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Optimization of cassava root sieviate medium to an enriched animal feed by Aspergillus niger

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Abstract: - Optimization of the media components cassava root sieviate with Aspergellus Niger was carried out using Face-Centered Central Composite Design (FCCCD) of the Response Surface Methodology (RSM) and the responses were measured in terms of protein and crude fiber contents. Statistical Analysis (ANOVA) of the result showed that time and substrate concentration had effect on biodegraded cassava root sieviate (p-value was 0.00) ie. 0.00 < 0.05. The optimum value of enriched cassava root sieviate with Aspergellus Niger were found to be on 10 days (time) and 6g/10ml concentration resulting to 9.42% from 1.85% enrichment in protein content and degrading crude fiber to 8.38%, from 70.3%, thus indicating the potency of Aspergillus niger for the production of economical livestock feed from renewable source.

Keyword: - *Cassava root sieviate, Aspergillus niger, Optimization, animal feed, Biodegradation.*

I. INTRODUCTION

Cassava root sieviate is the by-product left after peeled cassava tubers have been processed to foo-foo. It contain high fiber and low protein content hence utilization in animal feeding is limited (lyayi and Tewe, 1994, Iyayi and Losel 2004). It also contains high amount of non-starch polysaccharides mostly of non-digestible carbohydrate such as cellulose, hemicelluloses, lignin which have a high water holding capacity. This was observed to be poorly depressed and bio-utilized by laying birds, which results in, digressed 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 of ruminants and a 2% decrease for pigs (F.A.D, 1985, and Aderolu et al, 2002). Cassava root sieviate contains about 50% crude fiber, and 2.01% crude protein (Aderemi et al, 2004).

Nigeria is the world's largest producer of the crop, with an annual production capacity of 45million metric tones (www.foramfera.com/index.php/market). The processing of cassava into various uses generate large amount of wastes and environmental hazards of very serious concerns. These wastes generally cause air pollution and contamination of soil by release of cyanide because of fermentation, if not harnessed (FAO, 2001). In recent years, considerable emphasis has been placed on the improvement of fibrous crop by the growing of non-toxic fungi on straw. The ability of fungi to produce enzymes, which bring about catalytic transformations in the wide range of desirable reactions make them interesting to industrialists and agriculturists (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).This research is very important because livestock feed were sourced from renewable and relatively inexpansive food materials such as agricultural by-product and waste, peel, seed, chaff and root sieviate.

Aspergillus niger is safe for industrial, medical and agricultural use. It is readily available and secretes enzymes such as lipases, cellulases, proteases, amylases and xylanases when grown on selected agro industrial wastes. (Schuster et al, 2002, Vande Vandervoort et al, 2004). This fungus is widely used industrially to produce various types of enzyme and citric acid pectinase, alpha galactasidase, and glucose oxidase, among others. It is used for waste management and bio transformation (Schuster et al, 2002 & Mbah, G. O. et al, 2013). Exploiting the above characteristics, the nutritional value of cassava root sieviate could be enhanced by optimizing the

media constituents through solid state fermentation (biodegradation) which is a better choice in the bio conversion of agro industrial wastes to animal feed considering the nature of feed.
Optimization of media composition is a vital tool to an efficient cell growth and improved secretion of various enzymes that help in degradation. Sugar syrup was added to provide more carbon which influence the secretion of enzymes by Aspergillus niger. Hence, optimization of media constituents will go a long way in improving yield and reducing cost of production. Central Composite Design (CCD) have been described as a statistical technique that is widely used for the optimization of medium composition for growth and metabolite.

In this study, Face-Centered Central Composite Design (FCCCD) was used to determine the influence of medium constituent and identify the optimum level of the constituent in enriching the nutritional value of cassava root sieviate to animal feed by Aspergillus niger.

