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RESEARCH ARTICLE

IMPROVING THE NUTRITIVE VALUE OF CASSAVA PEELS AND CASSAVA ROOT SIEVIATE THROUGH BIODEGRADATION WITH ASPERGILLUS NIGER

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ABSTRACT

This paper discusses the effects of process parameters on the biodegradation of cassava peels and cassava root sieviate through the action aspergillus niger. The ability of aspergillus niger to increase the crude protein content and decrease the crude fiber content of the cassava wastes was investigated with time and substrate concentration as process parameters. Face-centered central composite design (FCCCD) of the experiment was applied in the determination of the two-parameter improvements of the nutritive value of the wastes. The experiment was designed in minitab version 16.0 while the analysis of variance (ANOVA) SPSS 16.0 was applied in the analysis of the raw data. The experimental data analysis showed that the crude protein percentage contents of the two wastes significantly increased, from 4.85 to 15.86 and from 1.85 to 9.42 for the cassava peels (CP) and cassava root sieviate CRS) respectively due to the action aspergillus niger. Similarly, crude fiber was decreased from 68.2% to 7.82% and 70.3% to 8.30% for CP and CRS. These remarkable improvements on the nutritive value of the cassava wastes were recorded at an optimum time of 10 days and substrate concentration of 0.6g/ml. From the results, it was obvious that Aspergillus niger was able to enrich the protein while decreasing the fibre contents of the cassava wastes significantly ($P < 0.05$). However from the interaction plots and multiple comparison tests, 6 days of biodegradation and 0.2g/ml substrate concentration have the optimum desirable effect.

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INTRODUCTION

Cassava (*manihot esculanta*), is native to South America. It is extensively cultivated as an annual crop in the tropical and subtropical regions of the world (Fawole and Oso., 1988). It is an edible, starchy, tuberous root, composed mainly of carbohydrates. It is the third largest source of food carbohydrates in the tropics. It is a major staple food in the developing world, producing a basic diet for over five hundred million people. Cassava is one of the drought tolerant crops, capable of growing on marginal solids. Nigeria is the world's largest producer of the crop, with an annual production capacity of 45 million metric tones. Apart from being a major staple food in most developing countries, cassava has many other commercial, pharmaceutical, industrial and agricultural values. (http://www.foramfera.com/index.php/membership_zone (2012). These include starch production for use in the textile industry, oil well drilling, pharmaceuticals, foods and beverage industries. The processing of cassava for these diverse uses generates large amounts wastes and environmental hazards of very serious concerns. These wastes generally cause air pollution (offensive order) and contamination of soil by release of cyanide because of fermentation if not harnessed (FAO, 2001). Cassava processing is generally considered to contribute significantly to environmental pollution and to the depletion of depletion of water resources due to the strong and unpleasant odor and the visual display of waste products. Cassava peels, leaves and starch residues constitute about 25% of the cassava plant (Iyayi and Losel 2000). Cassava peel is the skin of cassava tubers, which is removed on peeling, while cassava root sieviate is the

by-product left after peeled cassava tubers have been process to "foofoo", Aderemi *et al.* (2006). It contains high amount of non-starch polysaccharides mostly of non-digestible carbohydrate such as cellulose, hemicelluloses, which have a high water holding capacity. This was observed to be poorly digested and bio utilized by laying birds, which resulted in depressed weight gain and reduced egg production, Aderemi *et al.*, (2004). The digestibility of a feed for both ruminant and non-ruminant tend to decrease with crude fiber content. Typically, a 1% increase with crude fiber brings a 1% decrease in digestibility for ruminants and a 2% decrease for pigs (F A D, 1985, and Aderoru *et al.*, 2002). The data of Devendra (1977), Adegbola and Asaolu (1980) and FAO (2012) showed that cassava peel contains 10-30% crude fiber, 4-7% crude protein, high soluble carbohydrate (69%) and high levels of hydrocyanic acid. According to Aderemi *et al.*, 2004 cassava peels and cassava root sieviate which can be used as animal feed, for biogas and as a source of fiber, contain 50% crude fiber, 2.09 crude protein and high amount of non-starch polysaccharides mostly of non-digestible carbohydrate, (Iyayi and Tewe, 1994). The preponderance of fiber in their composition relative to their commotional counterparts has been documented (Longe, 1988; Longe and Fagbeno-Biyron 1980). There has been growing interest in the application of these cassava wastes in the production of monogastric animal feed, and this has in turn triggered of a number of researches towards the improvement of their nutritional values with respect to their protein, fibre, non-starchy polysaccharides and cyanide contents, (Iyayi and Losel, 2004; Ubalua, 2007; Aro, 2008). The concept of using microorganism in enhancing the nutritive value of plant and animal products is not entirely a new one. The ability of fungi to produce enzymes, which bring about catalytic transformations in the wide range of desirable reactions, makes them interesting to industrialists and agriculturists.

