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Incidence and prevalence of naturally occuring fungi on palm kernel sludge and its attendant *in vitro* digestibility

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Abstract

This study investigated incidence and prevalence of fungi isolates that grow naturally on palm kernel sludge (PKS). The *in vitro* digestibility of crude enzyme extract (CEE) from identified fungi on proximate composition, fibre fractions and reducing sugars of the substrate was also assessed using standard methods. Solid state fermentation (SSF) with inoculum concentration of 2.3 x 10^6 spores/mL was used to produce CEE from the most prevalent isolated fungi after which PKS was digested with the CEE. The fungal isolates with high prevalence of occurence on PKS were: *Aspergillus candida*(90%), *Aspergillus flavus*(90%), *Aspergillus niger*(90%), *Rhizopus microspororus*(90%), *Trichoderma harzianum* (80%), *Aspergillus brasiliensis*(80%), *Phythium* sp (80%) and *Cunninghamella* sp (70%). *Aspergillus candida* was consistent in significantly reducing lignin, Acid detergent fibre and Neutral detergent fibre of PKS relative to the control. *Aspergillus brasiliensis* was able to release reducing sugars from the substrate. Crude enzyme extract of isolates tested on PKS improved the substrate in one form or the other. The different results exhibited by different isolates point to the fact that tested fungal isolates explore different mechanisms in degrading the substrate however the utilization by animals can be confirmed when nutrient digestibility is carried out *in-vivo* with any species of livestock.

Introduction

Large quantities of agricultural and agro-industrial residue are generated from diverse agricultural and industrial processes. This has generated a lot of environmental problems and various efforts have been made to manage this problem including open burning (Ab *et al*., 2009). Agro-industrial by-products in Nigeria vary from primary processing of farm produce wastes to wastes from other agro allied industries. Some of these wastes are left unutilised, often causing environmental pollution and hazard (Iyayi, 2004). Agricultural and industrial waste products contribute to increased eutrophication due to excessive discharge of nutrients meanwhile these environmental wastes can be recycled and controlled by biodegradation and the product would be of nutritive value in compounding livestock feeds (Adenipekun *et al*., 2012).

The oil palm crop which gives high yield production can be cultivated in the tropical region such as Indonesia, Malaysia, Thailand and Nigeria (Wangrakdiskul and Yodpijit, 2015). The oil palm industry has been recognized for its contribution towards economic growth and rapid development, it has also contributed to environmental pollution due to the production of huge quantities of by-products from the oil extraction process (Rosnah *et al.*, 2010). The by-products are mostly fibrous in nature and may not be utilised in livestock diets. The by-products from oil palm processing consist of oil palm trunks, oil palm fronds, empty fruit bunches, palm pressed fibres, palm kernel shells, palm kernel cake (PKC), liquid discharge palm oil mill effluent (POME) (Singh *et al*., 2010; Sulaiman, 2010) palm kernel extraction residue (PKER) and palm kernel sludge (PKS) (Fafiolu *et al.*, 2015) amongst others. Information on utilization of PKC and PKM in animal nutrition have been reported by Ng *et al.* (2002), Ng (2003), and Ng (2004) but there is paucity of information on the use of Palm Kernel Extraction Residue (PKER) and PKS in monogastric animal nutrition (Fafiolu *et al.*, 2015). But Fafiolu *et al*. (2015) reported utilization PKS to replace maize in the diet of broiler birds.

PKS can be mistaken for PKC or PKM but the difference is in the oil content, particle size and texture. PKC or PKM contains more oil, has bigger particle size and is more rough than PKS. For comprehension on how PKS is produced, flow chart for production of PKS is presented in Figure 1. PKS can be used as

