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RESPONSE SURFACE OPTIMIZATION OF FERMENTATION CONDITIONS FOR CASTOR SEED (*RICINUS COMMUNIS*) BASED ON NITROGEN EVOLUTION

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ABSTRACT

Castor seed (Ricinus communis) is an oil-rich seed used in southeastern Nigeria for the production of fermented condiments such as ogiri. Because traditional fermentation is carried out under variable processing conditions, quantitative information on the combined effects of fermentation time, temperature and particle size is useful for process control. This study investigated the effects of fermentation time, temperature and particle size on nitrogen evolution during castor-seed fermentation and applied response surface methodology (RSM) to determine an optimum operating condition. Matured castor seeds obtained from Afor-Opi, Nsukka Local Government Area, Enugu State, Nigeria, were boiled, dehulled, oven-dried at 35°C, grated and sieved into particle sizes of 250–1200 µm. Fifty-gram portions were fermented at 25–45°C, with nitrogen evolution monitored at 24-h intervals for up to 120 h. A randomized three-factor design generated 20 experimental combinations. The fitted quadratic model was highly significant ($F = 112.55$, $p < 0.0001$) and explained 99.02% of the observed variation in nitrogen evolution ($R^2 = 0.9902$). The adjusted and predicted R^2 values were 0.9814 and 0.9212, respectively, and adequate precision was 41.206. Fermentation time, temperature, particle size, the time–temperature interaction and the quadratic time term were significant model terms. Numerical optimization selected 113.6 h, 43.67°C and 313.33 µm as the preferred combination, with a predicted nitrogen evolution of 0.984% and desirability of 1.000. The result demonstrates that the three operating variables can be jointly controlled to improve the predictability of castor-seed fermentation. Experimental confirmation of the predicted optimum is recommended before scale-up.

Keywords: Ricinus Communis; Castor Seed; Ogiri; Fermentation; Nitrogen Evolution; Response Surface Methodology; Process Optimization; Particle Size

1 INTRODUCTION

Castor seed (Ricinus communis) is an oil-rich crop with established industrial uses and a place in traditional food processing in southeastern Nigeria. Fermented castor seed is used to produce ogiri, an alkaline fermented condiment valued for its characteristic flavour and use in soups and sauces. Recent reviews continue to identify castor seed among the underutilized seeds used for traditional fermented condiments in Nigeria (Oladeji et al., 2025).

The food-processing use of castor seed requires appropriate pretreatment and controlled processing because the raw seed contains toxic constituents and is not consumed directly. Contemporary reviews describe boiling, dehulling and subsequent fermentation as important stages in traditional ogiri production, with processing conditions varying among communities (Oladeji et al., 2025; Singh et al., 2024).

Recent experimental studies have continued to examine castor-seed fermentation. Onyekwelu and Uzodinma (2024) evaluated proximate composition, amino-acid profile and acceptability of castor–moringa seed ogiri, demonstrating the continued interest in castor seed as a substrate for fermented condiment production. Nweze et al. (2023) investigated starter-culture production of ogiri from castor oil seed and reported the involvement of bacterial cultures in laboratory and traditional fermentation. Agbo (2024) also compared the nutritional and mineral characteristics of fermented castor seed with other Nigerian fermented seeds.

Although these studies provide useful information on the nutritional, sensory and microbiological characteristics of fermented castor seed, the specific combined effect of fermentation time, temperature and particle size on a quantitative fermentation response remains less developed. In particular, optimization of particle size together with time and temperature provides a process-engineering perspective that is not addressed by studies focused mainly on composition or starter cultures.

Response surface methodology provides a practical framework for examining several interacting process variables simultaneously and identifying operating conditions that maximize or minimize a defined response. In the present study, nitrogen evolution was used as the response variable, and fermentation time, temperature and particle size were treated as controllable factors. The objective was to develop a quadratic predictive model for castor-seed fermentation and determine the factor combination associated with maximum predicted nitrogen evolution.

2 LITERATURE REVIEW

Ogiri is a traditional Nigerian fermented condiment that can be produced from castor oil seeds (Ricinus communis), among other oil-rich seeds. The fermentation is generally an alkaline process and is associated with changes in microbial populations, pH, nutrient composition and flavour development. A recent review of Nigerian fermented condiments describes castor seed as one of the underutilized substrates used for ogiri and highlights variation in traditional processing practices (Oladeji et al., 2025).

