Proximate Analysis Of Maggot Flour Fermentation Results Using *Aspergillus niger* and *Trichodema viride*

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Abstract: The purpose of this study was to determine the nutritional properties of maggot flour fermented microbially using *Aspergillus niger* and *Trichoderma viride* fungi at different dosage, especially on increasing protein, decreasing fat and fiber. The maggot used is derived from culture in a mass on the oil palm cake media for 7-10 days, the maggot harvest is dried and then ground and sieved. As a fermentor material, *A. niger* is inoculated on PDA (Potato Dextrose Agar) media added with yeast extract 0.3% with a sloping agar method, for propagation using rice as a medium, while for *T. viride* using PCA (Plate Count Agar) media. Then taken as much as 15% and 20% for the fermentation process of maggot flour. Based on the results of proximate analysis, produced 43.27% crude protein, crude fat to 6.66% and crude fiber 14.11% in *A. niger* fungi, while *T. viride* results in protein content of 39.19%, the reduction in fat content is relatively small at 24.21%, the value of fiber content becomes lower that is 11.75. The highest percentage reduction in fat content in *A. niger*, and crude fiber in *T. viride*. The difference in dosage does not affect the results of proximate analysis of both *A. niger* and *T. viride*.

1. Introduction

The volume of aquaculture production at the level of ASEAN countries including Indonesia has increased [1]. Increasing aquaculture production thus automatically increases the need for fish feed. The Indonesian feed mill association estimates that the amount of fish feed production increased from 400,000 tons in 2006 to 1.38 million tons in 2013 [2]. As the population grows, aquaculture production continues to grow to meet the needs of the animal protein community. Increasing aquaculture production automatically increases fish feed requirements, especially in intensive aquaculture systems. This condition causes the price of feed to be expensive, while the cost of feed is the biggest component in fish farming. In addition, the feed provided must be of high quality, nutritious and meet the needs of the farmed fish, available continuously so that it does not require a production process and can provide optimal growth.

Fish meal as an important source of protein in feed formulations is quite large in its supply because most of it is still needed. Indonesia as one of the countries importing fishmeal (and fish oil) also depends on the global condition of aquaculture, which is limiting the amount of fishmeal and increasing its prices to continue to soar [3]. This problem is a concern in the provision of feed that needs to be made efforts by finding and developing other raw materials as a substitute for fish meal. The materials needed must be in sufficient quantities and not compete with human needs or must be from organic processing waste. But in general, the available organic waste is vegetable material which cannot be directly consumed by fish as monogastric animals [4,5]. One of the sources of animal protein sources that are developed as substitutes or used as fish feed ingredients is maggots (*Hermetia illuscens*) [6]. Maggots are larvae of Black Soldier Fly (BSF), known as habitat decomposers because of their habit of consuming organic materials. The production process is known as Bioconversion, a process that changes the shape of the product/material that is not comparable to products that use biological agents (living things: BSF
insects). Nowadays maggot has been made in bulk through spreading eggs and using various types of media such as palm kernel cake, waste generated from the process of maintaining pupa-parent stage (BFS) in shared cages. Nevertheless, to produce maggots as feed raw material, it still needs to improve its nutritional quality.

Maggots can be given as fish feed in the fresh form or in the form of pellets as artificial feed. As raw material for feed, grubs are dried into grubs flour with varying protein content that is 30-50% depending on the nature and type of media used. The fermentation of palm kernel cake media can increase the protein content of maggot flour to 42% but with relatively high fiber and fat content (23%) [7]. Other research results [8] showed that maggot flour as a substitute for fish meal only reached 20%, in rainbow ornamental fish up to 10% maggot flour did not produce significant color and growth. [9] argues that high fat causes rancidity that shortens the shelf life of feed ingredients, while high crude fiber will cause feed ingredients to be difficult to digest by fish in the digestive process. This problem causes the substitution rate of maggot flour for fish meal is not optimal. Thus the use of maggot flour as feed raw material to replace fish meal still needs to be improved in quality to increase its contribution to fish feed formulations. This increase can be made possible by microbiology fermentation technology using A. niger and T. viride molds. The goal is to determine the improvement of maggot flour nutrition based on proximate analysis.

