Bioconversion of Breadfruit Starch to Citric Acid by Fungus *Aspergillus niger*: A Microbial Fermentation Parameter Optimization Investigation

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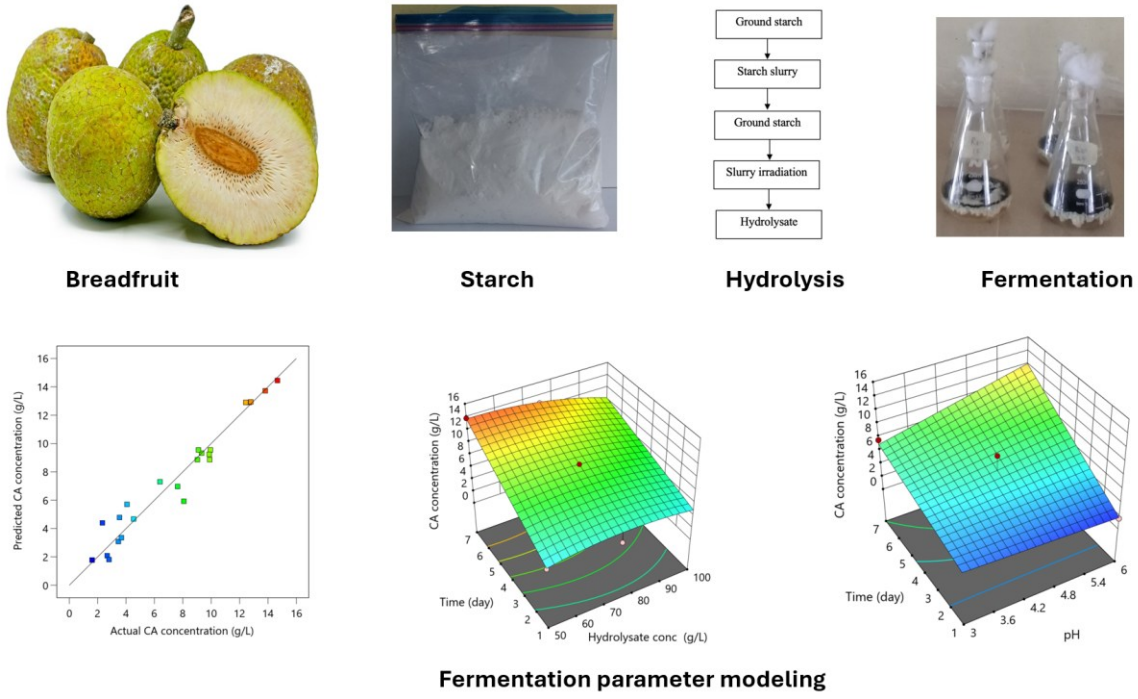
GRAPHICAL ABSTRACT



Bioconversion of Breadfruit Starch to Citric Acid by Fungus *Aspergillus niger*: A Microbial Fermentation Parameter Optimization Investigation

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Starch hydrolysate from breadfruit was used as the sole carbon source for citric acid (CA) biosynthesis by the filamentous fungus *Aspergillus niger* under surface fermentation conditions. The process was modeled and optimized by examining the influence of four critical factors: Starch hydrolysate concentration ranging from 50 to 100 g/L, medium pH between 3 and 6, nitrogen source comprising of $(NH_4)_2HPO_4$ or $NaNO_3$, and fermentation time from 1 to 7 days, on CA concentration. The results demonstrated that *A. niger* efficiently metabolized the hydrolysate, achieving a maximum CA concentration of 14.7 g/L after 7 days of fermentation. Statistical modeling predicted the optimal production conditions as a starch hydrolysate concentration of 50 g/L, pH of 5.4, $(NH_4)_2HPO_4$ as the nitrogen source, and a fermentation duration of 7 days. Under these conditions, the predicted CA concentration was 14.7 g/L, which was validated experimentally. Additionally, the process yielded 2.02 g/L of biomass and 15.2 g/L of reducing sugars. This study underscores the potential of breadfruit as a low-cost and sustainable substrate for CA biosynthesis. Applying response surface methodology with D-Optimal design proved effective in optimizing process variables and enhancing production efficiency. These findings provide a framework for developing cost-efficient and scalable fermentation processes, particularly in regions with abundant breadfruit resources.

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Keywords: Breadfruit; Fermentation; Starch hydrolysis; Optimization; D-Optimal design

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INTRODUCTION

Citric acid (CA) is a weak organic acid naturally occurring in citrus fruits, microorganisms, plants, and animal tissues. As an essential intermediate in the Krebs cycle, it plays a pivotal role in the metabolism of aerobic organisms (Akram 2014). The chemical formula of CA ($C_6H_8O_7$), molecular weight (210.14 g/mol), and acid dissociation constants (pKa of 3.4, 4.7, and 6.4) underline its multifunctional chemistry, which contributes to its widespread usage (Adeoye and Lateef 2021). Beyond its biological significance, the acid is extensively used across industries as a natural preservative, antioxidant, stabilizer, and acidulant. It also can participate in cross-linking reactions. Citric acid is valued for its biocompatibility, nontoxicity, high solubility, biodegradability, and palatability, making it

indispensable in the food, pharmaceutical, textile, cosmetic, agriculture, and biomedical industries (Adeoye and Lateef 2022; Li *et al*. 2022).

The global demand for citric acid continues to grow at an estimated 3.5% to 4% level annually, with production projected to reach approximately 2.91 million tons by 2026 (Adeoye and Lateef 2022). This surge is driven by its use as a preservative, acidulant, stabilizer, and antioxidant in food and beverages and its applications in detergents, pharmaceuticals, and other industries (Reena *et al*. 2022). Although CA can be synthesized chemically or extracted from fruits, these methods are hindered by high costs and low yields. Consequently, microbial fermentation has emerged as the most efficient and economically viable production method, leveraging advances in biotechnology to enhance yields and recovery rates while utilizing low-cost substrates (Ozdal and Kurbanoglu 2019; Perwitasari *et al*. 2023). The submerged fermentation process using *Aspergillus niger* accounts for over 80% of global CA production (Xu *et al*. 2024).

