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Response Surface Optimization of Fermentation Conditions for Mesquite Seed (Prosopis Africana) Based on Nitrogen Evolution

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Abstract- Mesquite seed (Prosopis africana) is an underutilized tropical seed with nutritional and economic potential and is traditionally processed into fermented condiments. Because traditional fermentation is carried out under different local conditions, the progress and outcome of the process can vary. This study examined fermentation time, temperature and particle size as controllable factors and used response surface methodology (RSM) to relate them to nitrogen evolution. Matured mesquite seeds obtained from Afor-Opi, Nsukka Local Government Area, Enugu State, Nigeria, were boiled, decarped, oven-dried at 35°C, grated and sieved into particle sizes of 250–1200 µm. Fifty-gram portions were fermented at 25–45°C for up to 120 h, and nitrogen evolution was monitored at 24-h intervals. The RSM design produced 20 randomized experimental combinations for the mesquite seed. The resulting quadratic model was significant ($F = 153.12$, $p < 0.0001$) and accounted for 99.28% of the variation in nitrogen evolution ($R^2 = 0.9928$). The adjusted R^2 and predicted R^2 were 0.9863 and 0.9456, respectively, while adequate precision was 52.011. Fermentation time, temperature, particle size, the time–temperature interaction, the time–particle-size interaction and the quadratic time term were significant. Numerical optimization gave 113.6 h, 44.33°C and 313.33 µm as the best combination within the experimental region, with a predicted nitrogen evolution of 0.915%. The result indicates that the three operating variables can be used together to control the fermentation response. The predicted condition should, however, be confirmed experimentally before it is considered for scale-up.

Keywords— Prosopis africana; mesquite seed; fermentation; nitrogen evolution; response surface methodology; process optimization; particle size.

I. INTRODUCTION

Mesquite seed (Prosopis africana) is available in many parts of Nigeria but remains less utilized than several better-known food crops. The seed is used after processing and fermentation and forms part of traditional food practices and small-scale trading activities. Aremu et al. (2015) reported that processing and fermentation influence the nutritional composition and amino-acid profile of P. africana seed, supporting its potential as a locally available food resource.

Fermentation is one of the established ways of processing P. africana seeds into indigenous condiments such as Okpeye. Recent reviews describe Okpeye as a spontaneously fermented, solid-substrate food condiment and emphasize the influence of fermentation on its nutritional characteristics, consistency and microbiological quality (Akpi et al., 2023). Recent experimental work has also shown that fermentation period significantly affects the nutrient and anti-nutrient characteristics of Okpehe seed, confirming fermentation duration as an important process variable (Uche & Umenne, 2022).

In practice, the fermentation process is not always carried out under closely controlled conditions. Differences in fermentation time, temperature, particle size, moisture, wrapping material and the organisms present can all affect the process. For a process intended to move from household or small-scale production toward more consistent production, these variables need to be better defined. A quantitative relationship between operating conditions and fermentation response would also make process control easier.

RSM was selected because it allows several process variables to be studied at the same time and, importantly, allows their combined effects to be examined. This is useful in fermentation because changing one variable can alter the effect of another. RSM has been used successfully to optimize fermentation time and temperature in other food systems (Adebo et al., 2018). Previous studies have examined the traditional fermentation of P. africana, its biochemical changes and its nutritional characteristics, while more recent work has applied RSM to P. africana using factors such as fermentation duration, inoculum concentration, pH and temperature (Asiru & Musbau, 2025). The present work takes a somewhat different process-engineering approach by considering fermentation time, temperature and particle size together and using nitrogen evolution as the measured response. The objective of this study was to develop a quadratic response-surface model for nitrogen evolution during mesquite-seed fermentation and to identify the combination of fermentation time, temperature and particle size that gives the highest predicted response within the experimental range.

II. LITERATURE REVIEW

P. africana is a lesser-known legume, but its seeds have a long history of use in fermented food products. Aremu et al. (2015) reported changes in the proximate, mineral and amino-acid composition of mesquite seed following processing and fermentation, including improvements in several nutritional indices. Other Nigerian work has also shown that the duration of fermentation affects nutrient and anti-nutrient characteristics of Okpehe (Uche & Umenne, 2022). Taken together, these findings support the need to pay attention to fermentation conditions when the seed is being processed for food use.

