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# Production and Characterization of Glucoamylase by *Aspergillus niger*

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## Abstract

**Background and Objective:** Glucoamylase is a potent starch degrading enzyme whose cheap production has been an area of research. Its production by *Aspergillus niger* in solid-state fermentation was studied using dried garden pea peel as a substrate, which enormously reduced the production cost. The current study intended to produce glucoamylase by a cost-effective strategy and exhaustively characterize the enzyme.

**Material and Methods:** Garden pea peel was used as a substrate in solid state fermentation by *Aspergillus niger* for the production of glucoamylase under process parameters. Response surface methodology, a statistical tool for optimization, was applied to setup the experimental design for glucoamylase production. Characterization studies of the enzyme were carried out with temperature, pH, metal salts and elemental composition analysis.

**Results and Conclusion:** The process parameters were temperature, amount of substrate and time of fermentation. Glucoamylase production was highest in the pH range of 5.4-6.2, was stable at pH 3.8, and maintained its maximum activity even at 70°C for 30 min. It showed higher catalytic efficiency when incubated with metal ions $Fe^{2+}$, $Cu^{2+}$, $Mg^{2+}$, and $Pb^{2+}$. $K_m$ and $V_{max}$ for glucoamylase were 0.387 mg of soluble starch $ml^{-1}$ and 35.03 U $\mu l^{-1}$ $min^{-1}$, respectively. Glycogen was also used as a substrate, which gave an increased $K_m$ by 2.585, whose $K_I$ was found to be 0.631. Energy-dispersive X-ray spectroscopy was performed for obtaining composition of the pea peel. C, N, and O were found to be 12.53%, 29.9%, and 55.27% by atomic weights, respectively. Cost- and time-effective production of glucoamylase was achieved by utilizing dried garden pea peel (a vegetable residue) powder as the substrate for production. Its high stability ensures efficient utilization under industrial conditions. This work provides a very good platform for the enzyme immobilization studies and scale up production in future.

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## 1. Introduction

Amylases present a great importance in processing of fermented beverages, food, textile, and paper industries. Despite being able to be extracted from diverse sources, including plants, animals and microorganisms, microbial enzymes, generally, find great industrial demand. Currently, great amounts of microbial amylases are available commercially, and their most important application is in starch hydrolysis in starch processing industries [1,2]. Amylases, which act on starch, glycogen and polysaccharides cleaving glycosidic α-1,4 bonds, may be produced by microorganisms with wide application in different industries [3]. The preferred amylolytic enzymes producing microorganisms are filamentous fungi, preferentially by *Aspergillus* (*A.*) *niger, A. oryzae, A. awamori, Fusarum oxysporum,* and *Trichoderma viride.* The fungal species that are used in the production of glucoamylase (GA) are *A. niger, A. fumigatus, A. saitri, A. terreus, A. foetidus* and *Rhizopus foetidus*. GA or Amyloglucosidase is the key enzyme used in food processing industries as well as in commercial purposes for production of glucose from starch [4-6]. GAs (1, 4- α-D-glucanglucohydrolase, EC 3.2.1.3) perform catalysis to release L-D-glucose from the non-reducing ends of starch and related poly- and oligo-saccharides [2,3,7-9,11-15]. GAs are found in two forms of iso-enzymes, which contribute to different specificities [10,16-20]. Fungal GA is widely used in the production of glucose and fructose syrups [2]. Traditionally, GA has been produced by submerged fermentation. In recent years, however, it has been increasingly applied by the process of Solid Sate Fermentation (SSF) [4]. Compared to submerged fermentation, SSF is simpler with lower energy requirements, superior productivity, cost-effective fermentation media, and without rigorous fermentation control parameters. Also

it uses less water, and its control for bacterial contamination is much easier [2,5]. Besides amylose and amylopectin fractions of starch, other molecules as maltose, dextrins and glycogen are hydrolyzed by the enzyme, which also acts on the α-1,3 bond. GA is an induction enzyme, which is produced in high amounts in the presence of maltose or starch in the media. However, as the enzyme shows a non-typical induction, the microorganism synthesizes it, even in the presence of glucose as carbon source [10,11-18].

The GA produced from wheat bran, paddy husk, rice processing waste and other starch containing wastes is also being used as a substrate [10]. Bioethanol production by GA producing fungi has earlier been studied [10]. The GA production under SSF using low-cost derivatives of agricultural residues as substrate has also been reported [10].

In the current study, optimization of physical parameters was done by a statistical tool (response surface methodology) utilizing agricultural waste of pea peel, followed by characterization of the crude enzyme and component analysis of the substrate by Energy-dispersive X-ray spectroscopy (EDX).

## 2. Materials and Methods

### 2.1. Microorganism & inoculum preparation

*A. niger* strain, isolated from soil (data not shown), was used to carry out the fermentation. The fungal culture was grown on potato dextrose agar (PDA) for 5 days before storage, and was maintained on PDA at 4°C with weekly sub-culturing.