II. MATERIALS AND METHODS

2.1 Aspergillus niger culture

The pure culture of Aspergillus niger was isolated from a bread sample spoilt by fungi using saboraud dextrose agar medium. Streak plate technique was employed for the isolation observing aseptic precautions. Incubation lasted for 72 hours at 30^oC

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2.2 Preparation of cassava root sieviate sample

Cassava tubers were procured from a local farm at Agbogugu, Enugu, Nigeria. They were peeled and washed. The peeled cassava samples were fermented for 72hours and later sieved. After sieving the fermented cassava, the cassava root sieviate chips were sundried (Tewe 1992) and ground into fine powder using Disc Mill. The ground cassava root sieviate were stored in airtight container. The chemical composition of unfermented cassava root sieviate was analyzed and recorded, such as Dry Matter, crude fat, Crude fiber (AOAC 2000), crude protein, Hemicelluloses (AOAC, 1990) total CHO, cellulose (FAO 2000), Lignin, NDF, ADF, ADL (Vine Soest and Wine 1967, AOAC 1990).

2.3 Substrate preparation for solid state fermentation (Biodegradtion)

The Face-Centered Central Composite Design (FCCCD) under the response surface methodology was used to determine the influence of medium constituents in enriching the nutritional value of cassava root sieviate and to identify the optimum levels. Two independent variables namely time and substrate concentration were investigated at three levels (low, basal, high) coded as (-1,0,1). The detailed experimental designs were presented in Table 1.

Thirteen experiments with five replications at center points were studied. The fermentation media were varied at 2g, 4g and 6g in duplicates. Then each sample was mixed with 10ml of 25% sucrose solution containing the fungal biomass. The fermentation medium was prepared in Petridish and incubated at 30^0C for different fermentation periods of 6,8 and 10days. Two dependent variables: protein and crude fiber content serve as the responses, Y_i. A linear regression equation given below was used to determine the relationships that exist between dependent and independent variables.

$$Y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 \ldots\ldots\ldots\ldots\ldots\ldots \quad 1$$

where Y is the dependent variable (protein and crude fibre content) X_1, and X_2 are independent variables (time and substrate concentration, β_o is the intercept term, β_1 and β_2 are the linear coefficients, β_{12} is the interaction coefficient.

III. RESULTS AND DISCUSSION

Table 1 summarizes the result obtained with the experimental design which was aimed at determining the conditions that favour maximum protein increase and maximum crude fiber degradation in cassava root seriate. Linear regression equation obtained from equation 1 and Table 1 for protein and fibre were fitted to the data model for predicting the responses as given below:

$$Y_{cp} = 6.86 + 1.285x_1 + 1.745x_2 + 0.2125x_1x_2 \ldots\ldots\ldots\ldots \quad 2$$

$$Y_{cf} = 20.27 - 5.91x_1 - 5.11x_2 - 0.0375x_1x_2 \ldots\ldots\ldots\ldots\ldots \quad 3$$

Table 1: FCCCD experimental design showing coded and actual valves with the experimental and predicted values for enrichment of cassava root sieviate

Run	Time (days) X_1	Conc. (g/mol) X_2	Crude Protein (%)		crude fibre (%)	
			Experimental	Predicted	Experimental	Predicted
1	8(0)	4(0)	7.21	6.86	20.50	20.27
2	6(-1)	2(-1)	3.62	4.04	30.50	31.24
3	10(+1)	2(-1)	5.77	6.19	18.85	19.52
4	8(0)	4(0)	7.21	6.86	20.50	20.27
5	8(0)	2(-1)	4.81	5.12	24.41	25.38
6	8(0)	6(+1)	8.83	8.61	14.70	15.16
7	6(-1)	4(0)	5.85	5.58	28.05	26.17
8	10(+1)	4(0)	8.41	8.15	16.10	14.37
9	8(0)	4(0)	7.21	6.86	20.50	20.27
10	6(-1)	6(-1)	6.42	7.11	20.10	21.10
11	10(+1)	6(+1)	9.42	10.10	8.30	9.22
12	8(0)	2(0)	7.21	6.86	20.50	20.27
13	8(0)	4(0)	7.21	6.86	20.50	20.27

Table 2:- Full design matrix and response result for the experiment variables

Standard	Run	X_1	X_2	X_1X_2	Y(CP)	Y(CF)
1	5	0	0	0	7.21	20.50
2	9	-1	-1	+1	3.62	30.50
3	1	+1	-1	-1	5.77	18.85
4	6	0	0	0	7.21	20.50
5	12	0	-1	0	4.81	24.41
6	10	0	+1	0	8.83	14.70
7	3	-1	0	0	5.85	28.05
8	4	+1	0	0	8.41	16.10
9	7	0	0	0	7.21	20.50
10	2	-1	+1	-1	6.42	20.10
11	13	+1	+1	1	9.42	8.30
12	8	0	0	0	7.21	20.50
13	11	0	0	0	7.21	20.50
Effect y (CF)		-11.8	-10.22	-0.075		20.27
Effect y (CP)		2.57	3.49	0.425	6.86	

