**EFFECT OF LIGNOSELULOLITIC FUNGUS TO ENZIMATIC ACTIVITY, FIBER FRCTION, AND DIGESTIBILITY ON FERMENTATION PROCESS OF COCOA POD**

***PENGARUH KAPANG LIGNOSELULOLITIK TERHADAP AKTIVITAS ENZIM, FRAKSI SERAT, DAN KECERNAAN PADA PROSES FERMENTASI KULIT BUAH KAKAO***

**Engkus Ainul Yakin1, Zaenal Bachruddin2, RistiantoUtomo2 & Ria Millati3**

1Faculty of Agriculture, Universitas Veteran Bangun Nusantara, Sukoharjo, 57514

2Faculty of Animal Sciences, Universitas Gadjah Mada, Yogyakarta, 55281

3Faculty of Food and Agriculture Product Technology,

Universitas Gadjah Mada, Yogyakarta, 55281

ABSTRACT

The study was conducted to determine the enzyme activity, fiber fraction and digestibility in the fermentation process of cocoa pod. The substrate was used the cocoa pod while the fungi used *Phanerochaete chrysosporium*, *Pleurotus ostreatus* and *Schizophyllum commune*. Preparation of cocoa pod was chopped, finely ground and then dried. Preparation of fungi by growing fungi in liquid medium. Research methodology was the fermentation conducted with different fungi used four treatments and five replications. T1 = fermentation of cocoa pod without fungi addition, T2 = fermentation of cocoa pod with *P. chrysosporium* addition, T3 = fermentation of cocoa pod with  *P. ostreatus* addition, and T4 = fermentation of cocoa pod with *S. commune.* Fermentation used Erlenmeyer 250 ml and weight of cocoa pod was 100 gram. Fungi added on 5% from substrat weight bassis dry matter. Variables observed was enzyme activity, fiber fraction and digestibility . This study was designed using research design completely randomized design with a unidirectional pattern analysis of variance. Significant variables followed Duncan’s multiple range test. The results showed fermentation used *P. chrysosporium* has highest lignin peroxidase enzyme activity of 0.52±0.04 U/mL and mangan peroxidase 0.06±0.00 U/mL, neutral detergent fiber 75,54±0,41%, acid detergent fiber 68,10±0,30%, lignin 26,86±0,19%, cellulose 27,17±0,25%, hemicellulose 6,77±0,52%, dry matter digestibility 69,70±0,43% and organic matter digestibility 69,59±1,03%. The conclusion from this research that the fermentation by using fungi *P. chrysosporium* addition has the best result to degradated lignin.

(Keywords : Cocoa pod, Digestibility, Enzyme activity, Fermentation, Fiber fraction)

***INTISARI***

*Penelitian ini dilakukan untuk mengetahui aktivitas enzim, fraksi serat dan kecernaan dalam proses fermentasi pod kakao. Substrat yang digunakan yaitu kulit buah kakao (KBK) sedangkan kapang yang digunakan Phanerochaete chrysosporium, Pleurotus ostreatus dan Schizophyllum commune. Kulit buah kakao dipotong dan ditumbuk halus kemudian dikeringkan. Persiapan jamur dengan menumbuhkan jamur dalam medium cair. Metodologi penelitian adalah fermentasi dilakukan dengan jamur yang berbeda digunakan empat perlakuan dan lima ulangan. T1 = fermentasi KBK tanpa penambahan kapang, T2 = fermentasi KBK dengan penambahan P. chrysosporium, T3 = fermentasi KBK dengan penambahan P. ostreatus, dan T4 = fermentasi KBK dengan penambahan S. commune. Fermentasi menggunakan Erlenmeyer 250 ml dan berat KBK yaitu 100 gram. Kapang ditambahkan 5% dari berat substrat berdasar bahan kering. Variabel yang diamati adalah aktivitas enzim, fraksi serat dan kecernaan. Penelitian ini dirancang menggunakan desain penelitian rancangan acak lengkap dengan analisis pola searah yang dilanjutkan dengan uji jarak Duncan apabila terdapat perbedaan. Hasil penelitian menunjukkan fermentasi digunakan P. chrysosporium memiliki aktivitas enzim lignin peroksidase tertinggi 0,52±0,04 U/mL dan aktivitas enzim mangan peroksidase 0,06±0.00 U/mL, neutral detergent fiber 75,54±0,41%, acid detergent fiber 68,10±0,30%, lignin 26,86±0,19% selulosa 27,17±0,25%, hemiselulosa 6,77±0,52%, kecernaan bahan kering 69,70±0,43% dan kecernaan bahan organik 69,59±1,03%. Kesimpulan dari penelitian ini bahwa fermentasi dengan menggunakan kapang P. chrysosporium memiliki hasil terbaik untuk mendegradasi lignin.*