### 2.2. Agro waste as substrate for production medium

In the present study, the substrate chosen is garden pea peel. In each 100 mL Erlenmeyer flask, desired amount of pea peel was taken (obtained from local market in Kolkata, India). It was mixed properly with required volume of distilled water.

### 2.3. Solid state fermentation

The fermentation medium was autoclaved at 121°C and 103.421 kPa. The sterile media was then inoculated with spore suspension and incubated at desired temperature for desired period according to the experimental method. Spore suspensions were duly prepared with 5 mL of autoclaved distilled water with one loop full of *A. niger* culture. This was then properly mixed with the fermentation medium.

### 2.4. Substrate composition analysis

Scanning electron microscope (SEM) attached with energy-dispersive X-ray spectroscopy (SEM-EDX) (JEOL, JSM 6360, Tokyo, Japan) was used at an accelerating voltage of 20 kV to view the substrate's elemental composition. The samples were mounted on brass stubs and then coated with a thin layer of palladium (approx. 8 nm thick) in an auto fine coater (JEOL, JFC 1600) before observation. EDX was done to analyze the chemical components in dried powdered garden pea peel particle under SEM-EDX.

### 2.5. Enzyme extraction

After desired period of incubation, the erlenmeyer flasks containing the fermented media along with the culture were subjected to soaking in distilled water in 1:6 (w $v^{-1}$) ratio (fermented media:distilled water). Then they were allowed to stay for 24 h at 4°C for effective diffusion of enzyme in the solution. The soaked solution was then filtered using Whatman filter paper no. 1 and subjected to centrifugation (Remi Sartorius Centrifuge, India) at 2500 ×g for 10 min. The supernatant, which was the crude enzyme, was then subjected for further assay.

### 2.6. Enzyme assay

All experiments were done according to the design matrix of response surface method (RSM) in 100 mL erlenmeyer flasks containing pea peel in required amounts as a solid substrate. The reaction mixture consisted of 2.5 ml of 10 g $l^{-1}$ soluble starch, 1 ml of 10 mM Mcllvaine buffer (pH = 3.8), and 0.5 ml of enzyme extract. After 10 min of incubation at 50°C, the liberated reducing sugars (glucose equivalents) were estimated spectrophotometrically by dinitrosalicylic acid (DNS) method of Miller [21]. The color developed was read at 540 nm using a spectrophotometer (Hitachi U-2000, Tokyo, Japan). Glucose (Merck Millipore, USA) was used as the standard. One unit of glucoamylase is defined as the amount of enzyme releasing 1 μmol of glucose equivalent/min under the assay conditions.

### 2.7. Optimization of environmental parameters by RSM

The levels of the significant parameters and the interaction effects between various medium constituents, which may influence the GA production significantly were analyzed and optimized by central composite design (CCD). The process parameters along with their ranges were selected from previous data obtained by one variable at a time. In the present study, the effects of incubation time (days), incubation temperature (°C), and substrate amount (g) on GA activity were optimized keeping the initial moisture, pH, and inoculum size constant. Each factor in the design was studied at 5 different levels (−α, −1, 0, +1, +α) as shown in Table 1. Enzymatic activity was measured in duplicate in 20 different experimental runs. The GA production was analyzed by using a second order polynomial equation, and the data were fitted into the equation by multiple regression procedure [1]. Later, an experiment was run using the optimum values for variables given by response optimization to confirm the predicted value, and amylase production was confirmed.

The GA activity was taken as the dependent variable or response, *y*. Empirical fitting of the experimental data was

by polynomial regression based on analysis of variance (ANOVA).

In order to fit an empirical second-order polynomial model, a central composite design with five coded levels was performed. The quadratic model for predicting the optimal point was expressed according to Eq. 1 [1]:

$$y = \beta_0 + \sum_{i=1}^{n} \beta x_i + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \sum_{i=1}^{n-1} \sum_{i=1}^{n} \beta_{ij} x_i x_j \qquad \text{Eq. 1}$$

Where, $y$ is the response variable, β is the regression coefficients of the model, and $x$ *is* the coded levels of the independent variable.

The response surface modeling helps to investigate the response over the entire variables' space and to identify the region where it reaches its optimum value. A response surface plot can provide information about the combination of process variables, which gives the best response.

### 2.8. Statistical analysis

The statistical significance of the second-order model equation was determined by F-test, and the proportion of variance explained by the model obtained was given by the multiple coefficient of determination, $R^2$. The optimization process searches for a combination of factor levels that simultaneously satisfy the requirements placed (i.e., optimization criteria) on each of the responses and process factors. The response was experimentally tested for the confidence limits between 95% and 99%. All the statistical analyses taking in account from the design of experiments to the optimization were performed using the statistical software 'Design Expert' (version 7.0.0; Stat-Ease, Inc. Minneapolis, MN USA).

### 2.9. Characterization of Glucoamylase

#### 2.9.1. Optimal pH for enzyme activity

The substrate (starch) was prepared at different pH values ranging from 3.0-8.0 (3.0, 3.8, 4.6, 5.4, 6.2, 7.0, and 7.8). Then it was assayed by DNS method [21].

