

# BIOCONVERSION PROPERTY OF TEA LEAVES WASTE BY *Aspergillus niger* AS FUNCTIONAL FIBER TO DECREASE BLOOD LIPID

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Received June 2, 2010; Accepted November 31, 2010

## ABSTRACT

This research was aimed to find out the change of tea leaves waste fiber on lipid absorption after being fermented by *Aspergillus niger* and also to be expected as a model to gain healthy livestock product. Tea leaves waste was fermented by *Aspergillus niger* during 6 day. Fifteen of rats with initial weight of 41.17±4.05 g were fed *ad libitum* by diets containing different levels of unfermented and fermented tea leaves waste as follows: basal diet (R1), unfermented tea leaves waste 5% (R2), unfermented tea leaves waste 10% (R3), fermented tea leaves waste 5% (R4), fermented tea leaves waste 10% (R5). The results indicated that bioconversion of tea leaves waste in diet (R4 and R5) decreased almost all of fiber fractions (NDF 75.55%; ADF 57.17%; celluloses 26.74%; lignine 31.19%), except hemicelluloses 18.38%, while the treatment did not change the lipid binding rate (2.07 vs 1.98). Also, the result showed that rats fed with diets containing fermented tea leaves waste (R4 and R5) had reduced cholesterol (86.25;87.4 mg/dl) and triglyceride (47.06; 46.03 mg/dl), but not on HDL-cholesterol (29.28; 26.51 mg/dl). It was concluded that bioconversion property of tea leaves waste by *Aspergillus niger* changed fiber fraction and decreased blood lipid.

*Keywords: Aspergillus niger, Blood, Cholesterol, Fiber Triglyceride, Tea*

## INTRODUCTION

Bingham (1987) defined that food fiber is unavailable carbohydrate, indigestible residue, as well as polysaccharide plants and lignin in which resistant enzyme of human digestion. Total suggested consumption of food fiber according to Life Science Research Office in 1987 is 20-35 g/day consisting of 70-75% of insoluble dietary fiber and 25-30% of soluble dietary fiber. The insoluble fiber consists of cellulose, some of hemicelluloses and lignin, whereas the soluble fiber contains of pectin, gum and some of hemicellulose.

Garzia-Diez *et al.* (1966) reported that pectin fiber can improve excretion bile acid in feces by increasing the synthesis of bile acid in hepatic and reducing cholesterol in liver and serum. Similarly, Matheson *et al.* (1995) states that the influence of hypocholesterolaemic for soluble fiber is directly adjusted by increasing the synthesis and pool ability of bile acid.

The tea leaves waste is the by product of tea

bottle industries that has its potential as the functional fiber. In Indonesia, tea leaves waste is approximately yielded as much 500 tons every year. It will seem to increase continuously in line with fast growth of tea bottle industries. Factory tea leaves waste contain high crude fiber; neutral detergent fiber (NDF) 36.8-54.1% and acid detergent fiber (ADF) 26.5-36.5% (Kondo *et al.*, 2004).

Fiber composition can be changed by processing. It was reported that the level of cellulose in *Phaseolus vulgaris* L. decreased when this cereal was steam-pressured (Martin-Cabrelas *et al.* 2004). Kahlil (2001) conducted research on mouse by giving them guar gum fiber processed by steam-pressuring, soaking with chemistry solution, and ripening in which then resulting the improvement of protein efficiency, digestibility and biological value.

Enzyme has an ability to hydrolyze fiber. Research resulted by Lopez *et al.* (1996) showed that there was a structure change on physical and chemical characteristics from various of fiber

sources those were already hydrolyzed by cellulolytic enzyme. The physical and chemical structures of pod cacao fiber was changed after being fermented by *Phanaerochaete chrysosporium* (Laconi, 1998). This fungi produces lignin peroxidase and manganese peroxidase (MnP) which be able to soften and to break fiber walls in releasing microfibril of fiber ribbons. *Aspergillus sp* can produce enzymes to hydrolyze polysaccharide such as cellobiohydrolase,  $\beta$ -fructofuranosidase (Wahyuni, 1995), but it also decrease crude fiber content (Nurhayati *et al.*, 2006). Modified fiber by fungi may improve to bind lipid in the digestive tracts so that the opportunity to enter in blood become low.

This research was aimed to find out the change of tea leaves waste fiber on lipid absorption after being fermented by *Aspergillus niger* and also to be expected as a model to gain healthy livestock product.

## MATERIALS AND METHODS

Mould media was made from a 250 g of bean sprout and was boiled in 1 L of water for 1 h. It was then filtered and taken 100 mL and added jelly as well as white sugar as much as 2 and 6 g. Furthermore, mixtured material was boiled until the solution colour became rather transparent and out it into 3 mL reaction tubes, covered by cotton and autoclaved. Then, it was cooled. The 10 of sterile aquades were entered into reaction tubes for stock culture and stirred until spore and its mycelium release. Mould media was inoculated into growth media and incubated for 3 d. Moreover, growth culture was diluted into 6 mL. Tea leaves waste was mixed with aquades in the ratio of 1:1 until homogeneous and autoclaved. After being cooled it was kept in plastic basin. 3 mL of culture solution was used for every 50 g tea leaves waste. It was then covered by plastic and after one day, the plastic cover was holed by sterile needle to get enough oxygen. Finally, fermentation was conducted for 6 d in order to obtain fermented product of tea leaves waste to be included and mixed into diet.