*(Kata kunci : Aktivitas enzim, Fermentasi, Fraksi serat, Kecernaan, Kulit buah kakao)*

**Introduction**

Waste food crops and plantations have an important role and potential in the supply of green feed for ruminants such as cattle, goats, sheep and buffalo, especially in the dry season. In the dry season forage grasses are stunted, making forage available is less in terms of both quantity and quality. Even in areas of specific fodder grass will dry up and die, causing a crisis forage feed. In addition, ruminant rearing system was still largely dependent on forage in the form of grasses and other forage with little or no additional feed.

Cocoa pod has an important role and potential in the supply of ruminant feed, especially goats, especially in the dry season. Cocoa pod utilization as animal feed can be given in the form of fresh or in the form of flour after processing. Judging from the composition, the cocoa pod contains 7.75% protein and energy of 3900 kcal/kg which exceeded the composition of elephant grass of 6.9% and a total energy of 3800 kcal/kg (Puastuti *et al*., 2009). Cocoa pod was an agro-industrial waste produced cocoa plant (*Theobroma cacao L*.). Cocoa consisting of 74% rind, 2% and 24% seed placenta. Proximate analysis results containing 22% protein and 3-9% fat (Nasrullah and Ella A., 1993).

Phanerochaete chrysosporium was a microorganism that has the ability to selectively degrade lignocellulose (Tuomelo *et al*., 2000) that degrades the lignin component first, followed by the cellulose component. Cellulose and hemicellulose utilized by fungi as a carbon source. This fungus also has the ability to grow at a relatively high temperature was 36-40o C so suitable for use in fermentation processes that produce a lot of heat (Tuomelo *et al*., 2000). Lignin degradation of high efficiency and minimal in utilizing cellulose polymers compared to other white rot fungi *P. chrysosporium* make the best choice in the treatment of lignin degradation.

Fungi degrade lignin are most active white-rot fungi, such as *P. chrysosporium* and Coriolus versicolor able to hemicellulose, cellulose and lignin from plant waste into CO2 and H2O (Paul, 1992; Limura, 1996). In general, white-rot basidiomisetes synthesize three kinds of enzymes, Lignin-peroxidase (LiP), manganese-peroxidase (MnP) and laccase. The all of these enzyme plays an important role in the degradation of lignin (Srinivasan *et al*., 1995).

**Materials and Methods**

**Preparation of the fungus**

The fungus was maintained at 37o C on potato dextrose agar (PDA) (200 g L-1 potato extract, 20 g L-1 glucose and 20 g L-1 agar) plates. The fungus was cultured in animmersed liquid culture system. The culture medium was prepared as described by Tien and Kirk (1984) but containing 20 mM acetate buffer (pH 4.4) instead of dimethyl succinate buffer. In addition, 1.5 mM vertryl alcohol (VA), 0.2 g L-1 yeast extract powder, and 1 g L-1 Tween 80 were added. The final spore concentration of 1 x 105spores mL-1 was fed into a 250 mL Erlenmeyer flask containing 100 mL medium. Then the flasks were incubated at 37o C in a rotary shaker with agitation of 150 rpm. The cultures were harvested at the time when the maximum activities of LiP was detected at approximately day-6 and centrifuged at 16200 g for 30 minute at 4oC. The supernatant was used directly as crude ligninolytic enzymes in the fermentation experiments.

**Cocoa pod**

Cocoa pod was obtained from Wonogiri Regency, Central Java Province, Indonesia. Cocoa pod was chopped into 2 cm in size and sun-dried until thewater content reached around 35%.