#### 2.9.2. Optimal pH for enzyme stability

The pure enzyme samples were incubated 1:2 (v $v^{-1}$) in different pH ranges (3.0, 3.8, 4.6, 5.4, 6.2, 7.0, and 7.8) at 4°C for 24 hrs. Then the samples were thawed and then assayed by DNS method [21].

#### 2.9.3. Optimal temperature for enzyme activity

The starch (substrate) was prepared in tubes; the tubes were incubated in different temperatures (30, 40, 50, 60, and 70°C) for 30 min followed by the addition of enzyme and assaying by the DNS method [21].

#### 2.9.4. Optimal temperature for enzyme stability

The pure enzyme was incubated in tubes in different temperatures (30, 40, 50, 60, and 70°C) for different times (10, 20, 30, 40, 50, and 60 min). Then the tubes were transferred freezing bath; next, the starch (substrate) (Merck Millipore, USA) was added. The tubes were incubated in water bath for 10 min. The activity of enzyme was estimated and the relation was drawn between remaining activity and different temperatures [4].

**Table 1.** Experimental data for a three factor five level response surface analyses

| Standard Order | Run | Block | Factor 1 A-Time (days) | Factor 2 B-Temperature (°C) | Factor 3 C- Substrate amount (g) | Response 1 Activity (U $gds^{-1}$) | Predicted value for Activity (U $gds^{-1}$) |
|---|---|---|---|---|---|---|---|
| 2 | 1 | Block 1 | 9 (+1) | 30 (-1) | 3 (-1) | 68.7 | 68.16 |
| 20 | 2 | Block 1 | 7 (0) | 37 (0) | 5 (0) | 65.62 | 65.59 |
| 19 | 3 | Block 1 | 7 (0) | 37 (0) | 5 (0) | 65.62 | 65.59 |
| 3 | 4 | Block 1 | 5 (-1) | 44 (+1) | 3 (-1) | 75.4 | 73.61 |
| 15 | 5 | Block 1 | 7 (0) | 37 (0) | 5 (0) | 65.62 | 65.59 |
| 8 | 6 | Block 1 | 9 (+1) | 44 (+1) | 7 (+1) | 68.042 | 65.88 |
| 17 | 7 | Block 1 | 7 (0) | 37 (0) | 5 (0) | 65.62 | 65.59 |
| 14 | 8 | Block 1 | 7 (0) | 37 (0) | 8.36 (+α) | 66.99 | 67.71 |
| 1 | 9 | Block 1 | 5 (-1) | 30 (-1) | 3 (-1) | 88.8 | 90.17 |
| 4 | 10 | Block 1 | 9 (+1) | 44 (+1) | 3 (-1) | 81.9 | 81.55 |
| 13 | 11 | Block 1 | 7 (0) | 37 (0) | 1.64 (-α) | 96.683 | 97.08 |
| 7 | 12 | Block 1 | 5 (-1) | 44 (+1) | 7 (+1) | 50.57 | 50.32 |
| 9 | 13 | Block 1 | 3.64 (-α) | 37 (0) | 5 (0) | 59.26 | 59.54 |
| 12 | 14 | Block 1 | 7 (0) | 48.77 (+α) | 5 (0) | 63.64 | 65.97 |
| 18 | 15 | Block 1 | 7 (0) | 37 (0) | 5 (0) | 65.62 | 65.59 |
| 10 | 16 | Block 1 | 10.36 (+α) | 37 (0) | 5 (0) | 53.28 | 54.12 |
| 16 | 17 | Block 1 | 7 (0) | 37 (0) | 5 (0) | 65.62 | 65.59 |
| 11 | 18 | Block 1 | 7 (0) | 25.23 (-α) | 5 (0) | 73.26 | 72.05 |
| 6 | 19 | Block 1 | 9 (+1) | 30 (-1) | 7 (+1) | 55.53 | 56.53 |
| 5 | 20 | Block 1 | 5 (-1) | 30 (-1) | 7 (+1) | 71.36 | 70.92 |

### 2.9.5. Effect of metal ions on Glucoamylase activity

The effect of metal ions on GA activity was carried out at 50°C for 30 min by addition of salt of different metal ions (KCl, $FeCl_3$, NaCl, $CaCl_2$, $BaCl_2$, $HgCl_2$, $MgSO_4$, $FeSO_4$, $ZnSO_4$, $MnSO_4$, $CuSO_4$, and $PbSO_4$) [all from Merck Millipore, USA] of final concentration of 1 mM to the GA reaction mixture vis. 10 mM Mcllvaine buffer, pH 3.8 containing 10 g $l^{-1}$ starch along with control, i.e., GA reaction without metal ions [5].