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There are recent advances in bioconversion of agro-industrial wastes to products of significance in livestock production (Iyayi, and Losel, 1999). Microbial fermentation has been reported as an effective means of breaking down non-starch polysaccharides of agro-industrial wastes to increase their metabolizable energy and their nutritive value in general. (Onilude, 1999).

Biodegradation is the breaking down of materials by biological activity with the aim of producing high quality products. It can be described as a process in which substrates are decomposed by known mono or mixed cultures of microorganism under controlled environmental conditions with the aim of producing high quality product. The substrate is characterized by relatively low water content (Zandrazil, *et al.*, 1990). Enzymes from microorganisms especially fungi has been indicated to be promising in degrading structural carbohydrates such as cellulose, hemicelluloses and lignin and in degrading or structurally modifying proteins and their anti nutritional properties and to liberate phosphorous complex compounds e.g. phytase (Rai *et al.*, 1988) By harnessing these natural forces of biodegradation, people can reduce wastes and clean up some types of environmental contaminations. (<http://www.gbenterprises.com/index.php>). *Aspergillus niger* is a widely distributed filamentous fungus that is responsible for the spoilage of many foods. This fungus is often found indoors and grows as black colonies, known as black mold. This fungus is widely used industrially to produce variety types of enzymes such as pectinase, amylase, cellulose and organic acid such as citric and gluconic acid (Benneth, 1985). In addition to its use in acid production, *aspergillus niger* can also be used in waste management and bio transformation (Schuster *et al.*, 2002). According to America Food and Drug Agency, *A. niger* is safe for industrial, medical and agricultural use. It is readily available and has the ability to produce enzymes such as amylases lipases, celluloses, xylanases and proteases. (Van de Vondervoort *et al.*, 2004).

MATERIALS AND METHODS

Samples Preparation

Aspergillus Niger culture

The medium used for the isolation of the fungus was supplied by the Resource concept laboratory in Emene, Enugu. The pure culture of *Aspergillus niger* was isolated from a bread sample spoilt by fungi using sabroud dextrose agar medium. Streak plate technique was employed for the isolation, observing aseptic precautions. The sample was incubated at 30°C for 72 hours.

Cassava peels, Sample Preparation and Analysis

Fresh cassava tubers were procured from a local farm at Agbogugu, Enugu (Nigeria), washed and peeled. The peels were sundried to 6.0% moisture content. It was later ground into fine powder using disc mill (Aro *et al.*, 2010). Sun-drying was used to reduce cyanogenic glycosides (Salami *et al.*, 2003; Tewe, 1992; Adegbola *et al.*, 1985). Dried samples were subjected to different preliminary analysis according to standard methods for the determination of dry matter, crude fat (soxhlet method), crude proteins (micro kjeldah, AOAC, 1990), crude fiber (Baker, 1977), total carbohydrate (AOAC, 1990), cellulose (FAO, 2000), hemicelluloses, lignin, Neutral Detergent Fibre NDF (NRC, 1989), Acid Detergent Fibre ADF, and ADL (Von Soest & Wine, 1968).

Cassava root Sieviate Sample Preparation and Analysis

The peeled cassava tubers were subjected to 72hours fermentation process and later sieved to produce foo-foo and the root sieviate which was subsequently sun-dried and ground to fine powder. The final product was analysed as in the cassava peels. The results of the two analyses are contained in Table 2.

