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Amylase Production from Cassava Root Fibre using *Aspergillus Niger* CFL5

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ABSTRACT

In this study, the use of *Aspergillus niger* isolated from a spoilt bread for the production of amylase using cheaply available agro residual waste as substrate was investigated. *Aspergillus niger* was isolated from a spoilt bread and was eventually screened for amylase production. The best amylase producer was used as choice isolate for further studies and was identified using ITS-region sequencing. Cassava root fibre (CRF), an agricultural waste was used as carbon source for amylase production. Enzyme production was carried out under solid substrate fermentation (SSF). Effects of Particle sizes, Nitrogen sources, metal ions, concentration of metal ions, surfactants, pH were studied using the conventional one-variable-at-a-time (OVAT) and response surface (RSM) method. The results showed that the best amylase producer was *Aspergillus niger* CFL5. Smallest particle sizes of CRF of 0.025mm favored maximal enzyme production while 0.1 showed the lowest enzyme production. The best nitrogen source which favored high amylase production was fish meal while soy bean meal decelerated the rate at which amylase is produced. Zn^{2+} at 5.0 mM concentration was the best metal ion which favored the high rate of amylase production. Tween-80 enhanced the rate of the enzyme production unlike Tween-40. Amylase production was optimum at a pH of 5.0. Response surface methodology showed a strong statistical relationship amongst amylase production and particle size, pH and Fish meal as strong influencing factors, in 25 runs. This research thus showed that cassava root fibre is an industrially important agro-waste product that can be utilized for sustainable amylase production, instead of being discarded as a waste material. Likewise, RSM is a time conserving, reliable and reproducible method for the optimization of amylase production as compared to the conventional OVAT procedure.

Key words: Amylase, *Aspergillus*, Cassava,

Introduction

Sustainable waste management is one of the core areas of environmental pollution management. Agro-wastes are serious contributors to environmental pollution, ranging from their odour to other toxic chemical compounds and some green house gases which they introduce into the environment (Opetubo *et al*., 2022a). Cassava fermentation is a procedure employed for the detoxification of linamarin compounds contained in *Manihot esculenta* Crantz. During this fermentation process, agro-wastes are generated which comprise of the cassava peels, cassava sieviates (cassava root fibre) and the fermentation waste water comprising majorly of hydrogen cyanide. Opetubo *et al*. (2022b) reported that substances that liberate hydrogen into the environment end up contributing to water formation as a result of binding to atmospheric oxygen. Different researchers (Lakshmi *et al*., 2011; Sharanappa *et al*., 2011; Mukherjee *et al*., 2017; Sugihatro, 2019) have posited and also reported the waste management methods for cassava agro-wastes. Some of these methods include the conversion of the waste water as substrate for fungal biomass and enzyme production; composting of the cassava peels and sieviates to serve as organic manure in agriculture; as well as other bioremediation processes involving species of *Aspergillus*, *Bacillus*, *Alcaligenes* amongst others. The fungal biomass and enzyme production conducted with cassava fermentation effluent is usually through a sub-merged fermentation procedure. This present study however, evaluated the use of cassava sieviates also known as cassava root fibre as a substrate for the production of amylase by employing a solid substrate fermentation technique by a strain of *Aspergillus niger*.

Methods

Isolation of *Aspergillus* sp.

The method of isolation employed by Suganthi *et al.* (2011) and that of Oyeleke *et al.* (2010) were used in this research. Pieces of bread were kept in a moist condition at room temperature (28±5°C) in dark for 4 days. The mouldy bread samples were serially diluted (10-fold) and different dilutions were plated out on Sabouraud's Dextrose Agar (SDA). The Petri-dishes were incubated at room temperature for 5 days. Fungal cultures were observed on SDA medium. Five different fungal colonies were selected and sub- cultured on SDA slants. All the 5 fungal strains were subjected to lactophenol cotton blue staining so as to study its morphology and the spore color. The culture isolates were designated MOB 1, MOB 2, MOB 3, MOB 4 and MOB 5.

Screening of isolates for amylase production by starch hydrolysis test.

The five isolates were tested for amylase production by starch hydrolysis using the method employed by Suganthi (2011). A starch agar medium (Peptone – 0.5g, Beef Extract – 15g, yeast extract – 0.15g, NaCl – 0.5g, Starch – 1g, Agar – 2g, Distilled water – 100ml) was prepared, sterilized at 121°C, and dispensed into Petri dishes. On cooling, the plates were inoculated with the respective isolates and incubated at room temperature for five days. Thereafter, iodine solution (0.2% iodine and 0.4% Potassium iodide) was used to flood the plates. Presence of blue-black colour indicated negative result for α-amylase production while a clear zone of hydrolysis with yellowish colour around a colony indicated a positive α-amylase result. Isolate MOB3 with the highest zone of starch hydrolysis (2.6 cm) was selected for further studies.

Enzyme production.

The enzyme production was carried out by solid substrate fermentation. The basal salt medium components according to Oyeleke *et al.* (2010) and Suganthi *et al.* (2011) were $(NH_2)_2SO_4$ –2g/l;

$KH_2\ PO_4$ –1g/l; $MgSO_4.7H_2O$ – 0.5g/l.

Five grammes of the cassava root fibre (CRF) was weighed and hydrated with 5ml of the mineral salt medium and adjusted with moisture content of 65%. This was autoclaved at 121ºC for 20 min and allowed to cool. The organism was inoculated into this sterile medium using a plug of luxuriant culture of the test organism. The Erlenmeyer flask containing this medium was plugged and kept at room temperature for 6 days. The flasks were periodically mixed by gentle shaking.