Different microbes have been exploited for citric acid biosynthesis, including *Yarrowia lipolytica*, *Candida oleophila*, *Bacillus licheniformis*, and *Aspergillus awamori*. *A*. *niger* is the preferred microorganism for large-scale CA production due to its high efficiency, ability to utilize diverse substrates, and capacity to thrive in low pH conditions, which minimizes contamination and byproduct formation (Behera *et al*. 2021; Betiku and Adesina 2013; Xu *et al*. 2024). Recent advances have focused on using unconventional substrates such as downgraded dates (Chergui *et al*. 2021), cocoa pod husks (de Oliveira *et al*. 2022), molasses (Ozdal and Kurbanoglu 2019), pineapple waste (Imandi *et al.* 2008), sweet potato starch (Betiku and Adesina 2013) and peel (Oyeniran *et al*. 2013), cassava starch (Betiku *et al*. 2010), cassava peel (Adeoye *et al*. 2015), apple pomace sludge (Dhillon *et al*. 2011), cashew juice (Adeoye and Lateef 2021), and banana pseudostem (Laltha *et al*. 2022) to reduce production costs and enhance sustainability.

The microbial fermentation process has been modeled, and the input variables have been optimized using various techniques to improve CA production yield. With surface fermentation, the optimum CA concentration of 83.0 g/L was achieved under the conditions of carbon substrate concentration of 154 g/L from sweet potato, KH_2PO_4 of 2.58 g/L, $(NH_4)_2HPO_4$ of 3.55 g/L, and fermentation time of 8 days using the central composite design (CCD) and response surface methodology (RSM) (Betiku and Adesina 2013). In the study using sweet potato peel as the carbon source with surface fermentation, the optimum CA concentration of 16.0 g/L was achieved under the conditions of a carbon substrate concentration of 151 g/L, methanol concentration of 3%, and fermentation time of 3.61 days using the Box Behnken design (BBD) and RSM (Oyeniran *et al*. 2013). In another study using sweet potato peel with submerged fermentation, a CA concentration of 4.36 ± 0.06 mg/mL was observed under the conditions of a carbon substrate concentration of 97.2%, nitrogen concentration of 1.25% w/v, pH of 6.4, and fermentation time of 7 days with the combination of the simplex mixture design and RSM (Aboyeji *et al*. 2020).

Breadfruit (*Artocarpus communis*) is a tropical fruit that serves as a staple food, providing high caloric and protein content. However, 60% to 80% of the fruit produced either deteriorates or remains underutilized. Unripe, mature breadfruit contains approximately 77.1% starch in its pulp, comparable to other carbohydrate-rich sources (Adewusi *et al*. 1995). It has been processed into flour and evaluated for use in unconventional bakery products (Bakare *et al*. 2016). Efforts to create value-added products from breadfruit included extensive hydrolysis studies of its starch flour (Betiku and Ajala 2010), with hydrolysates used for producing ethanol (Solomon *et al*. 1994;

Betiku and Taiwo 2015) and gluconic acid (Betiku *et al*. 2011). Betiku *et al*. (2023) demonstrated the potential of breadfruit as a carbon source for ethanol production with a yield of 4.99% (*V*) using fermentation conditions of pH of 4.7, hydrolysate concentration of 80 g/L, inoculum volume of 2%, and fermentation period of 20.41 h. Notably, no report in the open literature on citric acid production uses breadfruit as the carbon source. Furthermore, breadfruit leftovers after starch extraction include pulp and other fibrous materials, which could be used for animal feed and biofuel production.

This study uses hydrolysate from breadfruit starch as a carbon source for CA production. The fungal *A*. *niger* was cultured on hydrolysate enriched with additional nutrients. CCD coupled with RSM was employed to model and optimize the production process. The effects of key variables, including nitrogen sources, medium pH, hydrolysate concentration, and fermentation time, on CA production were established. This approach facilitated the identification of optimal conditions for achieving maximum CA concentration.

EXPERIMENTAL

Materials

Breadfruit and starch hydrolysate

The reducing sugar (starch hydrolysate) used as the substrate for this current study was obtained from breadfruit starch through microwave-supported acid hydrolysis (Betiku *et al*. 2023). The starch was dissolved in 0.1 M HCl solution to form a 10% (w/v) slurry. Five grams of breadfruit was weighed into 50 mL of 0.1 M HCl solution. A 0.15 M $CaCl_2$ solution was added to increase the conductivity of the solvent and accelerate the reaction rate (Kunlan *et al*. 2001). The mixture was homogenized for 10 min, followed by microwave irradiation treatment using 720 W and 6 min. Afterward, the product was cooled in an ice bath to room temperature. The hydrolysis was stopped with 2 M NaOH and 1 M HCl and centrifuged at 10,000 g for 10 min to obtain the supernatant. The reducing sugar in the breadfruit starch hydrolysate (BSH) was determined using the dinitrosalicylic acid (DNS) method.

Microorganism and inocula

The *A*. *niger* MC01, sourced from the Department of Microbiology at Obafemi Awolowo University, was maintained on potato dextrose agar (PDA). The PDA medium was prepared, sterilized, and inoculated with the microorganism, then incubated at 30 °C for 5 to 7 days. Cultures were stored at 4 °C with monthly sub-culturing. Spores were harvested aseptically using sterile distilled water, and 10 mL of the spore suspension was used to inoculate fermentation flasks (Betiku and Adesina 2013).

