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# Optimization of Invertase from *Aspergillus niger* Grown on Low Cost Agricultural Wastes by Response Surface Methodology (RSM)

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author FSI designed the study, performed the statistical analysis while author VJA wrote the protocol. Author VJA managed the analyses of the study. Author VE managed the literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** To optimize media components and cultural conditions using response surface methodology (RSM) in the production of invertase by *Aspergillus niger* grown on potato peels (P1) and pineapple peels (P2).
**Place and Duration of Study:** The study was conducted at Environmental Microbiology laboratory, University of Port Harcourt, Choba between September 2015 and January 2017.
**Methodology:** Chemical analyses of P1 and P2 were carried out using standard methods. Invertase production was screened using Fehling solution test coupled with invertase activity assay. Reducing sugar was estimated using dinitrosalicylic acid (DNS) procedure. The RSM optimization design involved four (4) independent variables (pH, temperature, pineapple peels concentrations, and potatoes peels concentrations) at five (5) levels, screened through thirty (30) different experimental runs using central composite design (CCD).

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**Results:** Optimal fermentation conditions that yielded maximum invertase (321.4 U/mL) and biomass (11.34 mg/mL) by *A. niger* was achieved with the combination of pH 9.0, temperature 35°C, pineapple peels concentration 10%, and potato peels concentration 50%. The model used gave a predicted R-Squared of -0.2258. Negative "Predicted R-squared" implies that the overall mean may be a better predictor of the response than the current model. "Adequate precision" of 6.190 was obtained, showing that the model can be used to navigate the design space.
**Conclusion:** This result has demonstrated the efficiency of RSM technique to optimize invertase production from *A. niger* using potato and pineapple peels as substrates. The use of local substrates can make invertase production economically attractive.

*Keywords: Invertase; Aspergillus niger; response surface methodology; pineapple peels; potato peels; solid state fermentation.*

## 1. INTRODUCTION

Invertases represent a group of industrially important enzymes that catalyse the breakdown of table sugar (sucrose) into fructose and glucose. They belong to enzyme family classified as glycoside hydrolases GH-32 [1,2,3], which comprises 370 different enzymes. Invertases are known by other names such as EC 3.2.1.26, glucosucrase, saccharase, sucrase, invertin, β-fructosidase, β-h-fructosidase, acid invertase, alkaline invertase, fructosylinvertase, maxinvert L 1000, and the systematic name, β-fructofuranosidase.

Commonly used carbon source for invertase production, especially by yeasts and fungi has been sucrose; however, other less conventional sources have included industrial agricultural residues, and other monosaccharide and disaccharide sugars (glucose, lactose, maltose etc.) [4]. Encouragingly, high yield of invertase have been reported with agricultural wastes such as sugarcane [5], Guar gum [6], soy bran [7] etc. as carbon source. For invertase production, a wide range of fungi preferentially utilise only sucrose as carbon source and as invertase inducer. This is probably because of the ability of most fungi to utilise sucrose hydrolysates for both growth and invertase production. The choice of preferred carbon source for invertase production seems to vary from one organism to another. Studies on the plausible use of varieties of agricultural wastes as substrate for invertase production have included the screening of the following: banana peels [8], lemon grass [9], fruit peels [10,11], sugar cane bagasse [12], wastes from food processing (orange pulp, red carrot residue, apple pomace, and okra) [13], corn cob and wheat bran [14], molasses media [15], and corn-steep liquor [16]. This study explores the use of potato peels and paw paw peels as substrates for invertase production. Interest in agricultural wastes as substrates for the production of enzymes in general and invertase in particular continues to increase. This is because of the need to avoid the food and feed crisis associated with the use of conventional substrates for enzyme production. Using wastes as substrate to produce invertase will not only provide cheaper sources of substrate to drive the enzyme industry but also will help in mitigating the negative effects of indiscriminate disposal of such wastes. Agricultural wastes traditionally are disposed by burying them in landfills; and these landfills represent very important source of land and air pollution. Therefore, in order to make the production of enzymes sustainable in line with sustainable development (SD) goal, substrates required for their production must come from renewable resource of which agricultural wastes are among.

In enzyme production in general and invertase production in particular, the fermentation conditions need to be optimized to obtain high growth of microorganisms and high yield of enzyme throughout fermentation. Classical optimisation methods involve identifying the various factors that may influence enzyme yield, and then, varying one of the factors at a time while keeping the rest constant. This approach is a single-dimensional enquiry procedure, which though simple, do not achieve optimised conditions as it fails to consider interactions between factors. Therefore, in finding solution to these obvious challenges, scientists designed statistical and mathematical procedures that circumvent these shortcomings. One of such statistical and mathematical procedure is response surface methodology (RSM). RSM is an experimental strategy that efficiently runs optimal conditions for multivariable schemes [17]. According to Ting et al. [18], RSM is an assemblage of mathematical and statistical techniques that designs experiments, builds

models, measures responses, evaluates effects and relationship between groups of controlled factors under experimental conditions, and searches for optimum conditions in bioprocess optimisation. In this study, RSM has been applied in optimizing media components and cultural conditions for the production of invertase by *Aspergillus niger* grown on potato peels (P1) and pineapple peels (P2) under solid state fermentation.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

Soil samples were obtained from decaying agricultural wastes in a waste dump site at University of Port Harcourt, Choba, Nigeria. The soil samples were collected from various points using a hand-held soil auger at depths between 0 and 15 cm. These various samples were made into composite sample. A sterile plastic bag was used to transport the samples to the Environmental Microbiology Laboratory, Department of Microbiology, University of Port Harcourt, Choba.

### 2.2 Isolation and Screening of Invertase-Producing Fungi

To isolate invertase-producing fungi, 1 g of the composite soil sample was dissolved in 10 mL of distilled water; this yielded the $10^{-1}$ dilution, from this, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, and $10^{-6}$ were generated. A volume of 0.1 mL from each of $10^{-3}$, $10^{-4}$, and $10^{-5}$ dilutions was spread onto a mineral salt agar containing (in g/L of distilled water): sucrose 20, yeast extract 10, $(NH_4)_2SO_4$1.0, $MgSO_4$ 0.75, $KH_2PO_4$ 3.5, agar 15, and pH 5.0. The medium was amended with 0.1 % (w/v) chloramphenicol to inhibit bacterial growth. The plates were incubated for three (3) days, at ambient temperature, and monitored for fungal growths. Fungal growths were purified on potato dextrose agar (PDA) and stored in agar slants.

