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Effect of beta-mannanase treatment on nutritive quality of palm kernel meal

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The aim of this study was to evaluate the nutritive quality of β-mannanase treated palm kernel meal (PKM). Beta-mannanase production was conducted using locust bean gum (LBG) as the sole carbon source; moisten with mineral salt solution, and enzyme activity determined by dinitrosalicylic acid. Crude β-mannanase was concentrated by ammonium sulphate. The chemical composition of enzyme treated PKM was determined according to standard chemical methods. The mineral composition of the enzyme treated PKM was determined using atomic absorption spectrophotometer method. The result obtained shows an increase in crude protein from 16.02±0.40 in non-enzyme treated PKM (NTPKM) to 23.26±0.13 in enzyme treated PKM (ETPKM). There were significant reductions (P>0.05) in ash and crude fibre contents in the ETPKM as compared to the control. The ETPKM showed a markedly reduction in crude fibre by 64.02%. The lignin, cellulose and hemicelluloses content decreased in ETPKM by 17.32, 76.85 and 11.74%, respectively. The phytate, tannin and cyanide contents of ETPKM had a reduction of 29.26, 6.99 and 58.44%, respectively. The mineral analysis of ETPKM showed that calcium, copper and potassium reduced in PKM after enzyme treatment. The percentage reduction of calcium, copper and potassium in ETPKM were 33.65, 81.28 and 29.12%, respectively. However, there was significant increase in zinc and phosphorus contents of ETPKM in comparison with the control. The treatment of PKM with β-mannanase resulted in decrease of complex fibre fractions in the PKM to increase its crude protein and certain minerals (zinc and phosphorus) contents.

Key words: Nutritive quality, palm kernel meal, β-mannanase, chemical composition, mineral composition.

INTRODUCTION

The utilization of agro-industrial wastes as feedstuff is one of the strategies involved in the reduction of cost of livestock production. Agro-industrial by-products in Nigeria vary from primary processing of farm produce wastes to wastes from agro allied industries. Some of these wastes are left unutilized, often causing environmental pollution and hazard. Those that are utilized do not have their full potentials harnessed. Agro-industrial wastes can be of tremendous use in the livestock industry for feeding animals; they include brewers dried grain, palm kernel cake, maize offal, wheat offal, rice bran and cassava peels just to mention few. Microbial bioconversion and associated enzymes, especially fungal bioconversion of wastes seems to be a practical and

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promising alternative for increasing their nutritional value, transforming them into animal feed and thus producing a value-added product (Villas-Boas et al., 2003; Agosin et al., 2006), fungal bioconversion of agro-industrial by-products is an environmentally friendly biotechnological process (Karunanandaa et al., 1995; Zhang et al., 2002; Mukherjee and Nandi, 2004). From an animal nutrition point of view, agro-industrial wastes are not suitable feed ingredients as they are deficient in digestible protein (Song et al., 2009), rich in β-mannan and anti-nutrient compounds (Khanongnuch et al., 2006).

Nigeria is one of the major producers of palm (*Elaeis guineensis*) oil in Africa. An important by-product 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 to be a moderate quality feed ingredient for ruminant but not suitable for monogastric animals (Alimon, 2004; Saenphoom et al., 2011). The fiber of PKE is mainly hemicellulose consisting of 58% mannans (Saenphoom et al., 2011), moderate amounts of cellulose and small amount of other polysaccharides (Swe et al., 2004). Most mannan, making up between 25 to 32% of PKE 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 their complex chemical structure, the fiber of PKM and PKE require a combination of enzymes including mannanases, galactosidases, glucosidases and xylanases to release the potential fermentable sugars to be of use for monogastric animals. As the use of PKM and 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 in solid state fermentation (SSF) to breakdown and reduce the hemicelluloses, cellulose and lignin in PKM (Noraini et al., 2001), and (ii) supplementation of exogenous enzymes into the diet containing PKM in poultry (Chong et al., 2003).