Methods

Media composition and fermentation studies

The fermentation media consisted of the starch hydrolysate obtained from breadfruit as the carbon source, along with 3.55 g/L of $(NH_4)_2HPO_4$ or $NaNO_3$, 2.56 g/L of KH_2PO_4, and 0.1 g/L of $MgSO_4{\cdot}7H_2O$ (Betiku and Adesina 2013), following the composition outlined in the experimental design in Table 1. The pH of each medium was adjusted using 1 M HCl or 2 M NaOH. All media and flasks were sterilized at 121 °C for 15 min before use. This study used the surface fermentation technique to produce citric

acid (Betiku and Adesina 2013). For the fermentation studies, 50 mL of the BSH was measured into 250 mL flask and the required nutrients were added, followed by sterilization at 121 °C for 15 min. A 5% (v/v) inoculum was aseptically introduced, and the flasks were incubated at 30 °C for the period stipulated in the design.

Citric acid concentration determination

Citric acid accumulation was measured using a titrimetric method. The supernatant from the fermentation broth was mixed with distilled water in a ratio of 1:1. The mixture was titrated with 0.1 N NaOH with phenolphthalein as an indicator until a pink endpoint was achieved (AOAC 1990). The percentage of CA was then calculated using Eq. 1:

$$Citrc\ Acid\ (\%) = \frac{\text{Volume of 0.1 N NaOH used} * \text{Normality} * \text{equiv. wt. of citric acid} * \text{dilution factor}}{\text{Weight of sample (g)} * 10} \quad (1)$$

Experimental design for citric acid biosynthesis

The D-optimal design approach was chosen for this study because it helps reduce the number of experiments compared to standard factorial and fractional factorial designs. Also, it accommodates both numeric and categorical factors, which traditional design methods cannot handle (Kang *et al*. 2023). The design was used to generate the experimental conditions for the study using Design-Expert software (version 13, State-Ease, MN, USA), with three input factors: hydrolysate concentration, pH, fermentation time, and nitrogen source. The $(NH_4)_2HPO_4$ and $NaNO_3$ were evaluated as nitrogen sources for the fermentation media used for the CA biosynthesis. The four parameters investigated with the actual experimental levels are shown in Table 1. The twenty-four experiments produced and conducted in the laboratory are presented in Table 2.

Table 1. Experimental Factors and Levels for Citric Acid Fermentation

Factor	Symbol	Type	Actual Factor Levels		
Hydrolysate concentration (g/L)	*A*	Numeric	50	75	100
pH	*B*	Numeric	3	4.5	6
Fermentation time (days)	*C*	Numeric	1	4	7
Nitrogen source	*D*	Categorical	$(NH_4)_2HPO_4$	$NaNO_3$	-

Statistical analysis and model validation

The data obtained in this work were statistically analyzed using multiple regression analysis and RSM. The data were fitted to the second-order mathematical model equation. An analysis of variance (ANOVA) was performed to test the significance of each parameter and their interactions. The reliability of the model was evaluated using fit statistics, *viz.*, coefficient of determination (R^2), adjusted R^2, predicted R^2, coefficient of variance (CV), and adequate precision. The model was validated using the optimum values for the four parameters to conduct three replicate experiments in the laboratory.

Table 2. Experimental Matrix, Observed and Predicted Citric Acid Concentration

Run Order	Hydrolysate Concentration (g/L)	pH	Time (days)	Nitrogen Source	Actual Value (g/L)	Predicted Value (g/L)
1	50	3	4	$(NH_4)_2HPO_4$	9.86	9.24
2	75	4.5	1	$(NH_4)_2HPO_4$	4.07	5.71
3	100	6	7	$NaNO_3$	9.95	9.55
4	100	6	1	$(NH_4)_2HPO_4$	3.68	3.36
5	100	3	1	$NaNO_3$	2.33	4.40
6	50	6	1	$(NH_4)_2HPO_4$	3.46	3.08
7	100	3	1	$NaNO_3$	2.67	2.08
8	50	4.5	1	$(NH_4)_2HPO_4$	4.53	4.68
9	50	3	4	$NaNO_3$	3.54	4.78
10	100	3	7	$(NH_4)_2HPO_4$	9.03	8.86
11	100	6	7	$NaNO_3$	9.10	9.55
12	75	6	1	$NaNO_3$	1.61	1.78
13	75	6	1	$NaNO_3$	1.61	1.78
14	50	6	7	$NaNO_3$	12.44	12.90
15	50	6	7	$NaNO_3$	12.68	12.90
16	75	4.5	7	$(NH_4)_2HPO_4$	12.80	12.94
17	100	3	7	$(NH_4)_2HPO_4$	9.90	8.86
18	75	4.5	4	$NaNO_3$	8.07	5.93
19	75	4.5	4	$(NH_4)_2HPO_4$	9.32	9.32
20	50	4.5	7	$(NH_4)_2HPO_4$	13.81	13.72
21	50	3	1	$NaNO_3$	2.80	1.81
22	75	3	7	$NaNO_3$	7.63	6.97
23	75	3	1	$(NH_4)_2HPO_4$	6.39	7.30
24	75	6	7	$(NH_4)_2HPO_4$	14.67	14.45

RESULTS AND DISCUSSION

The microwave-supported acid hydrolysis of the starch slurry (122 g/L) obtained from breadfruit yielded 109.8 g/L of reducing sugars. It has been demonstrated that 92 g/L of reducing sugar could be obtained from enzymatic hydrolysis of 100 g/L of the starch slurry in 1h 40 min (Betiku and Ajala, 2010).