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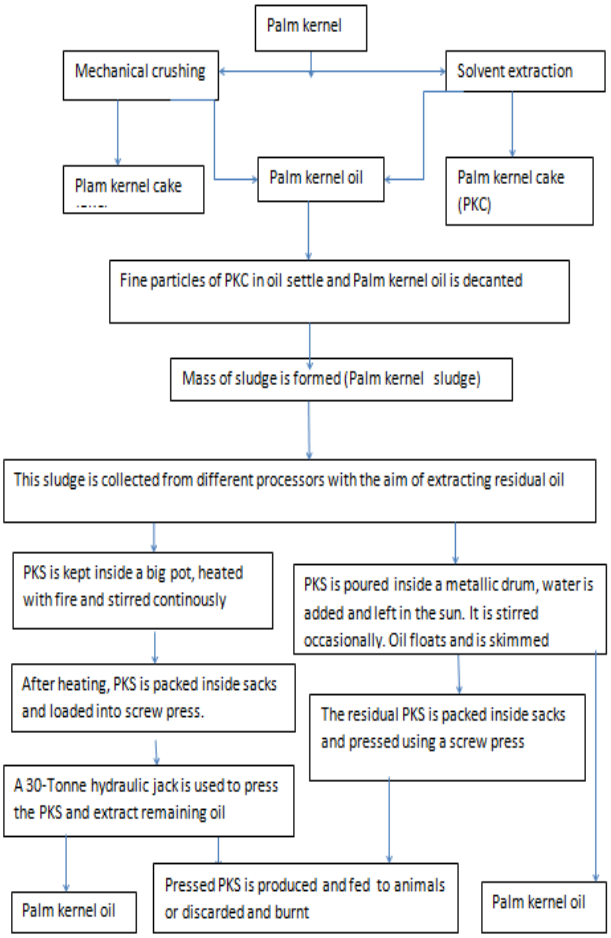

Figure 1. Flow chart for skimming of palm kernel sludge

an energy source in livestock nutrition but its usage and proportion to include in diet is hindered due high fibre and impaired digestibility. The high fibre and impaired digestibility can be overcome either by addition of exogenous enzyme or improvement in nutritive quality through use of crude or purified enzyme extract obtained from fungi using solid state fermentation process.

In studying the digestibility of lignocellulosic feed, strains of anaerobic fungi showing high fibrolytic enzyme producing ability have been isolated from the faeces of wild (hog deer, *Cervus porcinus*; blackbuck, *Antelope cervicapra*; spotted deer, Axis axis; nilgai, *Baselophus tragocamelus*) and rumen liquor of domestic (sheep, Ovies aries) ruminants (Paul *et al*., 2004). The genotypic and phenotypic diversity of microorganisms are being discovered day by day and it is likely that the strains currently held in culture collections represent a fraction of the existing diversity. So the objective of this research was to assess the incidence and prevalence of fungal isolates that grow naturally on Palm Kernel Sludge (PKS). Also, *in vitro* digestibility of crude enzyme extract (CEE) from the most prevalent among isolated fungi on proximate composition, fibre fractions, reducing sugars and organic carbon of fermented PKS was evaluated.

Materials and Methods

Collection of PKS for assessing Incidence and Prevalence of fungal isolates

Five hundred kilogrammes of fresh samples of Palm Kernel Sludge (PKS) was obtained from a palm kernel oil mill industry, along Ojoo – Iwo road express way, Agbowo, Ibadan, Nigeria on 8th of August, 2014. This was kept in ten sacks containing fifty kilogramms each for a period of six weeks. At the end of the sixth week, the contents of the sack was poured and lumps showing different colours of fungal growth were picked and scrapped to constitute a kilogramme of the sample from each of the ten bags. Each kilogramme of sample was kept in paper bags and transported to the laboratory. The different colours were scraped using sterile inoculating pin and the scrappings were used for inoculation on Potato Dextrose Agar (PDA). PDA was prepared as described by Atlas (1997). The remaining samples were then poured in distilled water to form a suspension inside sterilized mayonnaise bottles. The suspension was further used to innoculate duplicate petri dishes of PDA. The petri dishes were kept inside an inoculating chamber and were observed for fungal growth. The fungi observed were repeatedly sub cultured every week in order to obtain pure cultures of all fungi species that grow on PKS naturally. The fungi were isolated, identified and saved in PDA slants. At a later date the saved fungi were subcultured and multiplied for solid state fermentation of the PKS.

Identification of fungi to genera level was based on colony morphology and structural characteristics using both macroscopic characters (colony growth, colony surface, colony colour, colour on reverse side of plate) and microscopic characters (aseptate hyphae, branched hyphae, conidiophore, vesicle) as well as using the description given by Barnett and Hunter (1998) and Larone (2002). The fungal isolates observed and identified was used to assess the incidence (occurence) and prevalence (abundance) of fungal isolates in PKS. The prevalence was based on incidence of a particular isolate in a number of samples. Status of the prevalence was categorised as high (≥70%), medium (41 – 69%) and low (≤ 40%). The isolates with high prevalence were thus selected for *in vitro* studies while those with medium and low prevalence were discarded. The isolates with high prevalence were further sent to International Mycological Institute (IMI), United Kingdom for identification to species level. A unique cabi reference

number (IMI number) was assigned to each sample.

