Effect of β-mannanase on nutrient composition of palm kernel meal mixed with yeast cake

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Abstract The results of present study showed that % crude fiber were decreased when treated yeast-premixed palm kernel meal with β-mannanases in time- and concentration-dependent manner. Supplementation of β-mannanases at 0.02 and 0.04% significantly reduced the crude fiber content in 12 hours of incubation ($p<0.05$). The best improved palm kernel meal was obtained when 40% of yeast cake was mixed with palm kernel meal and 0.04% enzymes was added for 24 hours of incuabtion which contained % crude fiber equal to 4.84±0.85 and % crude protein equal to 40.86±1.87. In conclusion, improvement of palm kernel meal using enzymes and yeast cake increased % crude protein and reduced % crude fiber which could be further use as diets for non-ruminants.

Keywords: Palm kernel meal, β-mannanase, Feed improvement.

Introduction

Improving the quality of an agro-by-products as a promising feed ingredient could help to sheer the competition for food between human and livestock species and maximize the value of those available by-products. Palm kernel meal (PKM) is a locally available agro-industrial by-product and relatively inexpensive feedstuff in many tropical countries (Perez *et al*., 2000), including Thailand.

PKM contains a moderate level of crude protein (approximately 15 - 20%) but poor amino acid profile (deficient in lysine, methionine, and tryptophan) with a high level of fiber (approximately 13 to 20%) (Alimon, 2004; Abdollahi *et al*., 2015). Thus, it is considered as a moderate quality feed ingredient for ruminant but not suitable for monogastric animals (Alimon, 2004). Since non-ruminant animals such as poultry and swine have simple stomachs which limit in the digestion of fiber, the incorporation of the improper amount of PKM in non-ruminant diets is reported to be associated with impaired performance parameters (Sundu *et al*., 2006; Mardhati *et al*., 2011).

PKM contains β-mannan as the main fiber components, making up between 30 to 35% on dry matter basis of PKM (Sundu *et al*., 2006). It had been reported that PKM contained β-mannan content of up to 300 to 350 g/kg (Jackson, 2010). β-mannan is mostly consisted of water-insoluble glucomannan and a small amount of water-soluble galactomannan thus making it hard and water-insoluble (Knudsen, 1997; Sundu *et al*., 2006). Several studies have shown that supplementation of exogenous fibrolytic enzymes on diets containing PKM could improve its nutritive values by a great potential in releasing unavailable nutrients and energy (Sekoni *et al*., 2008; Chong *et al*., 2008; Natsir *et al.*, 2018). Since the main fiber of PKM is β-mannan, the major enzymes used to improve the nutritive value of PKM are mannanases, together with other fibrolytic enzymes including α-galactosidase and cellulase (Sundu and Dingle, 2003). However, studies investigating the effect of enzyme supplementation on PKM-containing broiler diets reported that fibrolytic hydrolysis to monomeric sugars is not very effective in the gastrointestinal tract of poultry and suggested that pre-treatment of PKM with enzymes before feeding could be an option in improving the nutritive values of PKM (Choct, 2006).

Besides the high fiber content, the moderate crude protein level and poor amino acid profiles also deteriorate the nutritive value of PKM. Single-cell protein (SCP) is the protein extracted from cultivated microbial biomass which can be used for protein supplementation replacing costly conventional protein sources like soymeal and fishmeal to improve protein quality in poor protein diets. Yeast *Saccharomyces cerevisiae* is well-known for its high protein content up to 40 – 50% and good amino acid profile with high essential amino acids especially leucine and lysine (Yamada and Sgarbieri, 2005). Yeast biomass is not only a source of proteins but also an excellent source of B-complex vitamins and nucleic acids (Ferreira e*t al.*, 2010). *S. cerevisiae* been accepted as safe as a food for humans and animals. Thus, yeast cake could be an excellent choice of protein source for improving the nutritive values of PKM.

The study aimed to investigate the efficacy of β-mannanase enzyme pre-treatment effect in lowering the crude fiber content of yeast-premixed PKM. We found that supplementation of β-mannanase effectively reduced

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the crude fiber while increased the soluble sugar content. Therefore, this improvement could be benefit to further application of PKM in non-ruminants.