The fungi were examined for invertase production using 50% sucrose-Cazpek liquid medium described by Mehta and Duhan [19]. The liquid medium was incubated for 10 days at 30°C and invertase production thereafter determined in the culture filtrate by Fehling's solution method. Positive results were indicated by brown and green precipitate. Isolates with the most pronounced precipitates were selected for further studies.

### 2.3 Biomass Collection, Communition, and Processing

The pineapple peels and potato peels used in this study were obtained from a local market in Choba, Port Harcourt, Nigeria. The pineapple peels and potato peels were washed and dried at atmospheric temperature for three days. The dry biomasses were further grinded with an electric blender (Philips blender HR2001, Japan), filtered with a 60 μm Mesh sieve and stored under dry conditions until further use [20].

### 2.4 Solid-state Fermentation (SSF)

Solid state fermentation (SSF) technique described by Malik et al. [21] was used in this study. Ten grams of each of the processed substrates (pineapple peels or potatoes peels) was added into a 250 mL Erlenmeyer flask. The substrates were moistened with 10 mL of the basal medium (containing in g/L: sucrose 20, yeast extract 10, $(NH_4)_2SO_4$ 1.0, $MgSO_4$ 0.75, $KH_2PO_4$ 3.5, and pH 5.0) with the flasks shaken properly. The flasks were plugged with cotton wool and sterilized in an autoclave at 121°C (15 psi) for 15 min. A 10% (v/v) seed culture of the fungus was used to inoculate each of the flasks containing the different substrates. The flasks were then incubated at 35°C for 7 days in a shaker incubator set at 150 rpm.

### 2.5 Invertase Assay

Invertase activity was determined using the method described by Qureshi et al.[22]. One millilitre cell-free supernatant obtained from SSF was mixed with an equal volume of an aqueous solution of sucrose (10 g/L). The mixture was dissolved in 20 mM acetate buffer (pH 5.5) and incubated at 35°C for 15 min. Two millilitres of dinitrosalicylic acid reagent (Biotech, India) was added to the mixture and the reaction mixture boiled for 5 min. Colour development was observed and the mixture cooled. Absorbance reading was taken at 540 nm using a spectrophotometer against a blank [23]. The amount of enzyme required to liberate 1 µg of reducing sugar at 35°C per minute was defined as one unit of invertase activity and calculated as given in Eq. 1.

$$\text{Invertase activity} = \frac{\mu\text{Mol of Glucose librated} * \text{df}}{\text{T x Volume of enzyme sample} * 2} \quad (1)$$

where, df = dilution factor; volume of enzyme sample = 1 mL; T = incubation time.

## 2.6 Experimental Design for Response Surface Methodology and Statistical Analysis

In this study, four (4) independent variables (pH, temperature, pineapple peels concentrations, and potatoes peels concentrations) at five (5) levels, screened through thirty (30) different experimental runs (Tables 1 and 2), with the insignificant ones eliminated to obtain a smaller and more fitting collection of factors were performed. Minimum and maximum values of variables investigated in cultivation medium are given in Table 1.The fractional factorial design comprised eight (8) factorial points, eight (8) axial points, and five (5) centre points. The centre point was repeated five times to obtain a reliable estimate of the experimental error. This ensured adequate estimation of the variation of the response, thereby providing the required number of degree of freedom for sufficiently testing the model [24]. On establishing the critical factors, the central composite design (CCD) was used to generate a quadratic model that comprised star points and factorial trials used in estimating quadratic effects and central points to determine the variability of the pure process with invertase production (Y) and biomass production as responses. Design Expert version 10 was used in analysing and interpreting experimental data obtained with CCD. The system's behaviour is explained by second-order polynomial equation given in Eq. 2.

$$\mathrm{Y} = \beta_0 + \sum_{i=1}^{K} \beta_i \mathrm{x}_i + \sum_{i=1}^{K} \beta_{ii}\, \mathrm{x}_i^2 + \sum_{i=1}^{\mathrm{K}-1} \sum_{i<jj=2}^{\mathrm{K}} \beta_{\mathrm{ij}} \mathrm{x}_i\, \mathrm{x}_j + e \quad (2)$$

Where: *Y* represents dependent variable, $x_i$ and $x_j$ independent variables, $\beta_0$, $\beta_i$, $\beta_{ii,}$ and $\beta_{ij,}$ represent the model's regression coefficients and e, model's error.

To test the estimation competence of the process, caparison between the actual responses and the predicted responses generated from RSM were drawn using Design Expert Version 10. ANOVA and $R^2$ statistic aided evaluation of significant differences between various factors and the model's adequacy, which is best when close to 1. Lack-of-fit (a model's adequacy test tool), compares the pure error from measurement replications to the other lack of fit from the performance of the model. Validation of the statistical model was based upon invertase production and biomass production at Erlenmeyer flasks' level under the predicted conditions by the model. Sampling was carried out at desired intervals and invertase production and biomass production determined.

## 2.7 Protein Estimation by Lowry's Method and Biomass Determination

The protein determination method used was a modification of Lowry et al*.* [25] method. A volume of 0.2 mL bovine serum albumin (BSA) working standard was pipetted into 25 mL test tubes and made up to 1 mL using distilled water. About 4.5 mL of Reagent I (48 mL of 2% $Na_2CO_3$ in 0.1 N NaOH, 1 mL of 1% NaK tartrate in $H_2O$, and 1 mL 0.5% $CuSO_4.5H_2O$ in $H_2O$) was added and incubated for 10 min. After incubation, 0.5 mL of Reagent II (1 part Folin-Phenol [2 N]:1 part water) was added and incubated for 30 min. The absorbance was measured at 660 nm and the standard graph plotted. The amount of protein present in the given sample was estimated from the standard graph. Fungal biomass was estimated according to the method of Dinarvand et al. [24] and the cell dry weight expressed in milligram per millilitre (mg/mL).