**Solid state fermentation**

The solid state fermentation was conducted in a 250 mL Erlenmeyer flask as the fermenter. Erlenmeyer flask was filled with 100 g of cocoa pod, which was then inoculated with mold as much as 5% of the weight of the substrate based on the dry matter. The fermentation process used a shaker incubator at a speed of 150 rpm and temperature of 30° C for 7 days. Before and after fermentation, cocoa pod were weighted. At the end of fermentation, cocoa pod was dried at 50° C for 4 days to stop the activity of microorganisms. Cocoa pod was milled and shieved using a Thomas-Wiley Mill type 4 with a diameter of 1 mm sieve. The research design uses a completely randomized design with four treatments and five replications.The treatments were T1= cocoa pod fermentation without fungi addition,T2= cocoa pod fermentation with *P. chrysosporium* addition, T3= cocoa pod fermentation with *P. ostreotus* addition, and T4= cocoa pod fermentation with *S. commune* addition

**Statistical analysis**

The activity of LiP and MnP was measured as described by Tien and Kirk (1984), fiber fraction analysis : NDF, ADF, hemicellulose, cellulose, lignin was measured as described by Van Soest (1982) and in vitro digestibility was measured as described by Tilley and Terry (1963) which has been modified by Utomo (2010). Analysis of data obtained from the treatment were then tested by analysis of variance (ANOVA) test pattern in line with Duncan's multiple range test.

**Result and Discussion**

**Enzime aktivity**

Results of Lignin peroxidase (LiP) enzyme activity during the study are listed in Table 1. The mean treatment in a row were T1 = 0.02±0.00 U/mL; T2 = 0.22±0.01 U/mL; T3 = 0.13±0.00 U/mL; and T4 = 0.16±0.00 U/mL. Statistical analysis using the Duncan Multiple showed results significantly different (P<0.05).

The results mean Manganese Peroxidase (MnP) enzyme activity are listed in Table 1. The mean treatment in a row were T1 = 0.02±0.00 U/mL; T2 = 0.09±0.00 U/mL; T3 = 0.05±0.00 U/mL; and T4 = 0.05±0.00 U/mL. Statistical analysis using the Duncan Multiple showed results significantly different (P<0.05).

The enzyme activity in T2 where the cocoa pod fermentation was using fungi *P. chrysosporium* produce LiP enzyme activity and MnP the highest among other treatments. Fungus *P. chrysosporium* was one of the white rot fungus that of the timber. These fungi produce extracellular enzymes LiP, MnP and laccase. Cocoa pod fermentation process was performed on all treatments by using temperature and fermentation time same proving that the fungus *P. chrysosporium* shows the results of enzyme activity LiP and MnP higher compared with treatment using other fungi.

Lignin peroxidase in treatment T2 was equal to 0.22 U/mL showed higher enzyme activity compared with the research (Ilmi, 2013), which conducts research LiP enzyme activity *P. chrysosporium* fermentation using corncob waste that is equal to 0.06 U/mL.

 Lignin peroxidase and MnP has the same degradation mechanism on lignin. Lignin peroxidase is a peroxidase enzyme and MnP extracellular using H2O2 to degrade lignin. while laccase is an enzyme containing copper using molecular oxygen to degrade lignin (Hattaka, 1994).

LiP and MnP role in the weathering of wood degrading waste and lignin. LiP issued by fungus because cocoa pod was a lignocellulosic organic waste that is on the lignin acts as LiP enzyme inducers. Moreover cocoa pod is also rich in sugars that are naturally easy to be metabolized by the white rot fungus.

**Fiber Fraction**

The results mean the fraction of the fiber fraction during the study are listed in Table 2.

**Neutral detergent fiber (NDF).** Neutral detergent fiber mean results are listed in Table 2. The mean of NDF were T1 = 80.91±0.80%, T2 = 75.54±0.41%, T3 = 7.78±0.56%, T4 = 78.63±1.08%. Statistical analysis using the Duncan Multiple showed highly significant results (P<0.01).

Table 2 shows that the average content of NDF on cocoa pod fermented with different fungus than the lowest was T2 treatment amounted to 75.54% and the highest was T1 amounted to 80.91%.