Recent work also confirms continued research interest in castor-based ogiri. Nweze et al. (2023) reported the production of ogiri from castor oil seeds using bacterial starter cultures and observed differences among fermentation approaches. Onyekwelu and Uzodinma (2024) reported that fermentation of castor–moringa blends affected proximate composition and amino-acid characteristics, while Agbo (2024) documented nutritional and mineral characteristics of fermented Ricinus communis seed obtained in southeastern Nigeria.

Fermentation performance depends on the interaction of several factors, including substrate preparation, temperature, duration, moisture and the available microbial population. Recent reviews emphasize that many Nigerian fermented condiments are still produced by relatively uncontrolled processes, creating variability in product quality and limiting reproducibility (Oladeji et al., 2025). Controlled manipulation of process variables therefore offers a route toward more consistent fermentation.

Particle size is particularly relevant to solid-substrate fermentation because reducing particle size changes exposed surface area and can influence moisture distribution and microbial access to the substrate. In the present dataset, the response generally declined as particle size increased, supporting the importance of including particle size as a process variable rather than treating it only as a preprocessing detail.

The recent literature on castor-seed ogiri has concentrated largely on nutritional composition, amino-acid profiles, sensory quality, microbiological characteristics and starter-culture performance (Nweze et al., 2023; Onyekwelu &

Uzodinma, 2024; Agbo, 2024). These studies do not directly provide an RSM-based optimization of fermentation time, temperature and particle size using nitrogen evolution as the response. The present work addresses this process-optimization gap.

3 MATERIAL AND METHODS

3.1 Raw material

Matured castor seeds were purchased from Afor-Opi in Nsukka Local Government Area of Enugu State, Nigeria. The original study processed the seeds together with the other local seed materials used in the broader investigation. For this paper, only the castor-seed dataset is considered.

Figure 1: Matured castor seeds used as the raw material in the study.

3.2 Seed preparation and fermentation

The seeds were boiled for four hours, after which the seed coats were removed. The processed material was dried in an oven at 35°C, grated using a blender and separated into particle-size fractions of 250, 500, 750, 900 and 1200 µm. Fifty-gram portions of the different particle sizes were wrapped in muslin material, heated in water at room temperature for 10 min, and transferred to fermentation chambers. Fermentation was carried out at 25, 30, 35, 40 and 45°C for up to five days, with nitrogen measurements taken at 24-h intervals. Nitrogen content was determined using a **Kjeldahl-based method**.

3.3 Experimental design and RSM

Response surface methodology was used to examine fermentation time (A), temperature (B) and particle size (C) as the independent variables and nitrogen evolution as the response. The Design-Expert trial version was used to generate a randomized three-factor design containing 20 experimental combinations. The factors were represented using coded levels from −2 to +2, and a quadratic model was fitted to the response.

Table 1: Experimental Factors and Coded Levels Used in the Response Surface Methodology (RSM) Design

Factor	**Symbol**	**Unit**	**−2**	**0**	**+2**
Fermentation time	A	h	24	72	120
Temperature	B	°C	25	35	45
Particle size	C	µm	250	725	1200

The quadratic model was evaluated using analysis of variance (ANOVA). Model adequacy was assessed using the coefficient of determination (R^2), adjusted R^2, predicted R^2, coefficient of variation and adequate precision. Numerical optimization was then used to identify the factor combination giving the highest predicted nitrogen evolution within the experimental region.

4 RESULTS AND DISCUSSION

4.1 Experimental response

The experimental data showed a clear increase in nitrogen evolution with increasing fermentation time and temperature, while the response generally decreased with increasing particle size. At 24 h, the castor-seed response at 25°C declined from 0.15% at 250 μm to 0.06% at 1200 μm. At 120 h and 45°C, the corresponding values increased to 1.03% and 0.76%, respectively. These data indicate that prolonged fermentation and higher incubation temperature promoted the measured response within the tested range, whereas larger particles were associated with lower nitrogen evolution.

4.2 Selected experimental observations

Table 2: Selected castor-seed nitrogen-evolution observations extracted from the experimental dataset.

Time (h)	Temp. (°C)	250 μm	500 μm	750 μm	900 μm	1200 μm
24	25	0.15	0.13	0.09	0.08	0.06
24	45	0.25	0.19	0.15	0.13	0.09
72	35	0.73	0.66	0.55	0.50	0.42
96	45	0.95	0.87	0.82	0.79	0.72
120	25	0.59	0.56	0.50	0.46	0.44
120	45	1.03	0.91	0.85	0.81	0.76

4.3 Quadratic model and ANOVA

The fitted quadratic model was highly significant ($F = 112.55$, $p < 0.0001$), indicating that the combined process variables explained a substantial proportion of the observed response variation. Fermentation time, temperature and particle size were individually significant, as was the time × temperature interaction. The time × particle-size interaction and the temperature × particle-size interaction were not significant at the 5% level, while the quadratic term for time was significant.