## 2.8 Identification of the Invertase-Producing Fungus

Fungal slide culture technique and the Colour Atlas for clinical fungi [26] were used to identify the fungus. A culture of the fungus was inoculated on the edge of sterilized 5 $cm^2$ block of PDA medium, cut out with sterile scalpel and placed on alcohol-sterilized glass slide. The inoculated agar block was covered with

**Table 1. Experimental variables' range and levels**

| Factors | Level of factors | | | | |
|---|---|---|---|---|---|
| | **-1.68** | **-1** | **0** | **+1** | **+1.68** |
| Initial pH | 4.5 | 6 | 7.5 | 9 | 10.5 |
| Temperature ($^oC$) | 20 | 25 | 30 | 35 | 40 |
| Pineapple peels (%) | 10 | 20 | 30 | 50 | 70 |
| Potatoes peels (%) | 10 | 20 | 30 | 50 | 50 |

**Table 2. Central composite design employed for independent variables and various experiments' composition**

| Run | Temperature (°C) | pH | Pineapple peels (%) | Potatoes peels (%) |
|---|---|---|---|---|
| **1** | 40 | 7.5 | 30 | 30 |
| **2** | 25 | 6 | 10 | 10 |
| **3** | 25 | 9 | 10 | 10 |
| **4** | 30 | 7.5 | 30 | 30 |
| **5** | 30 | 7.5 | 30 | 30 |
| **6** | 35 | 9 | 50 | 10 |
| **7** | 30 | 7.5 | 30 | -10 |
| **8** | 35 | 6 | 10 | 10 |
| **9** | 30 | 7.5 | 30 | 30 |
| **10** | 35 | 9 | 10 | 50 |
| **11** | 35 | 9 | 10 | 10 |
| **12** | 25 | 9 | 50 | 50 |
| **13** | 35 | 6 | 50 | 10 |
| **14** | 30 | 7.5 | 30 | 30 |
| **15** | 35 | 9 | 50 | 50 |
| **16** | 30 | 4.5 | 30 | 30 |
| **17** | 30 | 7.5 | 70 | 30 |
| **18** | 25 | 6 | 50 | 50 |
| **19** | 30 | 7.5 | 30 | 30 |
| **20** | 20 | 7.5 | 30 | 30 |
| **21** | 25 | 6 | 50 | 10 |
| **22** | 35 | 6 | 10 | 50 |
| **23** | 30 | 7.5 | 30 | 70 |
| **24** | 30 | 7.5 | 30 | 30 |
| **25** | 35 | 6 | 50 | 50 |
| **26** | 30 | 7.5 | -10 | 30 |
| **27** | 25 | 6 | 10 | 50 |
| **28** | 25 | 9 | 10 | 50 |
| **29** | 25 | 9 | 50 | 10 |
| **30** | 30 | 10.5 | 30 | 30 |

cover-slip and placed on top of the short piece of glass rod (8.9 cm) inside a petri dish whose bottom was lined with Whatman™ No 1 Qualitative filter paper. The filter paper was moistened with sterile distilled water to provide humid environment to the culture. The plate was covered and incubated at 30°C for 4 days. After incubation, the agar block culture was removed; the slide and cover-slip were stained with lactophenol cotton blue solution and examined under x40 and x10 objective lenses. Characteristics such as the presence or absence of conidia, phialides, septation, and colour of mycelia were noted and used to identify the isolate.

## 3. RESULTS AND DISCUSSION

### 3.1 Chemical Composition of the Agricultural Wastes Used for Invertase Production

Chemical composition of the two agricultural wastes (pineapple peels and potatoes peels) used in this study are presented in Table 3. The carbohydrate content of the pineapple peels (25.12%) was lower than that of the potatoes peels (30.7%). Analyses of mineral content of the potatoes peels and pineapple peels revealed traces of Zn, Fe, Mn, and Mg (Table 3).

**Table 3. Chemical composition* of the substrates**

| Parameters | Potatoes peels | Pineapple peels |
|---|---|---|
| Ash | 1.3 | 1.83 |
| Crude protein | 2.16 | 4.09 |
| Crude fat | 0.46 | 7.45 |
| Crude carbohydrate | 30.7 | 25.12 |
| Crude fibre | 0.92 | 24.83 |
| Moisture | 62.2 | 29.54 |
| Calcium | 7.1 | 19.4 |
| Zinc | 9.66 | 5.05 |
| Iron | 28.5 | 16.3 |
| Manganese | 10.3 | 11.51 |
| Magnesium | 5.5 | 7.79 |

** crude substrates and moisture in %, elements in ppm*

Many researchers have used different agricultural wastes materials as substrate to produce invertase. Some of the frequently used wastes include soya bran [20,27], guar gum [6], pineapple peel [10], orange peel [11,28], corn cob [21,27], pomegranate peel [10,29], sugarcane bagasse [11,20,27,30], banana peel, wheat bran [21], rice bran/straw [21, 27], cassava peel [27], and potatoes peels.

### 3.2 Isolation and Screening for Invertase Production

Out of the twenty four (24) isolates screened for invertase production, isolates VA-8 was selected because it gave positive result to the Fehling's solution test (i.e. the brown and green precipitate) and the best invertase production when assayed. Preliminary production of invertase by the *A. niger* was studied through solid state fermentation (SSF) and submerged fermentation (SmF) using potato peels in one set-up and pineapple peels in another set-up. The results revealed that SSF was the preferred route for invertase production using potato peels as substrate and this was eventually used in the final production set-up. Preference for the use of SSF for the production of invertase by *A. niger* has been widely reported. Romero-Gómez et al. [31] reported that *Aspergillus niger* preferred SSF to SmF for the production of invertase and attributed such observation to a better growth rate of the mould in SSF. However, the findings of this study have been contradicted by a few other studies. Uma et al. [29] reported that *Cladosporium cladosporioides* produced higher invertase in SmF than in SSF. This therefore means that the type of fermentation technique can greatly affect invertase production by moulds.