Substrate Preparation for Biodegradation

2.0g, 4.0g and 6.0g powdered samples of the cassava peels and cassava root sieviate were separately weighed each into three Petri dishes. They were each mixed with 10ml of 25% sucrose solution containing the fungal biomass. Each prepared sample was subjected to solid state fermentation for 6, 8 and 10days at an incubation temperature of 30°C. At the end of each fermentation time, the samples were dried in an oven at 80°C until a constant moisture content of 6.0%. After cooling, their proximate analyses were carried out as above.

EXPERIMENTAL DESIGN

The face-centered central composite design (FCCCD) was used to determine the influence of time and substrate concentration as parameters in enriching the nutritional value of cassava peel and cassava root sieviate. Two parameters were investigated at three levels (low, center, and high) coded as (-1, 0, 1). Thirteen experiments with five at center points were studied. The detailed experimental designs were presented at Table 1.

Table 1. Experimental Design Showing the Samples with Coded and Un-coded Units

S/N	Time (Days)	Concentration (g/ml)
1	1 (10)	-1 (2)
2	-1 (6)	1 (6)
3	-1 (6)	0 (4)
4	1 (10)	0 (4)
5	0 (8)	0 (4)
6	0 (8)	0 (4)
7	0 (8)	0 (4)
8	0 (8)	-1 (2)
9	-1 (6)	1 (6)
10	0 (8)	0 (6)
11	0 (8)	0 (4)
12	0 (8)	-1 (2)
13	1 (10)	1 (6)

Statistical Analysis

Data generated from laboratory analysis were fed into computer and response surface regression equation and RS plots obtained using Minitab version 16.0. Also SPSS version 16.0 was used to compare the means of sample that differed significantly using LSD, through two-way ANOVA. Significant difference was determined at P = 0.05.

Model of the analysis: $X_{ijk} = \mu + a_i + b_j + \lambda_{ij} + e_{ijk}$

X_{ijk} = Content of biodegraded cassava peel and root sieviate taken from the substrate concentration of gm/10ml at different time interval.

μ = the grand mean.

a_i = the *i*th effects of substrate concentration of gm/10ml on crude protein, crude fibre and totalcarbohydrate.

b_j = the *j*th effect of time.

λ_{ij} = the interaction between substrate concentration and time.

e_{ijk} = error associated in the observation.

RESULTS AND DISCUSSION

Table 2. Chemical Composition Of Undegraded Cassava Peel And Cassava Root Sieviate

Nutrient composition	Cassava Peel (%)	Cassava Root Sieviate (%)
Crude protein	4.85	1.85
Crude fiber	68.2	70.3
Total carbohydrate	80.2	80.3
ADF	8.5	14.2
Crude fat	3.5	2.5
Fermentable carbohydrate	12.0	12.0
Lignin	3.62	5.57
ADL	4.24	6.62
Cellulose	4.88	8.63
Hemicelluloses	11.7	8.0
NDF	20.2	22.2
Dry matter	75	75

DISCUSSION

Characterization Results

Presented in Table 2 are the nutrient composition of undegraded cassava peel and root sieviate. The raw sample values of crude protein, crude fiber and total carbohydrate content are 4.85%, 68% and 80.3%, and 1.85%, 70.3% and 82.3% for cassava peel and root sieviate respectively. The mean and standard deviation for nutrient composition were shown in Table 3 and 4 for 2g/10ml, 4g/10ml and 6g/10ml each at different biodegraded time of 6 days, 8 days and 10 days respectively. The highest crude protein content was observed at 6g/10ml at 10 days for both samples. The minimum crude fiber content was observed at 6g/10ml concentration at 10 days biodegraded time, however highest total carbohydrate content was observed at 2g/10ml concentration at 6 days biodegradation time. These results was in line with Aderemi and Nworgu (2007) and Iyayi and Losel (2001) who observed the ability of *Aspergillus niger* to breakdown the fiber and increase protein content. The appearance of the mycelia of the fungi on the substrate after 48 hours was on indication that degradation has commenced. This was in line with Ofoya and Nwajiuba (1990) thus confirms suitable environmental conditions for the fungi. The degradation of cassava peel and root seriate starts with the breakdown of polysaccharides into Oligosaccharides which can be hydrolyzed by glucosidase into their component monomer. The metabolism of these monomers can then give energy and carbon for the growth of the micro-organism as reported by Smith *et al.* (1996).

