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Effect of Substrate Composition and Inoculum Dosage to Improve Quality of Palm Kernel Cake Fermented by *Aspergillus niger*

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Abstract: An experiment was conducted to improve the quality of palm kernel cake (PKC) through fermentation by combination of substrate composition and inoculum dosage. The experiment used complete randomize design (CRD) with 4 x 3 factorial and twice replication. The first factor was substrate composition(A): (1) PKC 80% + 20% of rice brand, (2) PKC 80% + 20% of feses, (3) PKC 70% + 30% rice brand, (4) PKC 70% + 30% of feses. The second factor was inoculum dosage (B): (1) 5%, (2) 10% and (3) 15%. The parameters were protease and cellulase activity, crude protein and crude fiber of palm kernel cake fermentation. The result of study showed that there was significantly ($p<0.05$) interaction between substrate composition and inoculum dosage to protease and cellulase activity, crude protein and crude fiber of palm kernel cake fermentation. Every factor from substrate composition and inoculum dosage showed that there were highly significant ($p< 0.01$) effect to protease and cellulase activity, crude protein and crude fiber of palm kernel cake fermentation. It can be concluded that palm kernel cake which was fermented by combination of substrate composition and inoculum dosage showed that substrate composition 80% PKC + 20% rice brand and inoculum dosage 10% had a better nutrient content of Palm kernel cake fermentation. This condition can be seen in protease activity (18.10 U/ml) cellulase activity (22.84 U/ml) crude protein (20.84%) and crude fiber (10.64%).

Key words: Fermentation, palm kernel cake, substrate composition, inoculum dosage

INTRODUCTION

Indonesia is still at the top position as the biggest producer of palm oil in the word and the number is increasing every year. Palm Kernel Cake (PKC) as the major agroindustrial by product of the palm oil industry. PKC is the by product of oil palm production that can be used as feedstuff for poultry and ruminant. The nutrition content of PKC is dry matter 87.30%, protein 16.07%, fiber 21.30%, fat 8.23%, Ca 0.27%, P 0.94% and Cu 48.04 ppm (Mirnawati *et al*., 2008).

PKC has some constraints for poultry, first is high fiber of PKC, the second is low digestibility for poultry which is about 53%, the third is high copper (Cu) around 48.04 ppm (Mirnawati *et al*., 2010a), the fourth is its usage about only 10% in poultry ration (Rizal, 2000; Yeong *et al*., 1981) because of its has low quality (Garcia *et al*., 1999; Perez *et al*., 2000; Odunsei *et al*., 2002; Onwudike, 1986).

There has been done a research for improving the value of PKC by using fermentation method to use the microorganism of cellulose characteristic such *Aspergillus niger*. The activity of *Aspergillus niger* can produce cellulase enzyme, amylase and phytase that will hydrolyzed substrate complex molecules to be simple molecule so it could be digested (Purwadaria *et al*., 1998). Supriyati *et al*. (1998) has done the research about processing the PKC by *Aspergillus niger* that resulted in improvement of protein which is high enough as much as 52.04% and the decreasing crude fiber around 42.03%, but still limited to use in poultry ration. Mirnawati *et al*. (2010b) also has done processing the PKC by *Aspergillus niger* with 7 fermented time and add 100 ppm humic acid that gave the best result: the crude protein around 23.20%, crude fiber around 10.59% and dry matter 42.38%.

In a fermentation, there are some factors that needed to be observed which are substrate composition an inoculums dosage (Winarno *et al*., 1980). Substrate was really needed by microorganism for developmental and growth. Microorganism will grow well in a substrate that enough energy and nutrition for its growth. Another factors that determined the fermentation process is inoculums dosage. The more inoculums dosage given, the factor fermentation process taken place. So, this research will be done by combining the substrate composition and inoculums dosage that can improve the nutrient compound of PKC throughout the fermentation by *Aspergillus niger*.

