Research Article

Nutrient Composition and Cell-Wall Structure of Palm Kernel Cake Supplemented with Enzymes

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Abstract | This study aimed to determine the nutritional composition of palm kernel cake (PKC) and its cell-wall structure. PKC with and without shells samples were used to study the chemical composition of PKC, and PKC with and without enzymes were used to investigate cell-wall structure. The enzymes consisted of mannanase (182 g/tons), nonstarch polysaccharide degrading enzyme (200 g/tons), and protease (130 g/tons). The chemical composition included dry matter (DM), crude protein (CP), ash, ether extract (EE), nitrogen-free extract (NFE), crude fiber (CF), total energy, amino acids (AAs), and fatty acids (FAs). In this study, the chemical composition was analyzed T - test student, while AAs, FAs, and cell-wall structure was analyzed descriptively. The microstructure of PKC cell wall after *in vitro* digestibility was determined by scanning electron microscopy (SEM). The PKC with shell contained the following: 4,417.81 Kcal/kg gross energy, 95.45% DM, 4.51% ash, 15.95% CP, 8.36% EE, 21.21% CF, 49.78% NFE, 0.55% lysine, 0.09% methionine, and 47% lauric acid (LA). The PKC without shell had the following: gross energy of 4,555.43 Kcal/kg, 97.84% DM, 4.26% ash, 22.33% CP, 9.29% EE, 15.43% CF, 50.29% NFE, 0.62% lysine, 0.14% methionine, and 49% LA. The PKC without enzyme addition showed a whole and smooth cell-wall structure, and that of PKC with enzyme was looser, coarser, and more porous. In conclusion, PKC without shells had higher chemical components, AA, and FA than the PKC with shell. The results on the cell-wall structure of PKC with enzyme showed that enzymes could loosen the cell-wall bonds of PKC.

Keywords | Amino acid, Cell wall structure, Chemical composition, Enzyme, Fatty acid, PKC

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INTRODUCTION

Palm Kernel Cake (PKC) is a by-product of palm kernel oil (PKO) production. PKC production in Indonesia approximately reached 4.16 million tons in 2021 (BPS, 2023). Mechanical pressing leaves produced a large amount of oil (around 9.6%), which causes the rapid rancidity of PKC due to the relatively high level of fat oxidation. PKC can be potentially used as poultry feed because it had a crude protein (CP) content of 18.71% (Dairo and Fasuyi, 2007). However, it also contained high crude fiber (CF) (21.7%), including hemicellulose (mannan and galactomannan), neutral detergent fiber (66.8%-78.9%) (Alimon, 2004), and mannan (35.2%) (Fan *et al.*, 2014). Therefore, the utilization of PKC in poultry feed remains limited. PKC also has a low amino acid (AA) balance, with AAs consisted of 0.37% lysine and 0.22% methionine (Alimon, 2004; Azizi *et al.*, 2021), and digestibility

(Sinurat, 2012). Approximately 9.1% up to 22.8% of shell fragments contaminated the PKC. Shell fragments can be reduced via shell filtration through a sieve with a diameter of 2 mm, which effectively reduced PKC shells by up to 50% (Sinurat *et al*., 2013).

The different treatments for quality improvement (increase CP content, improve nutrient value, and decrease CF) of PKC included physical, chemical, and biological methods (Prasetya *et al*., 2021; Abdeltawab and Khattab, 2018). Physical treatment involved the sieving PKC using a 2 mm diameter sieve. Biological treatment includes the addition of commercially available enzymes, such as mannanase or bacteria-producing mannanase (Prasetya *et al*., 2021). Chemical treatment requires the processes of PKC via the addition of an acidic solution for delignification to eliminate PKC lignin (Prasetya *et al*., 2021; Sharmila *et al*., 2014). Biological treatment is easy to implement in the industrialization process by adding commercial enzymes in the feed containing PKC. Enzymes are a type of animal feed additives. The use of feed additives is essential to improve the feed efficiency of poultry production. Nonstarch polysaccharide degrading enzymes (NSPases) are added into diet to break the bonds between the glucose units of NSP and considerably reduced the digesta viscosity in the digestive tract of chickens to improve nutrient digestion, absorption, and health status. The addition of enzymes, such as xylanase and mannanase, promoted the utilization of NSP in the diet, while proteases increased protein utilization (Singh *et al*., 2015).