From this study, it was observed that there was increase in protein content compared to undegraded CRS from 1.85 to 9.42%, also cassava peels have an improvement from 4.8 to 15.95%. This implied that *Aspergillus niger* has a significant ($p < 0.05$) effect on the protein content. This increase in the crude protein observed was probably due to the additional crude protein produced in the fungal mycelia and thus is influenced by carbon to nitrogen ratio. Similar result had been reported by Aderemi and Nworgu (2007). Also this result was in line with Iyayi and Losel (2001), who reported enriched protein of cassava peel and pulp with different fungi types. The fiber component decreased over the period of biodegradation. The fungi secreted some enzymes (cellulose, fungal amylase, pectinase) on the substrate, resulting in decreased fiber content (Bolaski and Galantin 1976). Crude fiber decreased from 70.3% to 8.3% for CRS and 68% to 7.9% for CP. This result is in line with Chesson (1993) who reviewed the early claim that disruption of cell walls and their degradation by microorganism enzyme could be beneficial to host animal. He reported that the available cell wall carbohydrate not attacked by digestive enzymes now seem wildly optimistic after biodegradation. He then stressed that total breakdown requires the action not only of the enzymes responsible for the primary attack on the cell wall polysaccharide and glucan hydrolases but also of a second set of glucosidases able to reduce oligosaccharides to their monomer components. The total carbohydrate content decreased over the period of biodegradation from 82.3% to 9.75% for CRS and 80.36% to 8.10% for CP. It implies that the fungi made use of it during biodegradation as source of carbon % total. The ease of degrading any fiber component is a function of the enzyme composition of fungi and the physicochemical properties of the substrate.

Table 3. Nutrient Composition of Cassava Peel Biodegraded at Different Periods (Days) Sample

Time /Conc. (days) (g/10ml)	Crude Protein (%)	Crude Fiber (%)	Total Cho (%)
A 10:2	9.50±0.14 ^a	12.45±0.21 ^a	5.60±0.28 ^a
B 6:6	12.20±0.28 ^b	13.60±0.28 ^b	14.30±0.14 ^b
C 6:4	6.10±0.14 ^c	18.30±0.14 ^c	19.25±0.07 ^c
D 10:4	9.90±0.14 ^d	8.90±0.14 ^d	9.05±0.07 ^d
E 8:4	8.50±0.14 ^e	14.10±0.14 ^e	14.35±0.07 ^e
F 8:4	8.50±0.14 ^e	14.10±0.14 ^e	14.35±0.07 ^e
G 8:4	8.50±0.14 ^e	14.10±0.14 ^e	14.35±0.07 ^e
H 8:4	8.50±0.14 ^e	14.10±0.14 ^e	14.35±0.07 ^e
I 6:2	4.35±0.13 ^f	22.30±0.14 ^f	24.30±0.14 ^f
J 8:6	14.41±0.01 ^g	12.61±0.01 ^g	13.50±0.14 ^g
K 8:4	8.50±0.14 ^e	14.10±0.14 ^e	14.35±0.07 ^e
L 8:2	6.71±0.01 ^h	16.70±0.14 ^h	18.10±0.14 ^h
M 10:6	15.86±0.08 ^j	7.85±0.07 ^j	8.10±0.14 ^j

* Data are means of duplicate determinations + SD **Data on the same column bearing different superscripts are significantly different ($P < 0.05$). The means were compared with multiple comparisons and considered significant at $p < 0.05$.