### 2.10. Determination of the kinetic parameters

In order to determine which substrate was hydrolyzed most efficiently, the apparent kinetic parameters $K_m$ of the crude enzyme were determined using starch and glycogen. The reactions were carried out at 50°C, and the apparent $K_m$ values were calculated from Hanes plots using Eq. 2 (5):

$$V = \frac{V_{max}\,[S]}{K_M + [S]} \qquad \text{Eq. 2}$$

Soluble starch and glycogen were prepared in different concentrations: 1, 3, 5, 7, and 9 mg $ml^{-1}$, in 10 mM Mcllvaine buffer of pH 3.8 (Table 2). The assay was equally carried out [21]. The Michaelis-Menten constant ($K_m$) and the maximum velocity ($V_{max}$) of GA were obtained.

**Table 2.** Calculated values of kinetics analysis parameters

| $1/_{[s]}$($mg^{-1}mL$) | $1/_v$(Glycogen)[$\mu M^{-1}min$] | $1/_v$(Starch)[$\mu M^{-1}min$] |
|---|---|---|
| 1.000 | 39.38 | 35.85 |
| 0.330 | 32.84 | 31.70 |
| 0.200 | 31.36 | 30.76 |
| 0.143 | 30.07 | 29.29 |
| 0.111 | 28.80 | 28.65 |

## 3. Results and Discussion

### 3.1. Optimization of Glucoamylase production

The optimum level of the key factors and the effect of their interactions on GA production were explored by the central composite design of RSM. Experimental design and results are shown in Table 1. By applying multiple regression analysis on the experimental data, the following second-order polynomial equation was established (Eq. 3) to explain the GA production:

$$Y=65.59 - 1.61A - 1.81B - 8.73C + 7.49AB + 1.91AC - 1.01BC - 3.1A^2 + 1.21B^2 + 5.9C^2 \qquad \text{Eq. 3}$$

Where, Y is the predicted yield of GA, and A, B, and C are the coded values of incubation time, incubation temperature, and amount of substrate, respectively. ANOVA was conducted to test the significance of the fit of the second-order polynomial equation for the experimental data as shown in Table 2. The F-value of 130.32 implies that the model is significant. p-values ≤ 0.05 indicate that the model terms are significant. In this case, A, B, C, AB, AC, $A^2$, $B^2$, and $C^2$ are significant model terms. Parameters in ANOVA having p> 0.10 indicate that the model terms are not significant. These non-significant model terms help in choosing the terms, which have minimal statistical and practical contribution towards the design of experiments. The predicted $R^2$ was found to be 0.9321, which is in reasonable agreement with the adjusted $R^2$ of 0.9839. This further speaks for the experimental model to be efficient for the present optimization study. No abnormalities were observed in the analyses that were performed on the residuals. Thus, it can be concluded that the model was statistically sound. The response surfaces (Figs. 1, 2, and 3) indicate the nature and extent of the interaction between different factors.

Previous studies also reported that elliptical contours are obtained when there is a perfect interaction between the independent variables [7]. As analysis from the present study shows, the model predicted maximum GA production of 90.1728 U $gds^{-1}$.

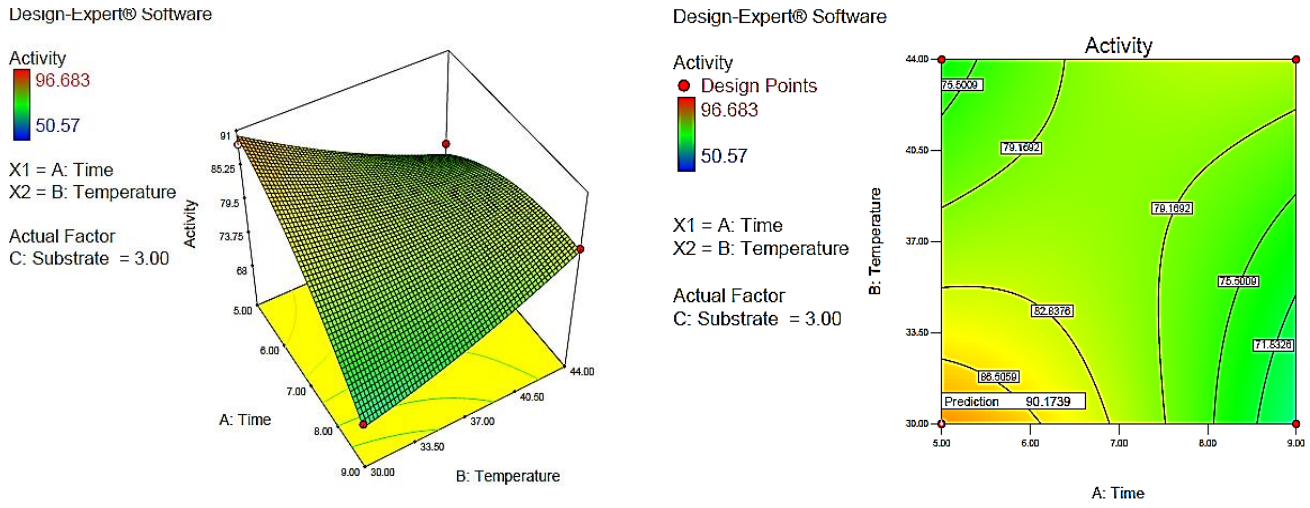



**Fig 1.** 3D and contour response surface plot showing the effect of incubation time and incubation temperature on glucoamylase activity

