Solid State Fermentation of Soybean Meal with *Aspergillus Niger* For Upgrading Nutritive Values

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ABSTRACT

The experiment was conducted to observe the effect of different fermentation times on nutrient compositions and phytase activity of fermented soybean meal (FSBM) used solid state fermentation (SSF) by Aspergillus niger. A completely randomized design (CRD) was used in this experiment which was included three treatments and replicated three times per treatment. Soybean meal was used as substrate and inoculated with A. niger spore suspension. Two hundred and fifty ml capacity of Erlenmeyer flask was used to cultivate the culture. The fermentation times were 0, 3 and 5 days. The contents of dry matter, ash, ether extract, crude protein, gross energy and phytase activity of fermented soybean meal were determined. The percentages of crude protein, gross energy and phytase activity of day 5 FSBM were significantly ($p<0.05$) higher than those of day 3 FSBM. Fermented SBM at day 5 had numerically increased in the ether extract contents. However, the ash and dry matter percentages of day 5 FSBM were significantly ($p<0.05$) reduced compared with those of day 3 FSBM. According to the obtained results, the effect of different fermentation times on nutrient contents and phytase activity of fermented soybean meal were significantly different. Therefore, fermented SBM incubated at day 5 is an appropriate alternative feed from SBM to be used as nourishable feed and phytase enzyme production in fermented soybean meal which will being economic benefits to farmers.

Keywords: *Aspergillus niger*, Nutritional value, Phytase activity, Soybean meal, Solid state fermentation

1. INTRODUCTION

Soybean meal (SBM) is the principal source of protein for the feed industry worldwide (Sara, 2003). The production of SBM in worldwide is over 160 million tons (Tadeusz, 2010). Large proportion of diets in poultry industry consists of plant derived feedstuffs in which soybean is good protein sources rather than meat protein. Soybean protein contains unsaturated, essential fatty acids such as linoleic acid and linolenic acid (Hirabayashi, 1998). The soybean protein products acceptance is being increased in animal feed for the low cost and high nutritive value with a good amino acid balance (Frias *et al*., 2007). But SBM contains anti- nutritional factors that limit its application in animal feed (Song *et al*., 2010). Anti- nutritional factors of SBM are trypsin inhibitors, protease inhibitors, lectins, phytoestrogens, stachyose and raffinose, phytates, allergens, etc (Liener, 1994). Phytate in plant origin presents as phytate- phosphorus in feedstuffs which is only partially utilized by monogastrics that is reflected in the poor bioavailability of total

phosphorus (P) for pig (Cromwell, 1993). Two- thirds of phosphorus in soybean is bound as phytate (Liener, 2000).

The various soybean products are processed by fermentation and non- fermentation methods. They have been applied to improve the nutrient bioavailability of feed and reduce the wastage of the feed (Bimrew, 2014). Fermentation process leads to offer new fermented products (Kim *et al*., 2010) that it can enhance the production of growth promote factors (Yamamoto *et al*., 2007). From enormous traditional fermentations, solid state fermentation (SSF) offers numerous advantages over submerged fermentation (SmF) (Ashok *et al*., 2003). SSF is a process involves cultivation of microorganism on solid substrate with minimal moisture content (Ashoke *et al*., 2017) to improve the nutritional quality and to obtain edible products with palatable sensorial characteristics (Larroche and Gros, 1997). The SSF systems have been reported to be an effective way to enhance phytase production (Mandviwala and Khire, 2000).

Objectives of the experiment

The experiment was guided by the following objectives;

i. To determine the effect of different fermentation times on nutrient values of fermented and unfermented soybean meal.
ii. To compare the phytase enzyme activity of fermented and unfermented soybean meal with different incubation times.

2. METERIALS AND METHODS

2.1 Preparation of spore suspension and substrate

Aspergillus niger was obtained from Myanmar National Health Laboratory (NHL) and the characterization of the obtained *A. niger* was known by the phenotypic identification with gram staining method. Then it was cultured on potato dextrose agar (PDA, HIMEDIA) at 37°C for 3 days. *A. niger* spores were washed by adding 100 ml distilled water in PDA flasks. The concentration spores grown in spore suspension were monitored by haemocytometer.