Solid state fermentation of PKS

A sterile inoculating pin was used to collect the spores and the mycelia of the earlier saved fungi and was inoculated on sterile PDA in an inoculating chamber. Inoculated plates was incubated at 34^0C for 14 days. On the fourteenth day, after the growth of the fungi on the plates has been fully established, the inoculum of each isolate was prepared by using the breadth of a sterilized glass slide to scrape the actively growing mycelium. The scraped mycelium was transferred into a 300 mL beaker after which 40 mL of distilled water was poured into the beaker. The mycelium was blended to break loose the conidia using a hand held Binatone food mixer cum blender model No HM – 350S for 30 seconds. After blending, it was passed through a muslin cloth to remove traces of agar, hyphal fragments and mycelium while the conidia was in the filtrate. Another 40 mL of distilled water was used to rinse the blender and the beaker.

The filterate was used for spore count using Neubaur improved haemocytometer. Spore count of approximately 2.3 X 10^6 per mL was obtained and this was used to inoculate the substrate as described by Lawal *et al*. (2010) and Mehboob *et al.* (2011). One hundred kilogramme of fresh PKS was collected on November 26th, 2014 and was dried, powdered and stored in a cool dry place. Subsequently, small quantity(100 g – 1 kg) of the stored PKS was gradually fetched and used as at when needed. Basal medium was prepared based on modification of Lawal *et al.*, (2010). The basal medium contained KNO_3, 0.05 g; KH_2PO_4, 2.0 g; $MgSO_4.7H_2O$, 0.5 g; Tryptone, 0.5 g; $FeSO_4.4H_2O$, 0.05 g per litre of distilled H_2O. After preparation, the pH of the basal medium was adjusted to 5.5 (Sharma and Pandey, 2010) using NaOH solution. This was kept overnight and used within 24 hrs.

Crude enzyme extraction

Preparation of fungal isolates for crude enzyme extraction using PKS as substrate was based on adaptation and modification of Lawal *et al.* (2010). Two hundred grams of the sieved and dried PKS was autoclaved at 121^0C and 15 PSI for 15 minutes in a 1 litre mayonnaise jar and then cooled to room temperature. During sterilization, the lid of the jars were turned such that it could be used to lift up the jar but not tightly sealed to avoid breaking of the jars. After cooling, each jar was moistened with 80 ml of the prepared basal medium and was inoculated with 4 mL of an aqueous spore suspension of each isolate. More distilled water was added to make 90 mL. This was inverted vigorously several times for thorough mixing and to achieve a uniform distribution of inoculums throughout the solid medium. All the flasks were tightly sealed and incubated at 34^0C for 7 days. Each of the jars was in triplicates along with the control. The control was not inoculated but was incubated along with the inoculated samples.

Phosphate buffer was routinely prepared while Dinitrosalicylic acid (DNS) solution was prepared as described by Miller (1959). At the end of the seventh day of fermentation, as much as 3 mL of 0.1M phosphate buffer pH 7.2 was added per gram of PKS substrate (i.e 600 mL for 200 gms). Initially, 350mL was added after which the residue was further rinsed with 250 mL of the same buffer. All the flasks were shaken on the rotary shaker at 150 rpm for 30 min at room temperature and swirled until it was homogeneous. The solid biomass was separated from the suspension by filtration through muslin cloth (Tuan Lah *et al*., 2012) and centrifuged at 3000 rpm for fifteen mins. The supernatant of the extract was used as the source of crude enzyme preparation (Silva *et al*., 2005) and was used immediately for *in vitro* digestion of PKS.