Materials and methods

Sample sources

Palm kernel meal was perchesed from a reputable feed mill in Pranburi, Prachuap Khiri Khan Province, Thailand in 2017. The samples were stored in moisture free container until use. Hemicell^{TM}-HT (Elanco, USA) and dried *Saccharomyces cerevisiae* yeast cake was provided by Behn Meyer Chemicals Co., Ltd. All reagents and solvents were analytical grade.

Preparation of β-mannanase treated yeast-premixed palm kernel meal

Palm kernel meal were pre-mixed with yeast cake at 3 different ratios including 100:0, 80:20, and 60:40 palm kernel meal: yeast cake (PKM100, PKM80, and PKM60, respectively) in the plastic bag at a total weight of 200 g in each bag. Commercial β-mannanase (Hemicell^{TM}-HT) was added at 0.00%, 0.02%, and 0.04% to every ratio of yeast-mixed palm kernel meal. The enzyme was mixed throughly before thightly closed the plastic bag. The β-mannanase treated yeast-premixed palm kernel meal were then incubated at 40°C in the temperature control incubator. Samples with different level of β-mannanase treatment were analysed for proximate composition and soluble sugar content at 0, 12, and 24 h after incubation. Each treatment was done in triplicates.

Determination of proximate nutrient composition

Proximate composition including dried matter (DM), crude protein content (%CP) and crude fiber content (%CF) of all treatments and test ingredients, palm kernel meal, and yeast cake, were determined according to the method of Association of Official Analytical Chemists [A.O.A.C.] (1990). Each sample were analysed in triplicates.

Soluble sugar content analysis

Soluble sugar contents were determined by the 3,5-dinitrosalicylic acid (DNS) reducing sugar assay (Miller, 1959). One gram of each sample was dissolved in 10 ml of distilled water. The samples were shaken throughly for 10 min and centrifuged at 10,000 g for 10 mins. The pellet was discarded and the supernatant was transferred into a new clean tube. Supernatant (0.2 ml) was diluted with 0.8 ml of distilled water then 2.0 ml of DNS solution was added. Samples were boiled for 10 mins prior to the addition of 7.0 ml of distilled water. Absorption at 550 nm was read using microplate reader (SPECTROstar Nano, Germany) and sugar content was calculated using glucose as standard. Each sample was done in triplicates.

Statistic analysis

One-way analysis of variance (ANOVA) were calculated using R-program and comparison of means was performed by Tukey's multiple range tests. A value of $P<0.05$ was considered statistically significant.

Results

Nutrient composition of PKM, yeast cake, and yeast-premixed PKM

Prior to mixing, the nutrient compositions of PKM and yeast cake used in this study were analyzed individually. As shown in Table 1, PKM contained high fiber content of up to 30.07% compared to yeast cake which has low in fiber (0.42%). Crude protein analysis results demonstrated the yeast cake has a high amount of protein about 2-fold higher than that of PKM. PKM mixed with yeast cake showed higher protein content. PKM80 and PKM60 contained 26.53 and 32.60% of crude protein, respectively.

Table 1. Chemical composition (%DM) of PKM and yeast cake

Chemical contents	PKM100	Yeast cake	PKM80	PKM60
Dry matter	94.88	94.40	94.79	94.58
Crude fiber	30.07	0.42	26.31	16.34
Crude protein	19.65	46.69	26.53	32.60

Crude protein composition of β-mannanase treated yeast-premixed PKM

PKM was mixed with yeast cake in order to improve its protein composition. Crude protein content (%CP) of yeast-premixed PKM was higher when increasing the ratio of yeast cake (21.40±2.62, 26.53±2.65, and 32.60±7.24 for PKM100, PKM80, PKM60 at 0 h with no enzyme, respectively). As shown in Figure 1, upon incubation time increase, the treatment of PKM100 plus 0.02% enzyme, PKM80 plus 0 and 0.02% enzyme and PKM60 plus 0 and 0.02% enzyme showed significant time-dependent increased of %CP ($P<0.05$). At 24 h, PKM100 supplemented with 0.02 and 0.04% enzyme showed %CP equal to 28.42±1.05 and 25.48±1.92 which were significantly higher than 0% enzyme (21.53±1.14). The highest amount of %CP was obtained from PKM60 incubated without enzyme for 24 h (45.45±3.91) (Figure 1).