Table 4. Nutrient Composition Of Cassava Root Sieviate Biodegraded At Different Periods (Days)

SAMPLE Time Conc. (days) (g/10ml)	Crude Protein (%)	Crude Fiber (%)	Total Cho (%)
A 10:2	5.77±0.04 ^k	18.85±0.07 ^k	20.30±0.14 ^k
B 6:6	6.42±0.03 ^l	20.10±0.14 ^l	22.2±0.28 ^l
C 6:4	5.85±0.07 ^m	28.05±0.07 ^m	30.10±0.14 ^m
D 10:4	8.41±0.01 ⁿ	16.10±0.14 ⁿ	18.10±0.14 ⁿ
E 8:4	7.21±0.01 ^p	20.50±0.14 ^p	22.20±0.28 ^p
F 8:4	7.21±0.01 ^p	20.50±0.14 ^p	22.20±0.28 ^p
G 8:4	7.21±0.01 ^p	20.50±0.14 ^p	22.20±0.28 ^p
H 8:4	7.21±0.01 ^p	20.50±0.14 ^p	22.20±0.28 ^p
I 6:2	3.62±0.03 ^q	30.50±0.14 ^q	32.45±0.07 ^q
J 8:6	8.83±0.04 ^r	14.70±0.14 ^r	15.80±0.00 ^r
K 8:4	7.21±0.01 ^p	20.50±0.14 ^p	22.20±0.28 ^p
L 8:2	4.81±0.01 ^s	24.41±0.01 ^s	18.85±0.07 ^s
M 10:6	9.42±0.03 ^t	8.30±0.14 ^t	9.75±0.07 ^t

* Data are means of duplicate determinations + SD ** Data on the same column bearing different superscripts are significantly different ($P < 0.05$)