Soybean meal used in this study was obtained from local market in Nay Pyi Taw, Myanmar. It was dried at 60°C overnight and the flasks contained soybean meal were sterilized in autoclave at 121ºC as well as cooled down in water bath.

There were three different incubation times (0 day, 3 days and 5 days). This experiment was consisted of three treatments and replicated three times in each treatment.

2.2 Solid state fermentation

Soybean meal substrates were mixed with distilled water to obtain 50% moisture. This substrate (50g DM) in each 300 ml conical flask was inoculated with 5 ml of spore suspension (contained at least 2 x 10^7). This

experiment was conducted in flasks covered with cotton plug to facilitate air transfer. This sample in flasks was incubated at 37'C for 3 days and 5 days and unfermented soybean meal (0 day) was served as control.

2.3 Proximate analysis

Dry matter, crude protein (using Kjeldahl method), crude ash (using Muffle- Furnace), crude fat (using Soxhlet extractor) and gross energy (using bomb- calorimeter) contents of unfermented and fermented soybean meal were determined by the method of AOAC (1995).

2.4 Phytase assay

The phytase activity of fungal culture in filtrate was assayed. The enzyme activity was determined by the release of phosphorus from sodium phytate substrate by the method Engelen *et al*. (1999). In this procedure, interval of adding reagents to every tube were completely coincided after the substrate was added to the reaction mixture.

2.5 Statistical analysis

The data collected in this study were analysed with ANOVA using general linear model (GLM) procedure of SAS® (2002) as a CRD experiment. The significant differences among treatment means were determined at $p< 0.05$ by Duncan's Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSIONS

3.1 The nutrient compositions of fermented and unfermented SBM

The dry matter (DM), crude protein (CP), ash, ether extract (EE) and gross energy (GE) contents of SBM and FSBM are shown in Table 1. There were significantly ($p<0.001$) different in the DM, CP, ash, EE and GE percentages of SBM and FSBM. The DM decreased in FSBM for different fermentation times with the minimum at day 5 and day 3 but the maximum DM was observed at day 0. It was agreed with Sardjono and Knol (1998) who concluded that the dry matter amount was apparently decreased (12.8%) in the fermented SBM with *Aspergillus oryzae* (after 96 hours). A similar observation was reported by Safari *et al*. (2012) also found that the dry matter percentage of fermented canola meal used *Aspergillus niger* was significantly reduced ($p< 0.001$) that compared with unfermented canola meal because the microorganism growth conditions optimized. Therefore, it might be due to exhibit the mycelia of microorganism growth in the biomass.

The CP percentages of FSBM and unfermented SBM were significantly different at $p< 0.001$. The CP was increased in FSBM for different durations of fermentation with the maximum at 5 days, 3 days and at 0 day after fermentation. This protein increase may be attributed to the carbohydrate and protein in the SBM used for microbial growth during fermentation. Increasing the protein content of fermented substrates that is why the fermentable carbohydrates with fungi, bacterial and yeast were consumed in order to produce protein biomass (Rozan *et al*., 1996; Pal vig and Walia, 2001).

The ash contents were significantly ($p< 0.001$) different among different fermentation times. The ash content of FSBM was increased in 5 days FSBM compared with that of unfermented SBM. The results of ash content obtained in this experiment are relevant to the data from Larine *et al*. (2012). They concluded that the different fermentation times had affected on the ash percentage of fermented rice bran by *Rhizopus oryzae* when compared with unfermented rice bran. Feddern *et al*. (2007) also found that the ash content of fermented rice bran was increased 10.5 % after 6 hr of fermentation with *Saccharomyces cerevisiae*.

The ether extract of FSBM did not significantly differ between fermentation times. There was numerically increase in ether extract contents of day 5 and day 3. The lowest amount of ether extract was found in day 0. Fermented SBM by *A. niger* also exhibited an increase in ether extract with increased the incubation times in this study. The increase in fat contents may be partly caused by the decreased carbohydrate content after fermentation. Carbohydrate in SBM may be used as substrates for energy and synthesis of fatty acids. The increase in the fat content might be due to the increase in the microbial mass, activities of lipolytic microorganisms to secrete extracellular enzyme (lipase), secretion of microbial oil into the fermenting medium and other products from the metabolism (Oboh *et al*., 2002). Similar finding reported by Hong *et al*. (2004) who demonstrated that crude fat contents in soybeans and soybean meal also increased after *A. oryzae* fermentation.