Palm kernel cake has high content of fatty acids, especially lauric acid, which is 37.75% (Freitas *et al*., 2017). Lauric acid is a medium chain fatty acid that has C:12 atoms. Medium-chain fatty acids are fatty acids that are easily absorbed into the blood and easily oxidized than lipids (Shah and Limketkai, 2017). The use of medium-chain fatty acids (lauric acid) in broiler feed has a positive effect on the gut health and performance of chickens (Zeitz *et al*., 2015).

Lysine and methionine are critical AAs for poultry nutrition. Lysine plays an essential role in protein synthesis. This AA is required for cell growth and maintenance and contributed to the utilization of dietary proteins by interacting with other AAs, such as threonine, through metabolic pathways. Therefore, AA availability in diet must be considered (Khwatenge *et al*., 2020). Methionine is sulfur-containing AA. Given their limited availability in plant-source feed ingredients such as corn and soya-based poultry diets, these compounds served as the first limiting AA in poultry diets. The level of methionine was high in poultry (0.49%) methionine because as a methyl donor, cofactor, and a precursor of cysteine, it is responsible for cellular metabolism (Bunchasak, 2009). Based on this theory, this research was conducted to investigate the nutritional composition of PKC, particularly AAs and fatty acids (FAs), and the structure of PKC fed to broilers with mannan-degrading enzymes. The addition of commercial enzymes such as mannanase, protease, and NSPase to feed containing PKC is expected to result in optimal growth when applied to poultry.

MATERIALS AND METHODS

Sample preparation

PKC with and without shells were used in this study and obtained from PTPN III Medan, North Sumatra. PKC was filtered using a 2 mm-diameter sieve. The PKC samples with and without shell were analyzed for their chemical composition, such as dry matter (DM), gross energy (GE), ether extract (EE), ash, crude protein (CP), crude fiber (CF), nitrogen-free extract (NFE), amino acids (AAs), fatty acid (FA), and the cell-wall structure. PKC was treated with enzyme as follows: mannanase (182 g/tons; Elanco Animal Health, Indiana, USA), NSPase (200 g/tons; Kemin Industries Asia Pte, Ltd., Singapore), and protease (130 g/tons; Jefo Nutrition, Quebec, Canada) while PKC without enzyme was used as control. Enzymes were mixed manually and stored in the refrigerator before analysis.

Chemical composition

Chemical composition of PKC included gross energy, DM, ash, CP, EE, CF, and NFE (AOAC, 2005).

Amino acid analysis (AOAC, 2005)

Two samples were used in this research. A total of 60 mg samples and 4 ml 6 N HCl were heated for 24 h at a temperature of 110 °C, and the results were obtained. Then, the samples were cooled to ambient temperature, neutralized with 6 N NaOH (pH 7), made up to 10 ml volume using aquabidest, and filtered using a 0.2 mm Whatman paper. Next, 50 µl samples were added with 300 ml orthophalaldehyde solution, stirred for 5 min, and injected (10 µl) into a HPLC (Ultimate™ 3000 Rapid Separation, Carlsbad, USA) injector. The column used was LiChrospher 100 RP-18 (5 mm) with mobile phases A (absolute methanol, 50 mM sodium acetate, tetrahydrofuran (2:96:2), pH 6.8) and B (65% methanol) at a flow rate of 1.5 ml/min. Seventeen AAs standard were used: aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, threonine, arginine, alanine, tyrosine, methionine, valine, phenylalanine, leucine, leucine, and lysine.

