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Improvements on the physicochemical characteristics of cassava root pulp and palm kernel mixtures under solid state fungal fermentation

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Abstract This study determined the effect of solid state fungal fermentation on the physicochemical conditions of cassava root pulp (CRP) and palm kernel cake (PKC) mixtures. Locally sourced CRP and PKC were mixed at a ratio of 1:1 on dry matter basis and subjected to different fungal inoculation methods. Treatment A (TA) was inoculated with a pure culture of *Aspergillus niger*, while treatment B (TB) was spontaneously inoculated and treatment C (TC) was inoculated with fermented samples from TA. The treatment D (TD) was neither inoculated nor allowed to ferment to serve as control. Each treatment was replicated twelve times in a Completely Randomized Block Design (CRBD). Each replicate comprised of a two kilogram (2 kg) substrate blend made by mixing 10 parts of ground fresh cassava tuber pulp (CRP), 3.3 parts of palm kernel cake (PKC), and 1 part of nutrient media or distilled water. Each substrate had a moisture content of 52 % and was kept in a plastic container (DANAPLAST DP-099). On the 7^{th}, 11^{th}, 15^{th} and 19^{th} day of fermentation, three replicates from each treatment were randomly sampled and air dried for physical (weight changes, particle size distribution, bulk density, specific gravity and water holding capacity) and chemical (proximate composition) analyses. Aggregate structures of the fermented materials were improved as shown by the complete removal of flouriness in the fermented cassava products, better particle size distribution, appeal and aroma. On the 7^{th} day of fermentation, the percentage crude protein content of treatments TA (13.77), TB (14.58) and TC (15.16) were significantly ($p< 0.05$) higher than the control (9.80), while crude fibre contents dropped from 21.75 in the control to 11.28 in TA, 11.62 in TB and 13.13 in TC. The calculated metabolizable energy of all fermented samples was however below that of the control value. It is therefore concluded that Solid state fermentation could lead to improvements in the physicochemical quality of CRP and PKC mixtures for use in animal feeds production.

Keywords: solid state fermentation, palm kernel meal, cassava root meal, *Aspergillus niger*.

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Introduction

The consumption of livestock products is expected to triple worldwide by the year 2020 due to several reasons such as increase in population, per capita income and urbanization as well as improvements in education, standard of living, technological development, trade and communication (FAO, 1999). These increases are expected to occur more in the developing countries of Africa, Asia and the Pacific regions, while it will be minimal in developed countries. In Nigeria and other sub-Saharan Africa, total meat production grew less than total meat consumption, indicating that these regions imported large amount of meat to keep up with growing demand (FAOstat, 2009). This shows that increase in per capital income, increases demand in animal food products even when production is deficient. This situation may have far reaching implications for the food security and economy of such countries.

Prime among the strategies proffered towards ameliorating the effects of imminent increases in animal food products demand are increases in livestock population and quality, improvement in technical/managerial skills of producers and enhanced feed efficiency. Of all these measures, improving the feed efficiency of livestock seems to be more realistic in the short term.

However, the current approach has focused on the search and investigation of alternative feed sources to substitute costly conventional feedstuff (Okeudo *et al*., 2005; Obun, 2013; Okah *et al*., 2013; Okoli *et al*., 2013). This is informed by the ever increasing demand for maize and other grains such as sorghum, soybean, groundnut etc, for human consumption, industrial production and livestock feed companies has resulted in escalating prices of these essential food grains in Nigeria in recent years (Esonu *et al*., 2005; Okeudo *et al*., 2005; Enyenihi., 2009). In addition, the seasonality of grain production due to a predominantly rain fed agrarian culture and inadequate storage technology has made their availability unreliable for livestock production. As a result of these, attention have being focused on non-conventional feed stuffs, such as cassava, palm kernel meal and other agro-industrial by-products, which are more readily available and cheaper (Okeudo *et al.,* 2005). Unfortunately, many of these non conventional feed resources seem to be inadequate as most of them have replaced maize and other grains only at the marginal level (Udedibie *et al*., 2004; Esonu *et al*., 2005).