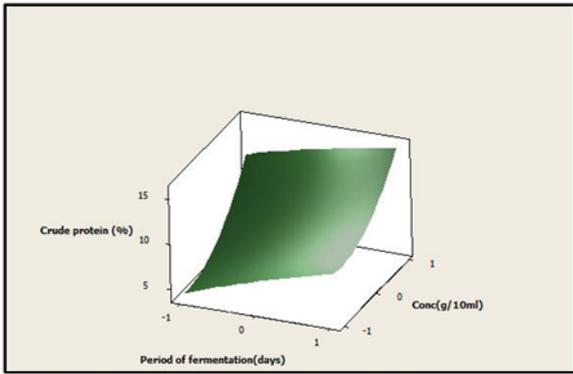


Fig. 3a RS plot of crude protein for cassava peel

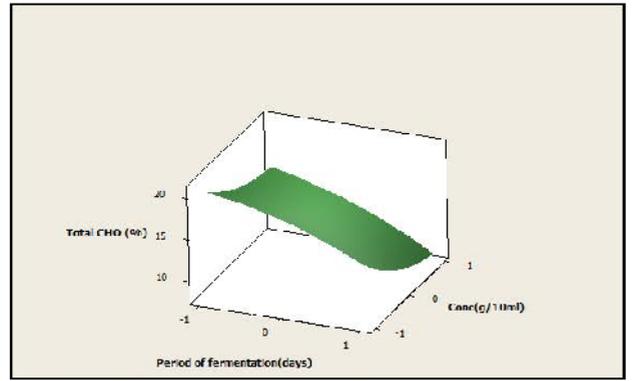


Fig. 5a RS plot of total CHO for cassava peel

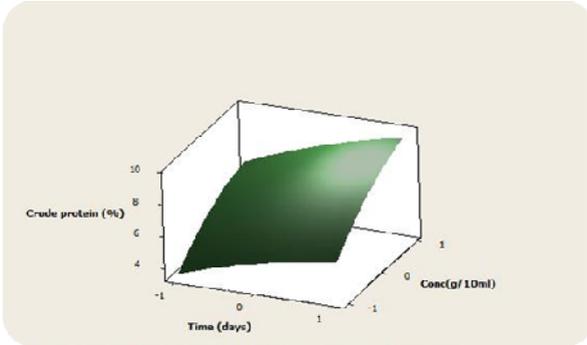


Fig. 3b RS plot of crude protein for cassava root sieviate

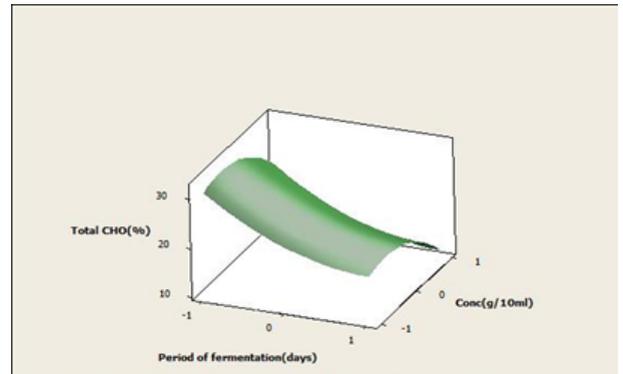


Fig. 5b RS plot of total CHO for cassava root sieviate

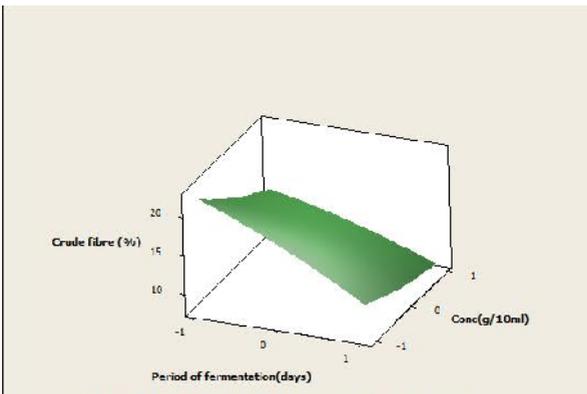


Fig. 4a RS plot of crude fiber for cassava peel

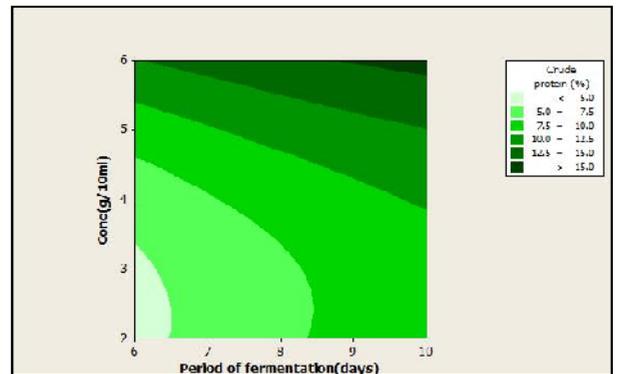


Fig. 6a Contour plot of crude protein for cassava peel

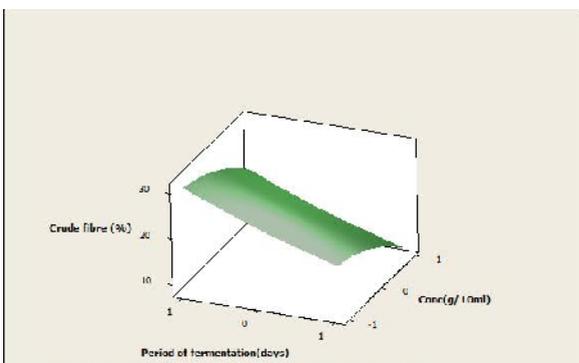


Fig. 4b RS plot of crude fiber for cassava root sieviate

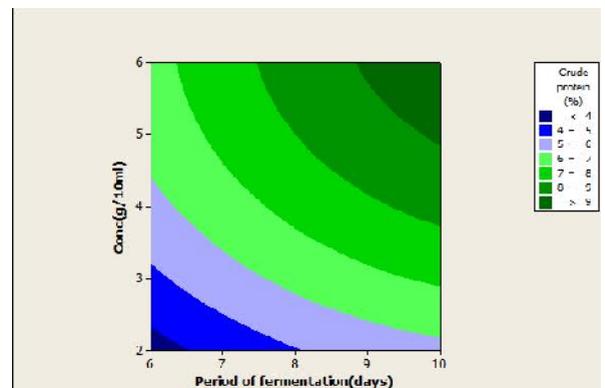


Fig. 6b Contour plot of crude protein for cassava root sieviate

Table 7. ANOVA Table for 0.2g/ml Conc.(CRS)

