantarum* Lp6 · Fermented soybean protein meal · Nutritional analysis · SDS-PAGE · Electron microscopy analysis

INTRODUCTION

Plant proteins are an abundant and relatively inexpensive source of proteins that are widely recognized due to their high nutritional value and excellent physicochemical properties. It is well known that plant proteins are an alternative to proteins from animal sources for human nutrition. Legumes are recognized as the best source of vegetable protein. Among legume proteins, soybean protein is one of the best proteins and has been extensively studied and processed [1]. Fermentation is one of the oldest technologies used for food preservation. However, in recent years, fermentation became one of the cheapest methods to improve nutritional values of legumes protein sources [2]. Free amino acids and bioactive peptides can be released by the microbial activity of fermented food or through enzymes derived from microorganism [3]. In the most cases effort has been directed towards improving the diets of people by the improvement of the protein content and quality of cereals through the addition of protein enriched foods, protein concentrates and/or essentials amino acids [4,5].

Studies have confirmed the degradation of soybean allergens during fermentation by microbial proteolytic enzymes in soy sauce, miso, soybean ingredients and feed-grade soybean meals [6-8]. Fermentation of legumes has been reported generally to improve nutritional and functional properties compared to original products [9]. Frias *et al.* [10] reported that soybean flour fermented with *Lactobacillus* sp. (*L. plantarum*) was able to further break down and use available proteins as nutrient sources thereby enriching the fermented product.

The objective of this study was to evaluate the physicochemical and nutritional composition of soybean protein meal before and after fermentation by *Lactobacillus plantarum* Lp6.

MATERIALS AND METHODS

Materials: Man-Rogosa-Sharpe (MRS) broth and commercial soybean protein meal were purchased from Shensi Biotech Co. Ltd (Shanghai, China) and Sun-Green Biotech Co. Ltd (Nantong, China), respectively. The strain *Lactobacillus plantarum* Lp6 was obtained from the culture collection of Jiangnan University (Wuxi, China)

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and molecular weight marker was purchased from Shanghai Institute of Biotechnology (Shanghai, China). All other chemicals were of analytical grade.

Fermentation and Preparation of Fermented Soy Protein Meal Hydrolysate Extract: The microorganism *L. plantarum* Lp6 used was stored initially at 4°C and cultured for 18 h at 37°C in MRS broth prior to use for fermentation. A 0.025 mL of *L. plantarum* Lp6 was prepared in sterilized distilled water and then mixed with 25 g of soybean protein meal (10^7 cfu/g) fortified with soluble starch (0.4 g/g of SPM) in polyethylene bag (140 mm × 200 mm) and vacuum sealed. Disodium phosphate (2 mg/g) was added to improve the activity of *L. plantarum* Lp6 and then solid-state fermentation was performed for 72 h at 37°C.

FSMP extract was prepared according to the method described by Ye *et al.* [11]. Five grams of fermented soy protein meal were mixed with 50 mL of distilled water, homogenized for 1 min and incubated at 37°C for 60 min. The incubated mixture was centrifuged at 9600 rpm for 2 min and the residue was washed with 20 mL distilled water, centrifuged again at the same speed and time and the combined supernatant was freeze-dried and stored at-20°C until further use.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE): SDS-PAGE of samples carried out using discontinuous system described by Laemmli [12] with 4% stacking and 12% separating gel. Separating gel was run at a constant current of 20 mA for about 3 h. The gel was stained in Coomassie Brilliant Blue R-250. Subunit Molecular Weight (MW) was estimated using low MW calibration kit (Shanghai Institute of Biochemistry, Shanghai, China) consisting of the following proteins: phosphorylase (97.4), bovine serum albumin (66.2), rabbit actin (43.0), bovine carbonic anhydrase (31.0), trypsin inhibitor (20.1) and hen egg white lysozyme (14.4) kDa.

Scanning Electron Microscopy: Scanning electron microscopic (SEM) studies of SPM and FSPM were carried out using scanning electron microscope (Quanta-200 FEI, Netherland). The samples were coated before loaded to the scanning electron microscopy. The coated samples were loaded into the system and the image was viewed under 5.0 KV potential using secondary electron image. The image was captured using 11.1 mm Ricoh Camera of 600x Mag.