Parameters for the optimization of amylase production using OVAT Model.

Several factors were considered for the optimum production of amylase using one variable-at-a-time (OVAT) procedure. The best factors which gave rise to higher yield of amylase were selected.

Effects of particle size.

CRF was sieved with standard sieves (Secor, India) of different pore sizes: 0.4mm, 0.8mm, 1.2mm, 1.6mm and 2mm labeled thus SA, SB, SC, SD and SE according to Sharanappa *et al.* (2011). The effects of particle size on the amylase production yield by *Aspergillus niger* was evaluated by weighing 10g of the different particle sizes (0.4mm – 2 mm) of the cassava root fibre and placing them in a 250ml Erlenmeyer flasks labeled SA1, SA2, SB1, SB2, SC1, SC2, SD1, SD2, SE1, SE2, SF1, SF2 from the measurements obtained from the sieves of different pore sizes. The CRF substrates were hydrated with 50 ml of basal medium and the flasks plugged with cotton wool, and thereafter sterilized at 121ºC for 15 mins. On cooling, the flasks were inoculated with 11mm agar plugs of luxuriant growth of *Aspergillus sp.* The flasks were then incubated at room temperature for 6 days. The flasks were periodically mixed by gentle shaking.

Effects nitrogen sources.

The effects of these organic nitrogen sources soy bean meal, bovine blood meal, fish meal, and groundnut cake on the production of amylase was assessed. The carbon to nitrogen ratio used was 2:1. The basal salt medium was supplemented with 10 g of the carbon source and contained in 250 ml Erlenmeyer flasks,, was separately added 5 g each of the nitrogen sources and sterilized in an autoclave at 121ºC for 15min. The organism was inoculated afterwards using the same method earlier described and kept at room temperature for 6 days.

Effects of metal ions on enzyme production.

The basal salt medium was supplemented with 10 g of the carbon source which was the cassava root fibre (CRF) and 5 g of the best supporting nitrogen sources for a high yield of amylase production. Five milli-Molar concentration of these metal ions: $CaCl_2.\ H_2O$, $CuSO_4.\ 5H_2O$, $ZnSO_4$, $MnSO_4$,$Pb(NO_3)_2$, $BaCl_2.\ H_2O$, $FeSO_4.\ 7H_2O$, were separately added to different flasks containing SSF medium and sterilized in an autoclave at 121ºC for 15mins. The organism was inoculated afterwards and fermentation carried out as described above. The control experiment was the basal medium without any of the listed metal salts.

Effect of concentrations of metal ions

The effect of different concentrations of the best metal ion which is the Zinc ion, on the production of amylase by *Aspergillus* was carried out. The different concentrations of $ZnSO_4$ used were 2.0mM, 5.0mM, and 7.0mM. The basal salt medium was supplemented with 10g of CRF and 5g of fish meal which was the best supporting nitrogen sources for a high yield of amylase production. The different concentrations of Zn^{2+} were separately added to different flasks containing SSF medium and sterilized in an autoclave at 121ºC for 15mins. The organism was inoculated afterwards and fermentation carried out at room temperature for 6 days.

Effects of surfactants

Different surfactants were analyzed for the best yield of amylase production. These surfactants were Tween 40, Tween 80, SDS, polyethylene glycol, stearic acid. 0.1% v/w of these surfactants were added to different flasks containing SSF medium and sterilized in an autoclave at 121ºC for 15 mins. The organism was inoculated afterwards and fermentation carried out at room temperature for 6 days.

Effects of pH

The effect of initial pH on enzyme yield by *Aspergillus* during solid state fermentation was studied by adjusting the pH of the mineral salts solution used to moisten the substrate to various pH levels. The pH values were 4.8; 5.1; 6.6; 7.1; 8.4; 9.5 and 11.3 using 1 molar sodium hydroxide (1M NaOH) and one molar hydrochloric acid (1M HCL). The different flasks containing the adjusted pH levels of the mineral salts medium for the SSF were sterilized at 121ºC for 15mins. The organism was inoculated afterwards and fermentation carried out at room temperature for 6 days.

Enzyme extraction.

A 22 ml portion of cold 0.1M phosphate buffered saline (pH 7) was added to each of the inoculated substrate flask and was vigorously shaken in rotary shaker for 15 minutes at 120rpm. The mixture was filtered through a Whattman No. 1 filter paper and centrifuged at 1000 rpm at 40°C for 15 min. The supernatant was used as the crude enzyme preparation (Oyeleke *et al.*, 2010). Enzyme amylase was assayed by Dinitrosalicylic acid method.

Enzyme assay

Amylase activity was assayed as described by Gupta *et al*. (2008). The reaction mixture consisted of 1 ml crude enzyme solution and 2 ml of 1% soluble starch in 2M sodium phosphate buffer of pH 6.8. The mixture was incubated for 30 min at 50°C. The liberated level of reducing sugars in the form of glucose equivalent was determined by adding 1 ml of dinitrosalicylic acid (DNS) method of Miller(1959).The blank contained 2 ml substrate and 1ml distilled water.

One unit of α-amylase activity was defined as the amount of enzyme that releases 1 µmol of reducing sugars per minute and is expressed as units per gram of dry substrate (U/g).

RSM Study

Response surface methodology was carried out using Box Behnken design in each of the conical flasks containing the three most influencing factors screened during OVAT optimization *viz*: carbon source x_1, Nitrogen source x_2 and appropriate pH x3. Their absorbance were read and subsequently converted to enzyme activity using the following procedure.

