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Quality Improvement of Durian Waste and Tofu Waste Fermented with *Pleurotus ostreatus*

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Abstract— *This study was conducted to determine the dose of inoculum and the duration of fermentation that is appropriate for the growth of Pleurotus ostreatus on a mixed substrate fermented of durian waste and tofu waste (DWTW) on crude fiber content, crude protein content and cellulase enzyme activity. This research was designed using Factorial Completely Randomized Design 3x3 with 2 replications. Factor A consisted of the dose of inoculum A1 (6%), A2 (8%), A3 (10%) and Factor B consisted of the duration of fermentation namely B1 (7 days), B2 (9 days), B3 (11 days). The variables observed were crude fiber content, crude protein content and cellulase enzyme activity. The results of the analysis showed that there was an interaction between the inoculum dose and the length of DWTW fermentation with Pleurotus ostreatus which had a significantly different effect (P <0.05) on cellulase enzyme activity, crude fiber content, and crude protein content. From the results of this study it can be concluded that the 8% inoculum dose and 9 day fermentation time are optimal and efficient conditions for increasing the quality of the mixture of durian fruit and fermented tofu waste with Pleurotus ostreatus. In this condition cellulase enzyme activity was 1.36 U / ml, crude fiber content was 14.69%, and crude protein content was 19.25%.*

Keywords— ***durian waste, fermented, Pleurotus ostreatus, tofu waste, quality.***

I. INTRODUCTION

The feed is a very important factor in determining the success of a livestock business, especially poultry farming. The availability of feed ingredients that are commonly used lately is increasingly difficult, due to the reduced land used for feed raw materials. The high cost of imported feed, such as corn, wheat, soybean meal, and fish meal is a significant problem faced by poultry farmers. Therefore, poultry feed must be diversified to maintain nutritional quality and reduce the use of imported feed ingredients, which in essence look for alternative feed sources that are easily available, low prices and have good nutritional value and do not compete with human needs.

Indonesia is a tropical country that is crossed by the equator so that it has a diversity of natural resources that can be used as food. One of the plants that have abundant byproducts is durian fruit (Durio zibethinus). Durian fruit production in Indonesia continues to increase every year. Indonesia has an area that stretches 5,000 km from 95 ° to 141 ° BT and has a varied agroecological zone (AEZ), combined with the distribution of durian plants in almost all regions of Indonesia will encourage the emergence of fruit in sequence. This situation provides the opportunity for a long harvest period supported by data in the field showing the average durian harvest period, in general, is around 8 months every year. Theoretically, if the durian's volume and distribution are evenly distributed along the 46 ° longitude, a uniform supply of durian will be obtained for most of the year. According to data from the Directorate General of Horticulture, Ministry of Agriculture in 2013, durian production in Indonesia was 759,055 tons and in 2017 it increased by 795,200 tons. The highest amount of production was in East Java Province, while West Sumatra was ranked 5th with a total production of 74,540 tons.

Durian processing will produce a lot of waste because the part that is generally consumed is fruit flesh which is around 20-25% and the remainder is the skin part 60-70% and the seeds 5-15% have not been maximally utilized (Untung, 2008). Durian skin contains essential oils, flavonoids, saponins, cellulose, lignin, and starch. According to Nuraini (2019), durian skin contains 4.25% crude protein, 29.50% crude fiber and 2,050 kcal/kg of metabolic energy. While the nutritional content of durian seeds according to Nuraini and Mahata (1998) is 9.79% crude protein, 2.41% crude fiber and 2,750 kcal/kg metabolic energy and durian seeds can be used up to the 24% level in broiler rations or can replace 42 % of corn.

The use of durian peel and seeds in poultry rations is limited by the high crude fiber content. The content of

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food substances durian waste (a mixture of 50% peel and 50% seeds), obtained crude protein that is 7.50%, 21.95% crude fiber and 2250 kcal/kg of metabolic energy (Guntoro, 2015). The high crude fiber in durian waste will affect the digestibility and absorption of food substances such as protein, vitamins, and minerals in poultry rations. Large amounts of crude fiber cannot be digested by poultry and are as a booster or bulky (Wahyu, 2004). Therefore, before it is given to poultry, durian waste needs to be processed to improve its nutritional quality. Methods that can be used to reduce high crude fiber and increase crude protein in durian waste can be done by the fermentation method.