The potential of cassava as a livestock feed material include high energy levels and non seasonal supply in Nigeria (Tewe, 1996). The use of cassava meals in livestock diets have been limited by its high cyanogenic potential due to high contents of linamarin and lostuastralin and the floury nature of its meals (Udedibie *et al.,* 2008; Enyenihi *et al.,* 2009). The former is easily removed using processing techniques such as soaking, grating, boiling and fermentation

(Tewe, 1996; Okoli *et al.,* 2012) as well as wetting (Bradbury, 2004), whereas the later is often controlled by blending with vegetable oils (Udedibie *et al.,* 2009), and gelatinization (Udedibie *et al.,* 2004 and 2008). Addition of vegetable oils to livestock feeds often leads to rancidity problems, thus, limiting shelf life and feed intake in animals (Ezeokeke *et al.,* 2008). Gelatinization produces very stable products but increases cost of feed production due to its high process fuel demand (Okoli *et al.,* 2012).

Palm Kernel Cake (PKC) has been reported to be a cost effective ingredient that can be utilized in ration formulation in livestock feeding (Zahan and Alimon, 2006; Adesehinwa, 2007; Okeudo *et al.,* 2005; Egenuka *et al.,* 2013). Its crude protein content is moderate, rich in methionine and cysteine but deficient in lysine (AMINODat 4.0, 2010). The use of PKC in livestock rations is further limited by its grittiness due to high content of. Mannan is a structural polysaccharide which constitutes up to 35% of the cell wall of PKC and can only be broken down when the enzyme mannanase has degraded it into short chain polysaccharide or oligomers.

However, most monogastric animals do not elaborate mannanase in their digestive systems. Some techniques such as use of feed additives (Lawal *et al.,* 2010) and/or modification of flora by incorporation of mannanase producing microbes (Ouhida *et al.,* 2002) have been employed in nutrients in PKC better available to monogastric animals. These have as well yielded marginal results due to factors intrinsic to the metabolism of farm animals (Ouhida *et al.,* 2002).

In recent years, some researchers have used solid state fermentation to improve the nutritive value of cassava products (Tewe, 1996; Vorachinda *et al.,* 2011; Iyayi and Aderolu, 2004) PKC (Abdeshahian *et al.,* 2010) as livestock feed with promising results. Specifically, Ramin *et al.* (2005), reported improvement in crude protein and nitrogen free extracts and neutral detergent fibre fraction with a concomitant decrease in acid detergent fibre, when PKC is inoculated with *Aspergillus niger.* Other authors have demonstrated that *Aspergillus niger* in single or mixed culture can improve the protein content of cassava root meals and its by-products (Raimbault *et al.,* 1977; Kompaiang *et al.,* 1992; Soccol *et al.,* 1994; Bhargav *et al.,* 2008). Soccol *et al.* (1994) also increase the crude protein content of cassava baggase using solid state fermentation.

Spontaneous inoculation and concomitant degradation of palm kernel cake, cassava and cassava by-products occur in naturally in conditions similar to solid state fermentation employed in reported experiments. A blend of cassava root meal and PKC in a ratio of 1:1 may therefore yield a product that is similar to maize in proximate composition. This product if subjected to solid

state fermentation is likely to yield a value added product with improved qualities as livestock feed.

This study assessed the physicochemical conditions of cassava root meal and palm kernel cake mixtures subjected to solid state fungal fermentation.

Materials and methods

Experimental materials: Fresh tubers were peeled, washed and grated using a conventional locally fabricated grating machine. Palm kernel cake was sourced from a commercial livestock feed mill based in Owerri. *Aspergillus niger* was isolated and sub-cultured using Sabround Dextrose Agar (SDA) at the Biotechnology Laboratory of the Department of Biotechnology, Federal University of Technology, Owerri.

The approximate dry matter composition of cassava tubers was determined by oven drying four 100 g samples of freshly grated cassava tubers to a constant weight to range from 32 to 35%, with a mean value of 33.3%. Nutrient medium was prepared such that it comprised an equivalent of 18 g of $(NH_4)_2SO_4$, 5.4 g of urea and 10 g of KH_2PO_4 in 100 g of distilled water.