2. Material and Method

The substrate used is maggot flour, which is a separate maggot on the palm kernel cake media that begins with the spreading of eggs from black flies (Black Soldier Fly). Maggot larvae are harvested and rinsed thoroughly, drained and then dried in the sun to dry and then ground to produce maggot flour. Mold microbes used were A. niger and T. viride, obtained from InaCC (Indonesian Culture Collection), Biological Research Center, Indonesian Institute of Sciences (LIPI) Cibinong. A. niger was inoculated on PDA (Potato Dextrose Agar) [10-12] media added with yeast extract 0.3% with the agar slant method. Then proceed to breed it in petri dishes (diameter 9 cm) at room temperature for 5 days and then harvested. The harvested A.niger was then reproduced on rice as a media (11). After being planted with A. niger isolate, rice is aerated (fermented) aerobically for 5-6 days at room temperature. Then dried in an oven at 40 °C, mashed and ready to use on maggot flour media.

The maggot flour is sterilized in an autoclave, cooled and mixed with 15 grams of A. niger culture and 20 grams, stirring until evenly distributed. The dough is then placed on a tray with a thickness of 3 cm and then covered with plastic and perforated, aerobically incubated at room temperature for 3 days. The fermentation conditions are changed to anaerobic atmosphere by covering the container again with plastic without being pierced and incubated until day 6. Then it is dried in an oven at 40 °C, and then proximate is analyzed. As for T. viride, using PCA (Plate Count Agar) powder [13] media was weighed 6.75 grams were put into the erlenmeyer which already contained 300 ml distilled water and stirred until homogeneous with a magnetic stirrer. The mixture is waited until it becomes clear and not cloudy. Then put into the autoclave for about 15 minutes with a temperature of 121 °C pressure of 1 atm for sterilization. Cultivation of pure isolates of T. viride mold was carried out using aseptic ose needles in PCA media in petri dishes and incubated for 6 days at room temperature. Making inoculum is done by taking a 6-day-old pure culture dish into the agar medium contains PCA that has been prepared previously. Pure T. viride culture spores grown by scratching the surface of the media (1 ounce of savings) and waiting until the age of 7 days. [14] research concludes that the rate production of fungal biomass during liquid media fermentation by T. viride at 30±1°C for 5 days of incubation. Furthermore, using a mixture of maggot flour (70%), soybean (20%), and molasses (10%) added to a water content of 70% and steamed 30 minutes and cooled, then mixed inoculants prepared as many as 15 and 20 ml / 100 substrates. The fermentation process is finished after 5-6 days and then proximate analysis is performed.

Proximate tests were carried out to determine the nutritional content of fermented maggot flour, namely crude protein, crude fat, crude fiber, carbohydrates, ash content, and solids. Proximate tests were conducted based on [15]. Fermented maggot flour without fermentation was also tested as a control. Then compared the percentage increase in levels of crude protein, decrease in crude fat and crude fiber.
between control and treatment. The study design used was a complete randomized design with 3 replications. The data obtained were analyzed with ANOVA.

3. Results and Discussion

Based on the results of proximate tests showed that fermentation of maggot flour using \textit{A. niger} and \textit{T. viride} can improve the nutritional properties both at doses of 15\% and 20\% (Table 1), where an increase in protein content and decreased levels of fat and crude fiber with different percentages. By using \textit{A. niger}, the protein content of maggot flour increases, fat and crude fiber levels decrease. Whereas \textit{T. viride} protein levels increase, fat levels do not change significantly, and crude fiber decreases. Overall the fermentation process with the type of mold in this study lasted for 7 days and an increase in media temperature on the second day after treatment and reached 38 degrees Celsius. The ongoing fermentation is also known by changes in the water content of the material which is released by water molecules through evaporation that is visible on the plastic part used to cover the media. Addition of carbohydrate sources to the media as an energy source substrate for mold growth, in addition to energy sources from fat. To multiply \textit{A. niger} microbes, rice media is used because the carbohydrate source of maggot flour is small, while the fat content is quite high. All treatments showed significant and significantly different levels of crude protein in \textit{A.niger}. Reduction in crude fat is significantly different for \textit{A. niger}, whereas the dosages used does not significantly affect proximate results for both \textit{A.niger} and \textit{T.viride} (Figure 1).