Fermentation processing has the advantage of extending storage time, eliminating unpleasant odors, better nutritional value than its original ingredients, fermented food is easier to consume and increases digestibility, and increases flavor (Trisnadjaja and Subroto, 1996). The fermentation process is a process of chemical changes in an organic substrate through the activity of enzymes produced by microorganisms (Hidayanto, 2017). One way to reduce the content of crude fiber, especially cellulose and lignin, is to utilize microbial activity through a biodegradation process, where microbes can degrade fiber components more economically and the results can be more beneficial.

One source of microorganism that can increase protein and reduce crude fiber that has been done by previous researchers is the fungus *Pleurotus ostreatus* (oyster mushroom) which is lignocellulolytic because it can degrade cellulose and lignin which are components of crude fiber. *Pleurotus Ostreatus* is classified as white rot fungi from the Basidiomycetes group which can degrade lignin more extensively because it produces extracellular ligninolytic enzymes consisting of lignin peroxidase (LiP) manganese peroxidase (MnP) and laccase (Hatakka, 2001). Besides, the fungus *Pleurotus ostreatus* also produces amylase and cellulase enzymes (Sudiana and Rahmansyah, 2002) and protease enzymes (Shaba, 2012). According to Alarcon et al. (2003), the advantage of fermentation using the fungus *Pleurotus ostreatus* is that it can produce lovastatin compounds that can inhibit the formation of mevalonates, which ultimately inhibits the formation of cholesterol.

The presence of ligninase enzyme activity from *Pleurotus ostreatus* was reported by Badarina et al. (2013) that coffee waste fermented with *Pleurotus ostreatus* can increase crude protein content by 17.2% and reduce lignin by 31.12%. The substrate fermentation of a mixture of palm sludge and bran with a ratio (80: 20) incubated by *Pleurotus ostreatus* has been tried by Nuraini et al. (2017) can reduce crude fiber substrate by 41.10%, from 23.84% to 14.04%.

In the process of fermentation, pH, temperature, oxygen, and substrate composition are some of the factors that influence its success (Desroisier, 1998). Fermentation using *Pleurotus ostreatus* (oyster mushroom) requires a substrate that contains a source of carbon, nitrogen, and minerals to support the growth and development of mycelium. Durian peel and seeds can be used as a source of carbon (C) in the fermentation media but must be supplemented with a nitrogen source (N) to get a C: N balance suitable for the growth of *Pleurotus ostreatus*.

The source of nitrogen (N) that can be used is tofu waste. Tofu waste is an easily obtainable industrial waste, its availability is continuous and has good nutritional value, namely crude protein at 28.36%, fat 5.52%, crude fiber 7.06% and BETN 45.44% (Nuraini et al., 2012). High crude protein content in tofu waste can be used as a source of N for microbial growth. Mahfudz (2006) states that tofu waste contains amino acid lysine and methionine, as well as calcium which is quite high.

This study utilizes durian waste as a whole which consists of 70% durian waste (75% peel and 25% seeds) and 30% tofu waste obtained by crude protein 5.64%, crude fiber 22.73%, lignin 12.70 and cellulose 16.01% and metabolic energy 2,225 kcal/kg. To reduce crude fiber, fermentation using *Pleurotus ostreatus* was used in this research.

Nuraini (2006) states that the success of fermentation depends very much on the optimum conditions given, such as the composition of the substrate, thickness of the substrate, the inoculum and the duration of fermentation. According to Fardiaz (2005) the speed of fermentation greatly determines the number of enzymes produced, the longer the fermentation time used will be more and more ingredients are overhauled by enzymes, but with increasing time of fermentation the availability of nutrients in the media runs out so that the fungus eventually dies.

This study aims to determine other optimum conditions such as inoculum dosage and the correct fermentation time on a fermented mixture of 70% durian peel and seed fermentation and 30% tofu waste using *Pleurotus ostreatus* in reducing crude fiber (lignin and cellulose) and increasing protein content.

II. MATERIALS AND METHOD

2.1. Materials Research

The materials used in this study were 70% durian waste consisting of (75% peel and 25% seeds), 30% tofu waste and the fungus used as *Pleurotus ostreatus*. Durian waste is obtained from the fruit and processed fruit sales

point at Iko Gantinyo Store, located in the Pondok City Region of Padang, West Sumatra Province. Tofu waste is obtained from the tofu factory in Rimba Datar area, Bandar Buat. *Pleurotus ostreatus* was obtained from LIPI, Cibinong, Bogor. Chemicals for proximate analysis and cellulase enzyme activity. The equipment used in this study is analytical scales, autoclaves, laminar airflow, ovens, a set of equipment for proximate analysis, cellulase enzyme activity.