Table 3: ANOVA for the quadratic castor-seed nitrogen-evolution model.

Model term	F-value	p-value	Interpretation
Model	112.55	<0.0001	Significant
Time (A)	681.38	<0.0001	Significant
Temperature (B)	120.80	<0.0001	Significant
Particle size (C)	80.57	<0.0001	Significant
A × B	41.62	<0.0001	Significant
A × C	3.03	0.1123	Not significant
B × C	3.79	0.0803	Not significant
A^2	52.22	<0.0001	Significant
B^2	0.0477	0.8316	Not significant
C^2	0.4881	0.5007	Not significant

Table 4: Fit statistics for the castor-seed quadratic model.

Model statistic	Value
R^2	0.9902
Adjusted R^2	0.9814
Predicted R^2	0.9212
Standard deviation	0.0345
Mean response	0.4885
Coefficient of variation	7.07%
Adequate precision	41.2062

The model explained 99.02% of the observed variation in nitrogen evolution. The predicted R^2 of 0.9212 was reasonably consistent with the adjusted R^2 of 0.9814, and the adequate precision of 41.206 was well above the commonly used minimum of 4. These statistics indicate a strong model signal within the studied design space, although they do not replace independent experimental validation.

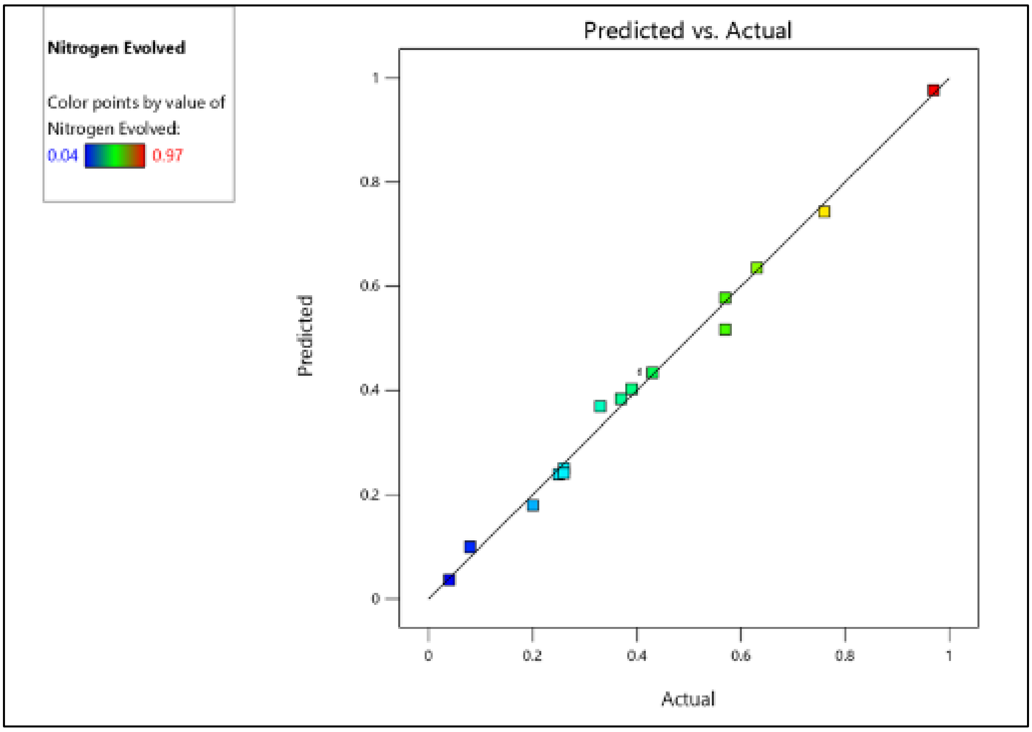

Figure 2: Predicted versus actual nitrogen-evolution response for the castor-seed model.

4.4 Regression equation

In coded variables, the fitted quadratic equation was:

$$Y = 0.5542 + 0.2850A + 0.1200B - 0.0980C + 0.0787AB - 0.0213AC - 0.0238BC - 0.1505A^2 + 0.0045B^2 + 0.0145C^2$$

where Y is nitrogen evolution, A is fermentation time, B is temperature and C is particle size in coded form. The positive coefficient for time was the largest linear coefficient, followed by temperature, while particle size had a negative coefficient. This pattern is consistent with the experimental data: longer fermentation and higher temperature increased the response, whereas larger particle size reduced it.