From analysis of variance (ANOVA) significant difference was determined at $P \leq 0.05$.
Model of the analysis: $X_{ijk} = \mu + a_i + b_j + \boldsymbol{\lambda}_{ij} + e_{ijk}$
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 ith effects of substrate concentration of gm/10ml on crude protein, crude fibre and total carbohydrate.
b_j = the jth effect of time.
$\boldsymbol{\lambda}_{ij}$ = the interaction between substrate concentration and time.
e_{ijk} = error associated in the observation.

TEST OF HYPOTHESIS
H_0 = Crude protein = crude fibre = Total CHO
H_1 = Crude protein ≠Crude fibre ≠ Total CHO
H_0 = Time has no effect on cassava peel.
H_1 = Time has effect on cassava peel.
H_0 = There is no interaction effect between time and substrate concentration
H_1 = There is interaction effect between time and substrate concentration.

TABLE 3: ANOVA Table for 2g/10ml 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 x Ferment. time	131.832	4	32.958	2230.238	C. 000
Error	0.133	9	0.015		
Total	8205.106	18			
Corrected Total	2014.229	17			

a. R. Squared = 1.000 (Adjusted R Squared =1.000)

TABLE 4: ANOVA table for 4g/10ml 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 x Ferment time	143.852	4	35.963	1899.455	C. 000
Error	0.170	9	0.019		
Total	6699.485	18			
Corrected Total	1255.371	17			

a R. Squared = 1.000 (Adjusted R Squared =1.000)

TABLE 5: ANOVA table for 6g/10ml 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 x Ferment time	153.522	4	38.381	2327.660	C. 000
Error	0.148	9	0.016		
Total	3469.057	18			
Corrected Total	503.530	17			

a. R Squared = 1.000 (Adjusted R. Squared = .999)

IV. DISCUSSION

4.1 Characterization Results

Presented in Table 1 are the FCCCD experimental design showing coded and actual values with the experimental and predicted values for enrichment of cassava root sieviate. The control values of crude protein and fiber for cassava root sieviate are 1.85% and 70.30%. The control value was gotten from preliminary analysis of unfermented cassava root sieviate. Table 2 showed the full design matrix and response result for the experimental variables. The highest crude protein and lowest fiber contents was observed at 6g/10ml at 10days for cassava root sieviate samples. These results were in line with Aderemi and Nworgu (2007) and Iyayi and Losel (2004) 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 an 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 root sieviate 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 for the micro-organism as reported by Smith et al (1996). From this study, it was observed there was increase in protein content compared to undegraded CRS from 1.85 to 9.42%. This implied that Aspergillus niger has significance ($p<0.05$) 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 (Onilude, 1994) and thus is influenced by carbon to nitrogen ratio, similar result had been reported by Abu (1997) using sweet potatoes in solid state fermentation. 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 decreased fiber content. (Bolaski and Galantin 1976). Crude fiber decreased from 70.3% to 8.3% for CRS. This result here 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 was 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 oligasaccharides to their monomer components.

Table 6: Validation Of Developed Quadratic Model And Optimum Medium Constituents

Time (day)	Conc. (g/mol)	Crude protein			crude fiber		
		Predicted (%)	Experimental (%)	Error (%)	Predicted (%)	Experimental (%)	Error (%)
6	2g/mol	4.04	3.62	1.04%	31.24	30.50	9.98
10	2	6.19	5.77	6.78	20.27	18.85	3.03
8	4	006.86	7.21	4.85	19.52	20.50	3.43
8	6	8.61	8.83	2.49	9.22	14.70	0.00
10	6	10.10	9.42	6.73		8.30	2.37

$$\text{Error \%} = \frac{\text{predicted} - \text{experimental}}{\text{Predicted}} \times \frac{100}{1} \quad \ldots\ldots\ldots\ldots \quad 4$$

Five combinations of the two independent valuable were experimented and the observed results were compared with the predicted results. The error analysis was computed to determine the closeness between the predicted and the observed results.