### 3.3 Composition of Various Experiments of the CCD for Independent Variables and Responses

The composition of various experiments of the CCD for independent variables (pH, temperature, potatoes peels, and pineapple peels) and responses (invertase production [U/mL] and biomass [mg/mL]) are presented in Table 4. The table reveals the actual and predicted values for both responses.

### 3.4 Fitting of Model and ANOVA for the Production of Invertase by *A. niger*

Summary of ANOVA for response surface quadratic polynomial models for the production of invertase and biomass by *A. niger* is given in Table 5. F-value of 2.87 for invertase production shows a significant model. This shows a 2.59% chance of an F-value this large occurring as a result of noise. Values of "Prob > F" that are less than 0.05 signify significant model. For this experiment, B, C, $D^2$ were significant model terms. When F-values are greater than 0.10, they signify insignificant model terms. Having many models with insignificant terms (excluding those essential for supporting hierarchy) may necessitate model reduction to improve the model. Lack-of-Fit F-value of 1.22, means that in relation to pure error, the Lack of Fit is insignificant. There is a 43.69% chance that a "Lack of Fit F-value" this large could occur due to noise. Lack of fit that is not significant is good as it helps in fitting the model. A negative "Predicted R-Squared" or "Predicted coefficient of determination" means that the overall mean may predict the response better than the current model. The model used gave a predicted R-Squared of -0.2258. "Adequate Precision" is a

measure of the signal to noise ratio. Ratios greater than 4 are desired; the ratio of 6.190 shows signal adequacy. This means that the design space can be effectively navigated with the model.

**Table 4. Composition of various experiments of the CCD for independent variables and responses by *A. niger***

| Run | Temp. (°C) | pH | Pineapple peels (%) | Potatoes peels (%) | Invertase (U/mL) | | Biomass (mg/mL) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Actual | Predicted | Actual | Predicted |
| **1** | 40 | 7.5 | 30 | 30 | 245.67 | 263.97 | 4.825 | 6.89 |
| **2** | 25 | 6 | 10 | 10 | 184.56 | 154.23 | 1.453 | 1.27 |
| **3** | 25 | 9 | 10 | 10 | 201.2 | 220.28 | 1.322 | 3.86 |
| **4** | 30 | 7.5 | 30 | 30 | 306.89 | 257.37 | 10.856 | 5.63 |
| **5** | 30 | 7.5 | 30 | 30 | 234.63 | 257.37 | 9.455 | 5.63 |
| **6** | 35 | 9 | 50 | 10 | 296.12 | 269.87 | 7.321 | 8.29 |
| **7** | 30 | 7.5 | 30 | -10 | 131.3 | 120.91 | 4.31 | 3.93 |
| **8** | 35 | 6 | 10 | 10 | 128.5 | 118.66 | 2.221 | 2.53 |
| **9** | 30 | 7.5 | 30 | 30 | 300.4 | 257.37 | 7.341 | 5.63 |
| **10** | 35 | 9 | 10 | 50 | 321.4 | 264.36 | 11.342 | 6.82 |
| **11** | 35 | 9 | 10 | 10 | 172.8 | 194.99 | 2.001 | 5.12 |
| **12** | 25 | 9 | 50 | 50 | 314.4 | 276.80 | 9.672 | 8.73 |
| **13** | 35 | 6 | 50 | 10 | 197.3 | 203.30 | 5.112 | 5.70 |
| **14** | 30 | 7.5 | 30 | 30 | 268.4 | 257.37 | 10.221 | 5.63 |
| **15** | 35 | 9 | 50 | 50 | 300.34 | 325.26 | 11.333 | 9.99 |
| **16** | 30 | 4.5 | 30 | 30 | 121.3 | 156.26 | 3.31 | 3.05 |
| **17** | 30 | 7.5 | 60 | 30 | 245.7 | 289.26 | 6.321 | 8.80 |
| **18** | 25 | 6 | 50 | 50 | 220.4 | 192.80 | 5.201 | 6.14 |
| **19** | 30 | 7.5 | 30 | 30 | 189.6 | 257.37 | 4.211 | 5.63 |
| **20** | 20 | 7.5 | 30 | 30 | 216.53 | 251.09 | 3.221 | 4.37 |
| **21** | 25 | 6 | 50 | 10 | 201.3 | 210.90 | 5.101 | 4.45 |
| **22** | 35 | 6 | 10 | 50 | 143.2 | 160.31 | 2.211 | 4.23 |
| **23** | 30 | 7.5 | 30 | 70 | 108.93 | 172.18 | 1.781 | 7.33 |
| **24** | 30 | 7.5 | 30 | 30 | 244.3 | 257.37 | 5.614 | 5.63 |
| **25** | 35 | 6 | 50 | 50 | 297.5 | 230.97 | 8.331 | 7.40 |
| **26** | 30 | 7.5 | -10 | 30 | 162.4 | 171.69 | 2.111 | 2.46 |
| **27** | 25 | 6 | 10 | 50 | 171.3 | 150.11 | 3.023 | 2.97 |
| **28** | 25 | 9 | 10 | 50 | 255.3 | 243.88 | 5.531 | 5.56 |
| **29** | 25 | 9 | 50 | 10 | 289.7 | 267.18 | 6.661 | 7.03 |
| **30** | 30 | 10.5 | 30 | 30 | 298.7 | 316.59 | 7.563 | 8.22 |

**Table 5. Analysis of variance (ANOVA) for invertase production and biomass *A. niger***

| Responses | Model | | Lack of fit |
|---|---|---|---|
| **Invertase production** | p-value | 0.0259 | 0.4369 |
| | F-value | 2.87 | 1.22 |
| | Coefficient of determination | 0.7279 | - |
| | Adjusted coefficient of determination | 0.4739 | - |
| | Predicted coefficient of determination | -0.2258 | - |
| | Adequate precision | 6.190 | - |
| **Biomass** | p-value | 0.0570 | 0.6695 |
| | F-value | 2.34 | 0.76 |
| | Coefficient of determination | 0.6859 | - |
| | Adjusted coefficient of determination | 0.3928 | - |
| | Predicted coefficient of determination | -0.2697 | - |
| | Adequate precision | 5.401 | - |

For biomass, F-value of 2.34 implies a 5.70% chance that an F-value this large could occur due to noise. The model was insignificant, but B, C, $D^2$ were significant model terms. The "Lack of Fit F-value" of 0.76 means the Lack of Fit is insignificant in relation to pure error. There is a 66.95% chance that a "Lack of Fit F-value" this large could occur due to noise. In addition, a negative "predicted coefficient of determination (predicted R-Squares)" was obtained. The "adequate precision" value was greater than 4 (5.401).