Experimental setup: Four experimental treatments were used to evaluate the effect of different fermentation techniques on the physical characteristics and chemical composition of the samples. Treatment A was designed to evaluate the effect of direct inoculation with *Aspergillus niger*. Treatment B was designed to study spontaneous fermentation techniques on the sample material, whereas Treatment C evaluated the effect of batch inoculation using already fermented samples from experiment 1. Treatment D served as control and was not subjected to any fermentation.

Treatment A: A two kilogram (2 kg) substrate blend comprising 10 parts of fresh cassava tuber pulp (CRP), 3.3 parts of palm kernel cake (PKC), and 1 part of Nutrient media solution containing 10^7 spores of *Aspergillus niger* per cm^3 was weighed out with a digital scale (Kenwood Electronic Scale Model 6s49). This substrate mixture was transferred into a 5 litres capacity plastic container and covered loosely to create a solid state aerobic fermentation environment. This setup was replicated twelve times and served as treatment A (TA). The fermentation baths were kept in an open hall and allowed to stand for 19 days.

On days 7, 11, 15 and 19 of the experiment, three replicates were selected at random, weighed, air dried by spreading them on cellophane sheets in an open space until they became crispy and friable and thereafter reweighed. The differences in weight were recorded as moisture lost. The samples were stored in air tight plastic containers ((DANAPLAST DP-099) until needed for analyses.

Treatment B: The substrate setup described in experiment 1 was repeated but this time the 1 part solution was made up of distilled water only. The 12 replicates were each spread in a plastic tray and allowed to stand under shade at ambient temperature (27^0C) and humidity (70 – 80%) for 24 hours. Thereafter, each sample was reweighed and moisture loss determined. The equivalent loss in moisture was replenished with distilled water to restore the substrate to its initial weight of 2 kg. Each sample was compressed into 3.25 litres plastic containers in order to exclude air, covered tightly and sealed with masking tape. The setup served as treatment B (TB) and represented spontaneously inoculated solid state fermentation experiment. On days 7, 11, 15 and 19 of the experiment, three replicates were selected at random, weighed, air dried by spreading them on cellophane sheets in an open space until they became crispy and friable and thereafter reweighed. The differences in weight were recorded as moisture lost. These were again stored in air tight plastic containers until needed for analyses.

Treatment C: For this treatment, 300 gram of a 19 days solid state aerobically fermented substrates as in TA was prepared. This was divided into four equal parts and transferred to Petri dishes and kept on a laboratory work bench at room temperature (28^0C). The dishes were opened every 2 days and the samples moistened by spraying with the nutrient media solution (Oxoid, England) until visible fungal mycelia growth became evident on the 14^{th} day. The four samples were thereafter pooled and thoroughly mixed to achieve a uniform distribution of fungal spores and left to incubate for a further 2 days. This was divided again into three equal parts to serve as the batch cultures.

Thereafter, three replicates of 500 g substrates comprising 10 parts CRP to 3.3 parts PKC and 1 part the aforementioned nutrient media was prepared. Each of these substrates was mixed thoroughly with one of the batch cultures and transferred into 3.5 L plastic containers and covered loosely as in treatment A.

On days 7, 11, 15 and 19 of the fermentation period, 100 g of the fermenting samples was scooped from each container, air dried and stored in cellophane bags until needed for analyses.

Treatment D (Control): The control substrates were prepared following the procedure described in treatment B (TB). These were however immediately air dried without fermentation and stored individually in a 5 L plastic container. On days 7, 11, 15 and 19 of storage period, 100 g of the samples was scooped from each container, air dried and stored in cellophane bags until needed for analyses.

Physical characteristics determination: The physical characteristics of the end products evaluated included substrate weight change, substrate particle size distribution, bulk density, specific gravity and water holding capacity.

Substrate weight change: This was determined as the residual weight of the samples when no significant weight changes occurred upon further drying.

Residual weight = (weight after drying/initial weight after fermentation) x 100.