In actual-factor units, the model was:

$Y = -0.154783 + 0.010274\text{Time} + 0.000631\text{Temperature} - 0.000058\text{Particle Size} + 0.000164\text{Time}\times\text{Temperature} - 9.32018\times10^{-7}\text{Time}\times\text{Particle Size} - 5.00000\times10^{-6}\text{Temperature}\times\text{Particle Size} - 0.000065\text{Time}^2 + 0.000045\text{Temperature}^2 + 6.44674\times10^{-8}\text{Particle Size}^2$

4.5 Response-surface effects

The response surfaces illustrate how the three variables interacted within the experimental region. The time–temperature surface shows a strong upward response toward longer fermentation times and higher temperatures. The time–particle-size surface shows higher nitrogen evolution at longer times and smaller particle sizes. The temperature–particle-size surface similarly indicates higher response values at elevated temperatures and reduced particle size.

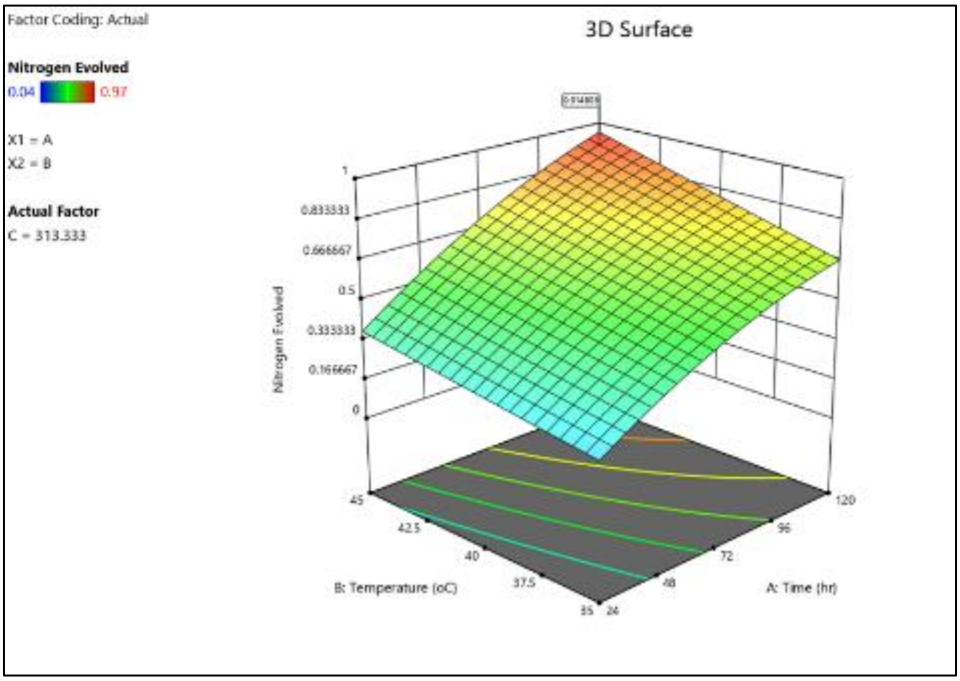

Figure 3: Response surface showing the combined effect of fermentation time and temperature on nitrogen evolution at a particle size of 313.33 µm.