 The decline shows the NDF content of lignocellulosic enzymes produced by the fungus *P. chrysosporium* able to loosen the lignin and hemicellulose. The decline in NDF content means the content of the substrate is reduced which then affect the composition of the fiber component. The decline occurred because the NDF content during fermentation of lignocellulosic bonds termination occurs and thriving microbial activity (Akmal, 2003).

Neural detergent fiber is the main part of the cell wall such as hemicellulose. cellulose and lignin (Van Soest et al., 1982). Changes NDF content of cocoa pod in this study followed by reduction of the content of other nutrients that are used by fungi to ferment activity. According Suparjo (2009) changes the content of the cocoa pod NDF due to the utilization of the contents of cell components that contain lipids, sugars, organic acids, non-protein nitrogen, pectin, soluble proteins, and other materials dissolved in the water by the fungus *P. chrysosporium*. In the process of fermentation. molds remodel or breaking the cell wall of the cocoa pod through the action of the enzyme selulasenya. Cracking walls of these cells will increase the digestibility of rumen fermentation when cocoa pod livestock consumption.

**Acid detergent fiber (ADF).** Acid detergent fiber mean results are listed in Table 2. The mean of ADF were T1 = 73.30±0.34%, T2 = 68.10±0.30%, T3 = 70.53±0.30%, and T4 = 69.70±0.55%. Statistical analysis using the Duncan Multiple showed highly significant results (P<0.01).

 Table 2 shows that the average content of ADF cocoa pod fermented with different fungus than the lowest was T2 treatment amounted to 68.10% and the highest was T1 amounted to 73.30%.

 The decrease in ADF content of the cocoa pod was due to the enzymes produced by the fungus *P. chrysosporium* capable of loosen the bonds of cellulase enzymes so that a strong bond that had become tenuous. The decline in ADF content was highest in treatment T2, because the fermentation process and the production of cellulase enzymes operate optimally.

 The decline in ADF content during fermentation caused by the use of components of the cell contents or overhaul of cell wall components. Fungi used utilize components of the cell contents as a source of nutrients for growth.

 Acid detergent fiber value was the estimated value of the fraction of the fibers that are difficult degraded by microbes in the rumen (Kustantinah *et al*., 2008). The fraction of fiber was difficult to degrade cellulose and lignin. But the white rot fungus has the ability to degrade lignin. This is supported by Mudgal and Pradhan (1988) which states that the kind of white rot fungi have the ability to break down tough fibers degraded fractions such as lignin. White rot fungus in addition to having activity lignolitik also have cellulase activity that can also degrade cellulose.

The decrease in ADF content on cocoa pod fermented caused by fungi during the fermentation process takes place also outlines and contents of the cells while utilizing the coca pod that begins with the enzyme activity cellulase to loose the cell wall of the coca pod.

**Lignin.** Lignin mean results are listed in Table 2. Average lignin were T1 = 36.00±0.19%, T2 = 26.86±0.12%, T3 = 28.45±0.22%, and T4 = 30.07±0.59%. Statistical analysis using the Duncan Multiple showed highly significant results (P<0.01).

Table 2 shows that the average content of lignin cocoa pod fermented with different fungus than the lowest was T2 treatment amounted to 26.86% and the highest was T1 amounted to 36.00%.

The decrease in lignin content of the cocoa pod to the treatment of fermentation with *P. chrysosporium* fungus was due to fiber degradation process run optimally, resulting lignin content also decreased. Fungus *P. chrysosporium* can degrade lignin effectively by producing extracellular enzymes peroxidase that LiP and MnP.

Fermented cocoa pod by using fungi *P. chrysosporium* provide the opportunity for mold to grow well so that the production of the enzyme produced was also high that affect lignin degradation in the fermentation process pod.

Lignin was a component of plant cell walls that had been developed after the plant experienced a maturation process. Cocoa pod as old crop waste has lignified advanced stages. Changes in lignin content of the substrate occurs due to overhaul the structure of lignin into simpler components. namely CO2 and H2O (Kaal *et al*., 1995).

The lignin content in the fermentation process relates to the production of the enzyme ligninase. The lignin degradation will pave the way for an overhaul of cellulose and hemicellulose. White rot fungus *P. chrysosporium* can degrade lignin so well that it becomes lebh low lignin content. Decreased levels of lignin by the fungus showed that white rot fungi able to degrade lignin. According Kaal *et al*. (1995), that white rot fungus has the ability to depolimerization lignin and lignin metabolizes into CO2 and H2O.