Particle size determination: Two granulated laboratory sieves of 2.00 mm and 0.85 mm was used to separate the feed samples into three particle size groups namely; greater than 2.00, ≤ 2.0 ≥ 0.85 and < 0.85. About 100 g of a sample was first sieved with the 2.00 mm sieve to separate feed particles greater than 2.00mm. The sievates were later passed through a 0.85 mm sieve to remove particles less than 0.85 mm. The weight of each category was expressed as a percentage of the original weight of the sample. This was replicated trice for each sample.

Bulk density: This was determined as weight of a feed sample divided by its volume, (weight of sample / its volume). This was repeated trice for each replicate of the experiment.

Specific gravity: This was determined as the weight of a volume of feed sampled for the determination of bulk density divided by the weight of equivalent volume water.

Water holding capacity: This was evaluated as the quantity of water retained by a feed sample. This was determined by passing 100 cm^3 of water through a 25 g sample of feed kept in a funnel which was plugged with a film of non absorbent cotton wool. The quotient of the quantity of water absorbed to the weight of the feed was recorded as the water holding capacity (WHC).

Proximate analysis of samples: The proximate compositions of the samples were analyzed according to the AOAC (1995) methods. Percentage moisture content (MC), ash content (AC), ether extract (EE), crude fibre (CF), crude protein (CP), nitrogen free extract (NFE) and calculated metabolizable energy (ME) were determined. Metabolizable energy values were calculated based on proximate composition using the prediction equation outlined.

$ME = (MJkg^{-1}) – 0.416CP + 0.0605EE + 0.367NFE-20.6.$
Where; ME = metabolizable energy, CP = crude protein, EE = ether extract, NFE = nitrogen free extract.

Experimental design and analysis: Data from the different parameters were collated and analyzed using a Completely Randomized Block Design (CRBD). TA, TB, TC and TD (control) served as treatments, whereas periods of fermentation served as blocks. The analysis of variance was carried out and

significantly different means were separated using the Least Significant Difference (LSD) method according to Steel and Torrie (1980).

Results and discussions

The final weights of the experimental samples after air drying are shown in Table 1. The dry weight of treatments A, B and C were all significantly higher than the control even on the 7th day of fermentation. This can be attributed to a possible increase in biomass and / or chemical changes in the nature of the fermented substrates. Until the 19th day of fermentation, the dry weights of treatment C was significantly higher ($p<0.05$) than those of treatment A (*A. niger* inoculation) and the spontaneously inoculated samples (TB).

Table 1. Weight changes (%) of the experimental materials after fermentation

Period of Fermentation	Method of Inoculation			TD (Control)	SEM
	TA	TB	TC		
7	59.30^{a}	55.43^{ad}	69.06^{e}	52.64^{ad}	1.470
11	57.56^{a}	57.33^{a}	65.00^{e}	50.97^{d}	
15	58.22^{ab}	56.05^{a}	65.25^{e}	50.25^{d}	
19	60.62^{ac}	63.33^{ce}	67.75^{e}	49.78^{d}	

$^{a, b, c, d}$, means in the same row and column with different superscript are significantly different ($p<0.05$). TA – *Aspergillus niger; TB* – Spontaneous, TC – Batch inoculation

Of particular note is the trend of increases across the treatment groups. Though there were no significant block (period of fermentation) effect ($p>0.05$) in *A. niger* inoculated samples, TA increased from 59.30 g on day 7 to 60.62 g on day 19, while TC decreased from 69.06 to 67.75 g within the same period. On the other hand, spontaneously inoculated samples increased significantly ($p<0.05$) from 55.43 to 63.33 g over the same period. This suggests that the agents and / or the products of fermentation may vary between the *A. niger* inoculated samples and the spontaneously inoculated ones. This obviously requires further investigation.

The particle size distributions of the fermented materials are shown in Table 2. There were no significant differences ($p>0.05$) in particle size distribution among TA, TB and TC. Though the data for the control was not evaluated, it is obvious that the treatments improved aggregate particle size distribution of the test material even at 7th day of fermentation. Cassava meals are very floury and could pass through 0.02 mm sieves and for optimal oil extraction from palm kernels, processors use mills capable of releasing particle sizes of 0.5 mm.