2.2. Research Implementation

Activities in this research include fermentation of durian fruit waste mixture and tofu waste using *Pleurotus ostreatus* and the quality test of fermented products.

2.2.1. The preparation of the substrate

The processing of durian waste is carried out referring to Guntoro (2015) where the collected durian waste is cleaned of attached impurities, carried out washing or disposing of slime and remaining durian meat to get fresh durian seeds. After washing the durian seeds are chopped small with a maximum size of 0.5-1 cm. The durian waste used consists of 75% peels and 25% seeds, which is the overall utilization of waste from durian. Tofu waste that has been obtained, squeezed and placed in a container.

2.2.2. Fermentation of durian fruit waste and tofu pulp with *Pleurotus ostreatus*.

This experiment aims to obtain the best dosage of inoculum and fermentation time with *Pleurotus ostreatus* for the content and quality of nutrition of durian waste. A comparison of the composition of the substrate used is 70% durian waste and 30% tofu waste. The dose of inoculum used according to treatment was 6%, 8%, and 10% and the level of fermentation time was 7, 9 and 11 days. The steps of researching at this stage are as follows:

- Weigh the substrate with durian waste composition and tofu waste that is 70%: 30% as much as 200 gr.
- The substrate in a plastic bag added with 7 ml of mineral Brook et al., Stirred in a plastic bag until homogeneous.
- Then the substrate is sterilized using an autoclave (temperature 121ºC with 15 minutes), allow it to drop to room temperature, then remove and cool in a sterile room (laminar airflow).
- Sterile substrate is inoculated with *Pleurotus ostreatus* inoculum according to treatment (6, 8, 10% of the amount of substrate) in a sterile room (laminar airflow).
- Then incubated according to treatment (7, 9 and 11 days)

- After the fermentation process ends, the fermented product is then weighed in fresh weight, taken 10 g for determination of enzyme activity. The remainder is dried at 80ºC for 2 hours to kill the fungus, then continue drying at 60ºC for 10-12 hours, until dry. After that, stir evenly, ground, and taken samples for analysis.

2.3. Observed variables

2.3.1. Crude Protein (%)

Crude protein analysis is based on the Kehjdal method, AOAC (Association of Official Analytical Chemists, 1990).

2.3.2. Crude Fiber (%)

Crude fiber analysis is based on the AOAC method (Association of Official Analytical Chemists, 1990).

2.3.3. Cellulase (U / mL) Enzyme Activity

Measurement of cellulase enzyme activity based on the Nelson method (1944).

III. RESULT AND DISCUSSION

3.1. Effect of inoculum dose and duration of fermentation durian waste on cellulase enzyme activity (U / ml) from *Pleurotus ostreatus.*

The average activity of cellulase enzymes from fermented durian waste products and fermented tofu waste by *Pleurotus ostreatus* (DWTW) for each treatment can be seen in Table 1.

Table 1. Mean cellulase enzyme activity (U / ml) Pleurotus ostreatus based on different inoculum doses and fermentation time on DWTW.

Dosage inokulum	Fermentation time			Average
	B1 (7day)	B2 (9day)	B3 (11day)	
A1 (6%)	1,17 c	1,28 b	1,30 b	**1,25**
A2 (8%)	1,18 c	1,36 a	1,37 a	**1,30**
A3 (10%)	1,19 c	1,38 a	1,39 a	**1,32**
Average	**1,18**	**1,34**	**1,35**	

Table 1 shows the activity of cellulase enzymes from a mixture of durian waste and fermented tofu waste by *Pleurotus ostreatus* (DWTW) ranging from 1.17 to 1.39 U / ml.

Based on the variance test showed that there was an interaction that gave a significantly different effect ($P < 0.05$) between factor A (inoculum dose) and factor B (fermentation time) on the cellulase enzyme activity of

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Pleurotus ostreatus on DWTW substrate. Likewise, the inoculum dose factor and fermentation time had a very significant effect (P <0.01) on the activity of cellulase enzymes.

DMRT test results showed that A2B2 treatment (8% inoculum dose and 9 days fermentation time), A2B3 (8% inoculum dose and 11 days fermentation time), A3B2 (10% inoculum dose and 9 days fermentation time) and A3B3 (10 % inoculum dose and 11 days fermentation time) had a significant effect (P <0.05) higher than other treatments.

The more inoculum doses (factor A) given into the substrate and the longer the fermentation (factor B) carried out is positively correlated to the high activity of cellulase enzymes produced, this is due to the more inoculum doses given in the substrate resulting in more *Pleurotus ostreatus* growing (fertile). This is supported by the opinion of Musnandar (2004) which states that the administration of doses with certain limits affects the increase in the number of microorganisms so that the substrate will be covered by mycelium, and result in increased activity of the enzyme.