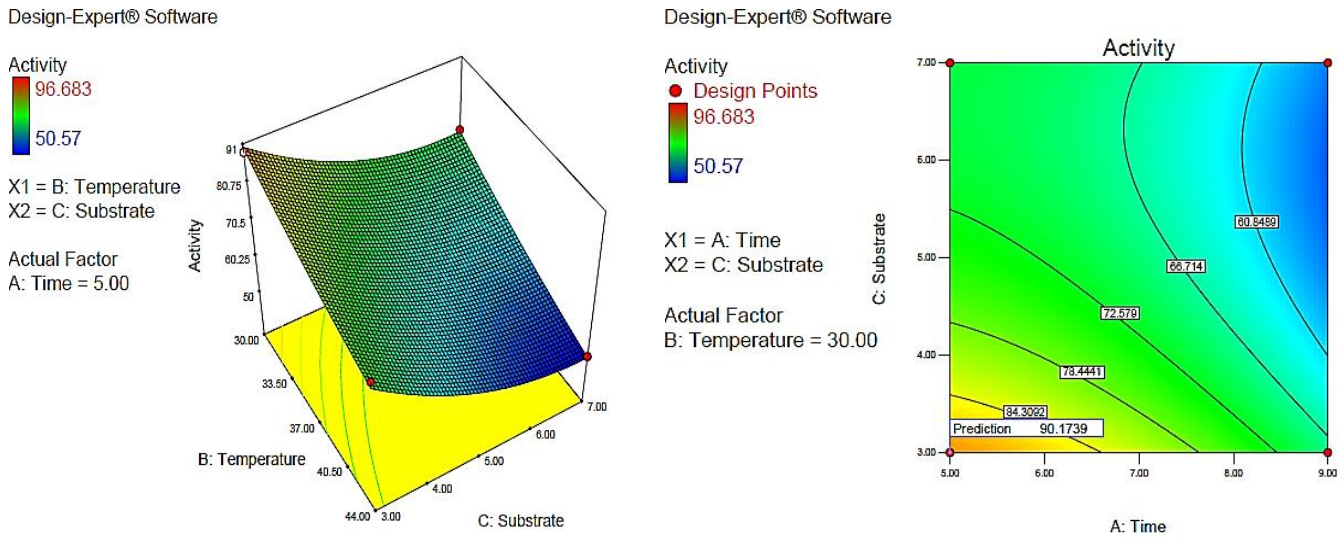



**Fig 2.** 3D and contour response surface plot showing the effect of incubation time and substrate amount on glucoamylase activity

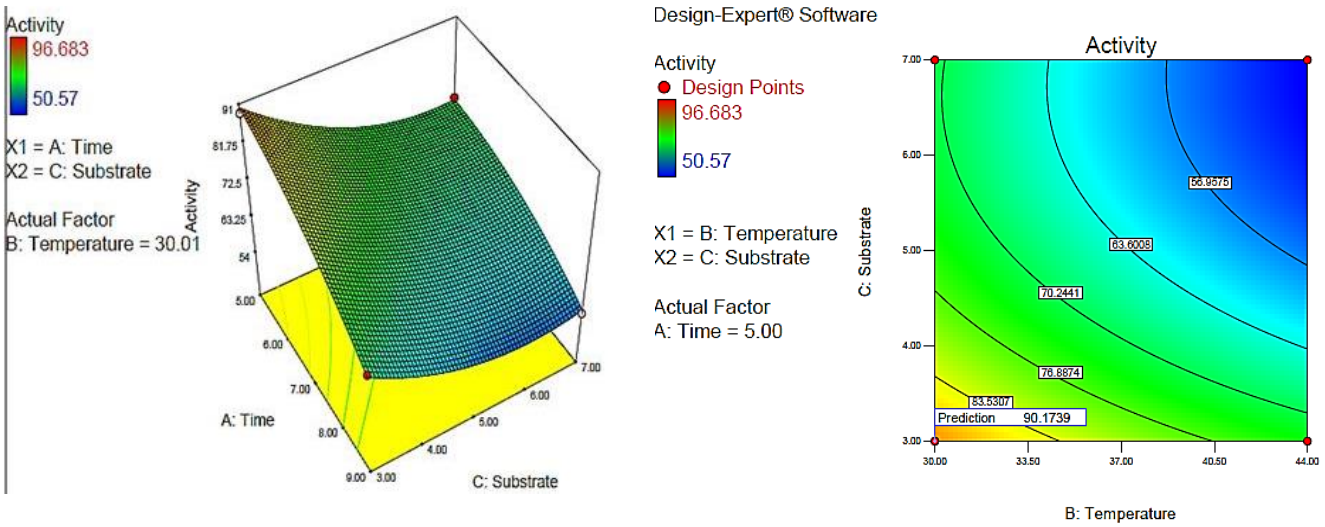



**Fig 3.** 3D and contour response surface plot showing the effect of incubation temperature and substrate amount on glucoamylase activity.