MATERIALS AND METHODS

This research was conducted to determine the quality of Palm Kernel Cake (PKC) through fermentation by

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combination substrate composition and inoculum dosage with *Aspergilllus niger*. Materials that are used on this research are: 1). Palm Kernel Cake from PT. Incasi Raya Jl. Baypass Padang, 2). *Aspergilllus niger* 3). The Media: PDA/Potato Dextrose Agar from Diffo-Becton Dickinson. 4). Aquades and mineral brooks which consist of $MgSO_4$ $7H_2O$, $FeSO_4$ $7H_2O$, $ZnSO_4$ $7H_2O$, $MnSO_4$ $7H_2O$, KH_2PO_4 and Thiamin hydrochloride. 6). Substrate which is the mixture of PKC and Chicken fesses and rice brand.

This research was used a completely randomized design with 4 x 3 factorial and twice replication. The first factor was substrate composition(A): (1) PKC 80% + 20% of rice brand, (2) PKC 80% + 20% of feses, (3) PKC 70% + 30% of rice brand, (4) PKC 70% + 30% of feses. The second factor was inoculum dosage (B): (1) 5%, (2) 10% and (3) 15%. The parameters were protease and cellulase activity, crude protein and crude fiber of palm kernel cake fermentation. The data was analyzed by using analysis of variance (ANOVA). If there is an effect of the treatment, the differences on treatments are determined by Duncan's Multiple Range Test (DMRT, Steel and Torrie, 1991).

RESULTS

Protease and cellulose activity: The average protease and cellulose activity of Fermented Palm Kernel Cake (FPKC) by *Aspergillus niger* on the interaction between combination substrate composition and inoculum dosage is shown in Table 1 and 2. The result of statistic analysis shows that there was significant interaction ($p<0.05$) among factor A and B each of the factor A and B shown significant difference ($p<0.05$) towards effect to protease and cellulose activity of fermented palm kernel cake by *Aspergillus niger*.

Crude Protein (CP): The average value of crude protein content of Fermented Palm Kernel Cake (FPKC) by *Aspergillus niger* at the interaction between substrate composition and inoculums dosage is shown in Table 3. The result of statistic analysis shows that there was significant interaction ($p<0.05$) toward effect among factor A and B. The factor A and B every were significant ($p<0.05$) effect to crude protein content of fermented palm kernel cake by *Aspergillus niger*.

Crude Fiber (CF): The average value of crude fiber content of Fermented Palm Kernel Cake (FPKC) by *Aspergillus niger* at the interaction between substrate composition and inoculums dosage is shown in Table 4. The result of statistic analysis shows that there was significant interaction ($p<0.05$) effect among factor A and B. The factor A and B every were significant ($p<0.05$) effected to crude fiber content of fermented palm kernel cake by *Aspergillus niger*.

Table 1: The average protease activity of palm kernel cake fermented by *Aspergillus niger* (Unit/ml)

	Inoculum dosage (B)			
Substrate composition (A)	5	10	15	Average
80% PKC + 20% rice brand	15.26^{bA}	18.10^{aA}	14.87^{bA}	16.08
80% PKC + 20% feses	15.36^{aA}	15.48^{aB}	14.45^{aA}	15.10
70% PKC + 30% rice brand	11.65^{bB}	14.45^{aB}	12.25^{bB}	12.78
70% PKC + 30% feses	10.56^{bB}	13.34^{aB}	12.42^{bB}	12.11
Average	13.21	15.34	13.50	

Note: Capital and small letter are different on the same column and row indicated significant ($p<0.05$)

Table 2: The average cellulase activity of palm kernel cake fermented by *Aspergillus niger* (Unit/ml)

	Inoculum dosage (B)			
Substrate composition (A)	5	10	15	Average
80% PKC + 20% rice brand	19.48^{bA}	22.84^{aA}	18.47^{bA}	20.26
80% PKC + 20% feses	16.66^{bB}	19.64^{aB}	17.85^{bA}	18.05
70% PKC + 30% Rice brand	15.55^{aB}	16.85^{aB}	16.55^{aB}	16.32
70% PKC + 30% feses	14.58^{bB}	15.74^{aB}	15.52^{aB}	15.28
Average	16.57	18.77	17.10	

Note: Capital and small letter are different on the same column and row indicated significant ($p<0.05$)