### 3.5 Final Equation in Terms of Factors

Table 6 shows the equation for invertase production and biomass by *A. niger* using coded factors. This can be used to make predictions about the response for given levels of each factor. Coded equations identify relative impact of factors by comparing the factor coefficients.

### 3.6 Effect of Reaction Parameters on the Production of Extracellular Invertase and Biomass

Interaction effects of different variables on the responses (invertase production and biomass production) were investigated by plotting 3D surface curves and contours against any two given independent variables, while another variable was kept at the central (0) level (Fig. 1 to 4). The results obtained from this study revealed agreement between the actual values and the predicted values (Table 4). Response surface methodology was employed in the optimization of cultural and environmental conditions necessary for the production of invertase. Applying RSM, allowed simultaneous determination of the main and interaction effects of different cultural factors on invertase production and biomass. The quadratic regression given in Eq. 2 generated the optimum values of the process variable. Analysis of variance (ANOVA) was used to justify the adequacies of the models. The use of response surface methodology (RSM) to optimize bioprocess techniques is gaining attention due to it suitability in effectively aggregating optimal conditions for multivariable schemes [17]. RSM technique has been further promoted due to the challenges of classical optimization technique and the need to maximize industrial processes. Many studies have attempted the enhancement of the production of bioactive substances using this technique. Sivakumar [32] used RSM technique to optimise keratinase production by *Bacillus cereus* and concluded that RSM can improve yield as well as the cost of enzyme production. The optimal fermentation conditions that yielded maximum production of extracellular invertase (321.4 U/mL) and biomass (11.34 mg/mL) was achieved by combination of pH 9.0, temperature (35°C), pineapple peels concentration (10%), and potato peels concentration (50%). The use of RSM in optimising bioprocess techniques has been heralded as an effective way of determining the most suitable parameters for production process.

**Table 6. The equation of the parameters (invertase production and biomass by *Aspergillus niger*) as the function of initial pH, temperature, potatoes peels concentration and pineapple peels concentration presented in terms of coded factors**

| Parameters | Responses | | | |
|---|---|---|---|---|
| | Invertase (U/mL) | Standard error | Biomass (mg/mL) | Standard error |
| **Intercept** | 257.37 | 19.27 | 7.95 | 1.00 |
| **A** | 3.22 | 9.63 | 0.63 | 0.50 |
| **B** | 40.08 | 9.63 | 1.29 | 0.50 |
| **C** | 29.39 | 9.63 | 1.59 | 0.50 |
| **D** | 12.82 | 9.63 | 0.85 | 0.50 |
| **AB** | 2.57 | 11.80 | 0.36 | 0.61 |
| **AC** | 7.00 | 11.80 | -0.06 | 0.61 |
| **AD** | 11.44 | 11.80 | 0.48 | 0.61 |
| **BC** | -2.44 | 11.80 | -0.00 | 0.61 |
| **BD** | 6.93 | 11.80 | 0.98 | 0.61 |
| **CD** | -3.49 | 11.80 | -0.30 | 0.61 |
| **$A^2$** | 0.039 | 9.01 | -0.76 | 0.47 |
| **$B^2$** | -5.24 | 9.01 | -0.41 | 0.47 |
| **$C^2$** | -6.72 | 9.01 | -0.72 | 0.47 |
| **$D^2$** | -27.71 | 9.01 | -1.01 | 0.47 |

*Legend: A: Temperature. B: pH. C: Pineapple Peels. D: Potatoes Peels*

### 3.6.1 Effect of temperature on production invertase and biomass by *A. niger*

Combined effects of temperature–pH, temperature-pineapple peel concentration, and temperature-potato peels concentration on extracellular invertase production and biomass are presented in Figs. 1 and 3. The maximum extracellular invertase production and biomass occurred at temperature (35°C), further