Amino Acid Analysis: The freeze-dried samples were digested with HCl (6 M) at 110°C for 24 h under nitrogen atmosphere. Reversed phase high performance liquid chromatography (RP-HPLC) analysis was carried out in an Agilent 1100 (Agilent Technologies, Palo Alto, CA, USA) assembly system after precolumn derivatization with o-phthaldialdehyde (OPA). Each sample (1 μL) was injected on a Zorbax 80 A C18 column (i.d. 4.6 × 180 mm, Agilent Technologies, Palo Alto, CA, USA) at 40°C with detection at 338 nm. Mobile phase A was 7.35 mmol/L sodium acetate/ triethylamine/tetrahydrofuran (500:0.12:2.5, v/v/v), adjusted to pH 7.2 with acetic acid, while mobile phase B (pH 7.2) was 7.35 mmol/L sodium acetate/methanol/acetonitrile (1:2:2, v/v/v). The amino acid composition was expressed as g of amino acid per 100 g of protein.

Parameters of Nutritional Quality: The estimated nutritional parameters of SPM and FSPM were determined by using their amino acid composition including (1) proportion of essential amino acids (E) to the total amino acids (T) of the protein; and (2) amino acid score (AAS) = (mg of amino acid /g of test protein/mg of amino acid/g of FAO/WHO/UNU standard pattern) × 100. The FAO/WHO reference pattern of essential amino acid requirements (g/100g of protein) (FAO/WHO 2007) was used as the standard. (3) Predicted protein efficiency ratio (PER) values. The estimated predicted PER values of SPM and FSPM were carried out in accordance with Alsmeyer *et al.* [13], using three regression equations.

PER-1 = -0.684 + 0.456 (Leu) -0.047 (Pro)
PER-2 = -0.468 + 0.454 (Leu) -0.105 (Tyr)
PER-3 = -1.816 + 0.435 (Met) + 0.780 (Leu) + 0.211 (His) - 0.944 (Tyr)

RESULTS AND DISCUSSIONS

SDS-Page: Figure 1 shows the SDS-PAGE profiles of fermented and unfermented soybean protein meal samples under reducing conditions. The control sample as well as fermented soybean protein meal (FSPM) showed similar banding patterns containing about seven polypeptides with estimated MWs ranging between 14.4 to 83.0 kDa. The fermented sample with *L. plantarum* Lp6 showed the degradation of the thicker bands than the control sample (SPM). The microorganisms have been long considered as a good enzyme source. However, the reduction of major polypeptides in the FSPM sample showed the effect of

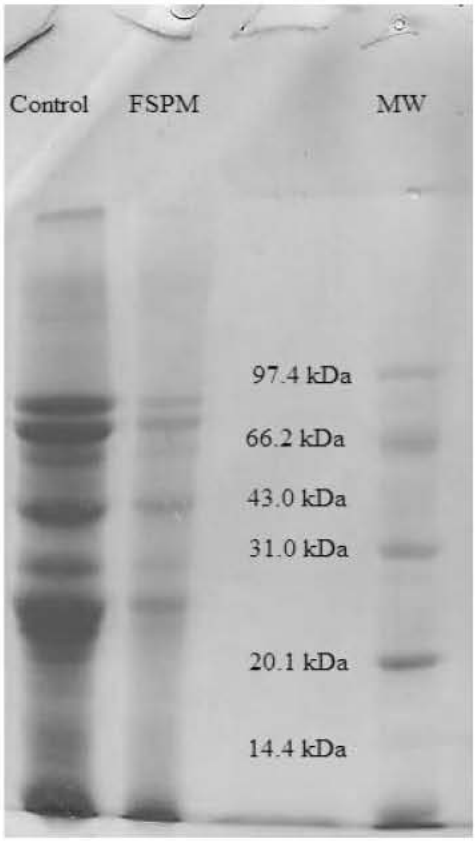

Fig. 1: SDS-PAGE profile of soybean protein meal before and after solid state fermentation. MW: standard molecular-weight marker; Control: unfermented soybean protein meal, FSPM: fermented soybean protein meal

fermentation on protein sizes (Figure 1), this is in accordance with results reported by Hong *et al.* [6]. The absence of high molecular weight polypeptides in FSPM may be attributable to the degradation of polypeptide chains by the proteolytic enzymes from *L. plantarum* Lp6.