In recent years, a β-mannanase produced from bacteria and fungi has been shown to improve feed conversion and performance of broilers, fish, turkeys and swine (Jackson et al., 1999). The important role of β-mannanase is hydrolyzing β-1,4-glycosidic linkages in β-mannan (Ooi and Kikuchi, 1995) and this can reverse the negative impact caused by β-mannan. For instant, Khanongnuch et al. (2006) reported an increase in the metabolizable energy (ME) and nutrient digestibility improvement of copra meal treated by β-mannanase. In addition, broiler chicks fed with enzymatic treated PKM by commercial enzymes increased in weight gain, feed conversion efficiency, dry matter digestibility and nutrient digestibility, while the jejuna content viscosity was decreased (Sandu et al., 2006).

Manno-oligosaccharides (MOS), one of the major end products of β-mannan hydrolysis by β-mannanase was found to be a substance which could prevent the colonization of *Escherichia coli* and Salmonellae, leading to an improvement of animal growth performance (Ishihara et al., 2000; Khanongnuch et al., 2006). The aim of this study was to evaluate the nutritive quality of β-mannanase treated PKM.

MATERIALS AND METHODS

Microorganisms

Penicillium italicum (Akinyele et al., 2013) previously confirmed to posses mannolytic property was obtained from the Research Laboratory, Microbiology Department, Federal University of Technology Akure (FUTA), Ondo State, Nigeria. The authenticity of the culture was confirmed by the method of Pitt and Hocking (1997) on the bases of cultural characters (colour, shape of colony, surface and reverse pigmentation and texture of the colony) as well as microscopic structure (septate or nonseptate hyphae, structure of hyphae and conidia). The fungal isolate was maintained on Malt Extract Agar (MEA) and sub-cultured at regular intervals. They were incubated at 30 ±2°C until the entire plates were covered by active mycelium and stored at 4°C in refrigerator on agar slants.

Sample sources

PKM was procured from a reputable feed mill in Akure, Ondo State, Nigeria. The samples were stored in air tight transparent plastic containers to keep it moisture free until use. Locust bean gum (LBG) was purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

Mannanase production

For the production of β-mannanase in solid state fermentation, the isolate was cultured at 30°C in 250 ml Erlenmeyer flasks containing 10 g LBG. The substrate was suspended in 33 ml Mandels and Weber's medium modified by El-Naggar et al. (2006). This medium (moistening agent) contained the following ingredients (g/L): Peptone 2, yeast extract 2, $NaNO_3$ 2, K_2HPO_4 1, $MgSO_4.7H_2O$ 0.5, KCl 0.5 and $FeSO_4.7H_2O$ traces. After sterilization at 121°C for 15 min, it was cooled and inoculated with 2 discs of 8 mm diameter of the organism from MEA culture plate using sterile cup borer. The flask was incubated at 30°C for 5 days at static condition.

Enzyme extraction

The solid state cultures were prepared by adding 10-fold (v/w) 0.1 M phosphate buffer (pH 6.8) and shaking (180 rpm) at 30°C for 60 min. The fungal biomass was separated by centrifugation (Centurion Scientific Limited) (6000 rpm, 15 min at 4°C).

Enzyme assays

β-Mannanase activity was assayed in the reaction mixture

Table 1. Proximate composition of mannanase treated PKM (% dry weight).