Fatty acids analysis

Two samples were used in this study. Fatty acid (FA) extraction was performed according to the AOAC Method (2005). A total of 5 g samples in a fat-free filter paper were placed in oven at 105°C overnight and weighed. Then,

each sample was placed in a Soxhlet and extracted with petroleum benzene for 16 h. The PKC oil was collected in a flask. The oil samples were evaporated using a rotary evaporator. A total of 0.25 ml of oil was added with 0.75 ml Na-methanol, heated in a water bath at 60 °C for 10 min, cooled and esterified with 1 ml boron trifluoride, heated at 60 °C, cooled again, and added with 2.5 ml n-hexane. Water-free hexane (1 µl) was injected for gas chromatography (GC) (Silalahi *et al*., 2018). GC–mass spectrometry (Agilent Technologies GC System 7890B, Santa Clara, USA), column Agilent HP88, and a flame ionization detector with standard supelcoR FAME Mix CRM47885 (Sigma Aldrich, St. Louis, Missouri, USA) were used to measure the peak area of FA methyl esters. The FA chromatographic peak area of standard was considered as 100%, and proportional calculations were performed for each FA peak value of samples (Demirci and Basalan, 2021).

In vitro digestibility (Bedford and Classen, 1993)

In vitro digestibility was determined via a two-step method. Two samples were used in this study, PKC with and without enzyme. A total of 15 ml 0.1 N HCl containing 2000 U pepsin/ml was incubated for 45 min at 40 °C with 0.5 g sample of PKC with and without shell (ground to 1 mm). Then, 3 ml $NaHCO_3$ (1 M) containing 2 mg/ml pancreatin was added. Incubation was continued for 2 h at 40 °C. After incubation, the samples were filtered using a filter paper (Whatman No. 1) to obtain the biomass. Sample of PKC biomass was observed by scanning electron microscopy (SEM).

Observation PKC cell-wall structure via SEM (Ezeilo *et al*., 2019)

A total of 0.5 g sample of PKC with and without enzyme after *in vitro* assay was added with 12.5 ml distilled water. Then, the samples were stirred at 150 rpm for 30 min and filtered using Whatman No. 1 filter paper. The residue was dried at 70 °C for 6 h. The samples were then sputter coated onto carbon tape, and a thin layer of gold was deposited using a JEOL JEC-3000FC Auto Fine Coater in a gold-plated tool. Two samples, PKC with and without enzyme were analyzed via field-emission SEM (JEOL 65110 LA series, Japan) at 1000× magnification.

Statistical analysis

The chemical composition was analyzed T - test student, and AA, FA, cell-wall structure of PKC was analyzed descriptively (Steel and Torrie, 1989).

RESULTS AND DISCUSSION

Chemical composition of PKC with and without shells

The results showed that there was a significant difference between PKC with shell and PKC without shell ($P<0.05$). The PKC with shells had lower DM, EE, and CF contents than the PKC without shells (Table 1). The chemical composition of PKC without shells was higher of CP than PKC with shells. This result suggests that PKC without shell is more effective as poultry feedstuff than that with shell. The PKC without shell contained 97.84% DM, 4.26% ash, 22.33% CP, 9.29% EE, and 15.43% CF and a gross energy of 4,417.81 Kcal/kg. The ash content in this study ranged between 4.21%–4.51%. The CF content of PKC with shell was higher than PKC without shell. Filtration of shell fragments increased the DM, CP, and EE contents by 2.44%, 28.57%, and 10.01%, respectively, and reduced the CF content by 27.25%.

Table 1: Results of chemical composition PKC with shell and without shell.

Nutrient composition	Palm kernel cake	
	With shell	Without shell
Gross energy (Kcal/kg)	4,417.81 ± 99.27	4,555.43 ± 32.23
Dry matter (%)	95.45 ± 0.71[b]	97.84 ± 0.28[a]
ash (%)	4.51 ± 0.41	4.26 ± 0.07
Crude protein (%)	15.95 ± 0.01[b]	22.33 ± 0.23[a]
Extract ether (%)	8.36 ± 0.16[b]	9.29 ± 0.09[a]
Crude fiber (%)	21.21 ± 0.01[a]	15.43 ± 0.01[b]
Nitrogen free extract (%)	49.78 ± 0.57	50.29 ± 2.66

[ab] Different superscripts in the same row indicate significant differences ($P<0.05$).

Amino acid content

The AA content of PKC without shells was higher than PKC with shells (Table 2). The increase in AA content ranged from 8.33% to 35.71%.