Modeling of Citric Acid Production Process

This study explored the potential of using hydrolysate from breadfruit starch as the sole carbon source for CA biosynthesis by *A. niger* under surface fermentation conditions. The process was modeled and optimized using D-Optimal design to evaluate the effects of four key input variables: hydrolysate concentration, fermentation medium pH, nitrogen source, and fermentation time. Table 2 presents the experimental and predicted CA concentrations. The findings revealed that *A. niger* efficiently metabolized the hydrolysate that served as the sole carbon source, converting 67.2% of the substrate within seven days. Citric acid production increased consistently during fermentation, with a concentration of 6.39 g/L after one day to 9.86 g/L after four days and 14.7 g/L after seven days (Table 2).

For comparison, Oyeniran *et al*. (2013) reported a CA concentration range of 11.4 and 16 g/L using 100 g/L of sweet potato peel hydrolysate without and with methanol

supplementation, respectively. The reported CA concentration range was based on 11.4% and 16% hydrolysate consumption rates, respectively. In contrast, this study achieved a higher CA concentration range corresponding to 12.8% and 29.3% hydrolysate consumption rates from breadfruit starch hydrolysate (50 g/L). The pH values used in this study (3, 4.5, and 6) were informed by literature. Optimal growth of filamentous fungi typically occurs at a medium pH of 3 to 6 (Anastassiadis *et al*. 2008). Betiku and Adesina (2013) identified a medium pH of 6 as the optimal condition for *A. niger*, which was consistent with this study, as media at a pH of 6 produced higher CA concentration compared to pH of 3 and 4.5 (Table 2). The impact of nitrogen sources on CA biosynthesis was also examined. Low nitrogen concentration is generally required for optimal CA production (Anastassiadis *et al*. 2008). Both nitrogen sources tested supported CA biosynthesis (Table 2), but $(NH_4)_2HPO_4$ yielded higher CA concentration (14.7 g/L) than with $NaNO_3$ (12.7 g/L). These findings underscore the importance of optimizing pH and nitrogen sources to enhance CA production.

The final equation in terms of coded factors for the response surface quadratic model is given in Eq. 2a. The quadratic expressions that best described the biosynthesis of CA in terms of nitrogen sources are expressed in Eq. 2b and 2c in terms of the actual values.

$$Y = 7.62 - 0.77A + 0.49B + 3.62C - 1.70D - 0.91AC + 1.55BC + 0.53BD - 0.89A^2 \quad (2a)$$

For $(NH_4)_2HPO_4$:

$$Y = 1.48284 + 0.23188A - 1.40783B + 0.55972C - 0.012085AC + 0.34492BC - 0.0014289A^2 \quad (2b)$$

For $NaNO_3$:

$$Y = -5.10506 + 0.23188A - 0.69846B + 0.55972C - 0.012085AC + 0.34492BC - 0.0014289A^2 \quad (2c)$$

where Y is the concentration of citric acid (g/L), A is the hydrolysate concentration (g/L), B is the medium pH, C is the fermentation time (days), and D is the nitrogen source concentration (g/L).

Table 3 shows the test of significance for all regression coefficients of the model. The results indicated that most model terms were significant, since the p-values were < 0.05. The three linear terms (A, C, and D) and two cross-products (AC and BC) were all significant model terms at a 95% confidence level. However, the linear term, B, cross-product, BD, and quadratic term A_2 were not significant, since the p-values were > 0.05. The F-value of 39.89 and $p < 0.0001$ of the models confirm that it was significant. The observation of this work is supported by a previous report on CA production using sweet potato starch hydrolysate as the sole carbon source. The medium pH, hydrolysate concentration, fermentation time, nitrogen source, and the interactions of hydrolysate concentration and medium pH and medium pH and fermentation time were significant model terms (Betiku and Adesina 2013). However, while the interaction between hydrolysate and fermentation time was significant in the current study, it was not significant when hydrolysate from sweet potato starch and peel was used (Betiku and Adesina 2013; Oyeniran *et al*. 2013).

The quality of the mathematical model was evaluated using various fit statistics (Table 4). The mean, standard deviation, and coefficient of variance of the model were 7.33%, 1.10%, and 15.0%, respectively, which suggest small deviations between the actual

and predicted values. A coefficient of variance of 15.03% was slightly higher than the acceptable threshold for a good model, which is < 10% (Betiku *et al*. 2023). Adequate precision representing the signal-to-noise ratio for the model was 18.77, demonstrating that it is appropriate for describing the CA production process because the acceptable value is > 4 (Ramaraj and Unpaprom 2019). The R^2 of the model was 0.9551, signifying that 95.51% of the variation observed was connected to the fermentation variables studied. The generally acceptable threshold for a good model is 0.8 (Betiku *et al*. 2016). The adjusted R^2 of 0.9312, which ignores the terms that are not significant, and the predicted R^2 of 0.8964 indicate the precision of the model in describing the process because the difference between the two metrics was < 0.2 (Ibrahim *et al*. 2022).

Table 3. Test of Significance for Regression Coefficients for CA Production

Source	Sum of Squares	Degree of Freedom	Mean Square	F- Value	p-Value
Model	387.47	8	48.43	39.89	< 0.0001
A	7.75	1	7.75	6.38	0.0233
B	3.84	1	3.84	3.16	0.0956
C	229.50	1	229.50	189.01	< 0.0001
D	64.49	1	64.49	53.11	< 0.0001
AC	8.92	1	8.92	7.35	0.0161
BC	32.82	1	32.82	27.03	0.0001
BD	4.47	1	4.47	3.68	0.0743
A^2	4.13	1	4.13	3.40	0.0850
Residual	18.21	15	1.21	-	-
Lack of Fit	17.45	11	1.59	8.33	0.0276
Pure Error	0.7617	4	0.1904	-	-
Corrected Total	405.68	23	-	-	-

Table 4. Fit Statistics for the Model

Parameter	value
Standard deviation	1.10
Mean	7.33
Coefficient of variance (%)	15.03
R^2	0.9551
Adjusted R^2	0.9312
Predicted R^2	0.8964
Adequate precision	18.77