$Y = M_x + C$ where

Y = Absorbance

X = Enzyme Activity

M = 0.0068 (constant)

C = 0.08 (constant)

Regression $R^2 = 0.9781$

For the Absorbances gotten from the RSM, each of them was converted with the formula

$$X \text{ (Enzyme Activity)} = \frac{\text{Absorbance} - C \text{ (constant)}}{M \text{ (constant)}}$$

RESULTS

Fungal isolation and identification.

Fungal cultures were isolated from bread sample by serial dilution on Sabouraud's dextroxe agar medium. Five cultures of *Aspergillus* sp. were isolated from bread. They were named as MOB 1, MOB 2, MOB 3, MOB 4, and MOB 5.

Starch Hydrolysis Testing of *Aspergillus* Isolates

All the isolates tested for amylase production using the starch plate method showed zone of clearance around the microbial growth which indicated the production of amylase. The isolate with the highest value of width of the clear zone (zone of hydrolysis) formed around its colonies on starch agar medium was MOB 3, with a diameter zone of 2.6 cm (Table 1). MOB 3 was made the choice isolate for further studies.

Molecular Characterization of the Choice Isolate

The isolate MOB 3, was confirmed as *Aspergillus niger* strain CFL5 through molecular typing.

Amylase Production using the Choice Isolate

The choice isolate, *Aspergillus niger* used for the production of amylase gave a highest amylase activity of 222.001 u/g at 48 h of fermentation as shown in Figure 1.

Optimization of Cultural Conditions for Amylase Production using the OVAT Method.

Effects of particle sizes.

Effect of particle size on enzyme activity(amylase) was studied. The results of this analysis showed that particle size of 0.8mm – 1.2mm favored maximal enzyme production. However, further reduction in size, resulted in a decrease in the enzyme activity. Lowest enzyme activity of 33.971u/g was attained with the substrates containing particles of 0.4mm. This is as shown in Table 2.

Effects of Different Organic Nitrogenous Sources.

Soy bean meal, bovine blood meal, fish meal, and groundnut bean cake were used as nitrogen source alongside the solid substrate. Fish meal showed the highest yield of amylase of 136.912U/g followed by groundnut meal which was 133.676 u/g, as shown in Table 3.

Effects of metal ions.

These metal ions were used to supplement the basal salt medium to enhance the amylase production. They are $CaCl_2$. H_2O, $CuSO_4.5H_2O$, $ZnSO_4$, $MnSO_4$, $Pb(NO_3)_2$, $BaCl_2$. H_2O, $FeSO_4$. $7H_2O$. It was observed that the medium containing Zn^{2+} gave the highest amylase activity (252.941 u/g), followed by Cu^{2+} (252.206 u/g) while the least was Mn^{2+} (133.088 u/g) (Table 4).

Effects of concentrations of Best metal ion.

The effect of different concentrations of Zinc on amylase production was analyzed. The concentrations in millimeter utilized were 1.0 mM, 2.0 mM, 3.0 mM, 4.0 mM, and 5.0 mM. High concentrations of Zinc ion stimulated the growth of *Aspergillus niger* thereby increasing the rate of amylase yield under solid substrate fermentation (Table 5).

Effects of surfactants.

The effect of surfactants (Tween 40, Tween 80, SDS, Polyethylene glycol and Stearic Acid) on enzyme synthesis by fungi during SSFwas evaluated by incorporating 0.1% (w/v) level of surfactants in the medium. The results presented indicate that among the various surfactants tested, Tween-80 gave the highest amylase yield of 258.823 u/g, while the least was Tween-40 (252.941 u/g) as shown in Table 6.

Effects of pH

The pH of the mineral salt medium was adjusted to the pH values of 4.8; 5.1; 6.6; 7.1; 8.4; 9.5 and 11.3 using 1 molar sodium hydroxide (1M NaOH) and one molar hydrochloric acid (1M HCL).The results presented showed that maximumamylase of 222.059 u/g was produced at pH range of 5.1. as shown in Table 7.

Optimization of Amylase Production using the Response Surface Model.

The optimization of amylase production studied with the Box-Behnken design in 15 runs using the best three factors that influenced amylase production at the OVAT model (particle size of carbon source (1.2 mm), best nitrogen source (5 g of fish meal) and optimum pH 5.1), statistically gave optimum amylase value of 245.588 u/g at pH 4.5 at the 9^{th} run designated as C9 as shown in Table 8. Contour plots showing clear relationships existing amongst the best particle size of carbon source, best nitrogen source (5 g of fish meal) and optimum pH 5.1 at C9, are shown in Figures 2-4 with an R^2 value of 0.9781, which signifies a strong relationship between these three variables for amylase production. These values obtained from the statistical run at C9 were used for another set-up for amylase production, and it gave an optimum amylase activity of 259.117 u/g in 48 h as shown in Figure 5.

Table 1: Starch Hydrolysis Reactions for the Presumptive *Aspergillus* Isolates.