Table 3: The average crude protein of palm kernel cake fermented by *Aspergillus niger* (%)

	Inoculum dosage (B)			
Substrate composition (A)	5	10	15	Average
80% PKC + 20% rice brand	18.38^{bA}	20.84^{aA}	18.37^{bA}	19.20
80% PKC + 20% feses	17.46^{aB}	18.64^{aB}	17.65^{aB}	17.92
70% PKC + 30% rice brand	17.55^{bB}	18.08^{aB}	17.35^{bB}	17.66
70% PKC + 30% feses	18.08^{bA}	17.74^{aB}	18.22^{bA}	18.01
Average	17.87	18.83	17.90	

Note: Capital and small letter are different on the same column and row indicated significant ($p<0.05$)

Table 4: The average crude fiber of palm kernel cake fermented by *Aspergillus niger* (%)

	Inoculum dosage (B)			
Substrate composition (A)	5	10	15	Average
80% PKC + 20% rice brand	14.18^{aB}	10.64^{bB}	14.37^{aA}	13.06
80% PKC + 20% feses	17.26^{aA}	16.44^{aA}	16.35^{aB}	16.68
70% PKC + 30% rice brand	17.35^{aA}	15.08^{bA}	17.25^{aB}	16.17
70% PKC + 30% feses	16.08^{aA}	17.54^{aA}	16.22^{aB}	16.61
Average	16.22	14.93	16.05	

Note: Capital and small letter are different on the same column and row indicated highly significant ($p<0.01$)

DISCUSSION

Protease and cellulose activity: The result of variant analysis showed that there was significant interaction ($p<0.05$) between substrate composition (factor A) and inoculums dosage (factor B) towards the activity protease and cellulose of palm kernel cake fermented. Factor A showed highly significant different ($p<0.01$) towards the activity of protease palm kernel cake fermented.

Based of DMRT test towards the interaction between substrate composition and inoculums dosage on activity of protease and cellulose showed that treatment A1B2 which is substrate composition 80% PKC + 20% rice brand and inoculums dosage 10% gave highest score of protease and cellulose activity compared to other treatment. In substrate composition 20% rice brand and 20% feces showed not significant difference ($p>0.05$) affected in inoculum dosage 5% and 15% but significant ($p<0.05$) affected in inoculum dosage 10% (A1B2). If substrate composition was added by 30% rice brand and 30% feses, it resulted decrease protease activity in inoculum dosage 5%, 10% and 15%. The same result at cellulase activity in A1B2 treatment was significant difference ($p<0.05$) than other treatment. In Inoculum dosage 5%, 10% and 15% with substrate composition 30% rice brand and 30% feses were not significant difference ($p>0.05$) affected cellulase activity. The higher activity of protease and cellulase on treatment A1B2 is caused by addition of 20% rice brand in the substrate that made the product become fertile that facilitate the growth and development of microbe. Besides that, rice brand contains nutrient and vitamin which is needed for molds growth. This is appropriate with Peppler (1973) that addition of nutrient source compound into the media of fermentation can support and stimulate the growth of mold.

Besides that, the high activity of protease and cellulose on A1B2 treatment also caused by 10% inoculum dosage which is an optimum dosage for PKC fermentation resulted microorganism better growth compared to other treatment. The better the growth of mold, the more enzyme will be produced such as protease and cellulose enzyme. This is appropriate with the Kassim *et al.* (1985) that there is a positive relation between molds growth and enzymes production. The high activity of protease and cellulose on 10% dosage is resulted in a fertile growth of molds as more amount of spore that can be seen compared to other dosage of treatment. Even though, the inoculums dosage was raison to 15%, it didn't resulted in a increase of protease and cellulose activity. This is appropriate with Shurtleff and Aoyagi (1979) which said that the more inoculums given, the more sporulation happened to mold instead of remodeling the nutrient.

Crude Protein (CP): The result of variant analysis showed that there is significant interaction ($p<0.05$) between substrate composition (A factor) and inoculum dosage (B factor) towards the crude fiber of PKC. A factor showed highly significant difference ($p<0.01$) whereas B factor showed significant difference ($p<0.05$) towards crude protein of PKC.