In vitro *digestion of PKS*

One kilogramme (1 Kg) of dried and powdered PKS earlier stored was weighed into polythene bags (Silva *et al.,* 2005) of 60 cm X 35 cm and was autoclaved at 121^0C and 15 PSI for 15 mins. Triplicate crude enzyme extracts from the same fungal inoculum prepared during fermentation was applied aseptically on triplicate polythene bags containing PKS at the rate of 250 mL/Kg (Lawal *et al*., 2010). The polythene bags containing the PKS were tightly sealed and allowed to stay at ambient temperature (Tuan Lah *et al*., 2012) for 7 days (Lawal *et al*., 2010). The ambient room temperature during the period was monitored using a mercury in glass thermometer and it was (26 ± 5^0C) while humidity was (45%). At the end of day 7, the samples were poured into paper bags (brown envelopes) and oven dried at 80^0C for 48 hrs to stop further action of the enzymes and inhibit spore growth if any. After oven drying, the paper bags were left to cool and the substrate were repacked in polythene bags.

At the end of *in vitro* digestibility assay, proximate composition of the samples was carried out using AOAC (2003). Fibre fractions which include neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) of the degraded PKS were also determined using Goering and Van Soest (1970). Hemicellulose was calculated as NDF - ADF while cellulose as ADF – ADL. Organic carbon

was determined using (Bray and Kurtz, 1945). Determination of reducing sugars which include: pentose (arabinose and xylose) and hexose (fructose, glucose, mannose and galactose) was by using modified dinitrosalicylic acid (DNS) method (Miller, 1959) and Saenphoom *et al.,* (2011). Metabolizable energy value of the PKS was calculated according to the procedure of Pauzenga (1985):

Data management and statistical analysis

The analysis of each triplicate representative sample was done in duplicates. This gave six values for each reading. Data collected during the study was managed using Microsoft Excel while statistical analysis was done using Statistical Package for Social Scientists (SPSS). The data was subjected to Analysis of Variance (ANOVA). Means of different treatments were separated using Duncan's Multiple Range Test (DMRT).

Results

Summary of fungal isolates found on PKS is presented in Table 1. All isolates listed were present (incidence) on PKS but the prevalent (abundance) ones were: *Aspergillus candida* (Ac), *Aspergillus flavus* (Af), *Aspergillus niger* (An), *Rhizopus microspororus* (Rm), *Trichoderma harzianum* (Th), *Aspergillus brasiliensis* (Ab), *Phythium* sp (Ps) and *Cunninghamella* sp (Cs). The genera *Aspergillus* accounts for 50% of the number of isolates with high prevalence found on PKS. Prevalence levels of the isolates were: Ac (90%), Af (90%), An (90%), Rm (90%), Th (80%), Ab (80%), Ps (80%) and Cs (70%). Proximate composition of PKS before and after degradation with crude enzyme extract of the isolates is presented in Table 2. Proximate composition include: Dry matter = DM, Crude Protein = CP, Crude Fibre = CF, Oil, Ash and Nitrogen Free Extract = NFE. The control sample is denoted as Ctr.

Range of values obtained for proximate composition were: DM (93.82-91.10%), CP (15.20-11.90%), CF (7.46-5.32%), oil (10.61-8.76%), Ash (19.33-13.72%) while NFE was (59.19-48.89%). There was no significant difference in the CP of PKS after digesting with CEE of Th, Cs, Ac, Ps, Rm, Af and An but PKS digested with CEE of Ab had a significantly lower CP (11.90%) than the undigested PKS (14.23%). Crude fibre and oil of PKS digested with CEE of fungal isolates were not significantly different from the control sample. The ash content of PKS digested with Ac (19.53%) was higher and significantly different from ash content of PKS digested with CEE of other isolates as well as the control (13.72%). The NFE composition of PKS digested with CEE of Ab (59.19%) was significantly higher than that of the undigested PKS (55.17%) while NFE of PKS digested with CEE of Ac (48.89%) was significantly lower than the ctr. The PKS fermented with Ab had a non significantly ($p>0.05$) lower organic carbon (58.52±10.67%) as compared to unfermented PKS (71.49±18.96%).