Scanning Electron Microscopy: Scanning Electron Microscopy was used to examine the micro structural changes of proteins hydrolysis after the fermentation. Figure 2 shows the SEM pictures of unfermented soybean protein meal and fermented SPM, respectively. The data shows that the protein has degraded into small fragments after the fermentation. Also there is a reduction in the particle size of the meal after fermentation (Figure 2b) compared to unfermented meal (Figure 2a). The results showed in SEM are normally empirical; Figure 2b is the decrease in protein aggregates.

Amino Acid Analysis: The amino acid composition of the control sample and that of FSPM are presented in Table 1. It was previously reported that lactic acid fermentation of soybean meal resulted in protein hydrolysis [14] and increased liberation of free amino acids. Since fermentation affected the protein size (Figure 1), the free amino acid content of fermented soybean meals increased significantly from 0.33 to 8.86 g/100g protein. However, fermentation of SPM did not affect the contents of most essential amino acids, including histidine, threonine, methionine and

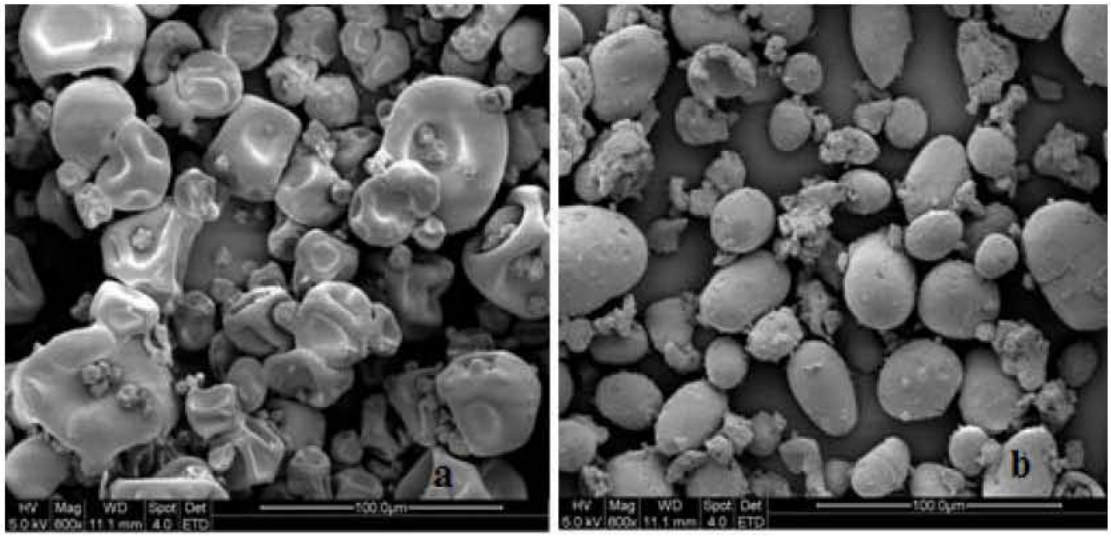

Fig. 2:

Table 1: Amino acid scores of Fermented soybean protein meal and Fermented soybean protein meal (g/100g sample)

Essential amino acid (EAA)			FAO/WHO/UNU[a]		Non-Essential amino acid (nEAA)		
EAA	[b]Control	[c]FSPM	Child	Adult	nEAA	Control	FSPM
Histidine	2.53	2.54	1.6	1.5	Alanine	4.27	4.29
Isoleucine	5.16	5.48	3.0	3.0	Arginine	7.80	7.09
Leucine	8.10	8.40	6.0	5.9	Aspartic acid[f]	11.23	11.59
Lysine	6.71	6.34	4.8	4.5	Cysteine-s[g]	0.50	0.38
Met + Cys[d]	2.48	1.89	2.3[d]	1.6[d]	Glutamic acid[h]	20.95	20.88
Threonine	3.56	3.26	2.5	2.3	Glycine	4.21	4.25
Phe + Tyr[e]	8.78	8.36	4.7[e]	3.8[e]	Serine	4.70	3.42
Tryptophan	1.06	1.00	0.66	0.6	Tyrosine	3.24	2.67
Valine	5.55	5.94	2.9	3.9	Proline	3.57	3.85

[a]FAO/WHO/UNU energy and protein requirements (2007), [b]Soybean Protein Meal, [c]Fermented Soybean Protein Meal. [d]Requirements for methionine + cysteine.[e] Requirements for phenylalanine + tyrosine. [f]Aspartic acid + asparagines, [g]Cysteine + cysteine, [h]Glutamic acid + glutamine.