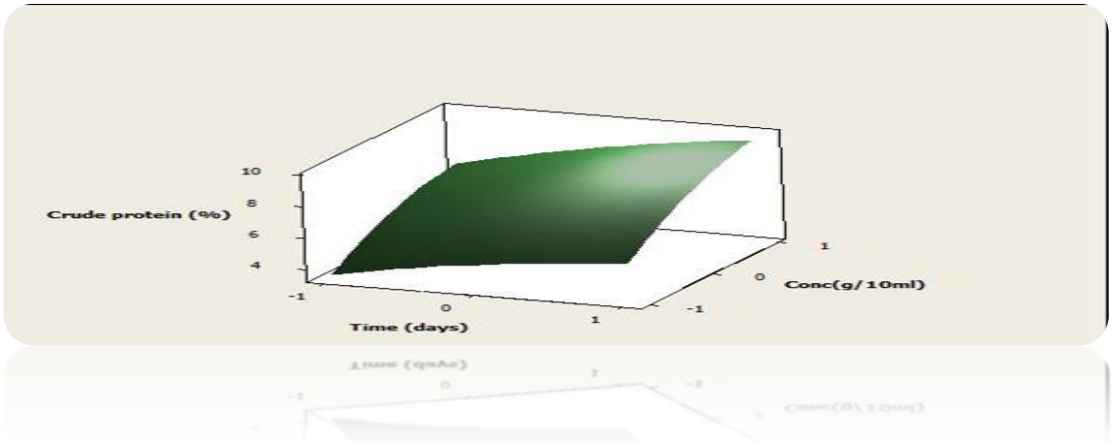

Fig. 1: RS plot of crude protein for cassava root sieviate

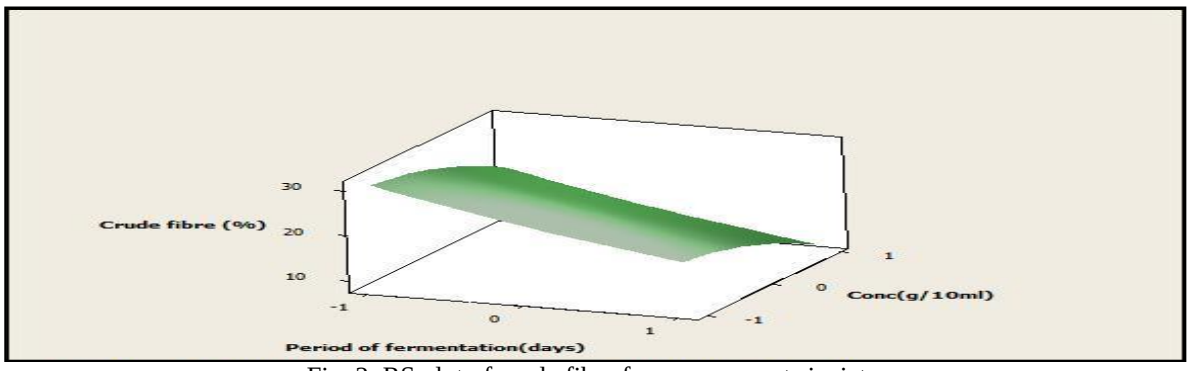

Fig. 2: RS plot of crude fiber for cassava root sieviate

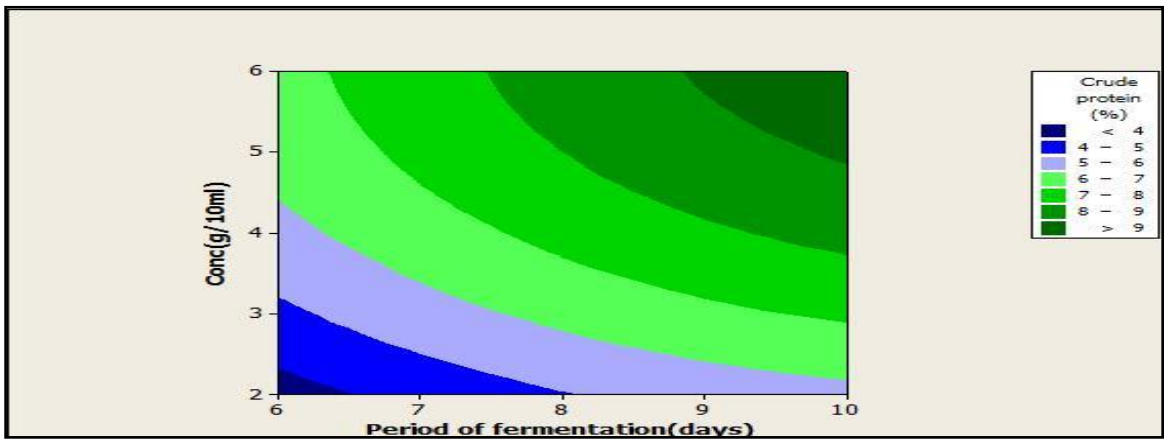

Fig. 3: Contour plot of crude protein for cassava root sieviate

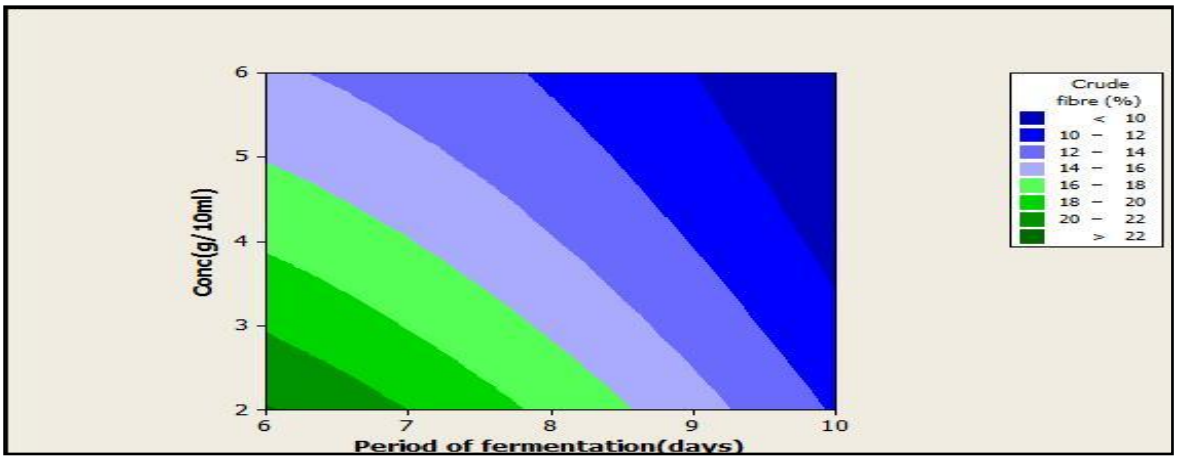

Fig. 4: Contour plot of crude fiber for cassava root sieviate

4.2 Response Surface Plot and Contour Plots

From response surface plot (Fig. 1 – 4), the crude protein content increases with increasing time and substrate concentration.

The corresponding contour plots show considerable curvatures for the figures, implying that the two interacting factors were interdependent. In other words, there were significant interactive effects on (CRS) between time and substrate concentration. It is pertinent to note that the values of output responses are tied to the intensity of the color of the plots. Hence, for the response surface plots and their corresponding contours, the best results are 6g/10ml concentration and 10 days.

4.3 Statistical Results

Biodegradation time and substrate concentration were validated by means of ANOVA to assess the goodness of fit. The test of hypothesis states that if P-value is less than 0.05, accept H_1, but if greater than 0.05, accept H_o(Myers and Montgomery,2002).

From the tables 3,4 and5 (CRS),,t P-value for substrate concentration, time and interaction effect was 0.00 which is less than 0.05 (0.00 < 0.05). It showed that time and substrate concentrate had effect on biodegraded CRS, and there was significant interaction. The fact that ANOVA report gave high R^2 value as 1.0, means that the correlation is perfect and model is adequate.

V. CONCLUSION

The optimum values of enriched cassava root sieviate with Aspergillus niger were found to be on 10 days and 6g/10ml conc., resulting in 9.42% enrichment in protein content and 8.30% crude fibre degradation in cassava root seriate. This suggest possible solution to utilization of cassava root seriate as animal feed and also solve environmental problem caused by their improper disposal.

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