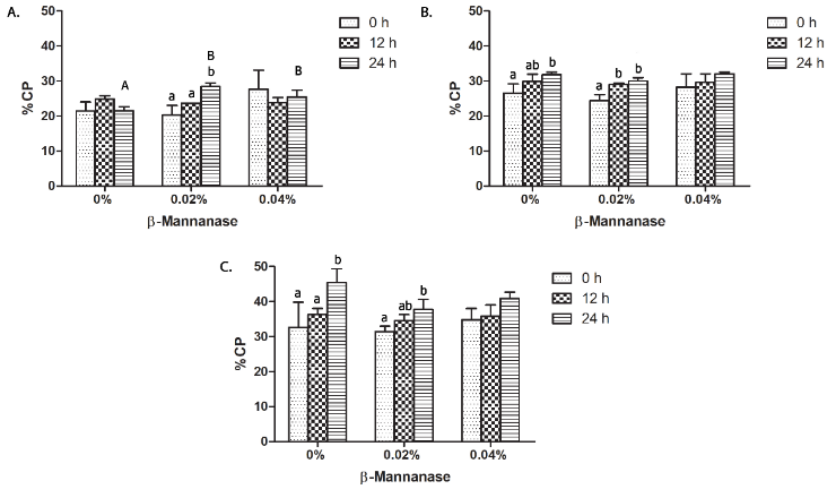

Figure 1. Crude protein composition (%DM) of β-mannanase treated yeast-premixed PKM at the ratio of PKM100 (A), PKM80 (B.) and PKM60 (C). The different small letter above the bar means significant different among incubation times of each enzyme level in each group of yeast-premixed PKM ($P<0.05$). The different capital letter above the bar means significant different among enzyme levels of the same incubation time in each group of yeast-premixed PKM ($P<0.05$).

Crude fiber composition of β-mannanase treated yeast-premixed PKM

Yeast-premixed PKM of 100:0, 80:20, and 60:40 ratio of palm kernel meal: yeast cake (PKM100, PKM80, and PKM60, respectively) were determined for β-mannanase effect on the reduction of fiber content in time- and dose-dependent. Three different levels of enzyme including no enzyme (0%), recommended level (0.02%) and high level (0.04%) were applied to the yeast-premixed PKM and incubated in an air-free plastic bag up to 24 h.

Generally, the crude fiber content of the yeast-premixed PKM was lower when more amount of yeast cake was mixed in PKM (Figure 2). Enzyme treatment of PKM100, PKM80, and PKM60 shown that %CF were reduced significantly at 24 h of incubation compared to time zero when 0.02 and 0.04% of β-mannanase enzyme were added (Figure 2).

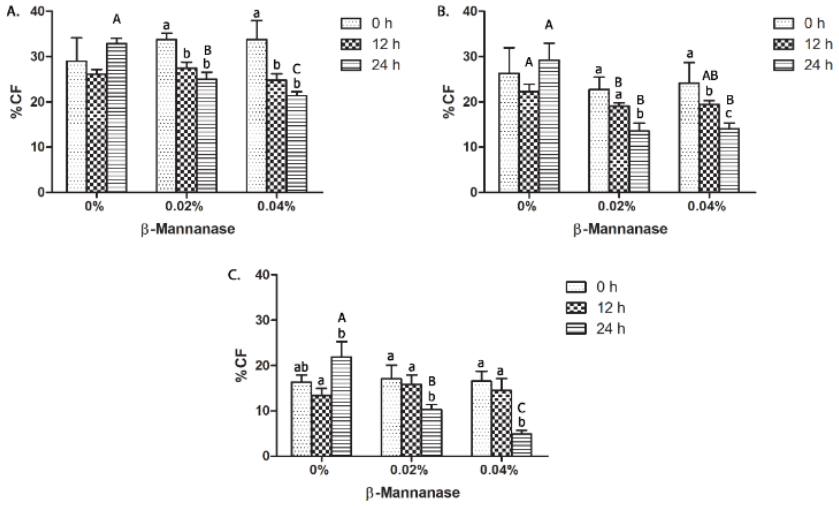

Figure 2. Crude fiber composition (%DM) of β-mannanase treated yeast-premixed PKM at the ratio of PKM100 (A), PKM80 (B.) and PKM60 (C). The different small letter above the bar means significant different among incubation times of each enzyme level in each group of yeast-premixed PKM ($P<0.05$). The different capital letter above the bar means significant different among enzyme levels of the same incubation time in each group of yeast-premixed PKM ($P<0.05$).

