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Full Length Research Paper

Effect of enzyme treatment on chemical composition and production of reducing sugars in palm (*Elaeis guineenis*) kernel expeller

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The primary objective of this study was to examine the effects of enzyme treatment on chemical compositions and production of reducing sugars in palm (*Elaeis guineenis*) kernel expeller (PKE). PKE was incubated in 80% water content at 55°C for 18 h with enzyme or without enzyme (as control). Crude protein (CP) and gross energy (GE) contents of PKE were not significantly different (P>0.05) between the control and enzyme treated PKE; averaged 17.95% for CP and 15.88 MJ/kg for GE. However, ether extract (EE) neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose and cellulose contents of enzyme treated PKE decreased (P<0.01) by approximately 34.6, 26, 20, 35.7 and 22.1%, respectively, compared with the control. Total content of reducing sugars (mannose, glucose and galactose) of the enzyme treated PKE increased by approximately 200 folds compared to the control (P<0.01). Enzyme treated PKE had higher (P<0.01) cellulase and mannanase activities but not α-galactosidase.

Key word: Enzyme treated palm kernel expeller, chemical composition, reducing sugars, palm kernel expeller.

INTRODUCTION

Identification and improving quality of unconventional feed ingredients including agro-industrial byproducts to offset the fluctuating global market price and supply of traditional feed ingredients for livestock production have been a priority for many developing countries. Malaysia and Indonesia are the world top producers of palm (*Elaeis guineensis*) oil. An important byproduct generated from palm oil industry is palm kernel meal (PKM) or palm kernel expeller (PKE) depending on the method used for the extraction of oil from the kernel 'with the latter normally containing slightly higher oil content'. Palm kernel expeller contains a moderate level of crude protein (14.5 to 19.6%) but a high level of fiber (13 to 20%) and poor amino acid profile (deficient in lysine, methionine and tryptophan) and thus it is considered be a moderate quality feed ingredient for ruminant but not suitable for monogastric animals (Alimon, 2004). The fiber of PKE is

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mainly as hemicellulose consisting of 58% mannans (Jaafar and Javis, 1992), moderate amounts of cellulose and small amount of other polysaccharides (Swe et al., 2004). Most mannan, making up between 25 to 32% of PKE (Yokomizo, 2004) consist of water insoluble glucomannan and small amount of water soluble galactosemannan thus making it resemble very much cellulose by being crystalline, hard and water insoluble (Knudsen, 1997; Sundu et al., 2006). Because of its complex chemical structure, the fiber of PKE requires a combination of enzymes including mannosidases, galactosidases, glucosidases and xylanases to release the potential fermentable sugars to be of use for monogastric animals. As the use of PKE for monogastric animals such as poultry, pigs and fish is limited due to the lack of the appropriate enzymes in these animals to hydrolyze the fiber, two most widely used methods to overcome this limitation are: (i) the use of fungi (particularly *Aspergillus niger*) in solid state fermentation (SSF) to breakdown and reduce the hemicelluloses, cellulose and lignin in PKE (Noraini et al., 2001), and (ii) supplementation of exogenous enzymes into the diet containing PKE in poultry (Chong et al., 2003).

The major exogenous enzymes used to improve the nutritive value of PKE are mannanase, α-galactosidase and cellulase (Sundu and Dingle, 2003). Supplementing exogenous enzymes directly into the diet of broilers, Chong et al. (2003) reported increased apparent metabolizable energy and true metabolizable energy in PKE diets while Sekoni et al. (2008) recorded increased retention of vital nutrients (protein, fat and nitrogen free extract) and metabolizable energy in broiler chickens. However, other worker (Choct, 2006) reported that enzymes were not very effective in breaking down the non-starch polysaccharides to monomeric sugars within the gastro-intestinal tract of poultry and suggested that pre-treating the PKE with enzymes before feeding could be a better option in improving the nutritive values of this byproduct. Ng et al. (2002) reported that fish fed with PKM pre-treated with enzymes had higher feed utilization efficiency and growth than those fed untreated PKM. However, large quantity of water (1 PKM: 2 water) was used in their treatment which could result in high cost of drying the product under commercial production. The objective of this study was to examine the effects of pre-treating PKE with a commercial enzyme of different source using solid state fermentation (SSF) on its chemical composition, particularly the fiber content which is the primary limiting factor in PKE as feed ingredient for monogastric animals. The enzymatic activities and the production of reducing sugars were also determined to assess the effectiveness of the treatment.

