The Influence of Fermentation Time in the Physical and Chemical Composition of Fermented Soybean Husk by Using *Aspergillus niger* on the Quality of Raw Feed Materials

Muhammad Ikhwan Ihtifazhuddin[1*], Happy Nursyam[2], Arning Wilujeng Ekawati[3]

[1]Master Program of Aquaculture, Faculty of Fisheries and Marine Sciences, University of Brawijaya, Malang, Indonesia
[2]Department of Aquaculture, Faculty of Fisheries and Marine Sciences, University of Brawijaya, Malang, Indonesia
[3]Laboratory of Fishery Safety, Faculty of Fisheries and Marine Sciences, University of Brawijaya, Malang, Indonesia

Abstract

Soybean husk (*Glycine max L*. Merrill) a soybean processing waste as raw material for *tempe* obtained after the process of boiling and soaking soybeans. The main problem in the use of soybean husk (*Glycine max L*. Merrill) as feed material is its crude fiber content which is fairly high. This study aimed to observe the fermented soybean husk using *Aspergillus niger* to improve the quality of the raw feed materials. This was conducted by using completely randomized design (CRD) analysis and repeated three times; the time optimization of *Aspergillus niger* in 2, 4, and 6 days based on chemical analyses (moisture, protein, fat, ash, crude fiber and feed containing carbohydrates (NFE) and physical assessment fermentation (smell, texture, moisture and hyphae) were analyzed descriptive qualitatively. The results showed that 4 days fermentation of soybean husk using *A. niger* is successful gives the highest score based on physical characteristics texture, aroma, moisture, and the formed hyphae and the most effective treatment for decrease in crude fiber is 13% and increase in NFE contained in the largest on 4 days fermented soybean husk by *Aspergillus niger* with a long time 4 days.

Keywords: *Aspergillus niger*, fermentation, soybean husk.

INTRODUCTION

Soybean husk is a waste that is produced from the process of boiling and soaking soybeans which were used as the materials to make *tempe*. After going through the process, the husk will be separated and will normally be thrown away by the *tempe* producer.

Based on the analysis in the Laboratory of Biochemistry and Nutrition Fish, Faculty of Fisheries and Marine Sciences, Brawijaya University that soybean husk (*Glycine max L*. Merrill) has a water content of 12.45%, 14.32% protein, 38.35% crude fiber, 2.32% fat, 4.14% ash and 2.42 kkal.g^{-1} energy. Therefore, soybean husk still has the potential to be used as a feed for animals considering that it has a high protein and energy [1].

The main problem in the use of the soybean husk (*Glycine max L*. Merrill) as a raw material is fairly high cellulose content of around 33.49% [2]. Further explained that the soybean husk (*Glycine max L*. Merrill) contains 10-20% hemicellulose, 29-51% cellulose, 1-4% lignin and 6 -15% pectin [3].

* Correspondence author:
Muhammad Ikhwan Ihtifazhuddin
Email : ichwannfeng@gmail.com
Address : Master Program of Aquaculture, University of Brawijaya, Veteran Malang, 65145

Technology to improve the quality of materials the feed is fermented [4]. Generally all fermentation end products usually contain compounds that are simpler and easier to digest than the original material thus increase the nutritional value [5]. The use of agricultural waste products as fermentation substrate is due to the mass-produced, the cost of which used lower and rich in nutrients [6].

Cellulase enzyme complex is composed of cellobiohidrolase, endoglucanase and β - glucosidases which all act synergistically to convert complex carbohydrates lignocellulosic biomass into glucose efficiently [7]. Cellulase can be produced by fungi, bacteria, and ruminants. Production of commercial enzyme normally uses fungi or bacteria. Fungi can produce cellulases include genus *Trichoderma*, *Aspergillus*, and *Penicillium* [8]. *Aspergillus niger* has been widely used because it produces the three fundamental enzymes required for cellulolysis [9].

Previous research the use of *A. niger* can decrease crude fiber is already done. Declared by the proximate analysis note that the content of crude fiber grout tofu before it is fermented in the amount of 24.03% and crude fiber content of the grout tofu out after fermentation between 0.04 to 0.16% [10]. This is supported by other research results [11], that the content of crude

fiber grout tofu 21.29% and after fermented decreased to 17.29%. A study mentioned that the fermentation 2 to 4 days can decrease the crude fiber [12].

The high fiber content and the lack of other nutritional content of the constraints of local feed use this as a source of alternative feed prospective. Thus, this study aims to observe different time duration in the physical and chemical composition fermentation soybean husk to improve the quality of fish feed as the feed materials.

MATERIALS AND METHODS

Soybean Husk Fermentation

Briefly, dried and ground soybean husk 100 g each were placed in a 600ml beaker glass and autoclaved at 121°C for 20 min [13]. After that, the soybean husk was densified by using dilution of 10^6 [14]. The soybean husk *(Glycine max L.* Merrill) was then added molasses in the ratio of 1:1 with the given mold dose [15] and the substrate was then stirred until it became homogeneous and mixed with sterile water until the water level reaches 70% [11]. Then, the tray was covered with *plastic wrap* and laid in the incubator at a temperature of 30°C with pH 5 [16].