Table 1. Fungal isolates identified from Palmkernel sludge, their prevalence status and IMI reference number of the isolates sent for identification to species level

S/N	Name of Fungus	Prevalence %	Status	IMI number
1	*Aspergillus candida*	90	High	604216
2	*Aspergillus flavus*	90	High	NA
3	*Aspergillus niger*	90	High	604212
4	*Rhizopus microspororus*	90	High	604213
5	*Trichoderma harzianum*	80	High	NA
6	*Aspergillus brasiliensis*	80	High	604214
7	*Phythium* sp	80	High	NA
8	*Cunninghamella* sp	70	High	NA
9	*Paecilomyces* sp	50	Med	NA
10	*Thielaia* sp	50	Med	604215
11	*Cladosporium* sp	40	Low	NA
12	*Penicillium* sp	40	Low	NA
13	*Gliocladium* sp	40	Low	NA
14	*Phoma* sp	30	Low	NA
15	*Peyronellaea* sp	30	Low	NA
16	*Helminthosporium* sp	30	Low	NA
17	*Sclerotium* sp	30	Low	NA
18	*Mucor* sp	20	Low	NA

NB:The organisms selected for *in vitro* digestibility was based on prevalence of ≥70%. NA were the isolates identified to genera level but not sent to IMI for identification.*Trichoderma harzianum* was identified to species level.

Fibre fractions of PKS after digestion with CEE of fungal isolates is presented in Table 3. Fibre fractions assessed and range of values observed were: lignin (11.76-8.62%), acid detergent fibre (ADF) (29.86-19.17%), neutral detergent fibre (NDF) (56.24-49.39%), hemicellulose (30.58-23.09%) and cellulose(18.51-7.42%). PKS digested with CEE from all the tested fungal isolates except Af (10.80%) and An (11.76%) had a reduced lignin content that was significantly different ($p<0.05$) to the control (Ctr) (11.45%). There was reduced cellulose content of PKS digested with CEE of Af (9.69%) and An (7.42%) as compared to ctr (13.00%).

Reducing sugars comprising arabinose, xylose, fructose, glucose, galactose and mannose content of PKS after degradation with crude enzyme extract of the isolates is presented in Table 4. PKS digested with CEE from Rm (0.61 mg/g) and Ab (0.65 mg/g) had

Table 2. Proximate composition of PKS after degradation with crude enzyme extracts of fungal isolates *in vitro*

Parameters: Organisms	DM(%)	CP(%)	CF(%)	Oil(%)	Ash(%)	NFE(%)	Organic Carbon(%)
Ctr	92.29±0.69^{bcde}	14.23±0.48^{ab}	6.59±0.57^{ab}	10.28±0.91^{ab}	13.72±1.05^{b}	55.17±0.52^{bcd}	71.49±18.96^{a}
Th	91.53±1.25^{cde}	14.53±1.77^{ab}	5.84±2.55^{ab}	8.79±0.53^{bc}	14.29±0.72^{b}	56.59±4.32^{abc}	75.14±10.48^{a}
Cs	92.57±0.77^{bc}	15.18±0.16^{a}	7.46±1.95^{a}	10.14±1.67^{ab}	14.82±0.26^{b}	52.39±1.93^{d}	84.46±11.47^{a}
Ac	91.70±1.01^{cde}	15.20±1.06^{a}	6.81±1.50^{ab}	9.77±1.62^{ab}	19.33±7.78^{a}	48.89±5.48^{e}	79.14±7.62^{a}
Ps	92.39±0.75^{bcd}	14.26±1.32^{ab}	5.57±0.62^{b}	9.59±1.40^{ab}	14.38±0.29^{b}	56.2±1.27^{abc}	75.81±16.96^{a}
Rm	93.42±0.69^{ab}	12.84±3.01^{bc}	6.20±0.63^{ab}	8.76±0.52^{bc}	14.57±0.45^{b}	57.63±3.13^{ab}	76.14±11.47^{a}
Ab	91.10±0.57^{e}	11.90±1.24^{c}	5.32±1.08^{b}	9.28±0.98^{abc}	14.44±0.59^{b}	59.19±1.67^{a}	58.52±10.67^{a}
Af	91.27±0.71^{de}	14.67±0.87^{ab}	6.47±0.51^{ab}	10.61±0.83^{a}	14.39±0.37^{b}	53.87±1.69^{bcd}	83.12±3.50^{a}
An	93.82±0.69^{a}	14.58±1.06^{ab}	5.82±0.81^{ab}	8.83±1.45^{bc}	14.26±0.62^{b}	56.91±3.49^{abc}	80.80±7.52^{a}