Table 2: Amino acid content of PKC with shell and without shell (%).

Composition of amino acid	Palm kernel cake	
	With shell	without shell
Aspartic acid	1.02	1.21
Glutamic acid	2.64	3.12
Serine	0.66	0.76
Glutamine	0.11	0.12
Glycine	0.62	0.73
Threonine	0.4	0.47
Arginine	1.87	2.27
Alanine	0.51	0.61
Tyrosine	0.68	0.87
Methionine	0.09	0.14
Valine	0.51	0.65
Phenylalanine	0.37	0.47
Iso leucine	0.28	0.39
Leucine	0.72	0.89
Lysine	0.55	0.62

Fatty acid content

Table 3 showed the FA contents of PKC with and without the shell. The PKC with shell contained approximately 47.0% lauric acid (LA), while PKC without shell had 49.0% of LA. A difference of 3.23% was observed between PKC with and without shell. Other high-content of FAs included myristic acid (16.7% in PKC with shell and 17.0% in PKC without shell), palmitic acid (9.01% in PKC with shell and 9.04% in PKC without shell), and cis-9 oleic acid (16.1% in with shell PKC and 14.2% in without shell PKC).

Table 3: The fatty acid content of PKC with shell and without shell (%).

Composition of fatty acids	Palm kernel cake	
	With shell	Without shell
Hexanoate	0.08	0.12
Octanoate	2.62	2.80
Decanoate	3.15	3.52
Laurate	47.0	49.0
Miristate	16.7	17.0
Palmitate	9.01	9.04
Stearate	2.81	2.79
cis-9 oleate	16.1	14.2
Linolelaidate	0.1	< 0.1
Linoleate	1.92	**1.43**

Microstructure profile of PKC

Microstructure profile of PKC with and without enzyme addition could be seen in Figure 1. The surface of PKC without enzymes (Figure 1a) appeared intact, smooth, and non-porous. However, after enzyme treatment, the surface became coarser and showed increased porosity (Figure 1b). Figure 1 showed that microscopically, enzyme treatment could break the bonds polysaccharide of cell wall structures PKC.

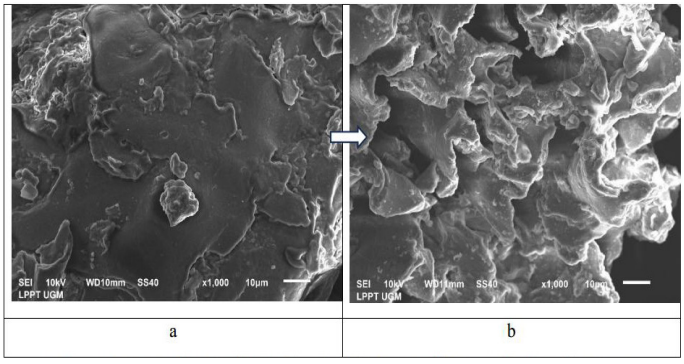

Figure 1: Images of PKC without (a) and with enzymes (b) at 1000× magnification.