Sample	Moisture	Ash	Crude fibre	Crude protein	Fat
PKM [a]	10.33^{b}±0.19	3.63^{c}±0.08	40.00^{c}±0.05	16.02^{a}±0.40	5.90^{a}±0.24
[b]	10.09^{b}±0.02	3.29^{b}±0.03	26.17^{b}±0.08	22.34^{b}±0.44	5.77^{a}±0.33
[c]	9.77^{a}±0.14	2.68^{a}±0.09	14.39^{a}±0.39	23.26^{c}±0.13	5.48^{a}±0.13

[a] Untreated, [b] during treatment [c] after treatment. Values are means± S.E (n=3). Means with the same superscript letters down the same column are not significantly different (P>0.05).

composing of 0.5 ml of 1% LBG prepared in 50 mM potassium phosphate buffer pH 6.8 and 0.5 ml of enzyme solution at 45°C for 60 min (modified from El-Naggar et al. (2006)). Amount of reducing sugar released was determined by the dinitrosalicylic acid reagent (DNS) (Miller, 1959). One unit of mannanase activity was defined as amount of enzyme producing 1 micromole of mannose per minute under the experimental conditions.

Preparation of enzymatic treated PKM (ETPKM)

Beta-mannanase was produced by *P. italicum* using LBG as a carbon source. The extracellular β-mannanase was harvested after 5 h of cultivation by centrifugation with 3.62×10^3 g for 20 min at 4°C and the supernatant was used as crude enzyme solution. The supernatant was brought to 30% ammonium sulphate concentration and centrifuged for 20 min. The supernatant collected was further brought to 70% ammonium sulphate concentration and re-centrifuged for 20 min (Adebiyi et al., 2008). The sediment, that is, the precipitate was taken as the enzyme. For every 100 ml of the solution that was spun in the centrifuge, the precipitate was suspended in 5 ml phosphate buffer at pH 6.8. The sample (PKM) was hydrolyzed with concentrated β-mannanase at 30°C for 60 h (36 g of PKM was suspended in 200 ml distilled water plus 12 ml of enzyme preparation with an activity of 69.36 U/ml) within a sealing system (modified method of Khanongnuch et al., 2006). After completion of the reaction, the product was completely dried by vacuum dryer at 65°C for 60 h to obtain the ETPKM (Khanongnuch et al., 2006).

Determination of proximate composition of ETPKM

The proximate composition of ETPKM and non-enzymatic treated PKM (NTPKM) were determined by standard methods according to AOAC (2005). Fiber compounds including acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL) contents were measured sequentially with a fiber automatic analyzer (Fibertec System, M, Tecator, Hoganas, Sweden) (van Soest et al., 1991). Hemicellulose was calculated as NDF - ADF, cellulose as ADF - ADL, while lignin content is obtained by the subtraction of residue after extraction from ash. Phytate was determined through the extraction of the samples with hydrochloric acid and sodium sulphate and absorbance measured at 660 nm (De Boland et al., 1975). Tannin was determined using the method of vanillinhydrochloric acid and absorbance was measured at 500 nm (Price et al., 1978). Oxalate determination was done according to the standard method of Day and Underwood (1986), while cyanide content was evaluated by the method of Obadeni and Ochuko (2001).

Determination of mineral composition of ETPKM

The mineral composition of the enzyme treated PKM was determined using the atomic absorption spectrophotometer method as described by AOAC (2005).

Statistical analysis

The statistical analysis was performed using the general linear model function of Statistical Package for Social Science (SPSS), version 16.0. All data generated was subjected to one-way ANOVA while statistical differences of treatment were determined using Duncan's multiple range.