**Table 3.** ANOVA for the response surface of a quadratic model*

| Response 1 | | | Activity | | | |
|---|---|---|---|---|---|---|
| Source | Sum of squares | df | Mean squares | F value | P-value Probe >f | |
| Model | 2326.78 | 9 | 258.53 | 130.32 | ≤0.0001 | significant |
| A-time | 35.49 | 1 | 35.49 | 17.89 | 0.0017 | |
| B-temperature | 44.52 | 1 | 44.52 | 22.44 | 0.0008 | |
| C-substrate | 1041.02 | 1 | 1041.02 | 524.76 | ≤0.0001 | |
| AB | 448.53 | 1 | 448.53 | 226.10 | ≤0.0001 | |
| AC | 29.04 | 1 | 29.04 | 14.64 | 0.0033 | |
| BC | 8.16 | 1 | 8.16 | 4.11 | 0.0701 | |
| $A^2$ | 138.22 | 1 | 138.22 | 69.67 | ≤0.0001 | |
| $B^2$ | 21.08 | 1 | 21.08 | 10.62 | 0.0086 | |
| $C^2$ | 508.86 | 1 | 508.86 | 256.61 | ≤0.0001 | |
| Residual | 19.84 | 10 | 1.98 | | | |
| Lack of fit | 19.84 | 5 | 3.97 | | 0.0191 | significant |
| Pure Error | 0.00 | 5 | 0.000 | | | |
| Core Total | 2346.62 | 19 | | | | |

*Model p-value suggests that the experimental design is significant for the chosen design of experiment. The large F-values suggests that the experimental setups were quite significant. The multiple binary terms were significant which confirmed stable interaction between the process parameters. Lack of fit was shown to be non-significant which assured the model to be fit again.

### 3.2. Reconfirmation experiments for RSM validation

On the basis of medium optimization, the quadratic model anticipated that the maximum production of GA was 90.1728 U $gds^{-1}$ when the incubation time, incubation temperature, and substrate amount were 5 days, 30°C, and 3g, respectively. To verify the predicted results, validation experiment was performed in duplicate tests. Under the optimized conditions, the observed experimental titer of GA was 90.102 U $gds^{-1}$, suggesting that the experimental and predicted values of GA yield were in good agreement. This result, therefore, substantiates the predicted values and the effectiveness of the model, indicating that the optimized medium favors the production of GA through solid state fermentation (Table 4).

**Table 4.** Point prediction for the validation of the RSM

| Factor | Name | Level | Low Level | High Level | Std. Dev. | Coding | |
|---|---|---|---|---|---|---|---|
| A | time | 5 | 5 | 9 | 0 | actual | |
| B | temperature | 30 | 30 | 44 | 0 | actual | |
| C | substrate | 3 | 3 | 7 | 0 | actual | |
| response | prediction | SE Mean | 95% CI low | 95% CI high | SE Pred | 95% PI low | 95% PI high |
| activity | 90.1728 | 1.15 | 87.6 | 92.74 | 1.82 | 86.12 | 94.23 |

*(CI= Confidence Interval; SE= Standard Error; PI= Prediction interval)[1]

The optimum level given by the statistical tool when the design of experiments were carried out between the low and high ranges. It points to the predicted highest value of GA's activity which lies in the range of 95% to 99% confidence limit. This also allows for validation studies.

### 3.3. Characterization of crude enzyme

The optimum pH for the highest activity of GA is in the range of 5.4-6.2, whereas the optimum pH for the enzyme stability is 3.8; this means that the highest activity can be measured in the pH range of 5.4-6.2 but it is short lived, while at pH 3.8, the enzyme's activity is at highest and it does not get destroyed due to the pH (Fig 4).

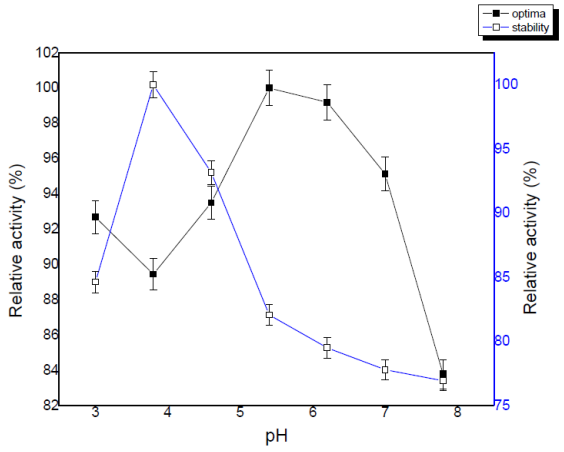



**Fig 4.** Effect of pH on optima and stability of Glucoamylase

In this study, the optimum temperature of GAs was 50°C. This also accounts for the fact that the enzyme is assayed at 50°C for the highest activity measurements (Fig 5). The thermostability of GAs was measured. It shows that the enzyme retains almost 90% of its activity at 60°C. This indicates that the enzyme can retain its activity even at 60°C for a period of 40 min (Fig 6). The GA activity was enhanced by the metal salts, which are of the common sulphate anionic group. Although $Zn^{2+}$ and $Mn^{2+}$ have shown inhibition but $Fe^{2+}$, $Cu^{2+}$, $Mg^{2+}$, and $Pb^{2+}$ had high activation rates. All the chlorine anion group metal salts had a detrimental effect on the GA activity (Fig. 7).