NB: Values presented are means of six (6) readings. Triplicate sample of all isolates were each done in duplicates. (n=6). Values followed by the same superscript in each column are not significantly different at 5% level of significance (DMRT). ($p<0.05$). Ctr=Undigested/Control, Th=*Trichoderma harzianum*, Cs=*Cunninghamella* sp, Ac=*Aspergillus candida*, Ps=*Pythium* sp, Rm=*Rhizopus microsporous*, Ab=*Aspergillus brasiliensis*, Af=*Aspergillus flavus*, An=*Aspergillus niger.*

Table 3. Fibre fractions and Metabolizable Energy (ME) of PKS after degradation with crude enzyme extracts of fungal isolates *in vitro*

Organisms	Lignin%	ADF%	NDF%	Hemicellulose%	Cellulose%	ME (Kcal/Kg)
Ctr	11.45±0.48^{a}	24.44±1.37^{cd}	52.60±1.63^{bcd}	28.15±2.85^{bc}	13.00±1.52^{d}	3326.20± 90.68^{a}
Th	9.07±0.28^{cde}	26.00±3.30^{bc}	51.13±1.54^{cdef}	25.13±2.81^{ef}	16.93±3.11^{bc}	3266.38±133.69^{a}
Cs	9.87±0.59^{bcde}	29.86±0.30^{a}	56.24±1.89^{a}	26.38±1.62^{cde}	19.99±0.30^{a}	3251.48±126.47^{a}
Ac	8.62±0.69^{e}	24.23±1.71^{d}	49.39±1.44^{f}	25.16±0.60^{ef}	15.61±1.05^{c}	3097.34±290.01^{b}
Ps	8.76±1.12^{e}	27.00±2.62^{bc}	53.37±3.31^{bc}	26.37±0.95^{cde}	18.24±1.51^{ab}	3306.96± 86.02^{a}
Rm	9.96±1.41^{bcde}	28.47±1.89^{ab}	53.79±3.88^{b}	25.32±2.12^{def}	18.51±0.57^{ab}	3237.31± 41.64^{a}
Ab	9.41±1.33^{cde}	27.91±0.97^{ab}	51.00±2.16^{cdef}	23.09±2.14^{f}	18.50±0.52^{ab}	3300.41± 75.54^{a}
Af	10.80±0.73^{ab}	20.49±0.83^{e}	51.08±0.95^{cdef}	30.58±1.71^{a}	9.69±0.60^{e}	3322.60± 43.02^{a}
An	11.76±1.50^{a}	19.17±2.35^{e}	49.47±0.82^{ef}	30.30±1.53^{a}	7.42±2.09^{f}	3281.89± 52.32^{a}

NB: Values presented are means of six (6) readings. Triplicate samples of all isolates were each done in duplicates. (n=6). Values followed by the same superscript in each column are not significantly different at 5% level of significance (DMRT). ($p<0.05$) Ctr=Undigested/Control, Th=*Trichoderma harzianum*, Cs=*Cunninghamella* sp, Ac= *Aspergillus candida*, Ps=*Pythium* sp, Rm=*Rhizopus microsporous,* Ab=*Aspergillus brasiliensis*, Af=*Aspergillus flavus,* An=*Aspergillus niger.*

higher but non significant arabinose content than Ctr (0.58 mg/g). This non significant increase was further observed in fructose, glucose, galactose and mannose content of PKS digested with CEE from Rm and Ab respectively.

Discussion

This study describes Incidence and prevalence of naturally occuring fungi on palm kernel sludge and its attendant *in vitro* digestibility. The occurence of fungal isolates on palm kernel sludge as observed in this study is an indication that PKS contains nutrients that support growth of fungi.This is simialr to report of fungal mycelia on kernels by Eggins and Coursey (1964) who noted profuse growth of fungal mycelia within stacks of undecorticated kernels at oil mills. The ubiquitous nature of all the fungal isolates found on PKS is well documented except for *Phythium* sp and *Cunninghamella* sp. However, it may be surprising that species like *Fusarium* sp. often generally found in soils and also found on oil palm was not encountered. Hence, the absence of Fusariumsp may not be unconnected to the fact that farmers in Nigeria grow fusarium resistant variety of oil palm (Ikuenobe, 2010). One of the isolates *Aspergillus niger* which has earlier been reported to grow from raw PKC and contain mannanase that can be used to degrade PKC (Asfamawi, 2013) has also been found in this study.