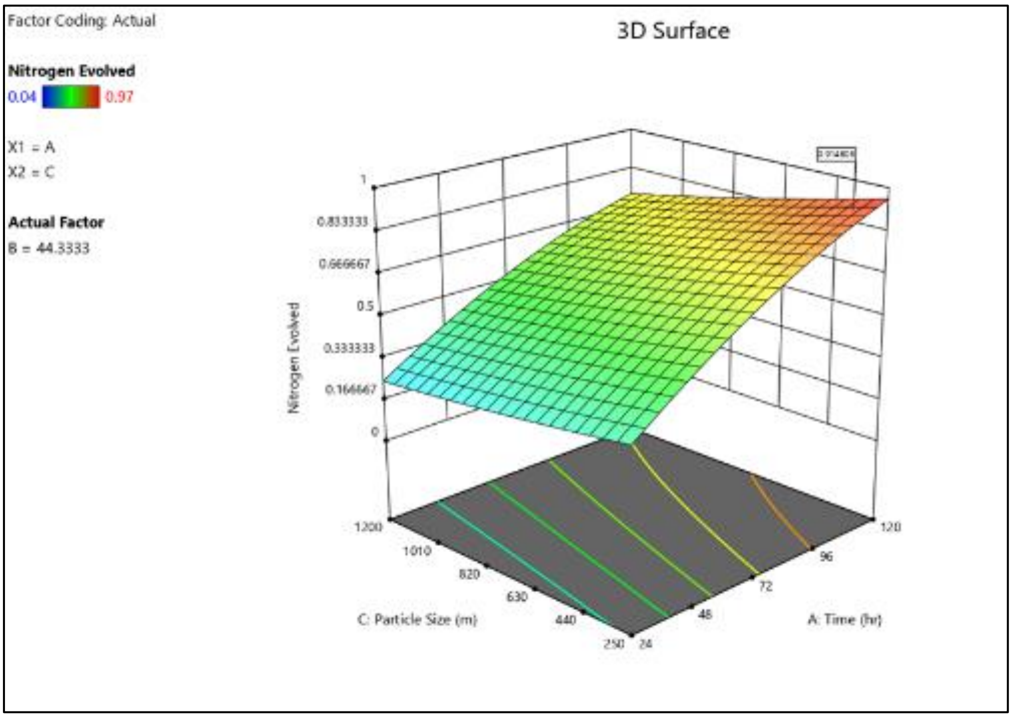

Figure 4: Response surface showing the combined effect of fermentation time and particle size on nitrogen evolution at 44.33°C.

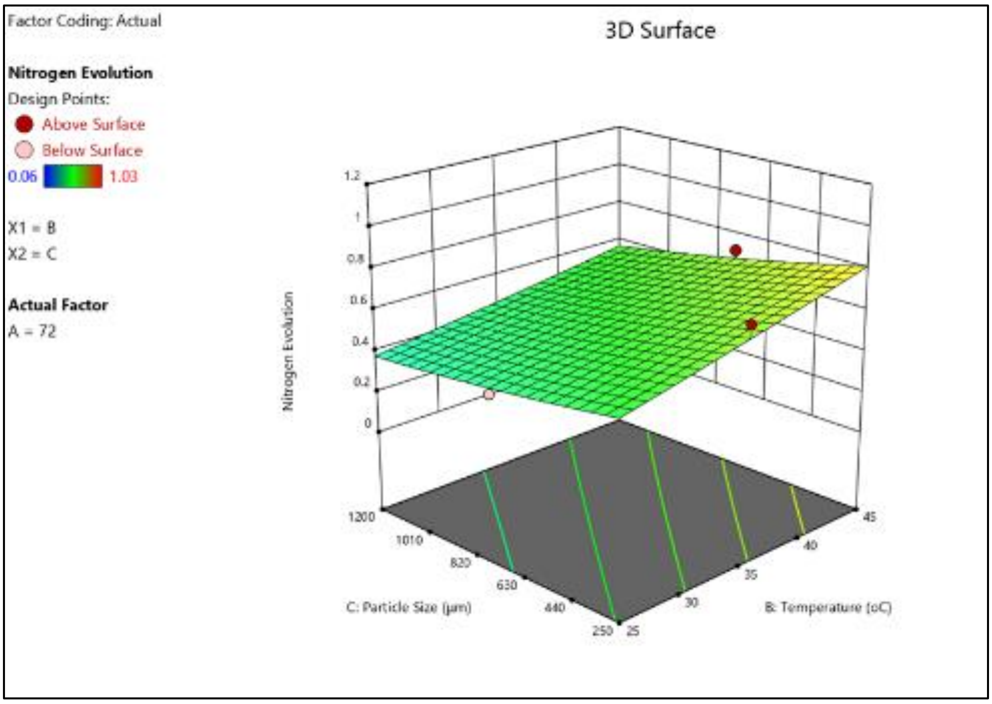

Figure 5: Response surface showing the combined effect of temperature and particle size on nitrogen evolution at 72 h.

4.6 One-factor effects

The one-factor plots provide a clearer view of the direction of the response when each variable is changed while the other factors are held at the specified values. Nitrogen evolution increased nonlinearly with fermentation time, increased with temperature across the tested range, and decreased as particle size increased. The particle-size effect is consistent with the larger exposed surface area expected from finer material, although the present study did not directly measure surface area, moisture transfer or microbial growth.