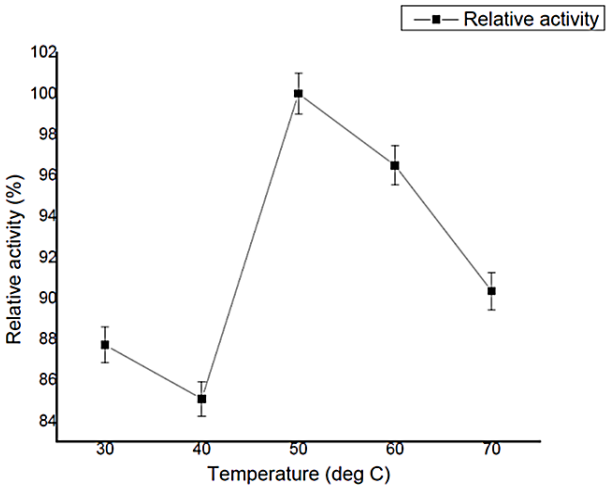



**Fig 5.** Effect of temperature on optimum of Glucoamylase

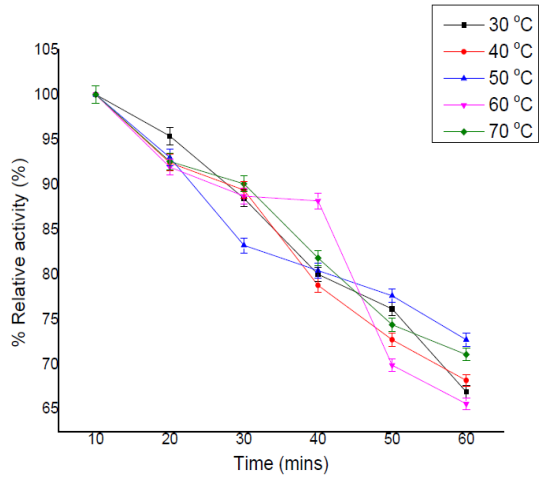



**Fig 6.** Effect of temperature on stability of Glucoamylase

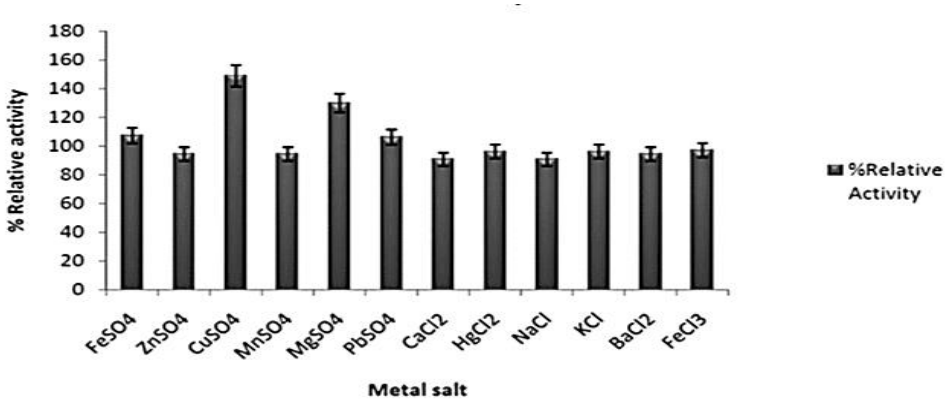



**Fig 7.** Effect of metal ions on Glucoamylase activity

The kinetic parameters and substrate specificity studies shown by Lineweaver-Burke Plot concludes that glycogen is an effective competitive inhibitor of soluble starch; where, $K_m$ and $V_{max}$ for GA were found to be 0.387 mg of soluble starch $ml^{-1}$ and 35.03 U $\mu l^{-1}$ $min^{-1}$, respectively at 50°C and pH 3.8 (Fig 8). The $K_m$ recently reported for *Fusarium solani* GA at 55 °C was 1.6 mg $ml^{-1}$ (20). The $K_m$ of GA produced by *A. niger* was about 4.13 times higher than that of *F. solani*, showing higher affinity of the enzyme towards starch hydrolysis. The agricultural substrate pea peel's component analysis done by EDX gave the idea that it has highly suitable fungal growth as it has 9.81% carbon, 27.32% nitrogen, 57.67% oxygen by weight and abundance of other minerals that supports for the microorganism growth (Fig. 9).