Isolates	Diameter Zone of Hydrolysis (cm)
MOB 1	0.8
MOB 2	1.6
MOB 3	2,6
MOB 4	2.0
MOB 5	1.7

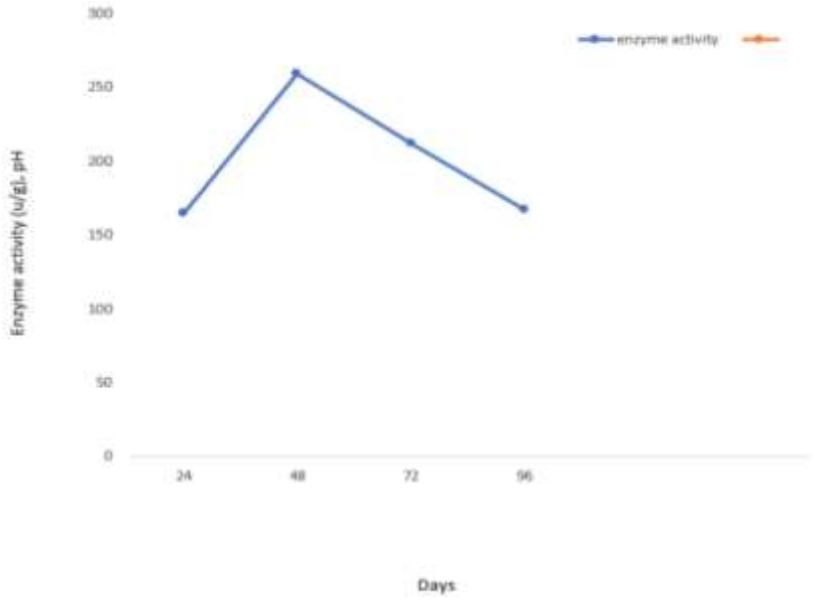


Figure 1: Amylase Production using Choice Isolate, Aspergillus niger strain CFL5

Table 2: Effects of particle size on amylase production.

Particle size (mm)	Absorbance	Enzyme activity (U/g)
0.4	0.311	33.971
0.6	0.407	48.088
0.8	0.511	63.382
1.2	0.623	79.853
1.6	0.413	48.971
2.0	0.391	45.735

Table 3: Effects of addition of organic Nitrogen sources on amylase production.

Nitrogen sources	Absorbance	Enzyme activity (U/g)
Blank	0.828	110.000
Soy bean meal	0.946	127.353
Cow blood meal	0.947	127.500
Groundnut meal	0.989	133.676
Fish meal	1.011	136.912

Table 4: Effects of Metal Ions on Amylase Production

Metal ions	Absorbance	Enzyme activity (U/g)
Blank	0.960	100.000
Mn^{2+}	0.985	133.088
Pb^{2+}	1.030	139.706
Ba^{2+}	1.315	181.618
Ca^{2+}	1.325	183.088
Fe^{2+}	1.350	186.765
Cu^{2+}	1.795	252.206
Zn^{2+}	1.800	252.941

Table 5: Effects of concentrations of Zn^{2+} ion on amylase production.

Concentration (mM)	Absorbance	Enzyme activity (U/g)
Blank	1.180	161.765
1.0	1.560	217.647
2.0	1.605	224.265
3.0	1.638	229.117
4.0	1.759	246.912
5.0	1.820	255.882

Table 6: Effects of surfactants on amylase production.

Surfactants (0.1% w/v)	Absorbance	Enzyme activity (U/g)
Tween 40	1.800	252.941
Polyethylene glycol	1.810	254.412
SDS	1.820	255.882
Stearic acid	1.830	257.353
Tween 80	1.840	258.823

Table 7: Effects of pH on amylase production.

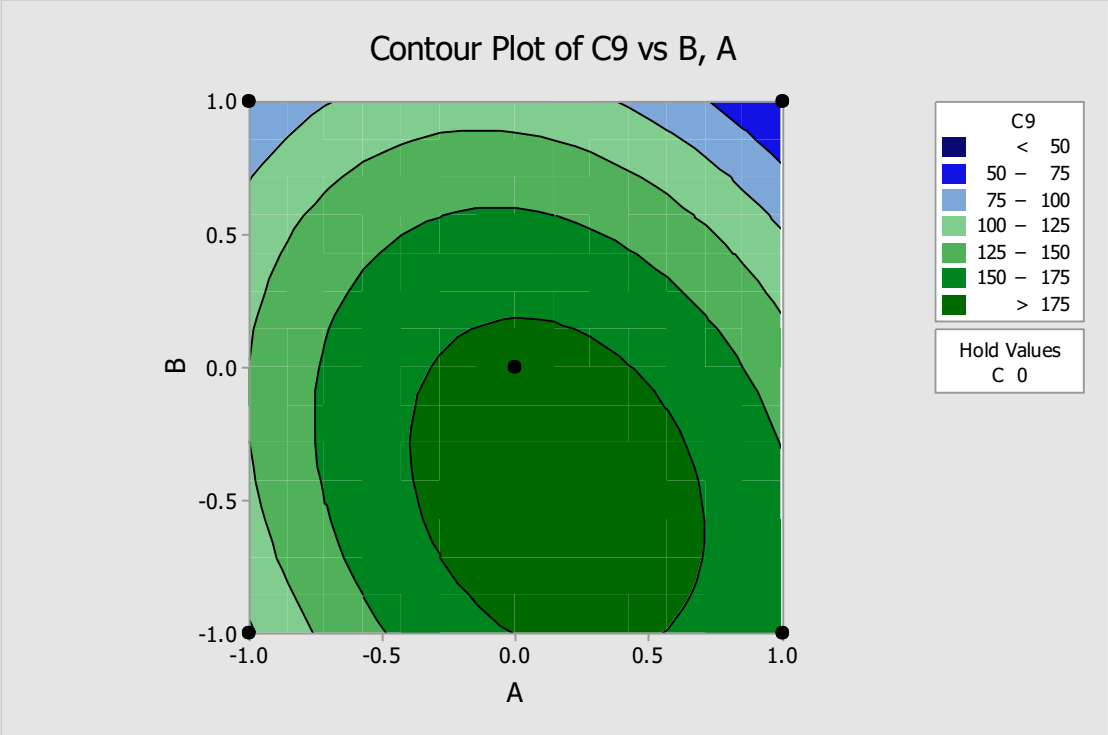
pH	Absorbance	Enzyme activity (U/g)
4.8	1.52	211.765
5.1	1.59	222.059
6.6	1.50	208.824
7.1	1.19	163.235
8.4	0.97	130.882
9.5	0.65	83.824
11.3	0.32	35.294