| Types of Fungi | Dosages (%) | Solids (%) | Ash (%) | Crude Fiber (%) | Crude Protein (%) | Crude Fat (%) | Carbohydrate (%) |
|---------------|-------------|------------|---------|-----------------|------------------|---------------|------------------|
| Control       | 0           | 92.45      | 14.87   | 21.13           | 31.68            | 28.66         | 10.47            |
| \textit{A.niger} | 15          | 85.05      | 13.63   | 14.05           | 43.27            | 6.66          | 22.66            |
|               | 20          | 90.06      | 14.65   | 14.11           | 43.23            | 5.82          | 22.21            |
| \textit{T. viride} | 15          | 90.41      | 11.41   | 11.75           | 39.19            | 24.21        | 11.45            |
|               | 20          | 90.28      | 11.17   | 9.56            | 34.11            | 23.86        | 17.89            |

Figure 1. Proximate Analysis results of maggot fermented flour using \textit{A.niger} and \textit{T.viride}.
The protein content of maggot flour with \( A. \text{niger} \) 15\% reached 43.27\% and at a dose of 20\% produced 43.23\%. Fat content of 6.66\% and 5.82\%, while crude fiber was 14.05\% and 14.11\%. The results of \( T. \text{viride} \) fermentation with lower protein content were 39.19\% and 34.11\%, fat content 26.21\% and 27.26\%, crude fiber became 11.75\% and 9.56\%. Both types of mold give different fermentation results on the nutritional component of maggot flour, at the same dose both 15\% and 20\% produce significantly different protein levels, while the fat content component has a very significant decrease in \( A. \text{niger} \) and by using \( T. \text{viride} \) fiber content has decreased more. Both in \( A. \text{niger} \) and \( T. \text{viride} \), the dose given did not give a different fermentation results on each component of the observed nutrients.

Through this fermentation process, nutritional properties can be improved. Crude protein increases higher in \( A. \text{niger} \) which is 36.58\% and 36.36\%, likewise crude fat was significantly reduced which reaches 76.04\% and 77.32\%. Whereas \( T. \text{viride} \) can reduced crude fiber higher than \( A. \text{niger} \) (Table 2 and Figure 2). In fresh and dried maggot product, high levels of fat and crude fiber become a problem in its use as fish feed.

### Table 2. Average increase in nutrient content of maggot fermented flour using \( A. \text{niger} \) and \( T. \text{viride} \)

| Type of Fungi | Dosage (\%) | Changes in Nutritional Parameters |
|---------------|-------------|----------------------------------|
|               |             | Crude Protein Increased (%)       | Crude Fat Decreased (%) | Crude Fiber Decreased (%) |
| \( A. \text{niger} \) | 15          | 43.27                            | 36.58                   | 6.66                        | 76.04                  | 14.05                      | 33.51                      |
|               | 20          | 43.23                            | 36.36                   | 5.82                        | 77.32                  | 14.11                      | 33.22                      |
| \( T. \text{viride} \) | 15          | 39.19                            | 23.64                   | 26.21                       | 15.65                  | 11.75                      | 44.39                      |
|               | 20          | 34.11                            | 17.63                   | 27.26                       | 16.75                  | 9.56                       | 54.75                      |

![Figure 2](image-url)  
**Figure 2.** Increased levels of crude protein and decreased levels of crude fat and crude fiber of maggot fermented flour compared to unfermented ones (control).

[12] put forward that the technology used to improve the quality of feed ingredients is through the fermentation process, where in general the fermentation end result produces compounds that are simpler and easier to digest compared to its original ingredients besides being able to remove toxic substances from an ingredient. Mold is widely used in the fermentation process to improve the quality of feed ingredients, including \( A. \text{niger} \), \( T. \text{viride} \), \( Rhizopus oligosporus \). Previous studies have been carried out using molds for vegetable raw materials, while the use for animal materials such as maggot is very small. Nevertheless, the results of this study indicate that the media animal ingredients as substrates can improve and improve the nutritional quality of maggot flour ingredients.
In preparing the substrate by adding a source of carbohydrates (rice) as an energy source for mold, because maggot flour contains low carbohydrates, while fat is quite high. Mold utilizes energy sources from carbohydrates and fats [16]. Other carbohydrate sources (palm sugar, tapioca, and molasses) added to the maggot flour substrate, in the research of [17] showed that the addition of molasses was 10% more effective in improving the nutritional quality of maggot flour.