Figures 1a-d depict the diagnostic plots to assess the performance of the regression model. The plot of the predicted and experimental CA concentrations is shown in Fig. 1a, with data aligning along the regression line. The plot of the normal probability *versus* the internally studentized residuals is displayed in Fig. 1b, demonstrating that the data followed a straight line and not an abnormal S-shape, implying a normal distribution of the residuals (Ibrahim *et al*. 2019). The plot of internally studentized residuals against the predicted CA concentrations is illustrated in Fig. 1c. The plot shows that the residuals were randomly spread, confirming the actual observation divergence, which does not change for the responses (Falowo *et a*l. 2019). The plot demonstrated the normality of the data and primary errors (Manmai *et al*. 2020). Figure 1d displays the plot of residuals *versus* experimental run numbers. Standardized residuals represented by d_i should be within -3 ≤

$d_i \leq 3$ (Myers *et al*. 2016). The data were within this threshold, signifying that the model correctly approximated all the data without errors (Falowo *et al*. 2019).

Effects of Fermentation Parameter Interactions on Citric Acid Production

The contour and response surface plots, showing the interactions between the process parameters for the bioprocessing of BSH to CA, are presented in Figs. 2 and 3. Figures 2a and 2b show the contour and response surface plots representing the interactive effect of the fermentation time and hydrolysate concentration on CA concentration using $(NH_4)_2HPO_4$ as a nitrogen source while keeping medium pH constant. The figures show that as the fermentation time increased, the CA concentration increased, while the reverse was the case with the hydrolysate concentration (Figs. 2a and 2b).

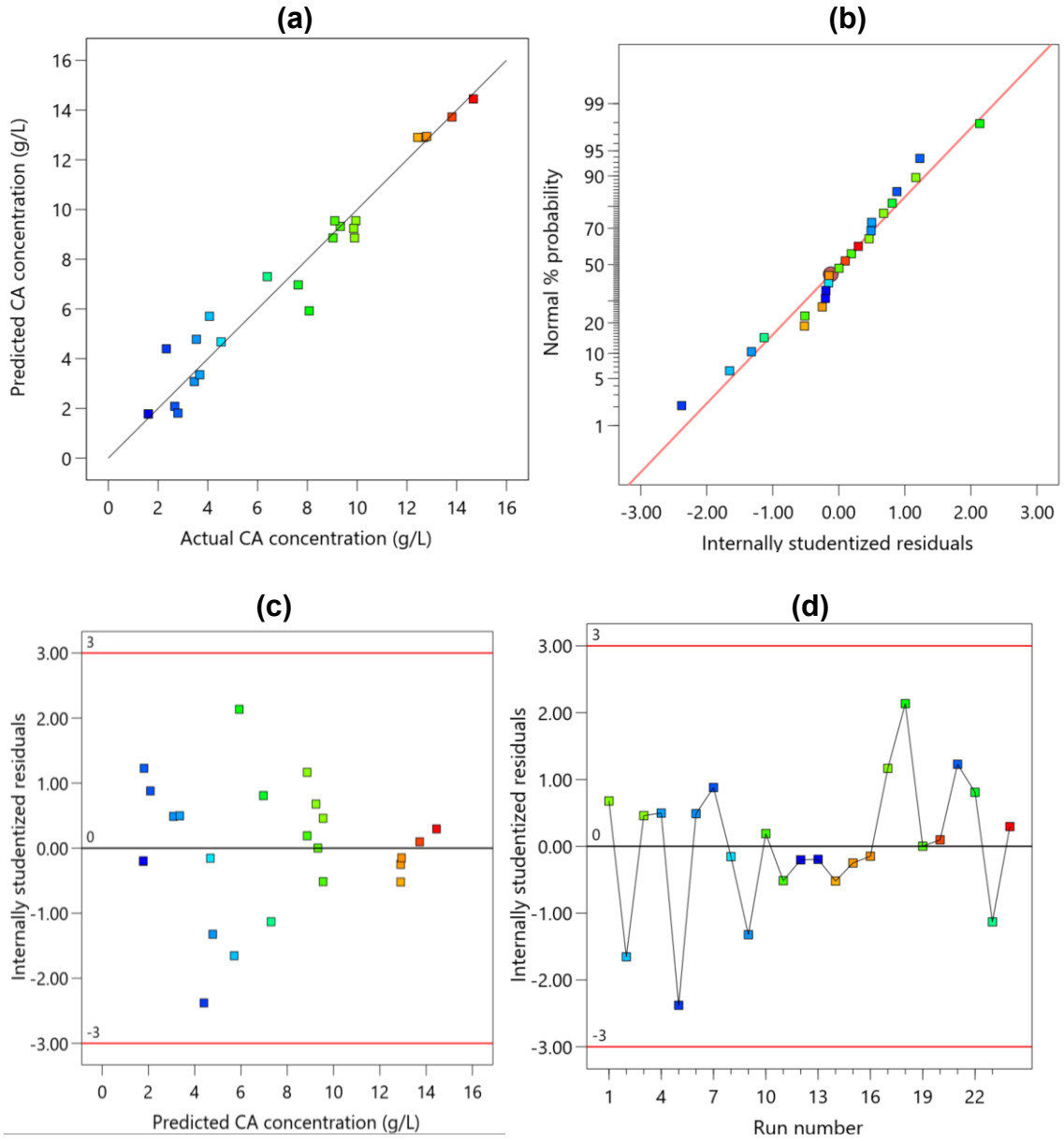

Fig. 1. Diagnostic plots for the mathematical model for the citric acid production process