Table 8: Statistical Optimization of Amylase Production using Response surface methodology

CODED VALUES

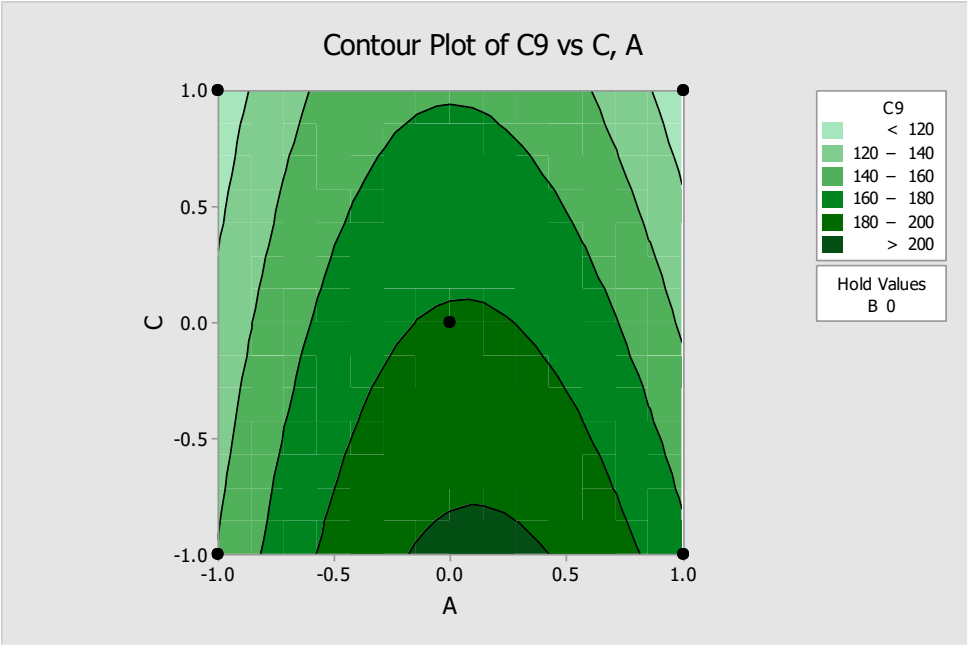
PARAMETER	- 1	0	+ 1
Carbon Source X_1	0.2	0.3	0.4
Nitrogen Source X_2	0.1	0.15	0.20
pH X_3	4.5	5.1	6

CODED VALUES				REAL VALUES			RESPONSE	
	X_1	X_2	X_3				ABSORBANCE	ENZYME ACTIVITY
1	-1	-1	0	0.2	0.1	5.1	0.467	56.912
2	-1	1	0	0.2	0.2	5.1	0.593	75.441
3	1	-1	0	0.4	0.1	5.1	1.131	154.559
4	1	1	0	0.4	0.2	5.1	0.672	87.059
5	-1	0	-1	0.2	0.15	4.5	0.932	125.294
6	-1	0	1	0.2	0.15	6	1.209	166.029
7	1	0	-1	0.4	0.15	4.5	0.809	107.206
8	1	0	1	0.4	0.15	6	0.920	123.529
9	0	-1	-1	0.3	0.1	4.5	1.750	245.588
10	0	-1	1	0.3	0.1	6	1.038	140.882
11	0	1	-1	0.3	0.2	4.5	1.171	160.441
12	0	1	1	0.3	0.2	6	0.263	26.912
13	0	0	0	0.3	0.15	5.1	1.602	223.824
14	0	0	0	0.3	0.15	5.1	1.625	227.206
15	0	0	0	0.3	0.15	5.1	1.729	95.441



