Study of Effect of Mixed Culture of Probiotic Microorganisms on the Efficiency of Fermentative Hydrolysis of Soybean Processing Products

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Abstract. Soybean meal (SM) is the main protein source for animals. However, certain anti-nutritional factors such as trypsin inhibitor, glycinin, raffinose, stachyose and others reduce its nutritional value, and inhibit the growth and development of animals. Fermentation of soybean meal is an economical alternative which improves nutritional properties of soybean meal due to both biodegradation of anti-nutritional factors, proteins, fibers, and probiotic and prebiotic production, which can subsequently improve taste and digestibility of nutrients. The purpose of this work is to study the effect of a selected consortium of probiotic microorganisms on the composition of fermented soybean meal (FSM). Experiments showed that the introduction of a mixed culture including strains Bacillus subtilis B7046, Aspergillus niger F1270, Saccharomyces cerevisiae Y4659, Lactobacillus plantarum K9 B546 led to an increase in the content of easily digestible protein in the fermented soybean meal and a decrease in the content of anti-nutritional factors.

1. Introduction
Fermentation of SM is the most economically efficient means which can improve the nutritional properties of SM not only by biodegradation of anti-nutritional factors (such as trypsin inhibitors, oligosaccharides and phytic acid), the increased content of easily digestible protein, but also by producing probiotics and prebiotics which can subsequently improve taste, digestibility of nutrients, and immunity of agricultural animals. In addition, it has been noted that the fermentation of SM increases the protein content in it, promotes antimicrobial and antioxidant activities [1]. The replacement of animal protein with forage one based on FSM is known to lead to improved intestinal microflora and weight gain in animals which are fed by this product [2]. These positive effects can be associated with the improved nutritional value of fermented soybean meal and eliminated anti-nutrients due to the microbial fermentation [3].

Fermented soybean meal can replace other protein isolates in the diet of animals, stimulating their growth and reducing the incidence of gastrointestinal diseases, allergy and mortality. Nutritional value of soybean meal can be improved through microbial hydrolysis which is a very important task for the agricultural industry [4].

By now, a large number of the microorganisms hydrolyzing soybean meal were tested, and it was found that the nutritional value of the obtained FSM varied depending on the microorganism type and
technological parameters [5]. Many strains of the microorganisms such as Lactobacillus spp., Bacillus spp. and Aspergillus spp. [6–8] which are used to ferment products and wastes have a high potential of using in the agricultural sector and aquaculture, since the efficiency of fermentation by these strains determines the final nutritional value of FSM [9].

The purpose of this work is to study the effect of a selected consortium of probiotic microorganisms on the FSM composition, in order to obtain a forage supplement with an increased protein content and reduced anti-nutritional factors.

2. Materials and methods

2.1. Soybean fermentation

Microbial fermentation of the SM was performed in the following way. Before the fermentation, a 30 g sample of the SM was placed in a 250 ml glass flask, and 130 ml of distilled water was added. Then the soybean meal was autoclaved at 121 °C for 15 minutes. After the sterilization, the sample was cooled and, under sterile conditions, 1 ml of culture liquid was added, which contained microorganisms (Bacillus subtilis B7046, Aspergillus niger F1270, Saccharomyces cerevisiae Y4659, Lactobacillus plantarum K9 B5466). The culture liquid was provided by the All-Russian Collection of Industrial Microorganisms National Bioresource Center. The fermentation was carried out at 37°C and constant stirring (180 RPM) for 3–5 days.

The parameters of the SM microbial fermentation were selected according to the methods described by foreign authors [10].

Then the FSM was freeze-dried and ground to a powder.

2.2. Analysis of crude protein content

The efficiency of the fermentation process was evaluated by the crude protein content in the sample for absolute dry substance using the Kjeldahl method (GOST (State Standard) 10846-91. Grain and Its Processed Products. Method for Protein Determination). The essence of the method lies in the mineralization of organic matter with sulfuric acid in the presence of a catalyst with the formation of ammonium sulfate, the destruction of ammonium sulfate with alkali with the release of ammonia, the distillation of ammonia with water vapor into a solution of sulfuric or boric acid, followed by titration. Then the mass fraction of nitrogen and the crude protein content are calculated (multiplied by a factor of 6.25).