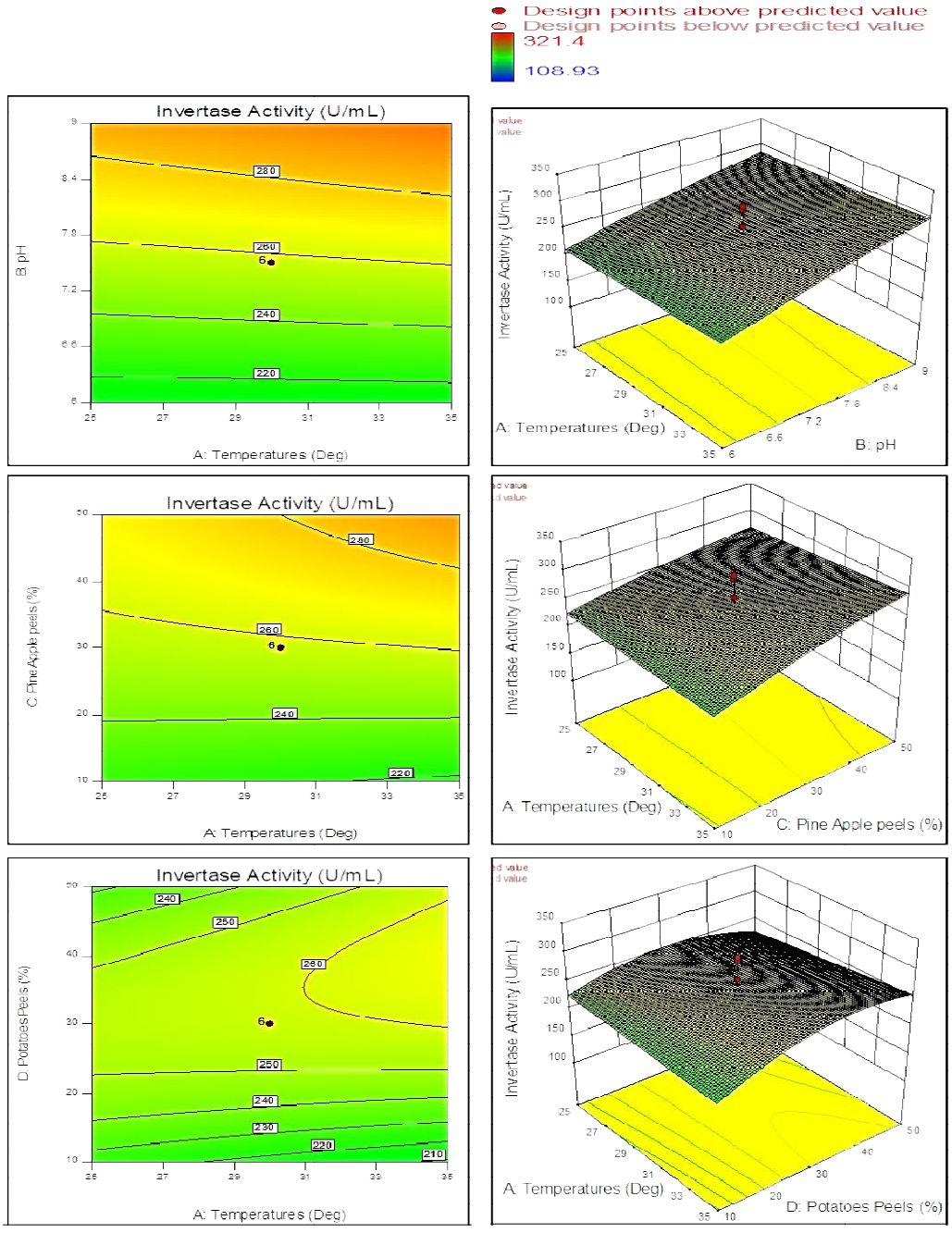


**Fig. 1. Response surface for invertase production (mg/mL) from *A. niger* in batch fermentation as a function of A and B, A and C, and A and D. (A: temperature (°C), B: pH, C: potatoes peels concentration (%), and D: pineapple peels concentration (%).Other variables were kept constant at their centre points. Numbers inside contour plots represent conversion yield (U/mL) of the invertase production.**

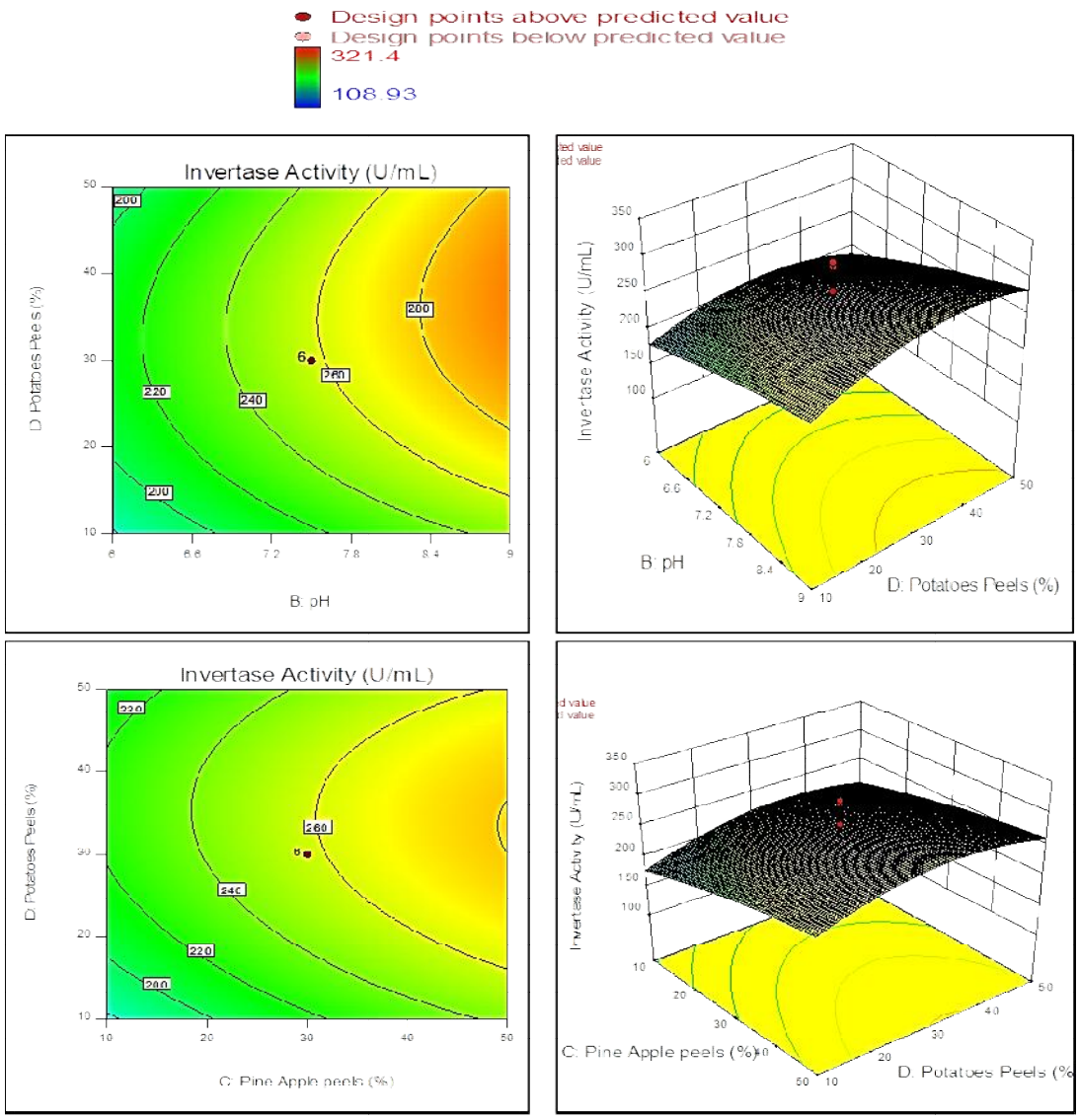


**Fig. 2. Response surface for invertase production (U/mL) from *A. niger* in batch fermentation as a function of B and C, B and D, and C and D. (A: temperature (°C), B: pH, C: potatoes peels concentration (%), and D: pineapple peels concentration (%). Other variables were kept constant at their centre points. Numbers inside contour plots represent conversion yield (U/mL) of the invertase production.**

decrease or increase in the temperature (35°C) neither increased extracellular invertase production nor biomass. The most suitable temperature for invertase production and biomass production was 35°C. Similar temperature has been reported by other studies. However, most studies involving fungi [5,7,12,29,33] have reported temperatures between 40 and 60°C as optimal for invertase production.