MATERIALS AND METHODS

Sample preparation

Raw PKE was purchased from a commercial kernel oil extraction factory in Klang, Selangor, Malaysia and ground to uniform size of about 2.5 mm before treatment. The PKE was treated in 2 L laboratory beaker in which water was added to achieve a moisture content of about 80% before adding the enzyme (0.67% of the weight of PKE). The PKE samples were incubated at 55°C in water bath for 18 h. After hydrolysis, the samples were dried for 48 h at 60°C and stored in a refrigerator for later use.

Proximate analysis

Samples of the enzyme treated and raw (as control) PKE were analysed for DM, CP, ash, EE (AOAC, 1985), NDF, ADF and acid detergent lignin (ADL) (Goering and Van Soest, 1970). Each of the aforementioned analysis was replicated nine times. Hemicellulose was calculated as NDF - ADF while cellulose as ADF - ADL.

Preparation of PKE filtrate

Approximately 10 g of the PKE was weighed into the Erlenmeyer flask. 100 ml of 0.01 M potassium phosphate buffer with pH 7 were added and shaken (121 rpm) at 4°C for 24 h (overnight), and filtered through whatman No 1 filter paper. The filtrate was stored at 4°C pending analysis of mannanase and α-galactosidase enzymes activities using procedures described by Khin (2004).

Determination of reducing sugar

1 g of PKE was added to 10 ml of 0.2 mM sodium phosphate buffer (pH ~7) in a 50 ml Falcon tube. The content was mixed thoroughly and vortex for 5 min (Foong et al., 2009). 1 ml of the supernatant was transferred into another Falcon tube followed with addition of 3 ml of dinitrosalicylic acid (DNS) reagent. This solution was boiled for 10 min after which 1 ml of Rochelle salts was added and left to cool to room temperature. The absorbance was measured in spectrophotometer (Barnstead turner Model SP-380 plus, USA) at 575 nm with distilled water as blank (Miller, 1959). Different concentrations between 0 to 2 mg/ml (0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.50, 1.75 and 2 mg/ml) of mannose, galactose, glucose and xylose, prepared using similar procedure as aforementioned were used to develop the standard curves for the respective sugars.

Substrate of mannanase enzyme preparation

This assay (as specified by Magazyme, Ireland) used 2 g of powder substrate (carob galactomannan from Magazyme, Ireland) to 80 ml of hot distilled water (85 to 90°C) and stirred on a hot plate. Stirring continued after the hot plate was turned off until the substrate was completely dissolved in approximately 10 min before allowed to cool to room temperature. The solution was added with 10 ml of 2 M sodium acetate buffer (pH 4) and the volume was adjusted to 100 ml with distilled water. After that, a few drops of toluene were added to prevent microbial contamination and stored at 4°C for enzyme activity assay.

β-Mannanase assay

The procedure described as follows followed the procedure specified by the Magazyme (Ireland). About 0.2 ml of the previously prepared PKE filtrate was added to 0.2 ml of the substrate (Azo-Carob Galactomannan) solution and stirred for 5 s on a vortex stirrer and incubated at 40°C for 10 min. After that, 1 ml of ethanol (~95%) was added to the mixture and was stirred continuously for another 10 s on the vortex stirrer. The mixture was allowed to equilibrate to room temperature for 10 min and then centrifuged at 3,000 rpm for 10 min. The supernatant solution was poured directly from the centrifuge tube into a cuvette and the absorbance was measured using spectrophotometer at 590 nm. Different concentrations of pure endo-1,4-β-mannanase (Megazyme, Ireland) was used for the preparation of standard curve following the same procedure as earlier described.

Cellulase assay

1 ml of the PKE supernatant (prepared in similar manner previously described for determination of reducing sugar) was added with 1 ml of 0.05 M citrate phosphate buffer and 0.05 g of whatman No.1 filter paper in Falcon tube (Foong et al., 2009). Samples were incubated in water bath shaker (Toyo thermo shaker Model TS-30G, Japan) at 39°C for 30 min after which 3 ml of dinitrosalicylic acid (DNS) reagent were added. The solution was boiled for 10 min and 1 ml of Rochelle salts was added and allowed to cool to room temperature. The absorbance was measured in a spectrophotometer at 575 nm with water as blank (Miller, 1959) and the activity was determined using pre-established glucose standard curve of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml. Cellulase activity was expressed in international units (IU) where one IU is the amount of enzyme required to release 1 µ mole glucose equivalent in 1 ml of enzyme solution in 1 min.