At 0 h incubation, yeast-premixed PKM showed no difference in crude fiber content regardless of the enzyme supplementation. The slight reduction in %CF was found at 12 h incubation of PKM80 treated with 0.02% and 0.04% enzyme. The %CF were significantly reduced from 22.23±1.67 of no enzyme to 19.10±0.69 and 19.41±0.86 of 0.02 and 0.04% enzyme ($P<0.05$). A sharp difference of %CF at different enzyme levels were shown at 24 h of incubation. Percentage of a crude fiber of PKM100 treated with β-mannanase were significantly reduced from 33.79±4.17 at no enzyme added to 24.76±1.46 and 21.36±0.84 for 0.02 and 0.04% enzyme, respectively ($P<0.05$). PKM80 also showed a significant β-mannanase effect at both 0.02 and 0.04% (13.59±1.71 and 14.07±1.26, respectively). PKM60 showed a significant decrease in %CF of 21.91±3.36 of no enzyme to 10.24±1.19 and 4.84±0.85 for 0.02 and 0.04%, respectively ($P<0.05$) (Figure 2).

Soluble sugar content of β-mannanase treated yeast-premixed PKM

Upon hydrolysis of β-mannan by the mannanase enzymes, the amount of β-mannan fiber is reduced while the soluble sugar is liberated which can be measured by determining of soluble sugar content in the sample. As shown in Figure 3, generally, the soluble sugar content of the yeast-premixed PKM was increased upon incubation time increase from 0 to 24 h. Even though the changes in soluble sugar could be seen without β-mannanase treatment; however, the effect of β-mannanase treatment can be distinguished.

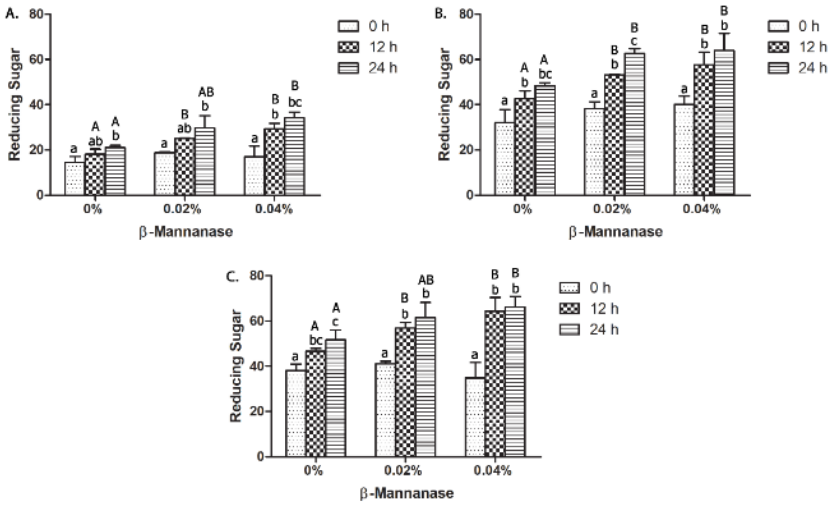

Figure 3. Soluble sugar content (mg/g dwPKM) of β-mannanase treated yeast-premixed PKM at the ratio of PKM100 (A), PKM80 (B.) and PKM60 (C). The different small letter above the bar means significant different among incubation times of each enzyme level in each group of yeast-premixed PKM ($P<0.05$). The different capital letter above the bar means significant different among enzyme levels of the same incubation time in each group of yeast-premixed PKM ($P<0.05$).