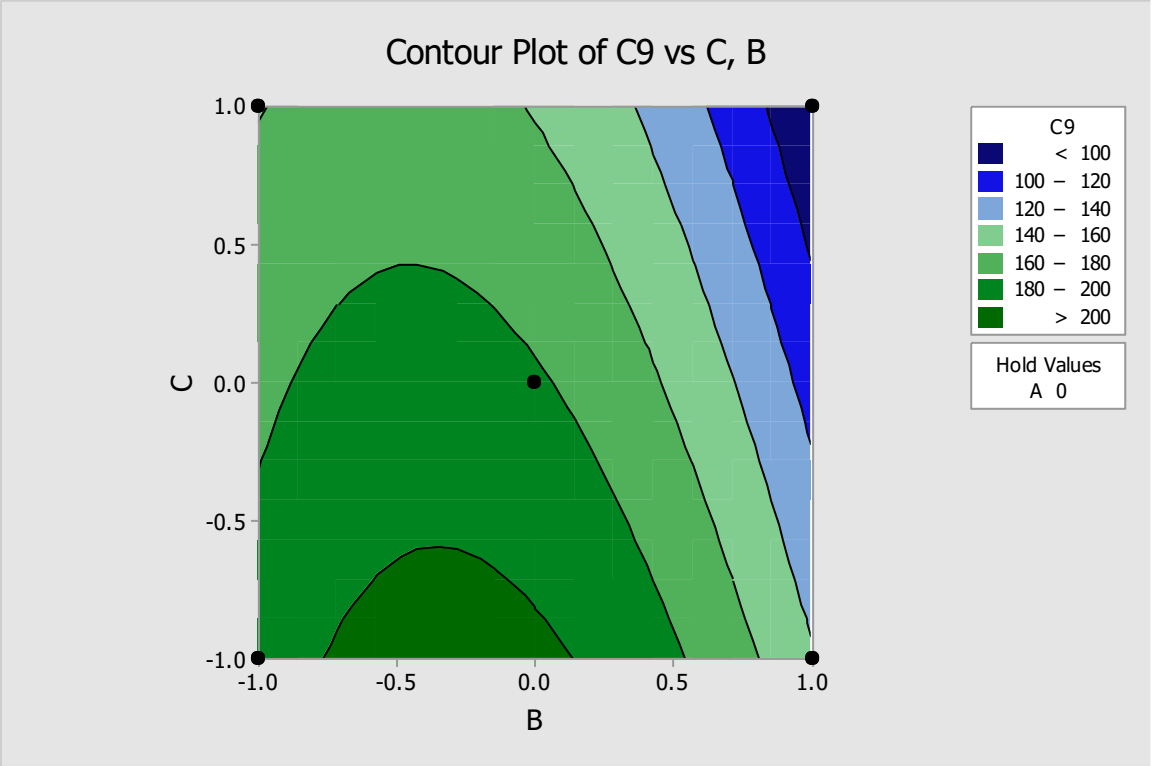
A: Particle Size of Carbon Substrate; B:Nitrogen source(Fish meal).

Figure 2: Contour Plot Showing Relationship amongst Code 9, Carbon and Nitrogen Sources.



A: Particle Size of Carbon Substrate; C: pH

Figure 3: Contour Plot showing Relationship amongst Code 9, Carbon source and pH.



B:Nitrogen source(Fish meal); C: pH

Figure 4: Contour Plot Showing Relationship amongst Code 9, Nitrogen Source and pH.

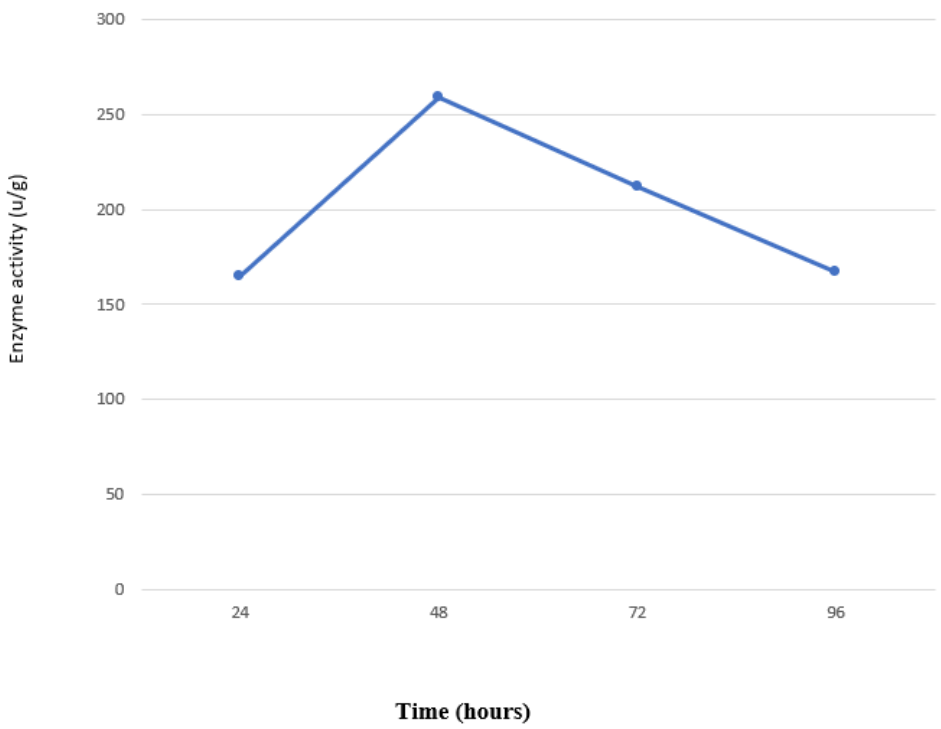


Figure 5: Amylase Production of A. niger strain CFL5 using Optimized Values from Response Surface Methodology (C9).

Discussion

The processes involved in gari processing from cassava are peeling and washing of the cassava roots, grating cassava roots into mash, de-watering and fermenting of the mash into wet cake, sieving wet cake into grits and roasting grits into gari, bagging and storing the gari and maintaining good hygiene compliance. The stage from which the substrate was collected was after sieving wet cakes into grits. Aspergillus cultures isolated from the substrate (cassava sieviates) have been reported by several authors (Adetunji et al., 2015; Abdulmajeed et al., 2016; Murkherjee et al., 2017; Sugiharto, 2019; Obong'O et al., 2020) to be indigenous to different cassava substrates. They also explained this phenomenon to be as a result of Aspergillus species to sporulate as well as their ability to produce amylase enzyme which helps them metabolize cassava substrates which are rich in carbohydrates. The choice isolate, Aspergillus niger CFL5 was the most amylase producing strain out of five assessed Aspergillus isolates. Some researchers (Murkherjee et al., 2017; Asrat and Girma, 2018; Obong'O et al., 2020) have reported different Aspergillus niger isolates producing good quantity amylase however, there has not been a specific record for strain CFL5 used in this study.