α-Galactosidase assay

The following procedure was specified by the manufacturer of the enzyme (Sigma assay, USA). 0.7 ml of 100 mM potassium phosphate buffer (pH 6.5 at 25°C) was added to 0.2 ml of 9.9 mM p-nitrophenyl α-D- galactopyranoside solution (PNP-Gal). The solutions were mixed by swirling and equilibrated to 25°C. 0.1 ml of α-galactosidase enzyme solution (0.05 to 0.1 unit/ml) or the extracted PKE filtrate was then added to the solution. Immediately, the solution was mixed by swirling and incubated at 25°C for exactly 5 min before 2 ml of 200 mM borate buffer (pH 9.8 at 25°C) was added. The solution was mixed by swirling and the absorbance was recorded at 405 nm by spectrophotometer. The same method as earlier mentioned was used for the blank sample except that the enzyme solution was added after addition of borate buffer (Sigma assay, USA).

Calculation of α-galactosidase

Concentration of α-galactosidase was calculated using the following equation:

$$\text{Units/ml enzyme} = \frac{(A_{405nm}\ \text{Test} - A_{405nm}\ \text{Blank})\ (3.0)\ (df)}{(18.5)\ (5.0)\ (0.1)}$$

Where, 3.0 = total volume of assay, df = dilution factor, 5.5 = conversion factor for 5 to 1 min and 18.5 = millimolar (in milliliter) of enzyme used (Sigma Assay).

Statistic analysis

Data were subjected to analysis of variance using Proc. ANOVA (SAS, 2002). Differences among means were tested using t-test (independent group) (Steel and Torrie, 1980). A significance level of $P<0.05$ was used.

RESULTS AND DISCUSSION

Chemical compositions

Chemical compositions of the control and enzyme treated PKE are shown in Table 1. CP and GE contents of the control and enzyme treated PKE were not significantly different; averaged 17.95% CP and 15.88 MJ ME/kg, respectively. Ng et al. (2002) reported that CP content of fungal fermented PKM increased nearly 2 folds (16.8 to 31.2%) while no significant increase in CP was detected in enzyme treated PKM. The authors suggest that the increased CP could be due to microbial protein synthesis during fermentation. Swe et al. (2003) also reported higher CP content in fungal fermented PKE (29.4%) compared to untreated material (16.9%), but the ratio of true protein (amino acids) to total CP for the fermented sample was much lower than the original PKE and further suggested that the increased CP in the fungal fermented PKE was due to non-protein nitrogen of the fungal cell wall which is non-digestible in poultry. Treatment significantly decreased ($P<0.01$) EE from 8.99 to 5.88% (34.59%), which is in agreement with Ng et al. (2002) and Swe et al. (2003) who reported that crude lipid content in enzyme and fungal treated PKM decreased by about 50% (from 6.82 to 3.36%) and 24% (from 6.82 to 5.15%), respectively. There were significant reductions ($P<0.01$) in NDF and ADF contents in the enzyme treated PKE compared to the control. Enzyme treatment decreased NDF content by about 26% (from 88.50 to 65.18%) and ADF content by about 20% (from 51.05 to 41.10%). Although enzyme treatment reduced ADL content by 2.22% units (from 17.36 to 15.14%), they were not statistically different. Enzyme treatment reduced hemi-cellulose content ($P<0.01$) by 34.81% while the reduction for cellulose ($P<0.05$) was 22.14%.

The aforementioned result is in agreement with Lawal et al. (2010) who found that the fungal enzyme treated PKC reduced crude fiber (CF), hemicelluloses and cellulose contents resulted in increased CP and soluble sugar (glucose, fructose, galactose and sucrose)

Table 1. Chemical compositions (% of DM) and gross energy of PKE with and without enzyme treatment.