Physical Assessment Fermentation

Physical Assessment Fermentation is used in order to determine differences in physical quality which appear, on the soybean husk that fermented and non-fermented. The scoring media of fermentation soybean husk started from 1 to 4 (Bad, Less good, Good and Excellent) where greater score indicated good fermentation [17]. In the scoring media of fermentation soybean husk is shown in table 1 below.

Table 1. Physical Assessment Fermentation

Score	Lumps (%)	Scent (%)	Water Steam (%)	Hyphae (%)
1	<10	>10	<10	<10
2	<10 - <25	>10 - <25	<10 - <25	<10 - <25
3	>25 - <40	>25 - <40	>25 - <40	>25 - <40
4	>40	<40	>40	>40

Notes: **Lumps** (Soft <10, Few <10-<25%, Some>25-<40%, A lot of >40%), **Scents** (No <10%, Slight >10-25<%, Normal >25-<40%, Strong <40%), **Water steam** (Dry <10%, Normal >10-<25%, Less >25-<40%, Moist >40%), **Hyphae** (No <10%, Few >10-<25%, Several >25-<40%, Many >40%).

Chemical analysis

The parameters observed in this research include chemical compounds contained in fermented soybean husk before and after being fermented through proximate test (moisture content, ash content, crude protein, crude fat, and crude fiber. The proximate analysis which was tested with the analysis of water content at a temperature of 105°C by using the oven for 6 hours, while the protein analysis was analyzed by using Kjeldahl method. Simultaneously, the fat was also analyzed by using soxhlet and petroleum ether in order to dissolve the fat. Crude fiber was assessed by using a solution of H_2SO_4 and NaOH as the solvent and the analyzation of the ash was carried out by using a furnace with a temperature of 600°C for 2 hours [18].

Statistical analysis

Statistical analysis used the analysis of variance (ANOVA). ANOVA was used to test the effect of the treatment and then further used the least significant difference (LSD) test at the level of 5%. In the other hand, the data of organoleptic test were analyzed descriptively qualitative.

RESULTS AND DISCUSSION

Successful Rate Fermentation

Based on the results, the best fermentation time was on the 4th day. The physical observation of the fermentation soybean husk includes texture, aroma, moisture, and the formed hyphae. The data scoring of fermentation soybean husk can be seen in Figure 1 below:

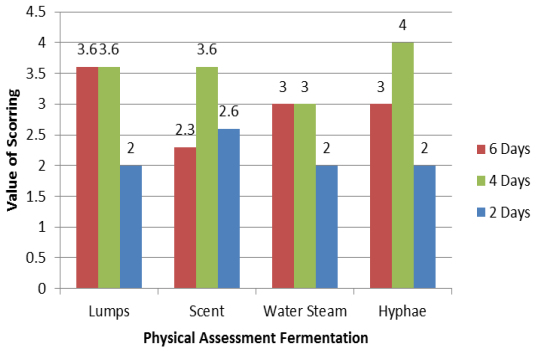

Figure 1. The average scoring value of physical assessment time fermentation containing ■ 6 days ■ 4 days ■ 2 days fermentation soybean husk.

The high scoring value indicated that the fermentation process has been going very well. The best result of the scoring occurred on the 4th day. On the 4th day gives the highest score based on physical characteristics lumps, scent, water steam, and the formed hyphae.

Moreover, the texture or lumps of the fermented soybean husk indicated the best result on the 4th day compared to other days. Fermentation causes the changing nature of the feed

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material including the texture; it is as a result of the content cleavage in the foodstuff caused by microorganisms that are in there [19]. Establishment of a texture is also influenced by water content, fat content, type and amount of carbohydrate food products [20].

Another thing is that in the 2nd and 4th day, the aroma of the fermentation soybean husk showed the best fragrance that is slightly sour and fragrant but it created an acid aroma and an odor of ammonia on the 6th day. Good fermentation has a sour and fragrant aroma [21]. Effect on the hydrolysis time increased levels of NH_3 (ammonia) giving rise to acid aroma [22]. Feed given additional fermentation may be associated with aroma and flavor that can affect the appetite of the animal [23].

In the fermentation with *A. niger,* water steam was formed on the 4 and 6th day because of an exothermic reaction when the process of organic material cleavage occurred. Fermentation process will produce CO_2 and heat as a result of the organic material breakdown [24]. The water content also affected the growth of mold and dynamics that occur during the process ensilase because water is required for the synthesis of protoplasm microorganisms and dissolved organic compounds [25].

Based on the results of the 2nd day, there were some very heavy hyphae throughout the fermentation media, and besides that, several spores grew well in some of the fermentation points. On the 6th day, hyphae began to decrease because the spores grew very much. Previous research also demonstrated that Coconut oil cake and palm kernel oil cake that were fermented with *A. niger* would have hyphae by 90% and spores by 10% on the edge of the spores on the 4th day [26]. Hyphae thrive but few spores that grow on the fourth day, so that the material is more easily digested and utilized because the spores increase fiber content material [5].