Recent literature describes Okpeye as a traditional spontaneously fermented condiment produced from P. africana seeds, with fermentation contributing to important nutritional and biochemical changes (Akpi et al., 2023). Ezeocha et al. (2022) further demonstrated that indigenous processing conditions, including fermentation conditions, affect the proximate composition, antinutrient levels and volatile compounds of the finished seasoning. These findings illustrate why fermentation time should be treated as a process variable rather than simply extended without control.

Fermentation time determines how long microorganisms and their enzymes act on the substrate. As fermentation proceeds, microbial growth, hydrolysis of substrate components and formation or release of metabolites change with time. Uche and Umenne (2022) demonstrated that changing fermentation periods produced significant differences in the nutrient and anti-nutrient characteristics of Okpehe seed. The appropriate fermentation time is consequently a balance between allowing the desired biochemical changes to occur and avoiding an unnecessarily long process. Temperature has a direct influence on microbial activity and on the rates of biochemical reactions occurring during fermentation. Particle size is also important because reducing the particle size increases exposed surface area and can improve contact between the substrate, microorganisms and enzymes. The interaction between process variables is one reason an experimental design that includes interaction terms is useful for fermentation optimization (Adebo et al., 2018).

Previous studies provide useful information on the nutritional and biochemical changes associated with P. africana fermentation, and RSM has already been shown to be useful for fermentation optimization (Adebo et al., 2018). Asiru and Musbau (2025) recently optimized P. africana fermentation using duration, inoculum concentration, pH and

temperature. The present study adds a process-control perspective by modelling fermentation time, temperature and particle size simultaneously against nitrogen evolution. The fitted response surface can therefore be used to examine how the operating variables work together and to locate a favorable operating point within the tested region.

III. MATERIALS AND METHODS

1. Materials and sample procurement

Matured mesquite seeds were purchased from Afor-Opi in Nsukka Local Government Area of Enugu State, Nigeria. The samples were taken to the laboratory and processed using the procedure described below before fermentation.

Figure 1. Mesquite seed material used in the study.

2. Seed preparation

The seeds were first boiled for four hours. They were then decarped and dried in an oven at 35°C. After drying, the material was grated with a blender and passed through sieves to obtain particle sizes of 250, 500, 750, 900 and 1200 µm.

3. Fermentation procedure

For each particle size, 50 g of the dried mesquite seed was wrapped in muslin sieve material and heated in water at room temperature for 10 min. The samples were then placed in fermentation chambers set at 25, 30, 35, 40 or 45°C. Fermentation was followed for 120 h, and measurements were taken every 24 h.

4. Determination of nitrogen evolution

Nitrogen content during fermentation was determined by the Kjeldahl method. The nitrogen-evolution value obtained from the analysis was used as the response variable in the response-surface analysis.

Important submission note: the supplied research document refers to a Kjeldahl calculation equation, but the equation itself is not present in the available text. Before submission, the exact equation, reagent concentrations, digestion and distillation conditions, and reporting basis should be confirmed from the laboratory record. This information is needed for another researcher to reproduce the analysis.

5. Experimental design and statistical analysis

Response surface methodology was used to study the effects of fermentation time (A), temperature (B) and particle size (C) on nitrogen evolution. The experimental ranges were 24–120 h, 25–45°C and 250–1200 µm, respectively. Design-Expert was used to generate a randomized three-factor design containing 20 experimental combinations. The factors were represented in coded form from −2 to +2, and a quadratic model was fitted to the response.

Factor	Unit	−2	−1	0	+1	+2
Time	h	24	48	72	96	120
Temperature	°C	25	30	35	40	45
Particle size	µm	250	500	750	900	1200

A quadratic polynomial model was fitted to the nitrogen-evolution response. Model significance was assessed by analysis of variance (ANOVA), while R^2, adjusted R^2, predicted R^2, coefficient of variation and adequate precision were used to evaluate model adequacy. Numerical optimization was subsequently used to identify the factor combination giving the maximum predicted nitrogen evolution within the experimental region.