According to [18] A. niger in its growth is directly related to food substances contained in the substrate, simple molecules contained around hyphae can be directly absorbed while more complex molecules must be broken down before being absorbed into cells, by producing some extra enzymes cellular such as protease, amylase, mananase, and α-glucosidase. Organic material from the substrate is used by Aspergillus niger for molecular transport activities, maintenance of cell structure, and cell mobility. Trichoderma is known for the production of enzymes in cell walls consisting of enzymes xylanase, chitinase and β-glucanases are associated with biocontrol roles while cellulases play a dual role [19]. The filamentous fungi Trichoderma is an important function used to produce enzymes by fermentation process. This genus secretes large amounts of cellulase and hemicellulase enzymes capable of degrading carbohydrate polymer [20,21]. With the ability of these two molds in the fermentation process, A. niger is able to increase protein 36.58%, besides being able to reduce fat content to levels of 5.82%. T. viride can only increase protein levels 23.64% even at higher doses (20%) increase in protein is only 7.63%. However, this fungus can reduce fiber levels greater (54.75% and 44.39%) compared to A. niger, this is because Trichoderma which secretes a number of cellulose and hemicellulose enzymes. [20] states that the ability of this mold to produce enzymes is influenced by the fermentation process conditions namely optimum pH 4-7, and the optimum temperature range was found between 30 - 40 degrees centigrade. In carrying out its activities as a producer of cellulase enzymes, T. viride influenced by several factors such as temperature, pH, nutrients, agitation, and fermentation time [13]. Cultivation of pure isolates of T. viride fungi was carried out with aseptic ose needles into PCA media in a petri dish and incubated for 6 days at room temperature. Making the inoculum is done by taking a 6-day-old pure culture dish into the agar medium, contains PCA that has been prepared previously. Pure spore of T. viride grown by scratching the surface of the media (1 ounce of savings) and waiting until the age of 7 days.

The results of this study indicate that the use of A.niger and T.viride molds on maggot flour as a substrate can improve the nutritional quality of maggot flour. Increasing the protein content of fish feed raw materials is important, especially in utilizing waste materials, as well as fat content and fiber content. The fat content of maggot flour is quite high depending on the growth media used, as well as the fiber content is quite high because the maggot media that is commonly used is vegetable waste material with high fiber content. High protein is needed from feed ingredients to support the growth of fish and can be used as a substitute or substitute for fish meal. The fat content is still needed, but with a limited amount to prevent oxidation that causes damage to food during storage. Likewise, crude fiber is restricted because of the ability of fish to digest low fiber.

4. Conclusions

Maggot flour can be improve the quality of nutrition by using A.niger and T.viride in fermentation process. A.niger besides increasing the level of crude protein also significantly reduce crude fat content in maggot flour, while T.viride decreases higher of fiber crude. Therefore fermented maggot flour has potential as a raw material for fish feed and can be an alternative substitute for fish meal.

Conflicts of interest

“There are no conflicts to declare.”

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References

[1] FAO (Food and Agriculture Organization) 2014 World review of fisheries and aquaculture (Rome, Italy) p 96

[2] Laining A, Usman, and Rachman Syah 2016 Aquatic weed Ceratophyllum sp. as a dietary protein source: its effects on growth and fillet amino acid profile of rabbitfish, Siganus guttatus. J. AACL BioFlux, Volume 9 Issue 2 pp 352-358.

[3] Fahmi M R 2016 Optimization Bioconversion Process Using Mini-Larva H Illucens For Fish Feed In Prosiding Seminar Nas Masy Biodiv Indonesia Vol 1 No 1 pp 139-144.