Item	Treatment		SEM
	Without enzyme	With enzyme	
Crude protein (%)	17.88±0.22	18.02±0.17	0.28
Ether extract (%)	8.99[a] ±0.84	5.88[b] ±0.44	0.95
Ash (%)	4.30±0.42	4.86±0.43	0.60
NDF (%)	88.50[a] ±1.31	65.18[b] ±0.80	1.53
ADF (%)	51.05[a] ±0.50	41.10[b] ±0.62	0.81
ADL (%)	17.36±1.26	15.14±0.62	1.54
Hemicellulose (%)	37.45[a] ±1.18	24.08[b] ±1.55	1.95
Cellulose (%)	33.69[c] ±1.40	26.23[d] ±2.08	2.51
Gross energy (MJ/kg)	15.73±0.65	16.03±0.18	0.54

[a,b] Values on the same row with different superscripts differ significantly (P<0.01); [c,d] Values on the same row with different superscripts differ significantly (P<0.05).
NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin.

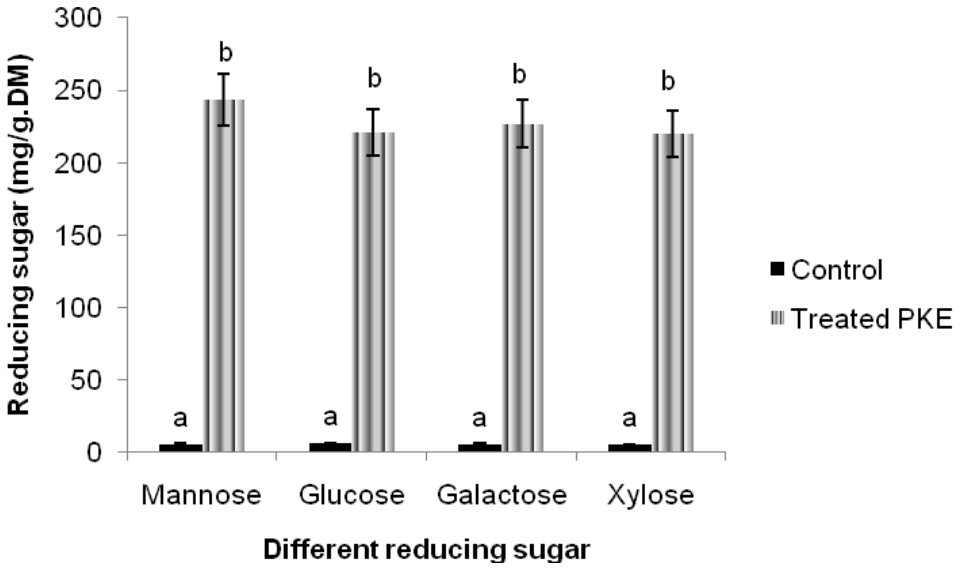

Figure 1. Concentrations of reducing sugars in the control and treated PKE.

contents. The significant reduction in hemicellulose content indicates that the enzyme is effective in breaking down mannan-hemicellulose, the main component of the fiber in PKE (Jaafar and Javis, 1992; Yokomizo, 2004).

Reducing sugars

Enzyme treated PKE had significantly higher (P<0.01) reducing sugars contents than the control (Figure 1). Mannose content increased 45 folds from 5.38 mg/g DM in the control to 243.4 mg/g DM in the treated sample. Similar increases in glucose (from 5.99 to 221.25 mg/g DM), galactose (from 5.44 to 226.84 mg/g DM) and xylose (from 5.07 to 219.58 mg/g DM) were also recorded between the treated PKE and the control (Figure 1). The aforementioned data clearly suggests that the enzyme effectively hydrolyzed the lignocelluloses in PKE to simple sugars which resulted in an increase in the total reducing sugar contents by proximately 200 folds. We know of limited studies reporting the effect of enzyme treatment on content of reducing sugars in PKE except