Table 2. Particle distribution of experimental samples

Parameter	Period of Fermentation in days	Method of Inoculation			SEM
		TA		TC	
Particle size distribution > 2.0mm (%)	7	22.31	5.520	16.41	5.520
	11	19.08		18.28	
	15	22.78		18.50	
	19	24.87		18.64	
Particle size distribution < 2.0≥ 0.85mm (%)	7	34.41	9.320	28.16	9.320
	11	32.96		29.37	
	15	29.96		28.91	
	19	40.15		28.91	
Particle size distribution < 0.85mm (%)	7	43.28	13.75	55.43	13.75
	11	47.96		52.35	
	15	47.26		52.59	
	19	34.98		52.45	

[a, b, c, d], means in the same row and column with different superscript are significantly different ($p<0.05$). TA – *Aspergillus niger; TB* – Spontaneous, TC – Batch inoculation

On the other hand, the dustiness characteristic of cassava meals was completely eliminated by the experiments. The exact mechanism responsible for this is not yet clear. It is possible that mycelia of *A. niger* must have served as a binding agent in samples in which they grew (A and C), but if the organisms responsible for fermentation in the spontaneously inoculated samples are not filamentous, this postulation will not be sufficient to explain the result.

Another proposition would be that the oil in palm kernel cake provided a binding effect. However, judging from the aroma of the fermented substrates the fatty acids in the fermented products were predominantly short chain volatile fatty acids. Though they are capable of providing some binding effect, it is expected that this will reduce significantly or even be lost completely during drying. Again the percent crude fat contents of fermented samples were generally low when compared to those used by Udedibie *et al.* (2009) to achieve a comparable result / effect. It is as well possible that the heat energy generated during fermentation may have provided a kind of partial gelling effect on the starch granules. This as well needs further investigation.

The results of the bulk density, specific gravity and water holding capacity measurements of the fermented samples are as shown in Table 3. The bulk density of the fermented samples decreased progressively with period of fermentation. However, the decrease was not significant until 19th day of fermentation except for the TA which became significant on the 15th day of fermentation. There were significant treatment differences ($p<0.05$) at all the periods of fermentation except at the 11th day when the values for TA (369.33)

was similar (p>0.05) to TB (381.10). The values are however worthy of note; 53.15 for TA, 22.98 for TB and 37.50 for TC.

The specific gravity of the *A. niger* inoculated samples (TA and TC) were similar at all the periods of fermentation studied. The values for spontaneously inoculated samples (TB) at 11th and 19th days of fermentation (371.04 and 381.01) were similar to those of TA (349.06 and 356.09) and TC (346.99 and 342.47) respectively at the same period. There were no significant block effect (p>0.05) in the specific gravity of TB and TC.

The water holding capacity (WHC) of treatments A and C decreased progressively with period of fermentation. The values however were not significantly (p>0.05) lower in TA but that of TC at 7th day of fermentation (2.65) was significantly lower than that at 19th day (1.91). Again, the WHC in spontaneously inoculated samples increased progressively though this did not produce any significant (p<0.05) result after 19 days of fermentation

Table 3. Bulk density, Specific gravity and Water holding capacity of experimental materials

Parameter	Period of Fermentation in days	Method of Inoculation			TD (control)	SEM
		TA	TB	TC		
Bulk Density (g/dm3)	7	373.70[a]	396.07[b]	350.00[c]	382.86[ab]	7.884
	11	369.33[a]	381.10[ac]	316.63[e]	382.87[ac]	
	15	321.31[b]	393.00[c]	300.00[e]	382.85[ac]	
	19	320.45[b]	366.09[d]	312.50[be]	382.78[a]	
Specific gravity	7	395.49[a]	385.10[a]	383.56[a]	417.45[c]	10.863
	11	349.06[b]	371.04[ad]	346.99[b]	416.01[c]	
	15	308.18[c]	383.01[a]	328.76[b]	420.02[c]	
	19	293.15[c]	356.09[bd]	342.47[b]	417.40[c]	
Water Holding Capacity (g/g of feed)	7	1.6[a]	1.96[c]	2.65[d]	1.83[ac]	0.109
	11	1.84[a]	1.99[ac]	2.04[a]	1.79[a]	
	15	1.39[b]	2.04[c]	1.94[c]	1.85[c]	
	19	1.21[b]	2.04[c]	1.91[c]	1.84[c]	