Table 1 Nutrient composition of fermented and unfermented soybean meal

Nutrients (DM % basis)	Treatments (Mean)			P-value
	Day 0	Day 3	Day 5	
DM (%)	85.17^{a}	37.48^{b}	33.95^{c}	0.000
CP (%)	52.07^{c}	56.43^{b}	65.03^{a}	0.001
Ash (%)	8.26^{c}	10.70^{b}	12.31^{a}	0.001
EE (%)	1.14	1.20	1.21	0.907
GE (kcal/kg)	4226.44^{c}	4380.71^{b}	4589.75^{a}	0.002

^{a,b,c} means with different superscript within the same column differ significantly ($p< 0.05$)

Gross energy value of fermented and unfermented SBM were significantly ($p<0.01$) different. The gross energy of unfermented SBM was significantly lower than that of 3 days FSBM. The highest amount of gross energy was observed in 5 days FSBM. The amounts of gross energy were significantly increased in fermented SBM that are expressed in Table 8. This finding is in line with Lawal *et al*. (2013) who suggested that the undegraded and degraded rice offal with SSF by *Aspergillus niger* reported improving the gross energy averagely 2% after 7 days biodegradation. The effect of prolonged biodegradation on gross energy of SBM might be due to the oxidation of ether extract which produced considerable amount of energy for the organisms

3.2 Phytase activity of fermented and unfermented soybean meal

The enzyme activities of SBM for different fermentation times are shown in Table 2. The phytase enzyme activity of different fermentation times were significantly ($p< 0.001$) different. The highest activity was observed at day 5 fermentation and the lowest at day 0 fermentation. The enzyme activity of FSBM at day 5 was significantly ($p< 0.001$) higher than that of FSBM at day 3 fermentation. Result of this study showed that phytase production increased progressively with different incubation times compared to unfermented SBM. Similar finding was recorded by Edune *et al*. (1995) who stated that the phytase production of fermented canola meal used SSF by A. ficuum was noticed at 36 hr of fermentation with an increase in homogenization time. Saithi and Tongta (2016) also reported that the phytase activity of fermented SBM by *A. niger* increased with prolonged fermentation time.

In present study, the phytase activity of FSBM was increased with prolonged incubation times and the optimum temperature (37°C) used by degrading the phytate which is a major nutrient inhibitor included in SBM. The incubation period and temperature influenced the metabolic activities of fungus (Ramachandran *et al*., 2005). However, the optimum temperature range for incubation of microorganisms in the high phytase production is 25- 37°C (Gautam *et al*., 2002). In the study of Tian *et al*. (2016), the potato waste incubated for different periods ranging from 48 to 144 hr. They concluded that the maximum amount of phytase activity was observed after 144 hr of incubation so incubation period had extremely significant influence ($p < 0.01$) on phytase activity. Soni and Khine (2007) observed the maximum phytase activity at 4-5 days of fermentation by *Aspergillus oryzae* K1. Contrasted with Ramachandran *et al*. (2005) and Wang *et al*. (2011), they found that longer incubation period did not result in significant increase in enzyme production because of reduction in nutrients in the substrate.

Table 2 Phytase activity of fermented and unfermented soybean meal

Descriptions	Treatments (Mean)			P- value
	Day 0	Day 3	Day 5	
Enzyme activity	1.40^{c}	28.99^{b}	32.06^{a}	0.000

^{a,b,c} means with different superscript within the same column differ significantly ($p< 0.05$)

4. CONCULSION

In this study the optimization of nutrient composition, gross energy contents and phytase activity at the fermentation times of day 5 were carried out by fungal strain of *A. niger* through solid state fermentation. Compared to the different fermentation times, day 5 fermented soybean meal proved a good substrate for use as animal feed and it could provide advantages to the nutrient enhancement for mono gastric animals. The enzyme results indicate that the fermented soybean meal at day 5 had the beneficial effects to increase the digestion of mono gastric animals and to reduce manure- born phosphorus pollution.

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