There was a non-significant increase ($p>0.05$) in CP of PKS fermented with CEE obtained from Th,

Table 4. Reducing sugars composition of PKS after degrading with crude enzyme extracts of fungal isolates *in vitro*

Parameters / Organisms	Arabinose mg/g DM	Xylose mg/g DM	Fructose mg/g DM	Glucose mg/g DM	Galactose mg/g DM	Mannose mg/g DM
Ctr	0.58±0.02^{bc}	0.53±0.02^{bc}	1.25±0.05^{bc}	0.61±0.02^{bc}	2.75±0.10^{bc}	2.94±0.11^{bc}
Th	0.52±0.01^{b}	0.47±0.01^{b}	1.11±0.03^{b}	0.54±0.01^{b}	2.52±0.07^{b}	2.70±0.07^{b}
Cs	0.53±0.01^{bc}	0.48±0.01^{bc}	1.14±0.02^{bc}	0.56±0.01^{bc}	2.58±0.05^{bc}	2.77±0.05^{bc}
Ac	0.55±0.07^{bc}	0.50±0.07^{bc}	1.18±0.15^{bc}	0.58±0.08^{bc}	2.68±0.35^{bc}	2.87±0.38^{bc}
Ps	0.56±0.07^{bc}	0.51±0.06^{bc}	1.20±0.15^{bc}	0.59±0.07^{bc}	2.73±0.33^{bc}	2.92±0.36^{bc}
Rm	0.61±0.06^{bc}	0.56±0.06^{bc}	1.31±0.14^{bc}	0.64±0.07^{bc}	2.98±0.31^{bc}	3.20±0.33^{bc}
Ab	0.65±0.02^{a}	0.59±0.02^{a}	1.40±0.05^{a}	0.68±0.03^{a}	3.18±0.12^{a}	3.40±0.13^{a}
Af	0.58±0.09^{bc}	0.53±0.09^{bc}	1.24±0.21^{bc}	0.60±0.10^{bc}	2.81±0.48^{bc}	3.01±0.52^{bc}
An	0.52±0.01^{b}	0.48±0.01^{b}	1.12±0.02^{b}	0.55±0.01^{b}	2.54±0.04^{b}	2.72±0.04^{b}

NB: Values presented are means of six (6) readings. Triplicate samples of all isolates were each done in duplicates. (n=6). Values followed by the same letter down each column are not significantly different at 5% level of significance (DMRT). ($p<0.05$). Ctr=Undigested/ Control, Th=*Trichoderma harzianum*, Cs=*Cunninghamella* sp, Ac=*Aspergillus candida*, Ps=*Pythium* sp, Rm=*Rhizopus microspororus*, Ab=*Aspergillus brasiliensis,* Af=*Aspergillus flavus,* An=*Aspergillus niger.*

Cs, Ac, Ps, Af and An, however the increase observed may be due to ability of fungi to produce enzymes that will increase the bioavailability of protein trapped within the cell walls with a resultant increase in mycelia protein. This is in line with reports of increase in crude protein of PKC after fermentation with enzyme obtained from *Aspergillus niger, Trichoderma viride, Rhizopus stolonifer* and *Mucor mucedo* (Lawal *et al*., 2010). Also, Bachtar (2005) reported that fungal enzymes have the potentials of improving Non Starch Polysaccharides (NSPs), protein as well as other dietary components such as fatty acids.

The non significance ($p>0.05$) in the increase of CP observed in this study may also be due to the fact that crude extract was used in this study as compared to purified enzyme extract of fungal isolates used by Lawal *et al.* (2010). The reduction of CP in the substrates fermented by protease secretion from Ab and Rm in this study may be due to solubilization and degradation of protein during fermentation (Shaalini and Julia 2011; Kranthi *et al.*,2012).

There was also a non significant decrease ($p>0.05$) in CF of PKS digested with CEE of Ab as compared with the undigested PKS. PKS digested with CEE from Ac had a significant increase ($p<0.05$) in ash and significant reduction ($p<0.05$) in NFE as compared to the control (Ctr). Increase in ash content and decrease in NFE of substrate degraded with CEE obtained from Ac is in line with report of Lawal *et al.* (2010) when *Rhizopus stolonifer, Mucor mucedo, Trichoderma viride* and commercial enzyme were used on PKC. Increased CP and reduced CF of PKS obtained with some of the tested fungal isolates is an indication of nutritive improvement of fermented PKS. This is in line with Adenipekun and Lawal (2011) who observed that the fungal biomass obtained after mycoremediation of crude oil and palm kernel sludge contaminated soils could be further exploited as spawn, fertilizer and fodder enrichment.