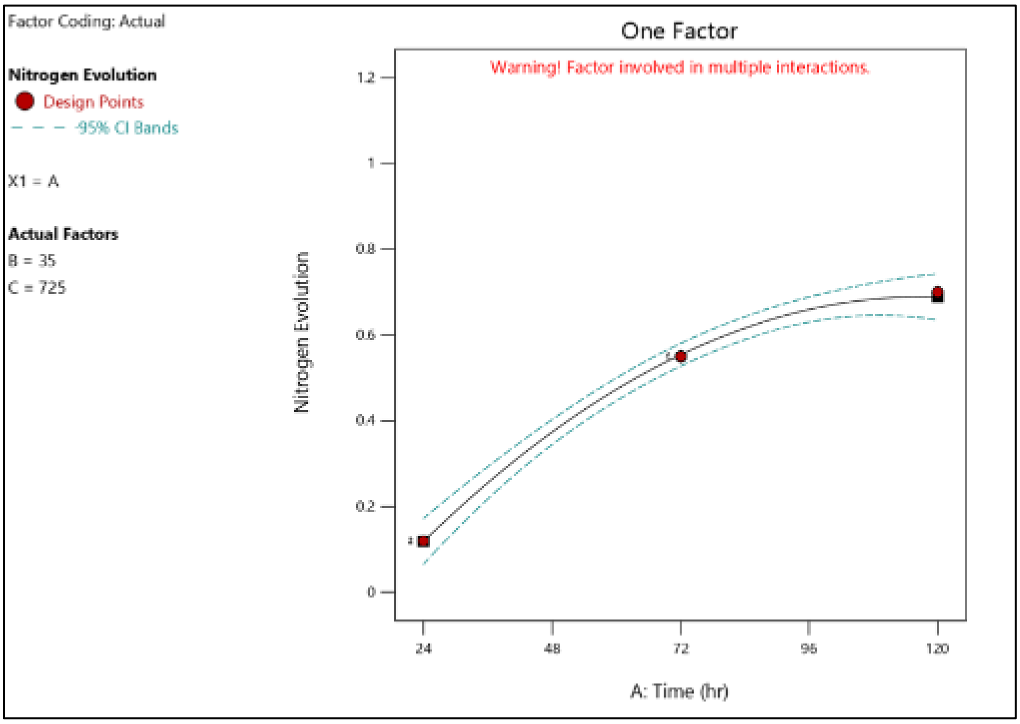

Figure 6: One-factor relationship between fermentation time and nitrogen evolution for castor seed.

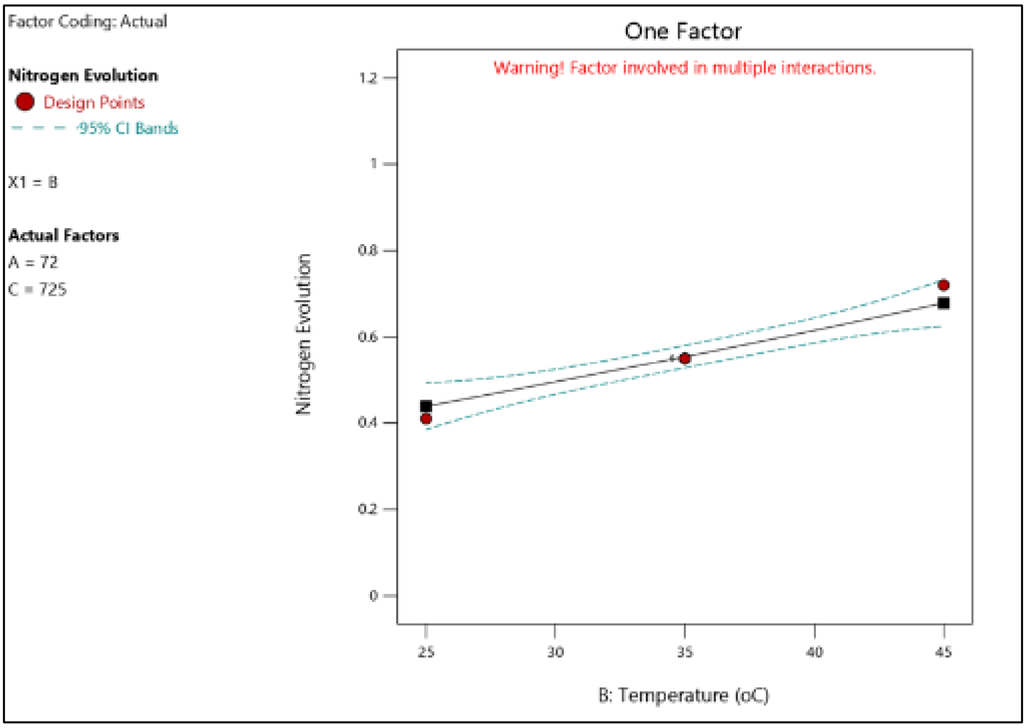

Figure 7: One-factor relationship between temperature and nitrogen evolution for castor seed.