Nutritional composition of PKC with and without shell

The PKC with shell had lower DM, CP, and EE contents and a higher CF content than the PKC without shell. Therefore, PKC without the shell is more effective as poultry feedstuff than PKC with shell. The PKC without shell contained 28.57% and 10.01% more CP and EE, respectively, than the PKC with shell. The ash and CF contents were 5.54% and 27.25% lower, respectively. These results agree with Sinurat *et al.* (2013), who observed that reduction the shell of PKC, increased nutrient contents of CP, EE, and AAs, and decreased CF. The DM content of PKC varies from 90.87% (Mustafa *et al.*, 2004) to 91.74% (Ezieshi and Olomu, 2009), 91.80% (Sharmila *et al.*, 2014), 93.21% (Wallace *et al.*, 2010), 88.57% (Keong, 2004), 91.42% (Alshelmani *et al.*, 2017), 96.3% (Zulkifli *et al.*, 2009), and 91.83% (Dairo and Fasuyi, 2007). Ash content serves an indicator of the total inorganic compound quality. Ash content of PKC was 4.74% (Alshelmani *et al.*, 2017), 4.3% (Zulkifkli *et al.*, 2009), and 7.56% (Dairo and Fasuyi, 2007). PKC contains 0.25% calcium (Ca), 0.6% phosphorus (P), and 0.3% magnesium (Mg) (Boateng *et al.*, 2013), and 0.16% Ca, 0.24% P (Wallace *et al.*, 2010). The CP content of PKC varies considerably from 13.60% to 20.56% (Sundu *et al.*, 2008; Pasaribu *et al.*, 2019; Akinyeye *et al.*, 2011; Wallace *et al.*, 2010; Keong, 2004; Alshelmani *et al.*, 2017; Zulkifli *et al.*, 2009; Dairo and Fasuyi, 2007). Enzyme-treated PKC contains 17.11% CP, 14.59% CF, and 5.15% EE (Keong, 2004). The shell reduction could increase the nutrient content (CP, EE, and amino acids) and decrease the CF content of PKC (Sinurat *et al.*, 2013). Shell reduction can reduce the CF content because the shell comprises 14.4% hemicellulose, 33.4% cellulose, and 46.3% lignin (Ninduangdee *et al.*, 2015). A shell observed in another study had a hemicellulose, cellulose, and lignin contents of 26.16%, 6.92%, and 53.85%, respectively (Okoroigwe *et al.*, 2014). PKC filtration using a 2 mm-diameter sieve reduced the CF content by 24.67%, increased the EE content by 13.66%, increased CP by 3.27%, and improved the AA content by 7.6% (Sinurat *et al.*, 2013).

Ezieshi and Olomu (2009) reported that PKC type Okomu, Presco, Envoy had 14.50%, 16.60%, 19.24% CP, respectively, had 10%, 12.29%, 17.96% CF, respectively, and had 9.48%, 7.59%, 1.30% EE, respectively. Okomu and Presco were mechanically-extracted PKC, while Envoy was solvent-extracted PKC. Crude fiber content of PKC was 14.6% (Zulkifli *et al.*, 2009), and 14.6% (Dairo and Fasuyi, 2007), while EE content of PKC was 8.03% (Zulkifli *et al.*, 2009) and 8.63% (Dairo and Fasuyi, 2007).

The PKC content varies due to different sources of PKC, types of palm kernels (Ezieshi dan Olomu, 2009), methods of separation of shells from palm kernels, the number of shells in the palm kernel before oil extraction (Omara *et al.*, 1999), and methods of processing palm kernels before use. Solvent-based oil extraction methods and mechanical methods affect the CF and EE contents of PKC (Mustafa

et al., 2004). Solvent-extracted PKC have a higher CF content than mechanically extracted cakes because of the high rate of oil extraction in the latter. The EE content of mechanically extracted PKC ranges between 8% until 9%, whereas PKC extracted with solvents reaches 1.3%. Solvent-extracted PKC have low levels of EE in PKC because it removed more fat than mechanical extraction (Ezieshi and Olomu, 2009). The DM content is used to determine the moisture content of the PKC to be stored. If the moisture content is higher than 14%, the PKC cannot be stored in large quantities and can be used for yeast growth, leading to decreased quality and nutrient content (Abdeltawab and Khattab, 2018).