Table 2: Nutritional evaluation of fermented and unfermented soybean protein meal

Parameters	Control[a]	FSPM[b]
Amino Acid Score (AAS)		
Histidine	158.13	158.75
Threonine	142.40	130.40
Valine	191.38	204.83
Met + Cys	107.83	82.17
Phe + Tyr	186.81	177.87
Isoleucine	172	182.67
Leucine	135	140
Lysine	139.80	132.08
Tryptophan	176.67	166.67
E/T (%)	43.06	43.26
Estimated PER		
PER-1	2.84	2.96
PER-2	2.87	3.07
PER-3	2.60	3.41
Total Free Amino Acid	0.33	8.86

[a] Control: Soybean Protein Meal, [b] FSPM: Fermented Soybean Protein Meal, AAS: amino acid scores, E/T: proportion of essential amino acids (E) to total amino acids (T), PER: predicted protein efficiency ratio.

phenylalanine, whereas the contents of leucine, isoleucine, valine, aspartic acid and proline increased after fermentation (Table 1). Phenylalanine, alanine and glycine also tended to be increased after fermentation. Similar results were reported by Hong *et al.* [6] who investigated Food Soybeans and Feed Soybean Meals from Korea. The amino acid profiles of the two samples were generally higher in essential amino acid (EAA) profiles compared with the suggested pattern of requirement by FAO/WHO/UNU [15]. This indicates that solid-state fermentation with *L plantarum* Lp6 of SPM under optimum conditions leads to interesting healthy food for both adults and children.

Nutritional Quality Based on Amino Acid Composition: Protein is one of the essential nutrients in the human diet. Both the amount and quality of protein provided by a food are important. Many benefits are attributed to fermentation. It preserves and enriches food, improves digestibility and enhances the taste and flavor of foods [16]. The protein quality, also known as the nutritional or nutritive value of a food, depends on its amino acid content and on the physiological utilization of specific amino acids after digestion, absorption, assimilation and minimal obligatory rates of oxidation. Because direct assessment of protein nutritional value in human subjects is impractical for regulatory purposes, methods based on *in vitro* (Chemical) and animal bioassays for assessment of protein quality have been developed.

The ratio of essential to total amino acid, amino acid score, PER of Control and FSPM are shown in Table 2. Control and FSPM had a higher ratio of essential to total amino acid than the pattern recommended by WHO (at least 38%). The control and FSPM predicted significant values as it would be expected, 43.06 and 43.26%, respectively. Though the two samples' essential amino acid and their AAS exhibited high values (Table 2).

Predicted PER values of both samples exceeded 2.00, which describes a protein of good to high quality [17]. FSPM has the highest PER value compare to that of Control (Table 2). The PER values of FSPM and Control were rather satisfactory compared with a standard casein PER of 2.5 [17]. PER values of beach pea proteins was reported to range from 1.12 to 2.99 [18], which corroborate with our results. Fermentation of soybean protein meal, not only improve the physiochemical quality but also the nutritional quality.

In conclusion, fermentation of soybean meal could improve the nutritional characteristics of soybean meal. The FSPM obtained through fermentation by *Lactobacillus plantarum* Lp6 showed substantial liberation of free amino acids and formation of low molecular weight peptides. These results have suggested that FSPM were most likely to contain some bioactive peptides with good nutritional properties. Further investigations are going on in the bioavailability of the fermented soybean protein meal extract with *Lactobacillus plantarum* Lp6.

ACKNOWLEDGEMENT

This research was financially supported by Governments of Niger Republic and People's Republic of China. The authors wish to thank Dr Sun Jin for his constructive advice.

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