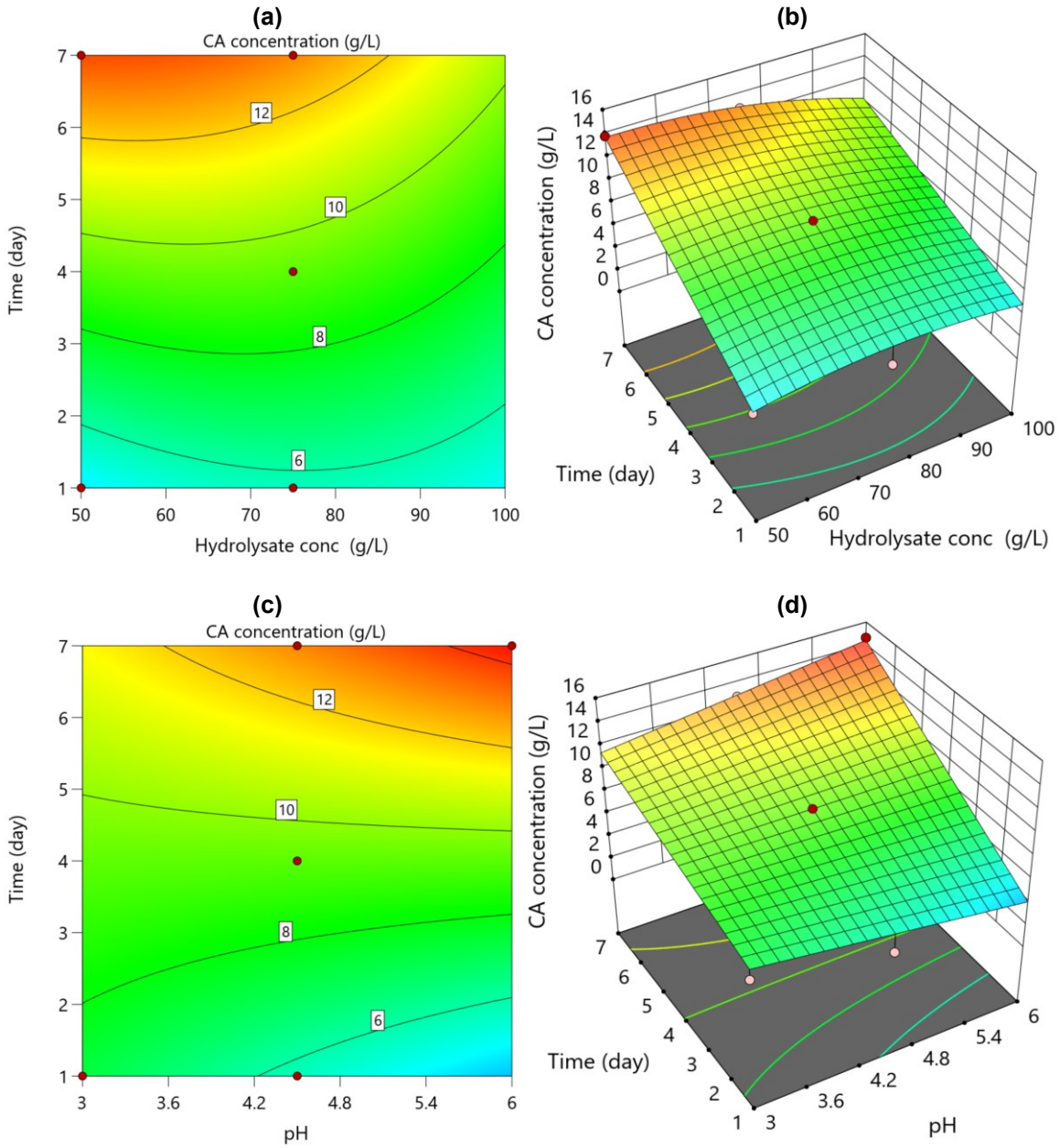

Fig. 2. Contour and surface plots of time, hydrolysate concentration, and pH on citric acid production using $(NH_4)_2HPO_4$ as the nitrogen source

The maximum CA concentration of approximately 12 g/L was observed at a hydrolysate concentration of 50 g/L and 7 days of fermentation time. Thus, the lower the hydrolysate concentration and the longer the fermentation time, the higher was the CA concentration. The observations reported when hydrolysate from sweet potato starch and peel were used for CA production modeling differ (Betiku and Adesina 2013; Oyeniran *et al*. 2013), which may be attributed to the carbon source.

Figures 2c and 2d show the contour and response surface plots representing the interactive effect of the fermentation time and medium pH on CA concentration using $(NH_4)_2HPO_4$ as a nitrogen source while keeping hydrolysate concentration constant. The figures show that the CA concentration increased as the fermentation time and medium pH increased (Figs. 2c and 2d). The maximum CA of about 12 g/L was observed at medium

pH in 6 and 7 days of fermentation (Fig. 2b). Thus, the higher the medium pH and the longer the fermentation time, the higher was the CA concentration.