IV. RESULTS AND FINDINGS

1. Experimental response

The raw experimental data showed a consistent increase in nitrogen evolution with increasing

fermentation time and temperature, while the response generally decreased as particle size increased. The original experimental records show, for example, that at 24 h and 45°C the nitrogen-evolution values decreased from 0.37 at 250 µm to 0.26 at 1200 µm, whereas at 120 h and 45°C they ranged from 0.97 to 0.76 across the same particle-size range. These trends are consistent with the fitted model and with the expected influence of substrate exposure during particle-size reduction.

2. Quadratic model

The fitted quadratic model for mesquite seed was highly significant ($F = 153.12$, $p < 0.0001$). The significant terms were time (A), temperature (B), particle size (C), time × temperature (AB), time × particle size (AC) and time^2 (A^2). The temperature × particle-size interaction and the quadratic terms for temperature and particle size were not significant at $p < 0.05$, although they were retained in the hierarchical quadratic model.

In coded variables, the fitted equation was:

$$Y = 0.4344 + 0.1990A + 0.1970B - 0.0740C + 0.0725AB - 0.0250AC - 0.0175BC - 0.0559A^2 + 0.0041B^2 + 0.0091C^2$$

where Y is nitrogen evolution, A is fermentation time, B is temperature and C is particle size in coded form.

3. ANOVA and model adequacy

Source	F-value	p-value	Interpretation
Model	153.12	<0.0001	Significant
Time (A)	606.19	<0.0001	Significant
Temperature (B)	594.07	<0.0001	Significant
Particle size (C)	83.82	<0.0001	Significant
A × B	64.37	<0.0001	Significant
A × C	7.65	0.0199	Significant
B × C	3.75	0.0815	Not significant
A^2	13.16	0.0046	Significant
B^2	0.0704	0.7961	Not significant
C^2	0.3479	0.5684	Not significant
Model statistic		Value	
R^2		0.9928	
Adjusted R^2		0.9863	
Predicted R^2		0.9456	
Standard deviation		0.0256	
Mean response		0.4130	
Coefficient of variation		6.19%	
Adequate precision		52.011	

The fitted model accounted for 99.28% of the variation observed in nitrogen evolution (R^2 = 0.9928). The adjusted R^2 was 0.9863 and the predicted R^2 was 0.9456. The difference between the adjusted and predicted R^2 values was less than 0.20, indicating reasonable agreement between the two measures. Adequate precision was 52.011, which is substantially higher than the commonly used value of 4 and indicates a strong model signal within the design region.

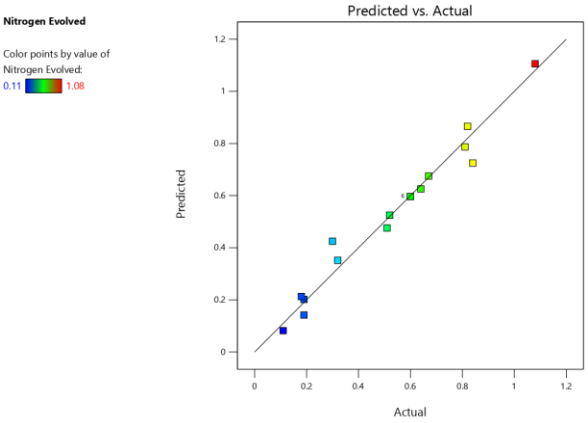

Figure 2. Predicted versus actual nitrogen-evolution response for the mesquite-seed model.

4. Effect of the independent variables

The regression coefficients show that fermentation time made a strong positive linear contribution to nitrogen evolution, with a coefficient of 0.1990. Increasing fermentation time therefore increased the response within the experimental range.

Temperature also made a strong positive linear contribution, with a coefficient of 0.1970. The positive time × temperature coefficient (0.0725) indicates that the effect of increasing temperature depended partly on fermentation time.

Particle size had a negative coefficient (−0.0740), showing that larger particles tended to give lower nitrogen-evolution values within the tested range. The negative time × particle-size coefficient

(−0.0250) similarly shows that the effect of particle size changed with fermentation time.