Amino acid content

The AA content increased, especially lysine (11.62%) and methionine (35.71%), due to the reduction of PKC shells. The AA content of PKC without shell was increased of 3% until 10% (Sinurat *et al*., 2013). PKC contains low amounts of AAs, especially lysine and methionine (Sundu *et al*., 2006). Lysine and methionine are essential for poultry. Fagundes *et al*. (2020) studied the effects of high levels of dietary methionine on broiler growth, digestibility, and AA transporter gene expression. Dietary imbalances can affect the digestibility of AAs through changes in the expression of AA transporter genes. Diets containing 0.48% methionine (control) were compared with low methionine content (0.28%), and the results showed that chickens fed with methionine-deficient diets exhibited stunted growth and lower feed efficiency than the control. Lysine is the second most limiting AA in corn- and soy-based poultry diets, and feed supplementation with lysine has been used since the 1970s (Kidd *et al*., 2013). Adequate levels of lysine can maintain immunity and gastrointestinal function in poultry (Vaezi *et al*., 2011). The AA contents in this study was similar with other findings in the literature. Lysine content of PKC reached 0.37% (Jose *et al*., 2022), 0.44% (Sinurat *et al*., 2013), 0.44% (Alshelmani *et al*., 2017), 0.49% (Marini *et al*., 2008), 0.53% (Manaf *et al*., 2022), 0.50% (Hakim *et al*., 2020), 0.36% (Stein *et al*., 2015), 0.56% (Boateng *et al*., 2013), and 0.38% (Mustafa *et al*., 2004); methionine contents was 0.17% (Sundu *et al*., 2008), 0.59% (Sinurat *et al*., 2013), and 0.22% (Jose *et al*., 2022; Alshelmani *et al*., 2017), 0.26% (Marini *et al*., 2008), 0.20% (Manaf *et al*., 2022), 0.30% (Hakim *et al*., 2020), 0.22% (Stein *et al*., 2015), 0.21% (Boateng *et al*., 2013), and 0.30% (Mustafa *et al*., 2004).

According to Sinurat *et al*. (2013), PKC has an AA composition consisting of 0.24% serine, 0.41% threonine, 2.01% arginine, 0.59% methionine, 0.70% valine, 0.73% phenylalanine, 0.52% isoleucine, 1.03% leucine, and 0.44% lysine. Jose *et al*. (2022) observed the following AA contents: 0.21% histidine, 0.36% threonine, 0.22% methionine, 0.63% valine, 0.52% phenylalanine, 0.42% isoleucine, 0.79% leucine, and 0.37% lysine. Alshelmani *et al*. (2017) reported AA contents of 1.12% aspartic acid, 2.48% glutamic acid, 0.56% serine, 0.23% histidine, glycine 0.60%, 0.41% threonine, 1.60% arginine, 0.62% alanine, 0.25% tyrosine, 0.69% methionine, 0.69% valine, 0.57% phenylalanine, 0.50% isoleucine, 0.89% leucine, and 0.37% lysine. Sundu *et al*. (2008) recorded 0.78% serine, 0.32% histidine, 0.65% glycine, 0.53% threonine, 1.92% arginine, 0.24% tyrosine, 0.17% methionine, 0.77% valine, 0.64% phenylalanine, 0.55% isoleucine, 1.04% leucine, and 0.04% lysine. The AA content results in this study were similar to those reported by Marini *et al*. (2008), which showed amino acid contents of 2.06% arginine, 0.52% threonine, 0.89% valine, 0.49% lysine, 0.63% isoleucine, 1.07% leucine, 0.68% phenylalanine, 0.11% tryptophan, 0.27% histidine, 0.26% methionine, 1.38% aspartic acid, 0.71% serine, 3.44% glutamic acid, and 0.74% glycine.

Content of fatty acids

The FA content of PKC was similar to PKO. PKO contained medium-chain FAs (MCFAs) and SFAs, with LA (46.6%) and myristic acid (16.6%) in large amounts (Santos *et al*., 2022). According to Rahman *et al*. (2022), PKO contained 49.25% LA and 16.30% myristic acid. PKC contained 47.4% LA and 16.67% myristic acid (Oliveira *et al*., 2015). PKC had LA content of 37.75% (Freitas *et al*., 2017), 45.37% (Boateng *et al*., 2013), 47.40% (Silva *et al*., 2013), 50.49% (Jose *et al*., 2022), 52.13% (Abubakr *et al*., 2015) and PKC had myristic acid of 19.51% (Freitas *et al*., 2017), 15.35% (Boateng *et al*., 2013), 16.67% (Silva *et al*., 2013), 10.92% (Jose *et al*., 2022), 15.38% (Abubakr *et al*., 2015). LA is a C12 saturated MCFA (Demirci and Basalan, 2021). MCFA is medium chain fatty acid from 6 to 12 carbon atoms, found in coconut oil and palm kernel oil. These MCFAs consist of capric acid (C6), caprylic acid (C8), capric acid (C10), and lauric acid (C12) (Roopashree *et al*., 2021). Lauric acid as a feed additive can improve food safety by reducing the levels of *Campylobacter coli* in broiler meat (Zeiger *et al*., 2017). LA accumulates in broiler meat to change the composition of medium-chain fatty acid in chicken rations, and it may have beneficial effects on humans and extend the shelf life of broiler meat (Demirci and Basalan, 2021). Lauric acid is also known as a healthy SFA that can act as an antimicrobial, antiviral, and antifungal agent. This LA can be converted to monolaurine, which damages the cell membranes of pathogenic to inhibit bacterial growth. The cell membrane is damaged by the interaction among hydrogen, hydrophobic bacterial lipids, and membrane functional groups (Nitbani *et al*., 2022).