The longer fermentation time affects the chance of mold to grow more optimally, this causes the activity of cellulase enzymes that occur higher. Setyawan (2005) states that fermentation is strongly influenced by the time used, the longer the time used by microbes to grow and develop properly, resulting in increased enzyme activity.

The high activity of cellulase enzymes in this treatment is caused by a combination of inoculum dosage and the right fermentation time so that molds grow better can remodel the substrate nutrition during fermentation. The substrate nutrition that is overhauled is cellulose, the more cellulose is regenerated into glucose as an energy source, the mold can produce cellulase enzymes with maximum activity. Following the opinion of Pujiati et al. (2014) states that the fermentation process with an appropriate dosage of inoculum and fermentation time can produce cellulase enzymes with maximum activity.

According to Santos et. al. (2012) states that cellulase enzymes are enzymes that can work synergistically remodel cellulose into glucose through a catalyst process. Furthermore, Murashima et al (2002) stated that cellulase enzymes consisted of 3 types of enzymes, namely endoglucanase (endo-1,4-β-D-glucanase), exoglucanase (ekso-1-4-D-glucanase) and cellobiose (β- D-glucosidase). These three enzymes work together to hydrolyze insoluble cellulose to be converted into glucose (Fikrinda, 2000).

The content of the best cellulase enzyme activity (which is efficient in terms of inoculum dosage and fermentation time) in this study is in the treatment of A2B2 (8% inoculum dose and 9 days fermentation time) which is 1.36 U / ml.

3.2. Effect of inoculum dose and duration of fermentation durian waste on crude fiber (%DM) from *Pleurotus ostreatus.*

The average crude fiber (%DM) from fermented durian waste products and fermented tofu waste by *Pleurotus ostreatus* (DWTW) for each treatment can be seen in Table 2.

Table 2. Mean crude fiber (%DM) Pleurotus ostreatus based on different inoculum doses and fermentation time on DWTW.

Dosage inokulum	Fermentation time			Average
	B1 (7day)	B2 (9day)	B3 (11day)	
A1 (6%)	18,45[a]	16,26[d]	15,99[d]	**16,90**
A2 (8%)	17,62[b]	14,69[e]	14,51[e]	**15,60**
A3 (10%)	17,28[c]	14,47[e]	14,35[e]	**15,36**
Average	**17,78**	**15,14**	**14,95**	

The results showed that the crude fiber content of DWTWF with *Pleurotus ostreatus* ranged from 14.35% to 18.45%. Based on the results of the variance test showed that there was an interaction that gave a significantly different effect (P <0.05) between factor A (inoculum dose) and factor B (fermentation time). Each factor, factor A (inoculum dose) and factor B (fermentation time) had a very significant effect (P <0.01) on the DWTW crude fiber content.

DMRT test results showed that A2B2 treatment (8% inoculum dose and 9 days fermentation time) had no significant effect (P> 0.05) with A2B3 treatment (8% inoculum dose and 11 days fermentation time), A3B2 (10% inoculum dose and 9 days fermentation time), A3B3 (10% inoculum dose and 11 days fermentation time), and significantly (P <0.05) lower than other treatments. The low content of DWTW crude fiber in A2B2, A2B3, A3B2, A3B3 treatments compared to other treatments due to cellulase enzyme activity in the 4 treatments was higher than other treatments. This is because the more doses are given and the longer the fermentation time is used resulting in the low fiber content of the substrate being low. The more doses of inoculum used in fermentation results in more ingredients that can be overhauled, to improve the nutritional content of a fermented product (Nurhaita et al, 2012). Furthermore, Musnandar (2004) states that the longer fermentation results in a longer chance of cellulase enzymes to be able to remodel crude fiber more optimally. Chesson (1993) also stated that the

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decrease in crude fiber was caused by the activity of extracellular enzymes produced by molds which caused the degradation of crude fiber cell wall components so that the crude fiber content decreased.

The longer fermentation time will cause the process of fungal metabolism to increase so that more energy is released by the fungus by degrading various energy sources contained in the fermentation durian waste extract, including crude fiber. Strengthening the results of this study Perez et. al., (2002) states that each microfungus has a different ability to decompose the substrate. The longer the incubation period, the more complex the compounds that are broken down by microbes into simpler compounds that can accumulate into energy.