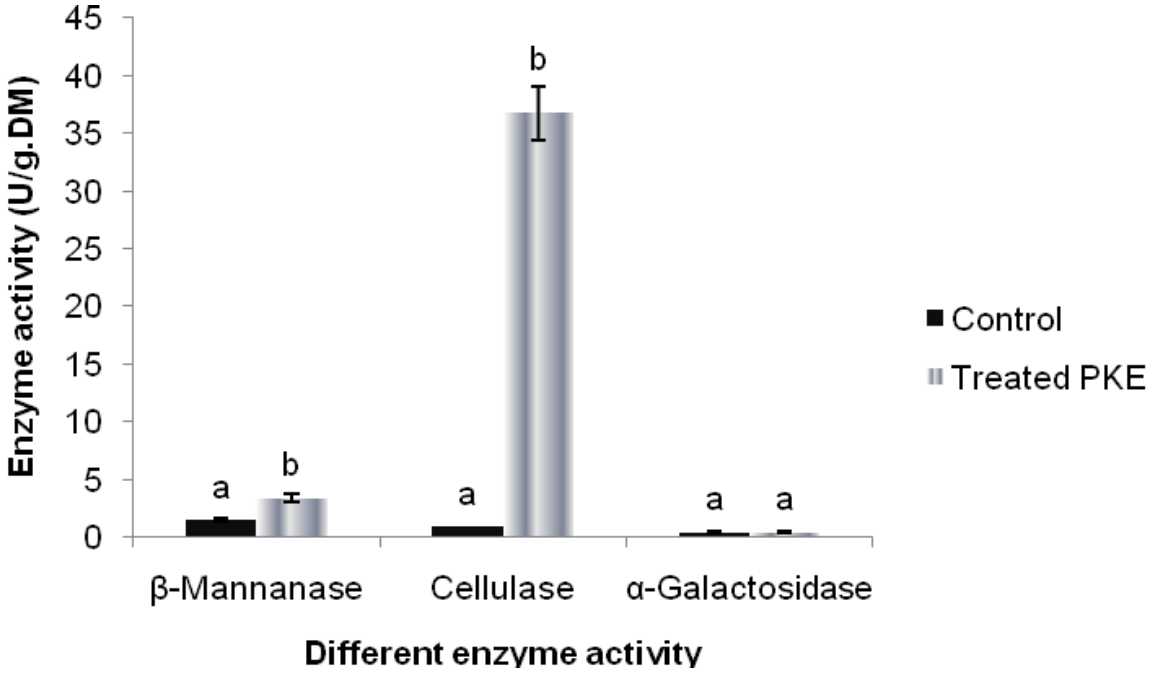

Figure 2. Concentrations of enzyme activity in the control and treated PKE.

Ng et al. (2002) reported that enzyme treated and fungal fermented PKM increased reducing sugars content by about 69% (from 2.87 to 9.25 mg/g) and 65% (from 2.87 to 8.09 mg/g), respectively, which were much lower than values obtained in this study. The hydrolysis of insoluble cellulose and hemicellulose into soluble sugars indicate an increase in usable energy in the enzyme treated PKE for monogastric animals such as chickens compared to the untreated PKE.

Enzyme activity

Activity of three enzymes; namely β-mannanase, cellulase and α-galactosidase in the control and treated PKE were compared (Figure 2). Enzyme treated PKE had higher ($P<0.01$) mannanase and cellulase than those in the control. Mannanase enzyme activity of the treated sample increased two folds ($P<0.01$) compared to the control while cellulase activity increased ($P<0.01$) about 37 folds. However, no difference ($P>0.05$) was detected in α-galactosidase between the control and treated PKE; 0.44 U/g DM. Based on the aforementioned results, the enzyme used for this study consisted mainly cellulase and mannanase. The low but detectable levels of the earlier three enzymes measured in the control samples could be due to the growth activities of microorganism found naturally in the samples.

Conclusion

Exogenous enzymes used in this study effectively hydrolyzed complex hemicellulose and cellulose and reduced their contents by 34.81 and 22.14%, respectively, compared to the control. There was significant increase, up to approximately 200 folds in the total reducing sugars (glucose, mannose, galactose and xylose) measured in the treated samples compared to the control, suggesting the effective hydrolysis of structural carbohydrates (hemicelluloses and celluloses) into monosaccharide sugars. The aforementioned reduction of fiber contents and the increased production of reducing sugars were due to the high activity of cellulase and mannanase present in the enzyme used. Several previous studies suggested that supplement of enzyme in broiler diets containing PKE improved growth performance, feed efficiency and metabolizable energy compared to raw PKE (Yahya et al., 2000; Chong et al., 2008; Sekoni et al., 2008; Iyayi and Davies, 2005), however, Choct (2006) reported that enzymes were not very effective in breaking down the non-starch

polysaccharides to monomeric sugars within the gastro-intestinal tract of poultry and suggested that pre-treating the PKE with enzymes before feeding could be a better option in improving the nutritive values of this byproduct.

A series of feeding trials are on-going in our laboratory to determine whether the reduction in fiber content and presence of higher content of soluble sugars in the enzyme treated PKE could enhance performance of broiler chickens.

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