Microstructure profile of PKC by SEM

Cell wall structure in PKC with enzyme recombinant β

mannanase KMAN 3 and MAN 6.7 suggests the weaker bonds of the loose structure and its more easily hydrolyzed into mono-oligosaccharides compared with PKCs without enzymes (Sritrakul *et al*., 2020). The weaker bond can also be attributed to the physical, chemical, and biological factors associated with PKC (Prasetya *et al*., 2021). Cell wall of PKC is made up of 4% xylan, 12% cellulose, and 58% mannan. Structure mannan of PKC is composed of hard and crystallized mannan. Mannanase could release nutrients by breaking down the structure of fibers containing mannan (Sathitkowitchai *et al*., 2018). The addition of enzyme mixture to PKC altered the structure of lignin and hemicellulose and reduced the crystallinity of cellulose (Ezeilo *et al*., 2019). The degradation of NSP by NSPase resulted in the release of monosaccharides and starch in the endosperm, which triggered the release of more sugars (Malathi and Devegowda, 2001). The mannanase enzyme targets the cell wall matrix or fiber components as its substrate (Ravindran, 2013). PKC has a nonporous surface, whereas its fermented counterpart exhibits a looser shape and better microbial penetration and aeration (Saw *et al*., 2012). Osorio *et al*. (2022) reported that using confocal immunofluorescence, showed a disruption in the structure of cellulose and galactomannan in the cell wall of PKC treated with β-mananase enzyme. This suggests that the use of β-mananase in PKC could convert mannan into mannan oligosaccharides, leading to the formation of prebiotic compounds. According to Shukor *et al*. (2016) reported that, PKC treated with β-glucosidase, cellulase, and mannanase exhibited porous cell walls, whereas PKC treated with no enzyme had robust cell walls. The cell wall's porous surface revealed that hemicellulose and cellulose were broken down during the hydrolysis of lignocellulosic components by enzymes. Lee *et al*. (2019) reported that PKC fermented using *Lactobacillus plantarum* and observed using Scanning Electron Microscope, showed that the surface of PKC became uneven and more porous compared to PKC without fermentation. This indicates that the bacteria were actively growing and secreting enzymes to degrade the fibre in the PKC. Mannanase-hydrolyzed PKC may be useful as prebiotic for Lactic Acid bacteria (Kalidas *et al*., 2017).

CONCLUSIONS AND RECOMMENDATIONS

The PKC without shell presented a higher nutrient composition especially CP, AAs, and FAs, and presented a lower CF than the PKC with shell. The enzyme-treated PKC showed a coarser and more porous cell-wall structure, which indicates that it was easily hydrolyzed by enzymes. PKC without shell and PKC with enzyme could be recommended for poultry feedstuff.

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NOVELTY STATEMENT

Several enzyme treatments were used to improve the digestibility of PKC. The difference between this study and previous studies is the use of three kinds of commercial enzymes consisting of mannanase, NSPase, and protease enzymes.

AUTHOR'S CONTRIBUTION

SZCarried out the experiment, carried out the laboratory analysis, analysed the data and drafted the manuscript. CH: Supervised the experiment and revised the manuscript. BA: supervised the experiment. APB: Designed and supervised the experiment. Z: Wrote and revised the manuscript. All authors were responsible for the reading and approval of the final manuscript.

Conflict of interests

The authors have declared no conflict of interest.

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