RESULTS AND DISCUSSION

Chemical composition of the control (NTPKM) and enzyme treated PKM (ETPKM) is shown in Table 1. As observed from the table, there was significant (P>0.05) increase in the crude protein of ETPKM when compare with control treatment (NTPKM). Crude protein increased from 16.02±0.40 in NTPKM to 23.26±0.13 in ETPKM. Liu and Baidoo (2005) reported that crude protein content of fungal fermented PKM increased nearly 2 folds (16.8 to 31.2%) while no significant increase in crude protein was detected in enzyme treated PKM. The authors suggest that the increased crude could be due to microbial protein synthesis during fermentation. Swe et al. (2004) also reported higher crude protein in fungal fermented PKM (29.4%) as compared to untreated material (16.9%), but the ratio of true protein (amino acids) to total crude protein for the fermented sample was much lower than the original PKM and further suggested that the increased crude protein in the fungal fermented PKE was due to non-protein nitrogen of the fungal cell wall which is non-digestible in poultry. The increase in crude protein value of the degraded PKC might partly be due to the ability of the enzyme to increase the bioavailability of the protein hitherto encapsulated by the cell walls (Ng et al., 2002). According to Bachtar (2005), the fungal enzymes have the potentials of improving not only the non starchy polysaccharides (NSPs) but also protein as well as other dietary components, such as fatty acids. Secretion of proteinase along side enzyme of interest by fungi invariably increases the protein content of feed materials. Many workers have reported similar increase in protein content. In his work, Iyayi and Aderolu (2004) reported increase in crude protein when *Aspergillus niger* was inoculated on sago fibre and cassava fibre resulting into 16.5 and 18.5% protein increase respectively. The author reported a 21.9% increase in the protein of cocoa shell when inoculated with *A. niger.* In their work, Ofuya and Nwajiuba (1990), reported increases in crude protein of 31, 36 and 41% with *A. niger*, 26, 33 and 38% with

Table 2. Fibre composition of mannanase treatment of *P. italicum* on PKM (% dry weight).

Sample	Lignin	Cellulose	Hemicelluloses
PKM [a]	10.64^{b}±0.50	62.11^{c}±0.49	12.91^{b}±0.64
[b]	10.38^{b}±0.13	24.01^{b}±0.24	12.42^{b}±0.33
[c]	8.80^{a}±0.20	14.38^{a}±0.37	11.40^{a}±0.14

[a] Untreated, [b] during treatment [c] after treatment. Values are means± S.E (n=3). Means with the same superscript letters down the same column are not significantly different (P>0.05).

Table 3. Anti-nutrient composition of mannanase treatment of *P. italicum* on PKM (mg/g dry) weight).

Sample	Phytate	Oxalate	Tannin	Cyanide
PKC [a]	15.63^{c}±0.83	0.36^{a}±0.01	0.14^{b}±0.01	9.84^{c}±0.02
[b]	12.24^{b}±0.07	0.36^{a}±0.00	0.14^{ab}±0.00	6.38^{b}±0.14
[c]	11.06^{a}±0.43	0.36^{a}±0.00	0.13^{a}±0.01	4.09^{a}±0.68

[a] Untreated, [b] during treatment [c] after treatment. Values are means± S.E (n=3). Means with the same superscript letters down the same column are not significantly different (P>0.05).

Aspergillus flavus and 27, 36 and 32% with *Penicillium* sp. in brewer's dried grain, maize offal and wheat offal, respectively after 14 days of their biodegradation. Similar results have been reported by Smith et al. (1996) when they cultured cassava peels with *Rhizopus* sp. The authors reported a 185% increase in the protein of the peels. Such high increase can be attributed to the fact that cassava peels are less fibrous than PKC. Results of other workers (Mikami et al., 1982; Balagopalan and Gregory, 1985; Manilal et al., 1985; Yokomizo, 2004) suggest the ability of fungi inoculated on low quality feed ingredients to increase the protein levels in such ingredients by the conversion of the carbon atom of the broken down carbohydrates into mycelia protein.

There was no significant decrease between the fat content of enzyme treated PKM and the control, however, enzyme treatment decreased fat content from 5.90±0.24 in untreated sample to 5.48±0.13 in ETPKM, which is in agreement with Swe et al. (2004) and Liu and and Baidoo (2005) who reported that crude fat 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>0.05) in ash and crude fibre contents in the ETPKM as compared to the control. The ETPKM showed a markedly reduction in crude fibre by 64.02%. The significant reduction in crude fibre content indicates that the enzyme is effective in breaking down mannan-hemicellulose, the main component of the fiber in PKM (Lawal et al., 2010; Saenphoom et al., 2011).