### 3.6.2 Effect of pH on extracellular invertase production and biomass by *A. niger*

Combined effect of temperature–pH, pH-pineapple peel concentration, and pH-potato peels concentration on extracellular invertase production and biomass is presented in Figs2 and 4. The maximum extracellular invertase production and biomass occurred at pH (9.0), further decrease or increase in the pH (9.0) neither increased extracellular invertase

production nor biomass. Thus, the *A. niger* produced maximum invertase and biomass at alkaline pH. From the result, pH had the most profound effect on invertase production; whereas pineapple concentration affected biomass production mostly. High impact of pH differences on invertase production has previously been reported [17,34]. In this study, invertase production by *Aspergillus niger* was induced more by alkaline pH. This finding is contrary to the reports by Kumar et al. [35]; Singh and Bhermi [34]; and Dinarvand et al. [17] that *A. niger* preferred acidic pH for invertase production. This development may be as result of the preference of the *A. niger* species to alkaline pH than acidic pH, which is supported by the higher biomass observed at alkaline pH.

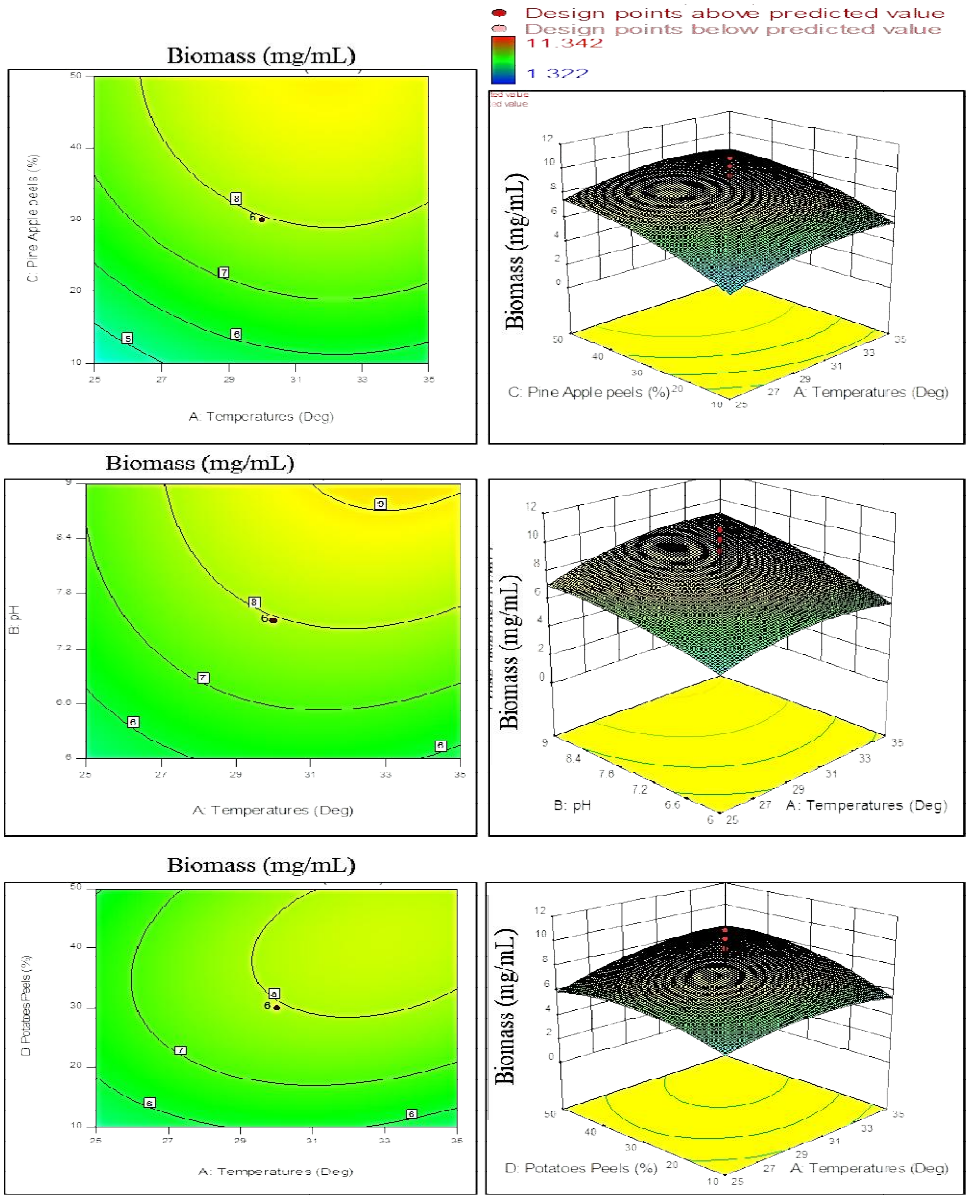


**Fig. 3. Response surface for biomass production (mg/mL) from *A. niger* in batch fermentation as a function of A and B, A and C, and A and D. (A: temperature (°C), B: pH, C: potatoes peels concentration (%), and D: pineapple peels concentration (%). Other variables were kept constant at their centre points. Numbers inside contour plots represent conversion yield (U/mL) of the invertase production**