In addition to producing cellulase enzymes that can break down cellulose to glucose (Belitz et al, 2008), *Pleurotus ostreatus* also produces extracellular ligninase enzymes consisting of Laccase, Manganese Peroxidase (MnP) and Lignin Peroxidase (LiP) which can remodel lignin (Hatakka, 1994).

In the treatment of A1B1, A1B2, A1B3, A2B1, and A3B1 the crude fiber content is still high. This is due to the small amount of inoculum used which is 6% and a short fermentation time which is 7 days. Short fermentation time causes a shorter chance of microbes in breaking down crude fiber components into simpler components (Fardiaz, 1989), in this case, less than the optimal activity of cellulose enzymes to remodel cellulose into glucose so that crude fiber is still high on the substrate.

The best crude fiber content (which is efficient in terms of inoculum dose and length of fermentation) in this study is in the treatment of A2B2 (DWTW with *Pleurotus ostreatus* at 8% inoculum dose and 9 days fermentation time) that is 14.69% (there was a decrease in crude fiber by 34, 44%).

3.3. Effect of inoculum dose and duration of fermentation durian waste on crude protein (%DM) from *Pleurotus ostreatus.*

The average crude protein (%DM) from fermented durian waste products and fermented tofu waste by *Pleurotus ostreatus* (DWTW) for each treatment can be seen in Table 2.

Table 2. Mean crude protein (%DM) Pleurotus ostreatus based on different inoculum doses and fermentation time on DWTW.

Dosage inokulum	Fermentation time			Average
	B1 (7day)	B2 (9day)	B3 (11day)	
A1 (6%)	16,50^{c}	18,05^{b}	18,39^{b}	**17,64**
A2 (8%)	16,93^{c}	19,25^{a}	19,31^{a}	**18,49**
A3 (10%)	17,01^{c}	19,38^{a}	19,66^{a}	**18,68**
Average	**16,81**	**18,89**	**19,12**	

Based on the results of the variance test showed that there were interactions and significantly different effects (P <0.05) between factor A (inoculum dose) and factor B (fermentation time). Each factor, factor A (inoculum dose) and factor B (fermentation time) had a very significant effect (P <0.01) on the DWTW crude protein content.

High crude protein content in A2B2 treatment (8% inoculum dose and 9 days fermentation time), A2B3 (8% inoculum dose and 11 days fermentation time), A3B2 (10% inoculum dose and 9 days fermentation time) and A3B3 (10 % inoculum dose and 11 days fermentation time) due to a large dose of inoculum and long fermentation time, causing microbial growth to increase and evenly distributed so that there is an opportunity for microbes to contribute a high enough protein, which causes crude protein to increase. The more inoculum doses used with optimum fermentation time, the more cell mass, so that the combination of optimum dosage of inoculum and fermentation time will increase the content and quality of food substances from fermentation products (Howard et al., 2003).

The increase in crude protein occurs because of the addition of protein donated by microbial cells due to growth that produces a single cell protein product (PST) or cell biomass that contains about 40-65% protein (Khrisna et al., 2005). *Pleurotus ostreatus* biomass which will contribute a lot of high crude protein in fermented products. Increased protein associated with additional protein from microbial cells that increase during the fermentation process (Bintang et al, 2009).The increase in crude protein is also caused by the presence of enzymes produced in the inoculum. The increasing dose of inoculum with long fermentation time, the increasing enzyme produced by *Pleurotus ostreatus* in the product so that the crude protein increases because the enzymes produced by microbes are also proteins (Noferdiman et. Al., 2008).

The low crude protein content in the treatment of A1B1, A1B2, A1B3, A2B1, and A3B1 is due to the inoculum dose given and the fermentation time is too short so that *Pleurotus ostreatus* grows little and the contribution of *Pleurotus Ostreatus* microbial body protein is small. The growth of molds that are infertile and uneven compared with A2B2, A2B3, A3B2 and A3B3 treatments is characterized by the low activity of cellulase enzymes acting on these treatments.

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The best crude protein content (which is efficient in terms of inoculum dose and length of fermentation) in this study is in the treatment of A2B2 (DWTW with *Pleurotus ostreatus* at 8% inoculum dose and 9 days fermentation time) is 19.25% (an increase in crude protein by 39, 12%).

IV. CONCLUSION

In this study, the best results were obtained on a mixture of durian fruit waste and tofu waste pulp with *Pleurotus ostreatus* with an inoculum dose of 8% and a fermentation time of 9 days. In this condition the crude fiber was 14.69%, the crude protein was 19.25% and the cellulase enzyme activity was 1.36 U / ml.

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