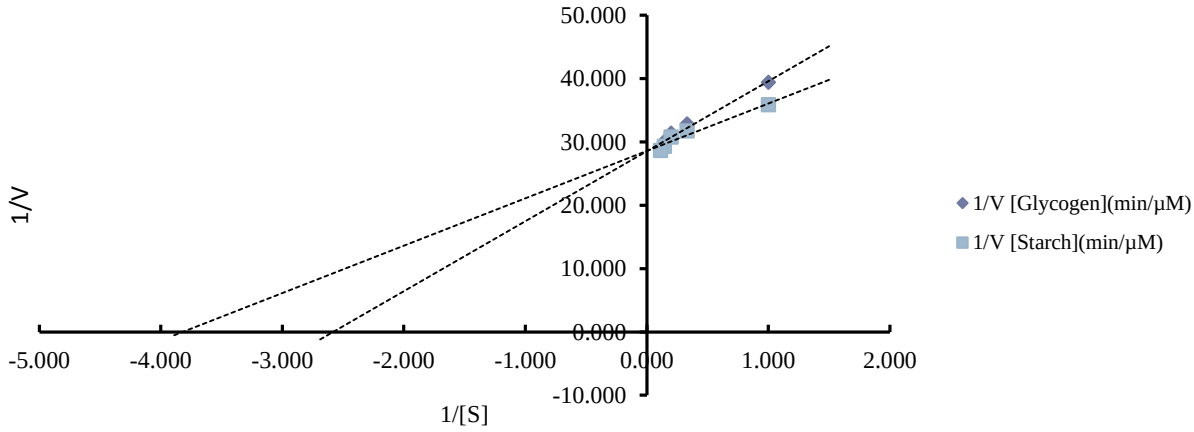
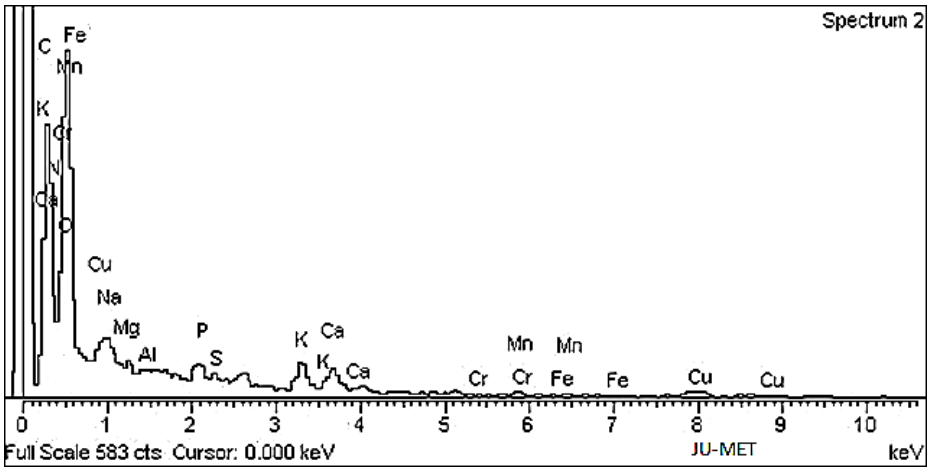



**Fig 8.** Effect of the kinetic analyses for competitive inhibition by glycogen on Glucoamylase activity




| Element | Weight% | Atomic% |
|---|---|---|
| C K | 9.81 | 12.53 |
| N K | 27.32 | 29.90 |
| O K | 57.67 | 55.27 |
| Na K | 1.20 | 0.80 |
| Mg K | 0.36 | 0.22 |
| AI K | 0.05 | 0.03 |
| P K | 0.23 | 0.11 |
| S K | 0.14 | 0.07 |
| K K | 1.09 | 0.43 |
| Ca K | 0.78 | 0.30 |
| Cr K | 0.07 | 0.02 |
| Mn K | 0.14 | 0.04 |
| Fe K | 0.23 | 0.06 |
| Cu K | 0.92 | 0.22 |
| | | |
| Totals | 100 | |

**Fig 9.** EDX analyses of the components of substrate pea peel showing the atomic percentages of different elements present in the pea peel

## 4. Conclusion

In the present study, CCD was proved to be effective in optimizing GA production by *A. niger*. The final fermentation composition optimized was incubation time: 5 days; incubation temperature: 30°C; and substrate amount: 3g, which resulted in GA production of 90.1728 U $gds^{-1}$. Thus the optimum fermentation parameters obtained in this experiment gave a basis for further study with large scale batch fermentation in a solid state tray fermenter for production of GA using this strain of *A. niger*. Maximum enzyme activity was reported in the pH range of 5.4-6.2, and the activity was found to be highly stable at 3.8. GA showed highest activity at 50°C, and was stable at 70°C for 30 min. The results showed that pea peel can be used as a good fermentation media for microbial growth as it contains all the necessary nutrients for the growth of the microorganism.

Hence, we can conclude that significant enzyme activity can be obtained utilizing pea peel, a vegetable residue, as a substrate for SSF. This provides us an opportunity to further investigate it in scale up fermentation processes and immobilization studies.

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## 6. Conflict of Interest

The authors declare that there is no conflict of interest.

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