Lignin, cellulose and hemicelluloses (fiber compounds) contents decreased significantly (P>0.05) in ETPKM as compared to the control (untreated PKM) (Table 2). The lignin, cellulose and hemicelluloses content decreased in ETPKM from 10.64±0.50 to 8.80±0.20, 62.11±0.49 to 14.38±0.37 and 12.91±0.64 to 11.40±0.14, respectively. The aforementioned result is in agreement with that of Albores et al. (2006) who found that the fungal enzyme treated PKC reduced lignin, hemicelluloses and cellulose contents resulting in increased crude protein and soluble sugar (glucose, fructose, galactose and sucrose) contents. The reduction in fibre compounds (lignin, cellulose and hemicelluloses) of ETPKM could be attributed to the ability of the fungi to secrete hydrolyzing and oxidizing enzymes, which could convert the recalcitrant compounds in the waste into utilizable compounds (Tanveer et al., 2000; Akinfemi, 2012).

The phytate, tannin and cyanide contents in the ETPKM were significantly lower than that of control treatment (Table 3). The phytate, tannin and cyanide contents of ETPKM had a reduction of 29.26, 6.99 and 58.44%, respectively. There was no significant different between the oxalate contents of the ETPKM and the control (NTPKM). The reduction in the anti-nutritional compounds in ETPKM could be due to the action of certain hydrolytic metabolites produced alongside the enzyme of interest (Nwafor and Ejukonemu, 2004; Cao et al., 2007; Safari, 2011). Reduction in the anti-nutrient compounds were reported by Ojokoh et al. (2012) for fermented groundnut and popcorn, fermented cassava tuber (Aro et al., 2008), fermented sorghum cultivars (Wedad et al., 2008) and fermented canola meal (Omid et al., 2012).

The mineral analysis of ETPKM and NTPKM are shown

Table 4. Mineral composition of mannanase treatment of *P. italicum* on PKM (ppm).

Sample	Calcium	Magnesium	Zinc	Phosphorus	Copper	Potassium
PKC [a]	1.15^{c}±0.00	0.23^{a}±0.00	0.08^{a}±0.00	0.39^{a}±0.01	0.39^{c}±0.00	2.98^{c}±0.00
[b]	0.91^{b}±0.01	0.23^{a}±0.01	0.08^{a}±0.01	0.62^{b}±0.05	0.33^{b}±0.03	2.73^{b}±0.02
[c]	0.76^{a}±0.03	0.24^{a}±0.02	0.12^{b}±0.01	1.21^{c}±0.03	0.07^{a}±0.01	2.11^{a}±0.14

[a] Untreated, [b] during treatment [c] after treatment. Values are means± S.E (n=3). Means with the same superscript letters down the same row are not significantly different (P>0.05).

in Table 4. It is shown in the table that calcium, copper and potassium reduced in PKM after enzyme treatment. The percentage reduction of calcium, copper and potassium in ETPKM were 33.65, 81.28 and 29.12%, respectively. The reason for decrease in certain minerals after enzyme treatment might be connected to the fact that some of these minerals could be utilized as co-factors for effective catalytic function of enzyme molecules. The reductions of certain minerals in fermented products have been documented (Aro, 2008; Akinyele et al., 2011; Ojokoh et al., 2012). The reduction in these minerals in fermented products could be as a result of their utilization by the fermenting micro-organisms as reported by Ojokoh et al. (2012). However, there was significant increase in zinc and phosphorus contents of ETPKM in comparison with the control. Similar observation was reported by Ojokoh et al. (2012) and Aro (2008) for fermented products, and it was attributed to the fact that some of these metals could be part of some biological macromolecules which were released into the solution from such structures during fermentation.

Conclusion

The treatment of PKM with β-mannanase resulted in decrease of complex fibre fractions in the PKM to increase its crude protein and certain minerals (zinc and phosphorus) contents. There was also reduction in the anti-nutrient compounds of enzyme treated PKM.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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