Tests of Between-Subjects Effects Dependent Variable: Content of biodegraded cassava root sieviate

Source	Type III sum of Square	df	Mean Square	F	Sig.
Corrected Model	2014.096 ^a	8	251.762	17036.523	.000
Intercept	6190.877	1	6190.877	418931.550	.000
Concentration	1725.976	2	862.988	58397.686	A. 000
Ferment time	156.288	2	78.144	5287.932	B. 000
Concentration*	131.832	4	32.958	2230.238	C. 000
Ferment time	.133	9	.015		
Error	8205.106	18			
Total	2014.229	17			
Corrected Total					

a.R. Squared = 1.000 (Adjusted R Squared =1,000)

Table 8. ANOVA Table for 0.4g/ml conc.(CRS)

Source	Type II sum of Square	df	Mean Square	F	Sig.
Corrected Model	1255.201 ^a	8	156.900	8286.978	.000
Intercept	5444.113	1	5444.113	287541.202	.000
Concentration	953.716	2	476.858	25186.149	A. 000
Ferment time	157.633	2	78.817	4162.853	B. 000
Concentration*	143.852	4	35.963	1899.455	C. 000
Ferment time	.170	9	.019		
Error	6699.485	18			
Total	1255.371	17			
Corrected Total					

a.R. Squared = 1.000 (Adjusted R Squared =1,000)

Table 11. ANOVA Table for 0.6g/ml conc.(CRS)

Tests of Between-Subjects Effects Dependent Variable: Content of biodegraded cassava root sieviate

Source	Type II sum of Square	df	Mean Square	F	Sig.
Corrected Model	503.382 ^a	8	62.923	3816.067	.000
Intercept	2965.527	1	2965.527	179850.005	.000
Concentration	198.661	2	99.330	6024.082	A. 000
Ferment time	151.199	2	75.599	4584.867	B. 000
Concentration*	153.522	4	38.381	2327.660	C. 000
Ferment time	.148	9	.016		
Error	3469.057	18			
Total	503.530	17			
Corrected Total					

R Squared = 1,000 (Adjusted R. Squared = .999)

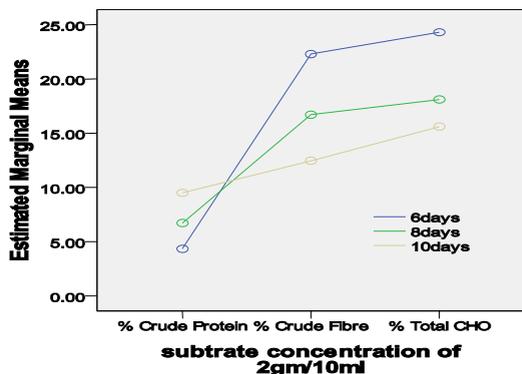


Figure 9. Interaction plot of CP at 0.2g/ml conc. At different days

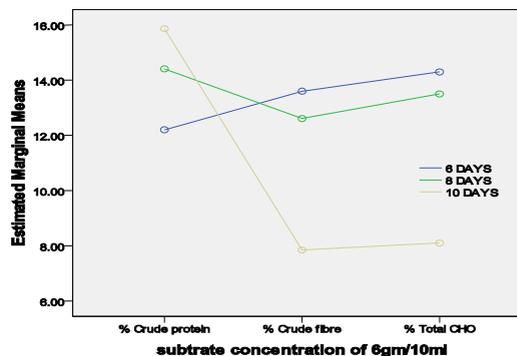


Figure 11. Interaction plot of 0.6g/ml conc. At different days (CP)

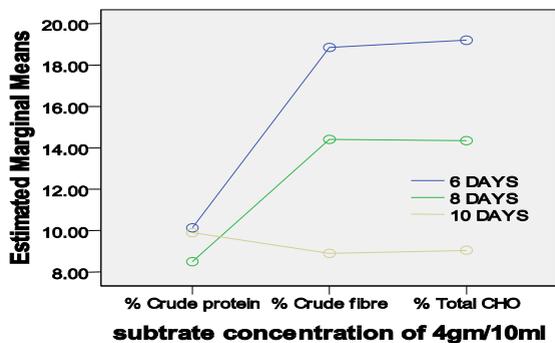


Figure 10. Interaction plot of 0.4g/ml conc. at different days (CP)