[a, b, c, d], means in the same row and column with different superscript are significantly different (p<0.05). TA – *Aspergillus niger; TB* – Spontaneous, TC – Batch inoculation

The proximate compositions of experimental materials are shown in table 4. The moisture contents of treatments TA, TB, and TC were all significantly (p<0.05) higher than the Control at 7, 11, 15 and 19 days of fermentation. These results are indicative of changes in chemical composition and consequently the ability of the substrates to retain water after air drying.

Crude protein: The fermented samples had a significantly higher crude protein contents than the unfermented samples (TD). This is expected due partly to increases in biomass probably caused by microbial reduction in carbohydrate fraction of experimental materials as a result of fermentation. Period of fermentation did not significantly affect the results across all the treatment groups ($p>0.05$). On the 7th day of fermentation, the results appeared as (15.16, 14.58, 13.77 and 9.8% for TC, TB, TA and TD respectively.

Table 4. Proximate composition of the experimental material after fermentation

Parameter	Period of Fermentation in days	Method of Inoculation			TD (control)	SEM
		TA	TB	TC		
Moisture (%)	7	33.36^{a}	26.59^{a}	32.32^{a}	13.85^{c}	3.140
	11	25.59^{a}	27.37^{a}	33.33^{a}	13.70^{c}	
	15	25.75^{a}	29.50^{a}	33.33^{a}	14.05^{c}	
	19	32.40^{a}	33.30^{a}	33.87^{a}	13.85^{c}	
Crude Protein (%)	7	13.77^{a}	14.58^{c}	15.16^{e}	9.80^{f}	0.136
	11	14.23^{b}	14.23^{bc}	15.40^{e}	9.60^{f}	
	15	14.23^{b}	14.93^{d}	15.46^{e}	9.85^{f}	
	19	14.41^{b}	14.93^{d}	15.46^{e}	9.74^{f}	
Crude Fibre (%)	7	11.28^{a}	11.62^{a}	13.13^{ce}	21.75^{f}	0.500
	11	12.30^{a}	12.87^{abd}	12.91^{cd}	21.60^{f}	
	15	11.59^{a}	14.86^{ce}	14.24^{e}	21.85^{f}	
	19	10.94^{a}	13.18^{b}	12.39^{bc}	20.98^{f}	
Fat Content (%)	7	3.95^{a}	4.45^{b}	5.91^{c}	8.15^{d}	0.100
	11	3.91^{a}	4.74^{b}	5.97^{c}	8.25^{d}	
	15	4.23^{a}	4.92^{b}	6.13^{c}	8.10^{d}	
	19	4.24^{a}	5.21^{b}	6.15^{c}	8.15^{d}	
Carbohydrate (%)	7	33.09^{a}	38.64^{a}	29.11^{ac}	49.70^{d}	3.490
	11	39.54^{a}	35.48^{ac}	27.90^{c}	49.60^{d}	
	15	39.69^{a}	31.89^{ab}	26.19^{b}	49.75^{d}	
	19	33.21^{a}	29.19^{a}	27.26^{ac}	49.65^{d}	

$^{a, b, c, d}$, means in the same row and column with different superscript are significantly different ($p<0.05$). TA – *Aspergillus niger; TB* – Spontaneous, TC – Batch inoculation

This shows that the microbes in the spontaneously inoculated samples were more effective in increasing the level of crude protein than the direct *Aspergillus niger* inoculation. Whereas the microbes under spontaneous inoculation are endogenous and must have been well adapted to the substrate either individually or through multiplicity of interactions, the screened and isolated *A. niger* must have needed time to adjust to the substrate. Crude protein content of batch treatment group was higher than both treatments TA and TB. It is probable that the microbes in the batch cultures adapted better to the

substrates and incubation environment which resulted in the higher crude protein level increase. This is in line with the earlier findings of Vorachinda *et al.* (2011) that the rate of protein and biomass turnover in batch or scale up experiments is higher than the controls. Sukaryana *et al*. (2010) also reported a 29.58% increment in crude protein when a mixture of palm kernel cake and rice bran on a ratio of 1:1 was inoculated with *Trichoderma viride*. Vorachinda *et al*. (2011) reported a 14.25% increment in crude protein of cassava pulp / soymilk residue using *A. niger*.