**Cellulose.** The results mean the cellulose contained in Table 2. The mean cellulose were T1 = 30.04±0.24%, T2 = 27.17±0.25%, T3 = 28.49±0.61%, T4 = 28.56±1.03%. Statistical analysis using the Duncan Multiple showed highly significant results (P<0.01).

Table 2 shows that the average cellulose content cocoa pod fermented with different fungi than the lowest was T2 treatment amounted to 21.17% and the highest was T1 amounted to 31.04%.

Cellulose binds tightly with hemicellulose and lignin. Cellulose consists of monomer units of D-glucose bonded through bonding β-1-4-glycoside. Fungus *P. chrysosporium* in addition to producing the enzyme ligninase also produce enzymes cellulase and hemicellulase group. each of which plays a role in the hydrolysis of cellulose and hemicellulose. Enzymatic cellulose degradation compounds produced oligosaccharide, a disaccharide and glucose monomers that are soluble. Enzymatic breakdown process occurs in the presence of cellulase enzymes.

Kregel and Dijkstra (2000) said cellulase enzyme was composed of three kinds of enzymes that work synergistically to degrade cellulose. The enzyme endo 1.4 β-glucanase. selobiohydrolase and β-glucosidase. The mechanism of cellulose degradation begins with the action of the enzyme endo glucanase followed by work selubiohydrolase enzymes and enzyme β-glucosidase to form glucose products.

**Hemicellulose.** Hemicellulose mean results are listed in Table 2. The mean hemicellulose were T1 = 7.61±0.45%, T2 = 6.77±0.52%, T3 = 7.25±0.75%, T4 = 8.92±0.53%. Statistical analysis using the Duncan Multiple showed highly significant results (P<0.01).

Table 2 shows that the average content of hemicellulose cocoa pod fermented with different fungus than the lowest was T2 treatment amounted to 6.77% and the highest was T4 amounted to 8.92%.

Hemicellulose was a heterogeneous group of polysaccharides with low molecular weight. Number of hemicellulose is usually between 15 and 30 percent of the dry weight of lignocellulosic material (Taherzadeh, 1999). Hemicellulose is relatively easy to be hydrolyzed with acid monomers containing glucose, mannose, galactose, xylose, and arabinose. Hemicellulose binds cellulose fiber sheets to form microfibrils which increases the stability of the cell wall. Hemicellulose. lignin was also crosslinked to form a complex network and provide a strong structure.

 Biodegradable hemicellulose into monomeric sugars and acetic acid by the enzyme hemicellulase. Hemicellulase like most other enzymes that can hydrolyze plant cell wall is a multi-domain protein. Xylan was the main carbohydrate constituent of hemicellulose and xylanase was a hemicellulase primary β-1.4 bond hydrolyze xylan chains. Fungus *P. chrysosporium* produces endoxylanase that play a role in the breakdown of xylan into oligosaccharides (Perez *et al*., 2002).

**In Vitro Digestibility**

The results mean dry matter digestibility and organic matter digestibility during the study are listed in Table 3. Dry matter digestibility were T1 = 64.63±0.55%, T2 = 69.70±0.43%, T3 = 67.04±0.60%, and T4 = 66.84±0.61%. Statistical analysis using the Duncan Multiple showed highly significant results (P<0.01). Organic matter digestibility were T1 = 64.11±0.12%, T2 = 69.59±1.03%, T3 = 67.11±0.58%, and T4 = 67.09±0.60%. Statistical analysis using the Duncan Multiple showed highly significant results (P<0.01).

Table 3 shows that the average of dry matter digestibility of cocoa pod fermented with different fungus than the lowest was T1 treatment amounted to 64.63% and the highest was T2 amounted to 69.70%. Pangola dry matter digestibility results in this study amounted to 64.18% showed different results with research Pramono *et al*. (2013) which states dry matter digestibility of pangola grass was 63.65%.

Table 4 shows that the average organic matter digestibility of cocoa pod fermented with different fungus than the lowest was T1 treatment amounted to 64.11% and the highest was T2 amounted to 69.59%. Pangola organic matter digestibility results in this study amounted to 64.78% showed different results with research Pramono *et al*. (2013) which states that the organic matter digestibility of pangola grass was 65.52%.