The product of fermented tea leaves waste was analyzed to find out the fiber fraction (Van Soest, 1963) and lipid binding rate, then it compared to unfermented tea leaves waste. Lipid binding rate of each substrate was measured by following steps : Entering 0.5 g of sample into weighted empty reaction tube and adding 3 mL of

palm oil. Then the mixture was put for 30 minutes. Every 5 minutes, it was shaken by vortex and centrifuged in 3000 rpm for 10 minutes. Separated oil was thrown away. Tube and its contents were reweighed.

$$\text{Lipid Binding Rate (g/g DM)} = [(b-a)-(b-c)] / [(a \times 0.01 \times \% \text{ DM})]$$

where:

a = initial sample weight

b = sample weight after oil added

c = sample weight after being centrifuged

DM = dry matter

Tea leaves waste was used as feed fiber in this experimental diets. Fifteen female white rats with body weight of  $41.17 \pm 4.05$  g used in this research. They were maintained for 5 weeks in individual cages in the size of  $30 \times 20 \times 10$  cm<sup>3</sup>. Treatment diets were consisted of 1) basal diet (R1), 2) diet with 5% of tea leaves waste (R2), 3) diet with 10% tea leaves waste (R3), 4) diet with 5% of fermented tea leaves waste (R4), 5) diet with 10% of fermented tea leaves waste (R5). The feed composition and nutrient content were presented in Table 1. In final experiment, blood sample was taken from heart to be tested on the level of blood lipid including cholesterol, triglyceride and HDL-cholesterol. Collected data were compared to their means and analyzed in descriptive method (Walpole, 1995) by using column chart type.

## RESULTS AND DISCUSSION

After fermentation process, there was a change in fiber fraction (Table 2). Fermented tea leaves waste had lower neutral detergent fiber, acid detergent fiber, cellulose, and lignin compared to unfermented one, but hemicelluloses content was higher for fermented tea leaves waste.

The chemical changes in tea leaves waste were caused by fermenting process of *Aspergillus niger*. Fermentation is microorganism activities to gain energy required for metabolism and growth through breaking (catabolism) organic substrate compounds. Enzyme produced by microorganism is able to stimulate oxidation reactions, reduction, hydrolysis and other chemistry reactions, thus there will be chemical changes at particular organic substrate to a certain product (Winarno, 1995). *Aspergillus niger* produces some enzymes, like  $\alpha$ -amylase, cellulase, glucoamylase, catalase, pectinase, lipase, and  $\beta$ -galactosidase. The above enzymes can digest organic matter (Banwart,

Table 1. Feed Composition and Nutrients Content of Experimental Diet

Type	R1	R2	R3	R3	R4
Feed Composition:					
Yellow corn (%)	64	59.69	56.76	60.83	59.05
Soy Bean Meal (%)	20	19.38	17.41	18.28	15.2
Fish meal (%)	10.5	10.5	10.5	10.5	10.5
Palm oil (%)	5	4.92	4.83	4.89	4.75
Premix (%)	0.5	0.5	0.5	0.5	0.5
Tea Leaves Waste (%)	0	5	10	0	0
Waste (%)	0	0	0	5	10
Nutrients:					
Dry Matter (%)	88.94	91.21	91.79	91.65	92.76
Ash (%)	5.46	5.39	5.67	5.72	5.46
Crude Protein (%)	21.77	22.25	22.24	22.26	22.26
Crude Fiber (%)	2.91	3.87	4.79	3.73	4.51
Crude Lipid (%)	9.18	9.36	8.85	8.84	9.72
Nitrogen Free Extract (%)	60.68	59.13	58.45	59.45	58.05
Ca (%)	0.62	0.59	0.67	0.66	0.67
P (%)	0.56	0.6	0.62	0.57	0.56

Tables 2. The Fraction of Tea Leaves Waste Fiber Before and After being Fermented by *Aspergillus niger*

Fraction Fiber	Unfermented	Fermented
NDF (%)	79.38	75.55
Hemicellulose (%)	1.64	18.38
ADF (%)	77.73	57.17
Cellulose (%)	42.35	26.74
Lignine (%)	35.38	31.19

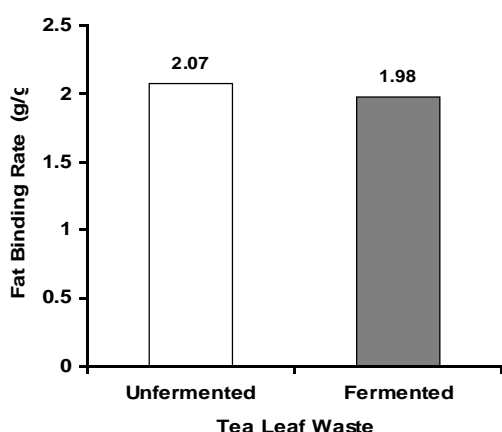


Figure 1. Lipid Binding Rate of Tea Leaves Waste Before and After being Fermented by *Aspergillus niger*

1989) of tea leaves waste.