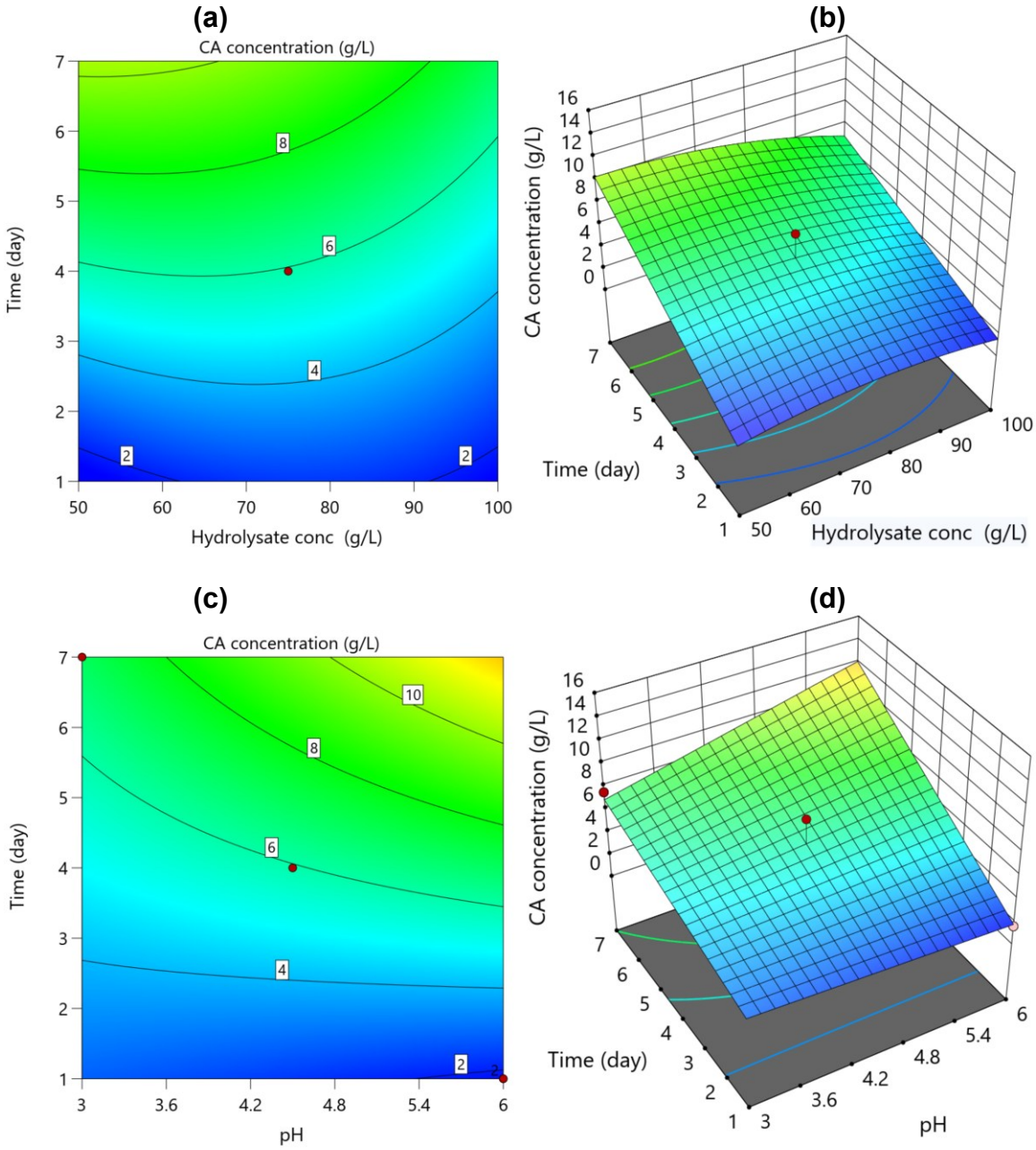

Fig. 3. Contour and surface plots of time, hydrolysate concentration, and pH on citric acid production using $NaNO_3$ as the nitrogen source

Figures 3a and 3b show the contour and response surface plots representing the interactive effect of the fermentation time and hydrolysate concentration on CA concentration using $NaNO_3$ as a nitrogen source while keeping medium pH constant. The figures followed the same pattern as those obtained for $(NH_4)_2HPO_4$ as nitrogen source (Figs. 3a and 3b), only that the CA concentration decreased to 8 g/L was observed at hydrolysate concentration of 50 g/L and 7 days of fermentation time (Fig. 3b). Thus, the lower the hydrolysate concentration and the longer the fermentation time, the higher the

CA concentration. Figures 3c and 3d show the contour and response surface plots representing the interactive effect of the fermentation time and medium pH on CA concentration using $NaNO_3$ as a nitrogen source while keeping hydrolysate concentration constant. Similar to what was observed when $(NH_4)_2HPO_4$ was used as a nitrogen source, the figures show that the CA concentration increased as the fermentation time and medium pH increased (Figs. 3c and 3d). The maximum CA of 10 g/L was observed at medium pH of 6 and 7 days of fermentation (Figure 3b). Thus, the higher the medium pH and the longer the fermentation time, the higher the CA concentration. The observations reported when hydrolysate from sweet potato starch and peel were used for CA production modeling were different (Betiku and Adesina 2013; Oyeniran *et al*. 2013). The maximum CA concentration was obtained at a medium pH of 7 and fermentation time of 4 and 7 days, respectively.

Optimization and model validation for citric acid production

The optimal values of the independent factors chosen for the fermentation process were obtained by solving the regression equation (Eq. 2a) using the Design-expert software package. The optimal conditions were statistically predicted as hydrolysate concentration of 50 g/L, medium pH of 5.4, $(NH_4)_2HPO_4$ as nitrogen source, and 7 days of fermentation time. The predicted CA concentration under the conditions was 14.7 g/L (Fig. 4). The optimal conditions were applied to three independent experimental replicates to verify the model prediction, and the average CA concentration obtained was 14.7 g/L, which confirms the predicted value by the model.