Crude amylase production by strain CFL5 was optimum at 48 h which agrees with works of Ubalua (2007) and Murkherjee et al. (2017) for other Aspergillus niger strains they examined; Murkherjee et al. (2017) examined Aspergillus niger RBP7. Optimization of culture conditions for the production of amylase by Aspergillus niger CFL5 was done using the one-variable-at- a-time (OVAT) method. OVAT method sought to give the optimal particle size, nitrogenous base, metal ion, surfactant and pH for amylase production using strain CFL5 in solid state fermentation (SSF). It was observed that fish meal, zinc ion, Tween-80, pH 5.1 and a particle size of 1.2 mm were best for optimal production of amylase.

Particle size of the substrate is a critical factor for enzyme production by SSF. For smaller particles, the surface area for growth is more, inter-particle space is less, while for larger particles the surface area for growth is less and the inter-particle space is more. This shows that the larger sizes of particles gave the organisms more space to easily break down the molecules of the substrate. Larger particles provide better respiration/aeration efficiency due to increase in inter-particle space. In contrast, a small substrate particle may result in substrate accumulation, which may interfere with microbial respiration/aeration and therefore result in poor growth and enzyme production. Aspergillus niger was able to breakdown starch easily due to the large size of the particles which provides a larger surface area for the organism to bind upon and act. This is in accordance with the research carried out by Sharanappa (2011). Optimal activity of 79.853U/g was seen in 1.2 mm particle size. Thus, the optimum particle size (1.2 mm) probably provides the most effective support material for attachment of the fungal strain (Lakshmi et al. 2011), or facilitates the mass transfer performance (gas and nutrient diffusion) greatly, resulting in a better respiration/aeration efficiency and an increased availability of nutrients (Balkan and Ertan 2007; Mazutti et al. 2007).These facts favor both the growth and enzyme production (Ertan et al., 2007).

Oshoma et al. (2009) and Kalairasi and Palvarhtam (2013) reported yeast extract as best nitrogenous substrate for Aspergillus sp. This work reflects that fish meal being an organic nitrogenous substrate stimulated optimum production of amylase, unlike most reported findings where inorganic nitrogen substrate (yeast extract) stimulates optimum amylase production over other organic substrates (fish meal, soy bean meal, groundnut cake and blood meal). In enzyme action, metallic cofactors are important because their presence or absence regulate its activity. The presence of specific metallic ions along with essential nutrient source can inhibit or enhance amylase activity. The effect of various metal ions stated above were evaluated and it was observed that the medium containing Zn2+and Cu2+ ions gave higher yield of the enzyme than others. These effects were in contrast to the research carried out by Sangeeta et al. (2009) who observed that confluent mycelial mat growth was seen in presence of Mg2+ and K+ but little or no growth with Cu2+ and Ca2+. Gupta et al. (2013) confirmed that tween 80 is most favorable for amylase production by A. niger under SSF and this corresponds with our own findings. The optimum pH of 5.1 observed in our study is in accordance with the studies carried out by Sindhu et al. (2009) which stated that amylase yield was significant over a range of pH 3 - 9, with optimum at pH 6.0.

The response surface method is a statistical estimation that provides reproducible and standardized optimum values for fermentation processes with minimal procedural stress as seen with the OVAT method. It actually reduces the number of trials involved in the OVAT method, while producing dependable results using interaction between independent variables. The variables chosen for the optimization of amylase production by strain CFL5 with the RSM are particle size, pH and Nitrogenous base (Fish meal) in a total of 15 runs (Table 8). The 9th run labeled as C9 gave the optimum amylase production of 245.588 u/g with a strong relationship existing amongst the variables and the enzyme as shown in the contour plots in Figures 2-4 with a regression coefficient (R2) value of 0.9781. The values obtained from C9 were used for the production of amylase whose optimum value of 259.117 u/g was obtained in 48 h (Figure 5). Murkherjee et al. (2017) reported an R2 value of 0.9873 for Aspergillus niger RBP7 for an RSM optimization for solid state fermentation using potato peels. They stated that amylase production by strain RBP7 had strong relationship (98.73%) with factors like pH, temperature, substrate concentration and inoculum concentration; while our own study of strain CFL5 also had strong relationship/dependence of 97.81% on the examined variables: particle size, fish meal and pH while other factors can occur due to chance.

Conclusion

The production of amylase through solid state fermentation by Aspergillus niger CFL5 using OVAT and RSM methods have shown that RSM has some extra advantages over the conventional OVAT method. These advantages center on the reduction of experimental time and number of trials required in using the OVAT procedure, while giving a reliable and reproducible result. This serves as a statistical approach to standardization of fermentation processes and is basically required in Nigerian fermentation processes which suffer standardization as a main constraint.

References

Abdulmajeed, B.O., Yusuf, B.A. and Abdullahi, I.O (2016). Efficiency of Aspergillus niger, Aspergillus flavus and Microsporum nanum to remove Heavy Metals from Refinery Effluent. Journal of Advances in Biology and Biotechnology6(3): 1-6.