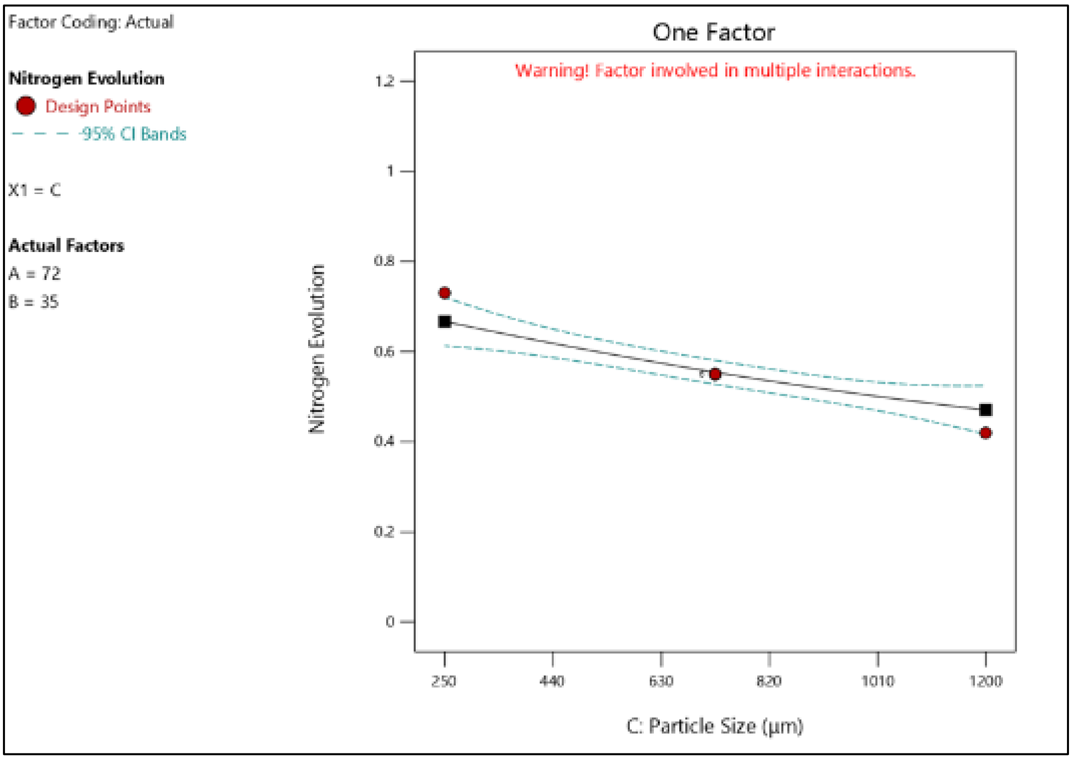

Figure 8: One-factor relationship between particle size and nitrogen evolution for castor seed.

4.7 Numerical optimization

Numerical optimization was used to maximize predicted nitrogen evolution within the experimental design space. The selected solution was 113.6 h fermentation time, 43.67°C temperature and 313.33 µm particle size. The corresponding predicted nitrogen evolution was 0.984%, with a desirability of 1.000. The selected values are within the tested ranges and therefore represent interpolation within the experimental design space rather than extrapolation.

Table 5: Numerical optimization of castor-seed fermentation.

Optimization parameter	Selected optimum
Fermentation time	113.6 h
Temperature	43.67°C
Particle size	313.33 µm
Predicted nitrogen evolution	0.984%
Desirability	1.000