Improvement Nutrient Content

The results of the chemical analysis can be seen in Table 2 below. Based on the results of proximate treatment C (fermented for 4 days) are considered better, it can be seen from the decline in crude fiber contained in the largest C treatment is 13%. This observation is not as good as the research of leaves lamtoro fermented with *A. niger* crude fiber decreased by 46.61% for 3 days [5]. The decrease in crude fiber occurred due to *A. niger* has three essential enzymes needed for celluloses [9]. The increased crude fiber during the final phase of fermentation vegetable waste was due to the utilization of the nutrients provided by the mold and then the reduction can be attributed to breaking the non-starch polysaccharide for mold protein [27]. Crude fiber is part of a carbohydrate that difficult to digest by the digestion of fish, the higher the fiber in the diet then lowers the energy [28]. High crude fiber will give a sense of satiety because of the composition of complex carbohydrates that stops the appetite that caused a decline in food consumption [29]. The fiber content is too high will suppress growth [30].

Based on the proximate result of an increase in the value of the protein with the length of fermentation time, testing crude protein fermented soybean husk is the highest by a long period of 6 days at 17.02%. Vegetable waste and fermentation by using *A. niger* S14 for 8 days increased the protein by 38% [27]. Increased protein content after the fermentation process probably derived from *A. niger* which has synthesized the urease enzyme to break urea into ammonia and CO_2. This ammonia was then used for the mold to form amino acids (protein) [31].

In the 6th day fermentation, higher *A. niger* content was given and it resulted in fat degradation. It happened because the mold has achieved an exponential growth [12]. The decreased fat in fermented palm kernel oil cake flour occurred due to the conversion of fat into a single protein biomass [32]. Lipase enzyme produced by fungi greatly affects crude fat content after fermentation substrate because the enzyme lipase will remodel fat to be used by fungi as an energy source [33]. Microbial lipases have been used as a catalyst in producing oleochemicals-based products include fats or oils such as triglycerides modified low-calorie [34], so even though the resulting reduced oils or fats rich in EPA and DHA [35].

Increased NFE in the 4th day fermentation due to increased glucose as a result of fermentation of *A. niger* hydrolyze cellulose. Glucose levels continued to rise from 8 up to 64 hours, but declined to 72 in rice straw fermentation using *A. niger* [36]. *Aspergillus niger* also produce ß-glucosidase enzyme that is strong that this enzyme serves to accelerate the conversion of cellobiose widened glucose [37]. Carbohydrates are used as energy source of non-protein replacing protein as an energy source. If the feed shortage of non-protein energy then the fish will use a portion of the protein to insufficient energy needs [38]. Feed containing carbohydrates (NFE)

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exact to reduce the use of protein as an energy source known as protein sparring effect [39].

In the trials, the dry matter loss was about 7% in the present study. 20% dry matter loss after 96 h of fermentation of mixed oil cakes with *A. niger* 616 [40]. In a similar study with wheat bran, has also reported significant ($p < 0.05$) reduction in dry matter throughout the fermentation period for wheat bran using *A. niger* S14, suggesting utilization of nutrients present in the substrate by fungi for its growth and metabolic activities [41]. Feed given additional fermentation may be associated with aroma and flavor that can affect the appetite of animal and an additional fermentation in feed also provides an additional element of essential amino acids [23].

Table 2. The results of the fermentation soybean husk proximate analysis

Composition	Treatment			
	A	B	C	D
Dry content (%)	87,34±1,56[a]	82,75±0,58[b]	83,21±0,57[b]	82.12±0,39[b]
Ash (%)	4,14±0,30[a]	3,67±0,37[a]	3,91±0,31[a]	3.84±0,70[a]
Protein (%)	14,32±0,64[a]	15.13±0,05[a]	15,55±0,11[a]	17.02±0,18[b]
Fat (%)	2,32±0,43[a]	2,13±0,09[a]	2,03±0,14[a]	2.03±0,1[a]
Crude fiber (%)	38,35±0,65[d]	37,52±0,28[c]	33,45±0,39[a]	35.72±0,30[b]
NFE (%)*	40,87±0,78[a]	41.55±0,73[a]	45,06±0,67[b]	41.39±0,71[a]

Table 2. Result of observation towards chemical composition including dry content, ash, protein, fat, crude fiber and NFE in different time fermentation soybean husk towards A. (0 days), B. (2 days), C. (4 days) and D. (6 days). Values are means±SEM of three replicates (n=3); means in the same row followed by the same superscript letter are not significantly different (DMRT, $p>0.05$). *NFE (Nitrogen Free Extract) = 100-Protein-Fat-Fiber-Ash.

CONCLUSION

The results show that 4 days fermentation of soybean husk using *Aspergillus niger* is successful gives the highest score based on physical characteristics texture, aroma, moisture, and the formed hyphae and chemical analysis result the best time fermentation is 4 days the most effective treatment for decrease in crude fiber and increase in NFE contained in the largest on 4 days fermented soybean husk by *Aspergillus niger* with a long time 4 days. All of these changes enhance the value of the soybean husk as an animal feed, including aquafeed.

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