[4] Warburton K and Hallman V Processing of material by the soldier fly, Hermetia illucens RIRDC Publication (Queensland) p197

[5] Hem S, Toure S, Sagbla C, and Legendre M 2008 Bioconversion of palm kernel meal for aquaculture: Experience from the forest region (Rep of Guinea) African Journal of Biotechnology 7 (8) pp1192-1198

[6] Subamia I W 2010 Maggot Application as an Alternative Source of Protein and Fish Feed Laporan Akhir Prog Intensif Riset Terapan, Balai Riset Budidaya Ikan Hias (Depok, Jawa Barat) p 12

[7] Bokau R and P Witoko 2017 Optimization of bioconversion process of coconut cake for production maggott H.illucens as a source of animal protein in fish farming J.Aquacultura Indonesiana Vol 18 No 1 June 2017 pp 20-25

[8] Priyadi A, Azwar Z.I, Subamia I W and Hem S 2009 The use of maggot as a substitute for fish meal in artificial feed for balashark fish seed (Balanthiocheillus melanopterus) Jurnal Riset Akuakultur p 12

[9] Pamungkas W 2011 Fermentation Technology, Alternative Solutions in Utilizing Local Food Materials Media Akua.Vol 6 No 1 pp 43-49

[10] Kusumaningati M A, S Nurhatika and A Muhibuddin 2012 Potency of Aspergillus sp in hydrolysis process to produce ethanol from vegetable and fruit wastes at Wonokromo Market Surabaya Univ Brawijaya p 16

[11] Palinggi N N 2013 Changes in Protein Content of Local Raw Materials Through Fermentation of Solid Substrates Using Aspergillus Konferensi Akuakultur Indonesia pp 315-320.

[12] Sari L and Purwadaria T 2011 Assessment of the nutritional value of Aspergillus niger fermented mutants on the substrate of coconut cake and palm kernel cake J.Biodiversitas 5(2) pp 48-51.

[13] Lailah R, Ahmad Syauqi, and Hari Santos 2017 Fungi Activity of Trichoderma viride in Substrat of Rind Powder Paste of Rambutan (Nephelium lappaceum) e-Jurnal Ilmiah Biosaintropis (Bioscience-Tropic) Volume 3 No.: 2, Oktober p 1-7

[14] Al-Taweil H I, Mohammad Bin Osman, Aidil Abdul Hamid and Wan Mohtar Wan Yusoff 2009 Optimizing of Trichoderma viride Cultivation in Submerged State Fermentation American Journal of Applied Sciences 6 (7) pp 1284-1288

[15] AOAC (Association of Official Analytical Chemists) 1993 Official Methods of Analysis, 16th edn. Association of Official Analytical Chemists International (Gaithersberg, Maryland, USA) pp 1141

[16] Azwar Z I and I Melati 2010 Quality Improvement of Maggot Flour Through the Use of Aspergillus Microbes and Their Utilization in Catfish Feed Prosiding Forum Inovasi Teknologi Akuakultur p 703-711.

[17] Melati I, Mulyasari, and Z I Azwar 2012 The effect of Fermentation using Trichoderma viride, Phanerochaete chrysosporium and the Combination of the Composition of Corn Flour Nutrients as Fish Raw Materials J. Ris. Akuakultur Vol. 7 No. 1 pp 41-47

[18] Madigan M T and Martinko J M Brock 2006 Biology of Microorganisms 11th ed. (New Jersey: Pearson Education) pp178-185.

[19] Shahid M, Mukesh S, Neelam P, Smita R and Srivastava AK 2012 Evaluation of Antagonistic Activity and Self life Study of Trichoderma viride (01PP-8315/11) Advances in Sciences 1 pp 138-140.
[20] Pandey S, M Srivastava, Mohammad Shahid, V Kumar, A Singh, S Trivedi and Y.K. Srivastava 2016 *Trichoderma species Cellulases Produced by Solid State Fermentation* J Data Mining Genomics Proteomics Vo 6 No 2 pp 1-4

[21] Ferdiansyah H, S H Sumarlan, and Bambang D A 2015 *Enzymatic Hydrolysis using Cellulase Enzyme from Trichoderma and Aspergillus in Bioethanol Production of Rice straw* Jur Keteknikan Pert Tropis dan Biosistem Vol 3 No 2 pp 211-216