Dry matter digestibility and organic matter digestibility of cocoa pod fermented with *P. crysosporium* has a higher digestibility values. This is because the fraction of the fiber was capable of optimally degraded by white rot fungus so that it resulted in an increase dry matter digestibility and organic matter digestibility.

Value dry matter digestibility and organic matter digestibility on coca pod fermentation process was closely related to white rot fungus enzyme activity. With the activity of this enzyme then the plant cell wall fraction fibers such as lignin. cellulose and hemicellulose can be degraded by either causing the rising value of the digestibility of the cocoa pod. The higher the value of the cocoa pod. the potential of the cocoa pod as animal feed ruminasia the better. High digestibility value which means the proportion of cocoa pod on the feed becomes larger.

Dry matter digestibility pattern consistent with organic matter digestibility because most of the dry matter consists of organic matter and the only difference being the ash content (Tillman *et al*., 1998).

**Conlusion**

Fermentation of cocoa pod with fungus *P. chrysosporium* produce LiP enzyme activity and the highest MnP consecutively 0.22±0.01 U/ml and 0.09±0.00 U/ml. Fermentation of cocoa pod with the addition of *P. chrysosporium* causes a decrease in content of NDF 75.54±0.41%, ADF 68.10±0.30%, lignin 26.86±0.19%, cellulose 27.17±0.25 %, and hemicellulose 6.77±0.52%. Fermentation of cocoa pod with *P. chrysosporium* produces the highest dry matter digestibility 69.70±0.43% and the highest organic matter digestibility 69.59±1.03%.

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Table 1. Average LiP and MnP enzyme activity and MnP

cocoa pod fermentation (U/mL)

|  |  |
| --- | --- |
| Variable | Treatment |
|  T1 | T2 | T3 | T4 |
| LiP | 0.02±0.00a | 0.22d±0.01d | 0.13±0.00b | 0.16c±0.00c |
| MnP | 0.02±0.00a | 0.09d±0.00d | 0.05±0.00c | 0.06c±0.00c |

a.b.c.d *Superscript* different on same line showed *significant* (P<0.05)

T1 = fermentation of cocoa pod without fungi addition

T2 = fermentation of cocoa pod + *P. chrysosporium*

T3 = fermentation of cocoa pod + *P. ostreatus*

T4 = fermentation of cocoa pod + *S. Commune*

Tabel 2. Average fiber fraction cocoa pod fermentation (%)

|  |  |
| --- | --- |
| Variable | Treatment |
|  T1 | T2 | T3 | T4 |
| NDFADFLignin Cellulosa Hemicellulosa | 80.91±0.80c73.30±0.34d36.00±0.66d30.04±0.24c7.61±0.45ab | 75.54±0.41a68.10±0.30a26.86±0.19a27.17±0.25a6.77±0.52a | 77.78±0.56b70.53±0.30c28.45±0.22b28.49±0.61b7.25±0.30ab | 78.63b±1.08b69.70b±0.55b30.07c±0.59b28.58b±1.03b8.92c±0.19b |

a.b.c.d *Superscript* different on same line showed *significant* (P<0.05)

T1 = fermentation of cocoa pod without fungi addition

T2 = fermentation of cocoa pod + *P. chrysosporium*

T3 = fermentation of cocoa pod + *P. ostreatus*

T4 = fermentation of cocoa pod + *S. commune*

Tabel 3. Average in vitro digestibility on Cocoa pod fermentation (%)

|  |  |
| --- | --- |
| Variable | Treatment |
|  T1 | T2 | T3 | T4 |
| DMOM | 64.63±0.55a64.11±0.12a | 69.70±0.43c69.59±1.03c | 67.04±0.60b67.11±0.58b | 66.84±0.61b67.09±0.60b |

a.b.c *Superscript* different on same line showed *significant* (P<0.05)

T1 = fermentation of cocoa pod without fungi addition

T2 = fermentation of cocoa pod + *P. chrysosporium*

T3 = fermentation of cocoa pod + *P. ostreatus*

T4 = fermentation of cocoa pod + *S. commune*