At 12 and 24 h incubation, 0.02% and 0.04% β-mannanase treatment produced a significantly higher amount of soluble sugar compared to no enzyme ($P<0.05$) (Figure 3). For PKM100 at 24 h, the amount of soluble sugar of 0.02 and 0.04% were 29.69±5.31 and 34.08±2.66 mg/g dwPKM which were significantly higher than no enzyme treatment (21.03±0.95 mg/g dwPKM). For PKM80 at 24 h, the soluble sugar content of 0.02 and 0.04% were 62.61±2.16 and 63.99±7.59 mg/g dwPKM which were significantly higher than 48.35±1.37 mg/g dwPKM of no enzyme treatment. For PKM60 at 24 h, 0.02 and 0.04% β-mannanase contained soluble sugar of 61.53±6.67 and 66.08±4.83 mg/g dwPKM while no enzyme contained lower soluble sugar of 51.65±4.27 mg/g dwPKM. The significant effect of enzyme treatment found was correlated to the reduction of the crude fiber content of the enzyme-treated PKM.

Discussion

An abundance of agro-by-products triggers the research invention of making these massive materials more values. PKM which has a moderate profile on proteins, but contains a high content of fiber is of interest for use as a feed ingredient for ruminants; however, it is not suitable enough for monogastric animals. Thus, improving the nutritive values of PKM to increase protein level, but lowering the fiber is one of the promising approaches. In this study, PKM was successfully improved its protein and fiber content by pre-mixed with yeast cake *Saccharomyces cerevisiae* followed by hydrolyzing the mannan fiber by β-mannanase enzymes.

The results demonstrated that PKM mixed with 40% yeast cake had a high protein content about 32% CP and was found increasing up to 45% when incubated for 24 h. The %CP obtained from an improved PKM is almost comparable to the %CP of soybean meal (SBM) which is about 45 to 50% (Baker and Stein, 2014; Lagos and Stein, 2017). Increasing time of incubation might result in higher protein content. Yeast fermented of poor protein feedstuffs have been showed to improve protein content. Cassava ships fermented with live-cell *Lactobacillus* and *S. cerevisiae* for 3 days showed improve protein content from 8.2% of untreated to 21.5% (Oboh, 2006). Swe *et al.* (2004) reported the higher crude protein in fermented PKM (29.4%) compared to untreated materials (16.9%). However, fermentation may take time and burdensome since it needs a special procedure for bacterial culture conditions. Thus using yeast cake agro-industrial wastes may help reducing time and avoid the troublesome of bacterial culture fermentation to obtain the desired amount of protein content. In addition to level up the protein content, since yeast contains a low level of fiber contents, our yeast cake mixed PKM showed reducing fiber as shown by approximately 50% reduction of crude fiber in PKM60 compared to without yeast, PKM100.

Enzyme treated yeast-mixed PKM significantly reduced the fiber content. The level of the enzyme at 0.04% was more effective than 0.02% in reducing crude fiber even though the soluble sugar of 0.02 and 0.04 is generally not different. Saenphoom *et al*. (2011) reported that pre-treating PKM with exogenous enzyme degraded

cellulose and hemicelluloses components by 22.14 and 35.70%, respectively, and increased availability of reducing sugars than the raw PKM. Enzyme treated PKM enhanced nutrient digestibility, retention of vital nutrients, and metabolizable energy (Iyayi and Davies, 2005; Sekoni *et al.*, 2008; Seanphoom *et al*., 2013). Several studies also showed that incorporation of enzyme-treated PKM in feed diets provided improve growth performance in poultry production (Esuga *et al.*, 2008). Reports indicated that inclusion of hemicell mannanase improved the performance and health of broilers fed with PKM-based diets (Daskiran *et al.*, 2004; Zou *et al.*, 2006). However, the efficacy of enzyme hydrolysis *in vivo* is difficult to extrapolate since the stability of additive enzyme may vary between different sources of enzymes. Therefore, pre-treated of PKM and evaluation of nutritive composition before incorporation in feed diets is advantage.

The results of β-mannanase treated yeast-premixed PKM shown in this study provide the alternative choices for improving PKM nutritive values with easy–to–make procedure avoiding the conventional bacterial fermentation. Evaluation of the nutritive composition of pre-treated PKM could help in the estimation of a suitable level of improved PKM in the formulation of feed diets. this improved yeast-premixed PKM could be further formulated evaluation for its efficacy in replacing of SBM in feed diets on growth performance and quality of meat products.

Acknowledgement

We thank Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, for financial and instrumental support of this work. We also would like to offer a particular thanks to Ms. Thitirat Pawaphutanon and Mr. Thanyaboon Limsakul for technical assistances.

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