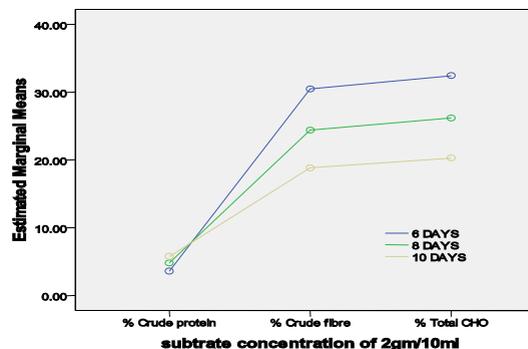


Figure 12. Interaction plot of 0.2g/ml conc. at different days (CRS)

Interaction plot for CP and CRS

The interaction plots between the estimated marginal means and the three contents, crude protein, crude fiber and total carbohydrate at different time (days) were shown in Fig 9-14. The interaction plots for CP and CRS in Fig 9,11,12,13,14 showed that biodegradation affected the crude protein content, crude fibre content and total carbohydrate content. The plot showed that there was interaction because the three lines met at a point. However, 6 days of biodegradation has the highest effect on the contents. Interaction plot for CP in Fig 10, showed no interaction, because the three lines did not meet at a point. Test showed that 6 days and 10 days are significant because P value was less than 0.05 (0.008<0.05). Therefore 6 days and 10 days time effect has significant on 4g/10ml concentration but 8 days time effect is insignificant.

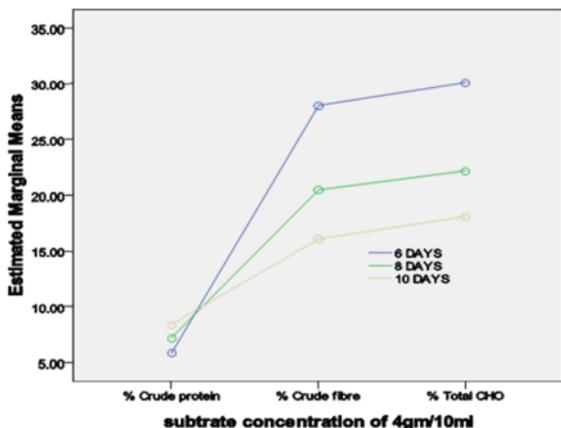


Figure 13. Interaction plot for 0.4g/ml at different days(CRS)

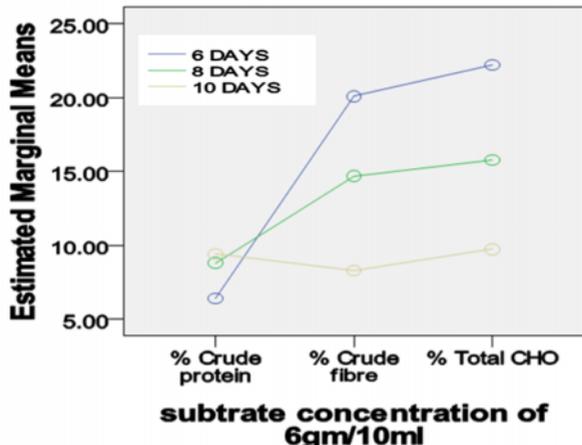


Figure 14. Interaction plot for 0.6g/ml at different days (CRS)

Conclusion

Aspergillus Niger has the ability to increase the crude protein content of the cassava peel and root sieviate and degrade the crude fiber content. However, the Aspergillus niger used up the total carbohydrate content hence the feed should be supplemented with energy giving food such as wheat flour, shea-butter. From the multiple comparison Table, the crude protein content has greater effect than other contents when compared. However, 6 days of biodegradation and 0.2g/ml substrate concentration had more effect on the biodegraded cassava peel and root sieviate. Therefore, cassava peels (CP) and cassava root sieviate (CRS) can be used as animal feed as this will also assist in solving some environmental waste problems.

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