Also, Bradford protein assay was used for comparison. This method is simple to use, inexpensive, however, it allows quantifying small amounts of protein. It is a colorimetric analysis method based on the reaction of a Coomassie dye with arginine and hydrophobic amino acid residues.

2.3. Polyacrylamide gel electrophoresis of proteins

Extracts of crude protein isolated from the FSS by the method based on simultaneous precipitation and denaturation with trichloroacetic acid and 2-mercaptoethanol in cold acetone [11] were separated by electrophoresis in polyacrylamide gel with sodium dodecyl sulfate. The Thermo Scientific Unstained Protein Molecular Weight Markers (Thermo Fisher Scientific Inc.), a mixture of seven purified proteins from 14.4 kDa to 116 kDa, was used as a standard for molecular weights of proteins. After the electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250 (Thermo Fisher Scientific Inc.).

3. Study of effect of microorganisms on protein content in soybean meal

The effect of the consortium of probiotic microorganisms as well as of particular strains of microorganisms contained in it on the FSM protein content was studied. From the presented materials obtained by the Kjeldahl method, it can be seen that the introduction of the consortium of microorganisms increases the protein content in comparison with the non-hydrolyzed SM by more than 33%. Among the particular strains, the highest protein content was observed in the FSM with the
addition of *Bacillus subtilis* B7046: the protein content growth increased by 31.9% compared to the native SM. Also, with the introduction of isolated cultures of microorganisms of the strains *Aspergillus niger* F1270, *Saccharomyces cerevisiae* Y4659 and *Lactobacillus plantarum* K9 B5466, the content of crude protein increased by 19.8%, 26% and 25.15%, respectively.

Protein was quantified using the Bradford protein assay. The highest protein content is observed in the FSM fermented by the consortium of microorganisms. This value is 28.21%, which is almost two times higher than this value in the untreated SM. With the introduction of the particular strains of microorganisms, the protein content in the FSM increased by more than 20% (figure 1).

![Figure 1](image1.png)

**Figure 1.** Effect of consortium of probiotic microorganisms, as well as particular strains of its contained microorganisms on the protein content in FSM samples.

When studying the protein profile of the fermented soybean meal on electrophoregrams, low molecular weight peptides with a mass of 6–10 kDa are observed in the samples of the FSM. In the sample of the unfermented SM there are a large number of high molecular weight fractions which include allergens and anti-nutritional factors (figure 2).

![Figure 2](image2.png)

**Figure 2.** Electrophoregram of fermented soybean meal (1 – marker of protein molecular weight, 2, 3 – control without microorganisms; 4, 5 – fermented soybean meal (*A. niger*); 6, 7 – fermented soybean meal (*B. subtilis*); 8, 9 – fermented soybean meal (*S. cerevisiae*); 10, 12 – fermented soybean meal (mixed culture); 11 – marker of protein molecular weight; 13 – unfermented (native) soybean meal).
4. Conclusion

In the course of the study we demonstrated that the introduction of a consortium of probiotic microorganisms (Bacillus subtilis B7046, Aspergillus niger F1270, Saccharomyces cerevisiae Y4659, Lactobacillus plantarum K9 B5466) led to an increase in the content of crude protein in FSM in comparison to the action of isolated strains. Thus, during hydrolysis by the consortium of microorganisms the content of crude protein increased by more than 30%, while during hydrolysis by the isolated strains the content of crude protein increased by more than 19%. Also, during the process of the SM fermentation high molecular weight peptides are degraded to low molecular peptides with the weight of less than 6–10 kDa. In the unfermented SM there is a large number of high molecular weight peptides which include allergens.

These results allow us to suggest that FSM is a promising source of easily digestible forage protein for the livestock industry as it can improve the productivity of animals.

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