Adetunji, A.R., Isadare, D.A., Akinluwade, K.J. and Adewoye, O.O. (2015). Waste-to-Wealth Applications of Cassava- A Review Study of Industrial and Agricultural Applications. Advances in Research4(4): 212-229.

Asrat, B. and Girma, A. (2018). Isolation, Production and Characterization of Amylase Enzyme

using the Isolate Aspergillus niger FAB-211. International Journal of Biotechnology and Molecular Biology Research, 9(2):7-14.

Ertan, F., Yagar, H. and Balkan, B. (2007). Optimization of α –amylase Immobilization in Calcium Alginate Beads. Preparative Biochemistry and Biotechnology, 37(3): 195-204.

Gupta, A., Gupta, V.K., Modi, D.R. and Yadva, L.P. (2008). Production and Characterization of α-amylase from Aspergillus niger. Biotechnology, 7(3):551-556.

Gupta. R., Gigras. P., Mohapatra. H., Goswami, V. K. and Chauhan, B (2013) Microbial α-Amylases: A Biotechnological Perspective. Process Biochemistry, 38: 15-16.

Kalaiarasi, K. and Palvartham, R. (2013). Optimization of Process Parameters for Amylase Production under solid-state Fermentation by Bacillus cereus MTCC 10202. African Journal of Microbiology Research7(45): 5166-5177.

Lakshmi, G.S., Paruchuru, L.B. and Shetty, P.R. (2011). Sustainable Bioprocess Evaluation for Xylanase Production by Isolated Aspergillus terreus and Aspergillus fumigatus Under Solid-State Fermentation using Oil Palm Empty Fruit Bunch Fiber. Current Trends in Biotechnology and Pharmacy, 5(4): 1434-1444.

Mazutti, M., Ceni, G., DILuccio, M. and Treichel, H. (2007). Production of Inulinase by Solid-State Fermetation: Effect of Process Parameters on Production and Preliminary Characterization of Enzyme Preparations. Bioprocess and Biosystems Engineering, 30:297-304.

Miller (1959). The use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Analytical Chemistry, 31:426-428.

Mukherjee, R., Paul, T., Soren, J.P., Halder, S.K., Mondal, K.C., Pati, B.R. and Das Mphapatra, P.K. (2017). Acidophilic α-Amylase Production from Aspergillus Niger RBP7 using Potato Peel as Substrate: A Waste to Value Added Approach. Waste Biomass Valor, DOI 10.1007/s12649-017-0114-8.

Obong'O, B.O., Ayodo, G., Kawaka, F. and Adalla,M.K. (2020). Fungi and Aflatoxin Levels in Traditionally Processed Cassava Products in Homa Bay County, Kenya. International Journal of Microbiology, https//doi.org/10.1155/2020/3406461.

Opetubo, O., Ibitoye, A. I., Oyinbo, S. T., & Jen, T. C. (2022b). Analysis of hydrogen embrittlement in palladium–copper alloys membrane from first principal method using density functional theory. Vacuum, 205(August). https://doi.org/10.1016/j.vacuum.2022.111439

Opetubo, O., Oyinbo, S. T., Oviroh, P. O., Ayotunde, I., & Jen, T. C. (2022a). Investigation of adsorption, dissociation, and Hydrogen diffusion through V-Ni-Zr Alloys Surface for Hydrogen purification: First principle method. ASME International Mechanical Engineering Congress and Exposition, Proceedings (IMECE), 6, 1–7. https://doi.org/10.1115/IMECE2022-96856

Oshoma, C.E., Imarhiagbe, E.E., Ikenebomeh, M.J. and Eigbaredon, H.E. (2009). Nitrogen Supplements Effect on Amylase Production by Aspergillus niger using Cassava Whey Medium. African Journal of Biotechnology 9 (5): 682-686.

Oyeleke, S.B., Egwim, E.c. and Auta, S.H. (2010). Screening of Aspergillus flavus and Aspergillus fumigatus strains for Extracellular Protease Enzyme Production. Journal of Microbiology and Antimicrobials, 2(6): 2141-2307.

Sangeeta, S., Gangadharan, D., Nampoothiri, K. and Soccol, C. (2009). Alpha-Amylase Production by Aspergillus oryzae Employing Solid-State Fermentation. Journal of Scientific and Industrial Research, 8:62-67.

Sharanappa, A., Wani, K.S. and Pllavi, P. (2011). Bioprocessing of Food Industrial Waste for α–amylase Production by Solid Fermentation. International Journal of Advanced Biotechnology Research, 2: 473-480.

Sindhu, R., Suprabha, G.N. and Shashidhar, S. (2009). Optimization of process parameters for the production of α-amylase from Penicillium janthinellum (NCIM 4960) under solid state fermentation. African Journal of Microbiology Research, 3(9): 498–503.

Suganthi, R., Benazir, J.F., Santhi, R., Ramesh Kumar, V., Hari, A., Meenakshi, N., Nidhiya, K.A., Kavitha, G., Lakshmi, R. (2011). Amylase production by Aspergillus niger under solid state fermentation using agroindustrial waste. IJEST. 3(2), 1756–1763 (2011)

Sugiharto, S. (2019). A Review on Fungal Fermented Cassava Pulp as a Cheap Alternative Feedstuff in Poultry ratio. Journal of World's Poultry Research, 9(1):01-06.

Ubalua, A.O. (2007). Cassava Waste:Treatment Options And Value Addition Alternatives. African Journal of Biotechnology, 6(18):65-73.