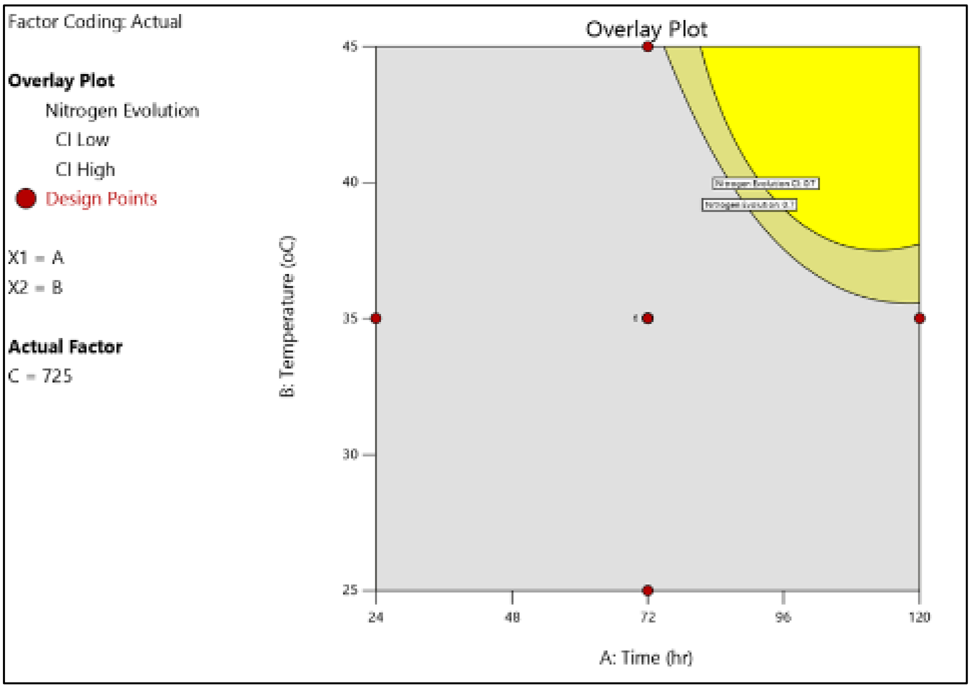

Figure 9: Overlay plot showing the castor-seed fermentation design space.

5 DISCUSSION

The Castor seed results demonstrate that fermentation time was the dominant linear contributor to nitrogen evolution, followed by temperature, while particle size exerted a negative effect. The significance of the time × temperature interaction indicates that the influence of temperature was not independent of fermentation duration. This is consistent with the general behaviour of alkaline seed fermentation, in which microbial and enzymatic activity changes as fermentation proceeds and substrate conditions evolve.

The observed increase in nitrogen evolution with fermentation time agrees with the broader literature on fermented castor-seed condiments. Lawrence and Mmadubuike (2023) reported progressive changes in protein and amino-acid composition during fermentation of castor-seed ogiri, while Nweze et al. (2023) demonstrated that bacterial starter cultures can influence castor-seed ogiri production. These studies, however, focused primarily on product composition and microbiological performance rather than simultaneous optimization of time, temperature and particle size.

The negative particle-size coefficient is also important from a process-engineering perspective. Finer particles expose more substrate surface and may facilitate microbial access and mass transfer. The present experiment supports this interpretation through the consistent decline in nitrogen evolution as particle size increased. Nevertheless, because the study did not independently measure surface area, porosity or moisture distribution, the mechanism should be treated as a plausible process explanation rather than a directly demonstrated causal pathway.

The optimized condition of 113.6 h, 43.67°C and 313.33 µm falls near the upper end of the investigated time and temperature ranges and toward the finer end of the particle-size range. This combination provides a quantitative operating target for further experimental testing. Importantly, the value is model-predicted; an independent fermentation run under the predicted conditions is needed to establish the prediction error before the condition is recommended for scale-up.

6 CONCLUSION

This study developed a response-surface model for nitrogen evolution during castor-seed (Ricinus communis) fermentation using fermentation time, temperature and particle size as process variables. The quadratic model was highly significant and explained 99.02% of the variation in the measured response. Time, temperature and particle size were significant main effects, while the time × temperature interaction and the quadratic time term were also significant. Numerical optimization identified 113.6 h, 43.67°C and 313.33 µm as the preferred operating condition, with a predicted nitrogen evolution of 0.984% and desirability of 1.000. The result provides a process-engineering basis for improving the reproducibility of castor-seed fermentation, but independent experimental validation is required before scale-up.

Recommendations

- The predicted optimum of 113.6 h, 43.67°C and 313.33 µm should be validated experimentally using independent fermentation batches.
- Future work should measure pH, moisture, microbial population and relevant enzyme activities alongside nitrogen evolution to explain the mechanisms behind the observed response.
- The fermentation process should be evaluated under controlled hygienic conditions to improve reproducibility and product safety.
- Further studies should examine the relationship between particle size, moisture transfer and microbial access to determine whether an even finer or intermediate particle size provides practical advantages.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

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