The lipid binding rate of each tea leaves waste substrate before and after fermentation process is presented at Figure 1. That illustration showed that the lipid binding rates relatively had the same result, with value difference of only 0.09 g/g. Possibly, this was caused by examined feed particle had the same size and fine and it was initially soaked for 30 minutes so that it may give the same opportunity to bind lipid.

Nevertheless, the result of lipid binding rate was not parallel with application of fermentation product as feed in experimental diet (Figure 2). The level of blood cholesterol and triglyceride were lower on fermented tea leaves waste compared to unfermented, even though the level of HDL-cholesterol was not significantly different. The different activities when fermented tea leaves waste became a part of diet tested was predicted as a consequence of discrepancy of bounding lipid source, where at lipid binding rate test palm oil was binded, whereas at bounding biological test bile acid was binded in wich bile acid is a compound that has an effect on lipid absorption in digestive tract. The two compounds above have different chemical structures causing a the difference response to fiber activity.

The change of fiber fraction due to fermentation may allow a change happening on binding lipid. Fermented tea leaves waste had higher hemicellulose content (Tables 2) in

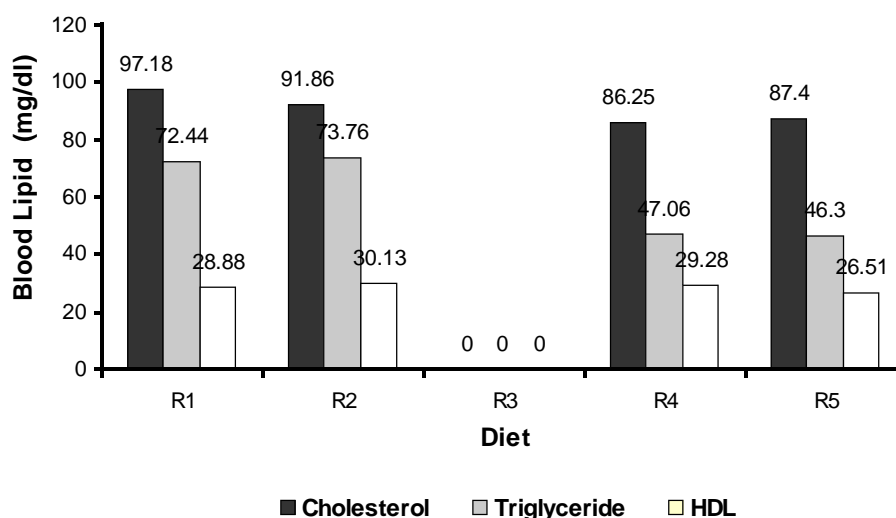


Figure 2. Level of Rat Blood Lipid Fed Diet Consisting of Tea Leaves Waste Before and After being Fermented by *Aspergillus niger*

comparison with tea leaves waste before being fermented (18.38% vs 1.64%). Some hemicelluloses fraction are readily dissolved. It is more effective to bind lipid, because soluble fiber is more distributed to bind bile acid compared to insoluble fiber. Beside that, each fiber has a broadly different viscosity resulting in different abilities to bind other metabolites. Binded bile acid is further released from the body together with feces. Bile acid binding will greatly reduce lipid absorption at liver and it is able to reduce cholesterol in serum. Miettinen (1987) stated that most of soluble fiber increased the release of bile acid up to 70% compared to insoluble fiber only 1.5%. This increase was followed by degradation of serum cholesterol, but not HDL-cholesterol.

The data blood lipid on R<sub>3</sub> treatment could not be obtained because there was lysis at the sampel when blood was taken. In prediction, this condition was happened due to higher tannin content on R<sub>3</sub> treatment resulted from 10% of tea leaves waste inclusion in comparison with 5% of tea leaves waste inclusion, 5% and 10% of fermented tea leaves waste. Tannin is natural compound existed on tea leaf. Tea leaves infusion processing still leaves over high tannin content in the waste. Kondo *et al.* (2004) reported that factory tea leaves waste contain total extractable tannin 5.58-8.25%. Tannin influences ferrum absorption from foods especially those in the category of heme non-iron, such as grains, vegetables, and legumes. Low Fe absorption

bother red blood cell stability, resulting in easy lysis. Fungi fermentation can break and lower tannin level. Binding tannin Fe can be prevented by utilizing EDTA compound (South and Miller, 1998).

## CONCLUSIONS

Bioconversion of tea leaves waste by *Aspergillus niger* resulted in altered composition of fiber fraction and decreased level of blood lipid.

## ACKNOWLEDGMENTS

This research was supported by The Higher Education Directorate General, Department of National Education trough fundamental research fund No. 027/SPPP/DP2M/IV/2005.

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