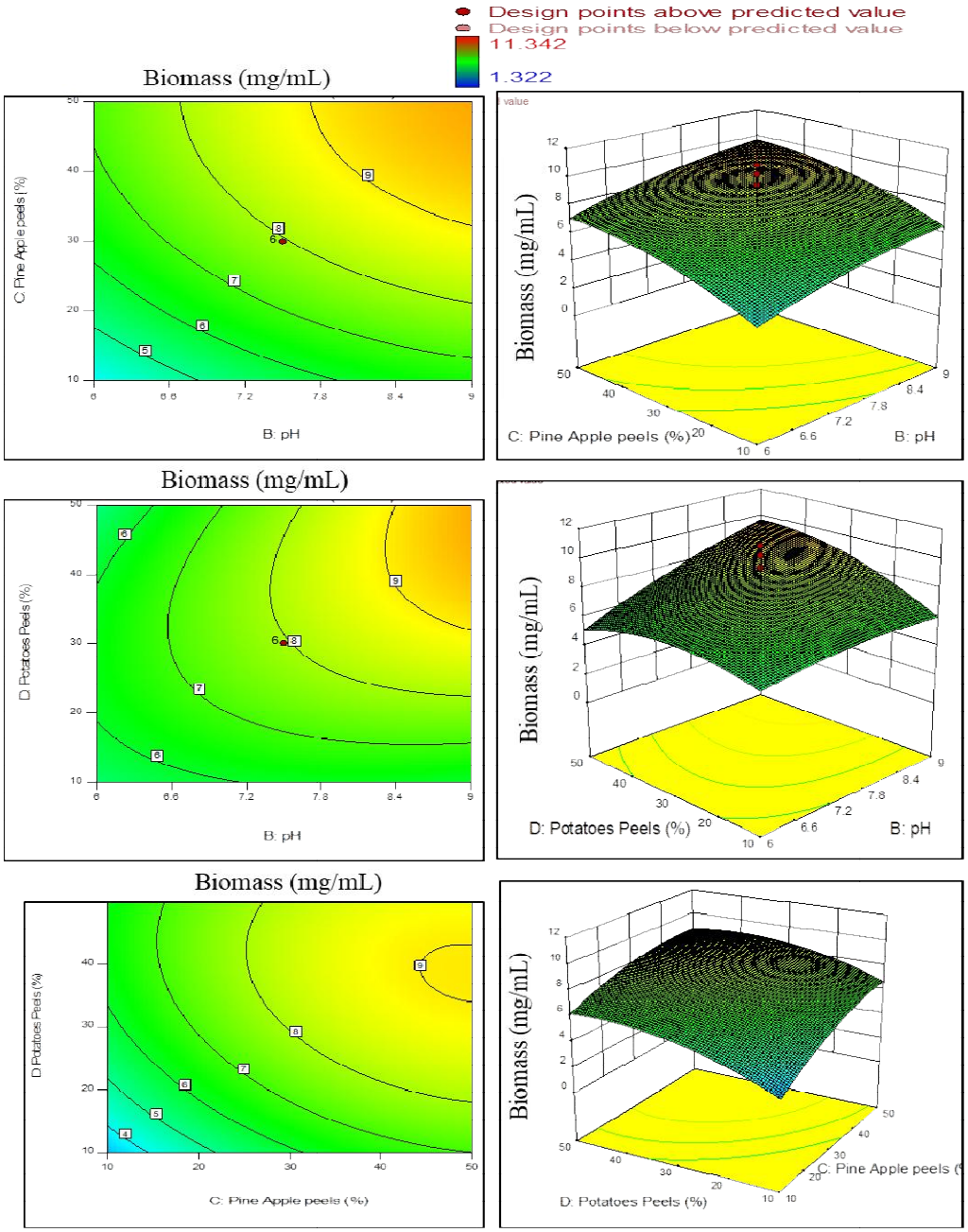


**Fig. 4. Response surface for biomass production (mg/mL) from *A. niger* in batch fermentation as a function of B and C, B and D, and C and D. (A: temperature (°C), B: pH, C: potatoes peels concentration (%), and D: pineapple peels concentration (%). Other variables were kept constant at their centre points. Numbers inside contour plots represent conversion yield (U/mL) of the invertase production**

### 3.6.3 Effect of pineapple peels concentration on extracellular invertase production and biomass by *A. niger*

Combined effects of temperature–pineapple peels concentration, pH-pineapple peels concentration, and pineapple-potato peels concentration on extracellular invertase production and biomass are presented in Figs. 1 to 4. The maximum extracellular invertase production and biomass occurred at pineapple peels concentration (10%, w/v), further decrease

or increase in the pineapple peels concentration (10%, w/v), did not increase extracellular invertase production and biomass.

#### 3.6.4 Effect of potato peels concentration on extracellular invertase production and biomass by *A. niger*

Combined effect of temperature–potato peels concentration, pH-potato peels concentration, and pineapple-potato peels concentration on extracellular invertase production and biomass is presented in Figs. 1 to 4. The maximum extracellular invertase production and biomass occurred at pineapple peels concentration (50%, w/v), further decrease or increase in the pineapple peels concentration (50%, w/v), did not increase extracellular invertase production and biomass.

### 3.7 Identification of the Isolates Used for Invertase Production

The fungus was identified as *A. niger*. The fungus showed characteristic dark surface colonies on PDA plates, with erect hypha and clustered conidia. The reverse view was white to cream. Microscopic examination revealed septate hypha with conidiophores and vesicles.

## 4. CONCLUSION

This study demonstrated the efficiency of RSM technique to optimize invertase production from *A. niger* using potato and pineapple peels as substrates. The use of local substrates in producing invertase can make invertase production economically attractive. Based on central composite design of RSM the parameters for optimum invertase production (306.89 U/mL) by *Aspergillus niger* were temperature, 30°C; pH 7.5; pineapple peels, 30%; and potato peels, 30%; whereas, parameters for optimum biomass production (11.34 mg/mL) were temperature, 35°C; pH 9; pineapple peels, 10%; and potato peels, 50%. Because coded equations identify relative impact of factors by comparing the factor coefficients, the final equation obtained with the help of central composite design of the response surface methodology from this study can be used to predict responses for given levels of each factor. This finding validates the claim that Nigeria can achieve complete indigenization of her enzyme production industry using readily available low-cost substrates.

## ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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