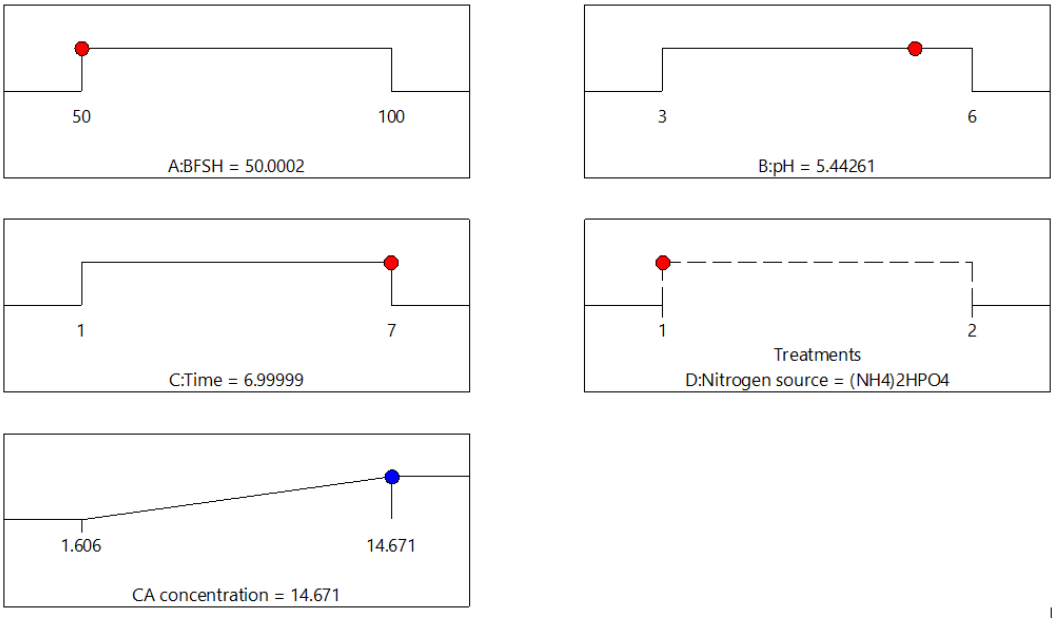

Fig. 4. Model predicted optimal conditions for maximum citric acid production

Table 5 compares optimal conditions reported in the literature with the current study. For instance, the optimal conditions established when sweet potato starch was used were a hydrolysate concentration of 154 g/L, medium pH of 6, KH_2PO_4 of 2.58 g/L, $(NH_4)_2HPO_4$ of 3.55 g/L, and fermentation time of 8 days, with a CA concentration of 83 g/L (Betiku and Adesina 2013).

Table 5. Optimization Parameters for Citric Acid Production by *A. niger*

Design and Modeling Tools	Fermentation Type	Substrate Source	Substrate Level (g/L)	Inoculum Size (%)	pH	Temperature (°C)	Fermentation Time (day)	Predicted Citric Acid (g/L)	Experimental CA (g/L)	Reference
OFAT	Submerge	Algerian dates (GHARS and MECH DEGLA)	85% and 80.47%	5	3	30	8	-	42.25 ± 0.91 and 36.6 ± 1.27	Chergui *et al*. (2021)
RSM	Submerge	Cassava peel-malted sorghum blend	5	6	4.5	30 ± 2°C	3.5	78.70	88.73	Adeoye *et al*. (2015)
CCD and RSM	Surface	Sweet potato starch	153.77	5	6	-	8	83.03	83.00	Betiku and Adesina (2013)
BBD and RSM	Surface	Sweet potato peel	150	5	6	-	3.61	15.97	15.98	Oyeniran *et al*. (2013)
Simplex mixture design and RSM	Submerge	Sweet potato peel	97.25%	-	6.5	-	7	32.2	43.6	Aboyeji *et al*. (2020)
Full factorial face-centered CCD and RSM	Surface	White grape pomace	100	1×10^4 spores/mL	5.2	30	15	87.8	85.4 ± 3.2	Papadaki and Mantzouridou (2019)
CCD and RSM	Submerge	Beet molasses	274.4	10^7 spores/mL	4	31.5	6.33	88.6%	87.81%	Lofty et al. (2007)
D-Optimal design and RSM	Surface	Breadfruit starch	50	5	5.4	30	7	14.671	14.67	Current study

The optimal conditions obtained when sweet potato peel was used were a hydrolysate concentration of 150 g/L, fermentation time of 3.61 days, and methanol concentration of 3% (volume), with a CA concentration of 16.0 g/L (Oyeniran *et al*. 2013).

When two Algerian date varieties were used, the optimal conditions observed were temperature of 30 °C, medium pH of 3, temperature of 30 °C, and fermentation time of 8 days, with a citric acid concentration of 42.25 ± 0.91 and 36.6 ± 1.27 g/L for GHARS and MECH DEGLA, respectively. The media were supplemented with 4% methanol (Chergui *et al*. 2021). The medium pH and fermentation time obtained in this study were 5.4 and 7 days, respectively, compared to ranges of 3 to 6.5 and 3.5 to 15 days reported in the literature. The lower citric acid yield obtained in the current study may be due to the surface fermentation used compared to submerged fermentation and the low substrate level. Substrate levels from other studies in Table 5 used over 100 g/L compared to the 50 g/L in the current study. Fermentation time, pH, and inoculum size used in the current study were similar to literature values and did not adversely affect the citric acid yield (Table 5).

CONCLUSIONS

This study investigated the possible use of reducing sugars from breadfruit starch hydrolysate as the sole carbon source for citric acid biosynthesis by *A*. *niger* under surface fermentation conditions. The process was modeled and optimized using D-Optimal design, which assessed the impact of four critical input variables: hydrolysate concentration, medium pH, nitrogen source, and fermentation time. The results obtained demonstrated the feasibility of producing citric acid using *A*. *niger* which effectively utilized the BSH to produce CA. Optimal fermentation conditions were identified as a hydrolysate concentration of 50 g/L, medium pH of 5.4, $(NH_4)_2HPO_4$ as the nitrogen source, and a fermentation duration of 7 days. Under these conditions, the process achieved a CA concentration of 14.7 g/L, demonstrating the efficiency and potential of this approach for CA biosynthesis. The results obtained in this work showed that RSM with appropriate experimental design can be effectively applied to model and optimize process variables in CA biosynthesis using *A*. *niger* and breadfruit. This study has provided valuable information regarding developing inexpensive and efficient fermentation processes.

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