Based on DMRT test towards crude protein showed that substrate composition 80% PKC + 20% rice brand and 10% inoculum dosage (A1B2) had higher point of significant difference ($p<0.05$). On 10% inoculums dosage, the crude protein showed significant difference ($p<0.05$) between substrate composition. On 5% and 15% inoculums dosage showed not significant difference ($p>0.05$) on every substrate composition.

The high crude protein on this treatment (A1B2) is caused by addition of 20% rice brand in substrate composition and Inoculum dosage 10% which have made the product to be more fertile that facilitate the development and growth of microbe. Besides that, rice brand also contained nutrient and vitamin which was needed for molds growth. This is appropriate with explanation of Peppler (1973) that the addition of nutrient sources into the fermentation media could support and stimulate the growth of mold. The higher crude protein on 10% inoculum dosage resulted good condition for molds to growth compared to other which can be seen from the more amount of spore produced compared to other dosage. The higher growth of mold the more enzyme would be produced, the more loss of dry matter on fermentation. This is appropriate with the explanation of Wang *et al.* (1979) which stated that the increase of protein during fermentation was caused by the increase of mold cells mass and the loss of dry matter during fermentation (Halid, 1991; Reade and Gregory, 1975; Rodriquez *et al.*, 1985).

The high crude protein after fermentation was caused by the addition the protein from molds body because mold also contained single cell protein. Appropriate with

Fardiaz (1992) which stated that mold had high amount of crude protein which was about 35-40%. Also, mold produce enzyme which was a protein (Saono, 1981). Added by Siregar and Mirwandhono (2004) that from their research reported that the of *Aspergilus niger* in the fermentation process was the best way compared to other mold such as *Rizopus oryzae* and *Tricoderma viridae*. This also appropriate with the result obtained (Mathot *et al*., 1992; Iyayi, 2004).

Crude fiber: The results of variant analysis showed that there was significant interaction ($p<0.05$) between substrate composition (A factor) and inoculum dosage (B factor) towards crude fiber of palm kernel cake fermented. A factor showed highly significant difference ($p<0.01$) whereas B factor showed significant difference ($p<0.05$) towards crude fiber of palm kernel cake fermented.

Based on DMRT test toward crude fiber showed that substrate composition 80% PKC + 20% of rice brand with inoculum dosage 10% had higher point of significant difference compared to other. The crude fiber showed significant difference ($p<0.05$) between substrate composition with inoculums dosage 10%. The 5% and 15% of inoculums dosage showed not significant difference ($p>0.05$) on every substrate composition.

The low crude fiber on substrate composition 80% PKC + 20% of rice brand with Inoculum dosage 10% showed that this mold grew more fertile, showed from the more amount of spore produced compared to other treatment. The high growth of *Aspergilus niger* on this treatment was caused by the addition of 20% rice brand in substrate composition which have made the product to be more fertile that facilitate the growth and development of microbe. Also rice brand contained nutrient and vitamin which was needed by mold for growth. This is appropriate with Peppler (1973) that the addition of nutrient source into fermentation media could support and stimulate the growth of mold. The more growth of mold the more cellulose would be produced so the more crude fiber would be degraded. This is appropriate with Kassim *et al*. (1985) which explanation that there was a positive relation between growth and cellulose production, the fertile of growth of *Aspergilus niger* the more enzymes would be produced to turn cellulose into glucose effected in the decreased of crude fiber in the end of fermentation (Damude *et al*., 1996; Ofuya and Nwajiuba, 1990; Iyayi and Losei, 2001). Susanto (2002) and Smith and Aidodo (1988) which stated that *Aspergilus niger* could decrease the amount of crude fiber.

Conclusion: The conclusion was palm kernel cake which was fermented by combination of substrate composition and inoculum dosage showed that substrate composition 80% PKC + 20% rice brand and inoculum dosage 10% had a better nutrient content of Palm kernel cake fermentation. This condition can be seen in protease activity (18.10 U/ml) cellulase activity (22.84 U/ml) crude protein (20.84%) and crude fiber (10.64%).

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