Crude fibre: The crude fibre contents of treatments A, B and C (11.28, 11.62 and 13.13 respectively) were all significantly lower ($p<0.05$) than the control values (21.75) on day 7. Treatment A generally gave lower values throughout the period of experiment. This indicates that *Aspergillus niger* was better able to utilize or breakdown the crude fibre of test material. This is expected considering the fact that *A. niger* elaborates mannanase and other cellulolytic enzymes (Kheng and Omar, 2005; Abdeshahian *et al.*, 2010). The results here represent 40 to 50% decrease in crude fibre content of the raw CRM and PKC mix. Sukaryana *et al.* (2010) reported a decrease in crude fibre content of 22.53%.

Fat content: There were significant differences ($p<0.05$) in fat contents across all treatment groups. It seems that *Aspergillus niger* used up more of the fat in the substrates than the organisms in spontaneous inoculation and then batch (3.95, 4.45, 5.91 respectively). Period of fermentation had no influence on the fat content of samples. It is possible that some equilibrium mechanism was established which controlled evolution, utilization or retention of fat within the system.

Carbohydrate: The residual carbohydrate level of the *Aspergillus niger* inoculated samples were not significantly higher ($p>0.05$) than the spontaneously inoculated samples and batch fermentation samples after 15 days of fermentation. The results for treatments A, B and C were significantly lower than the control. This indicates that significant proportion of the fermentable carbohydrate must have been used up by the organisms. This is as well expected considering that the principal reaction in involved in fermentation is the conversion of soluble highly fermentable carbohydrates to volatile fatty acids and / or alcohol, carbon dioxide and water.

Metabolizable energy: The calculated metabolizable energies of the experimental materials are shown in table 5. Metabolizable energy of treatments A, B and C were significantly ($p<0.05$) lower than the control through all the periods of fermentation.

Table 5. Metabolizable Energy (Kcal/g)

Period of Fermentation in days	TA	TB	TC	TD (control)	SEM
7	222.99[a]	252.93[a]	230.27[a]	311.65[c]	13.41
15	250.27[a]	241.50[a]	226.27[a]	311.05[c]	
15	253.75[a]	238.06[a]	221.77[a]	311.05[c]	
19	228.64[a]	223.37[a]	222.23[a]	310.91[c]	

Though the values here are calculated, the result is expected considering the fact fermentation of a glucose molecule leads to the evolution of 9 ATPs. Synthesis of biomass protein also requires energy. This is confirmed by the huge evolution of metabolic heat characteristic of fermentation, especially solid state fermentation techniques (Raimbault, 1999).

Microbial fermentation has played a significant role in the nutritional enhancement of the underexploited agro-industrial by-products generated through the harvesting and processing of cassava root (Soccol, *et al.*, 1994). Spontaneous inoculation technique is therefore judged suitable for improving the feed value of CRP-PKC blend within 7 days. Extending the period of fermentation to 19 days neither improve nor leads to any deterioration of product quality. If efficient methods of drying are employed, a product of up to 18 % Crude Protein and 2500 Kcal/Kg is achievable within 7 days of fermentation. More research is however needed to properly understand the microbiology and biochemical transformations of the process at each stage of fermentation; the amino acid profile of the end products, possible development of toxins as well as feeding trials to determine its actual value as livestock feed.

Conclusion

Based on the result of this work, it is therefore concluded that subjecting cassava root pulp and palm kernel cake mixtures to solid state condition either *Aspergillus niger*, batch or spontaneous inoculation improved the physical characteristics of the mixed feedstuff, increased the crude protein content by about 70% and led to a complete removal of the floury nature of cassava root meals.

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