Aspergillus candida improved fibre fractions of the substrate. It gave the lowest lignin (8.62), NDF (49.39) and hemicellulose (25.16) which were significantly ($p<0.05$) lower than the undegraded PKS. *Rhizopus microspororus* crude enzyme extract also gave a significantly lower lignin and hemicellulose than the control. Significant reduction in lignin and hemicellulose of PKS digested with CEE from Th, Cs, Ac, Ps, Rm and Ab implies that CEE from these isolates have the ability to degrade lignin and hemicellulose but CEE of An and Af could not degrade lignin and hemicellulose content of PKS. However, CEE obtained from An and Af degraded the cellulose present in PKS. Cellulose content of substrate was reduced by An and Af in this study as earlier reported by Lawal *et al.* (2010) but there was inability of An and Af to reduce lignin content of PKS in this study contrary to Lawal *et al.* (2010). The inability to reduce lignin content may be due to crude extract used in this study,

Crude enzyme extract of *Aspergillus brasiliensis* showed a consistent but non significant ($p>0.05$) improvement in the pentose (arabinose and xylose) and hexose (fructose, glucose, galactose and mannose) content of the degraded PKS. The higher values of sugars observed for PKS digested with CEE of Ab and attendant reduction in CF is an indication that the reduction in CF may have led to release of soluble sugars.This is as reported by Lawal *et al.* (2010). Also, Sae-Lee (2007) indicated production

of extracellular enzymes such as mannanase, cellulase and xylanase from *Aspergillus wentii* TISTR 3075, *Aspergillus niger, Aspergillus oryzae,* and *Trichoderma reesei* TISTR 3080 in solid-state fermentation using palm kernel meal (PKM) as a substrate and concluded that the degradation of non-starch polysaccharides (NSPs) in PKM by the fungal strains was indicated by the increased mannanase, cellulase and xylanase activities which correlated with the increase in reducing sugar content. Furthermore, Saenphoom *et al.* (2011), reported that reduction in fibre contents of palm kernel expeller may be due to activity of cellulase and mannanase present in the enzyme used. Thus, in the case of this present study, enzyme produced by the fungal isolates being mostly *Aspergillus* may be responsible for reduction in fibre fractions while the increment in reducing sugars may be as a result of effective hydrolysis of structural carbohydrates (hemicelluloses and celluloses) into monosaccharide sugars.

The reduction in organic carbon of PKS fermented with CEE from Ab (58.52%) which was lower than the control (71.48%) could be due to utilization of glucose and other reducing sugars present as a source of carbon. This is also evident in the increased quantity of reducing sugars present in PKS fermented with CEE obtained from Ab, this is in line with Sati and Bisht (2006) that found glucose and sucrose to be suitable sources of carbon for growth of *Tetracheatum elegans, Tetracladium marchalianum, Pestalotiopsis submersus* and *Flagellospora penicillioides.*

Conclusion

The crude enzyme extract of all the isolates tested on PKS improved the substrate in one form or the other. *Cunninghamella* sp and *Aspergillus candida* increased the crude protein of the fermented PKS. *Aspergillus brasiliensis* and *Rhizopus microspororus* decreased the CP. *Phythium* sp and *Aspergillus brasiliensis* decreased the crude fibre of the PKS. *Aspergillus candida* was consistent in significantly reducing the fibre fractions of fermented PKS in terms of lignin, NDF and hemicellulose. *Aspergillus brasiliensis* was consistent in releasing reducing sugars from the substrate thereby increasing the reducing sugars content of the substrate. Crude enzyme extract of *Rhizopus microspororus* gave a significant reduction in the lignin and hemicellulose of the substrate.These promising results exhibited by different isolates point to the fact that the different fungal isolates explore different mechanisms in degrading the substrate however the utilization by animals can be confirmed when nutrient digestibility is carried out *in vivo* with any species of livestock.

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