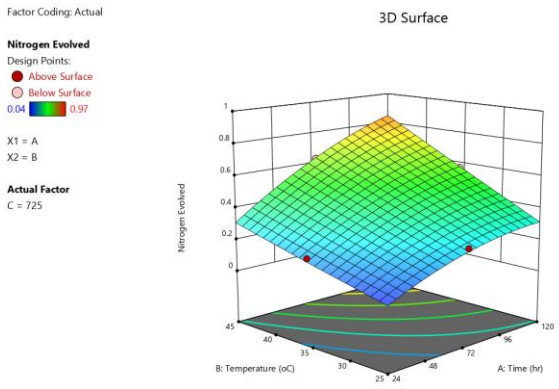

Figure 3. Response surface showing the combined effect of fermentation time and temperature on nitrogen evolution.

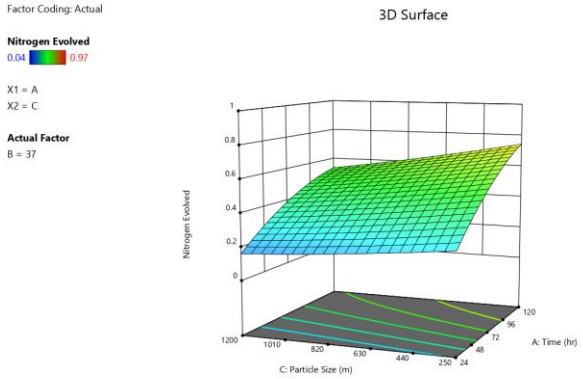

Figure 4. Response surface showing the combined effect of fermentation time and particle size on nitrogen evolution.

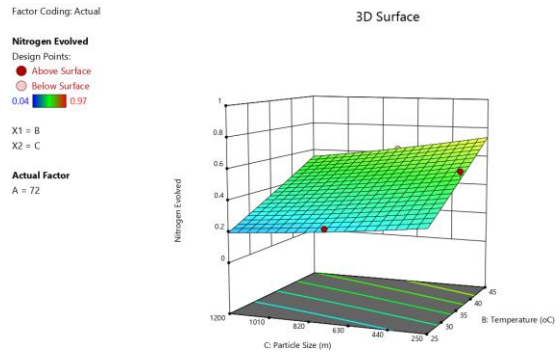

Figure 5. Response surface showing the combined effect of particle size and temperature on nitrogen evolution.

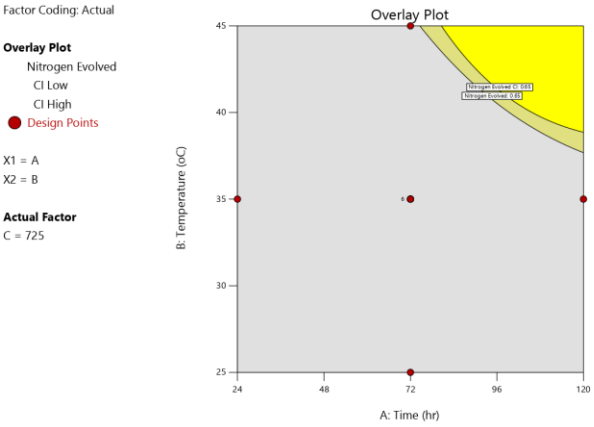

Figure 6. One-factor relationship between fermentation time and nitrogen evolution.

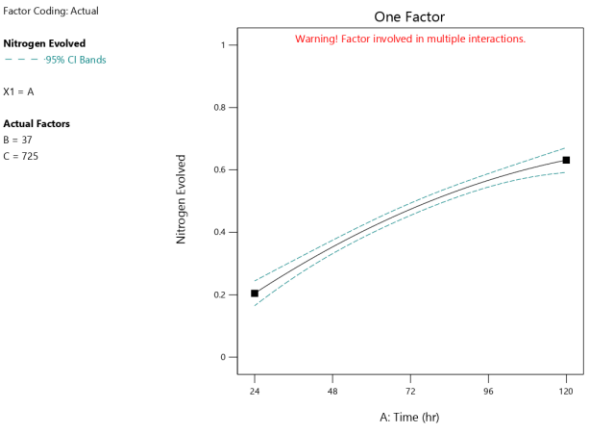

Figure 7. One-factor relationship between temperature and nitrogen evolution.

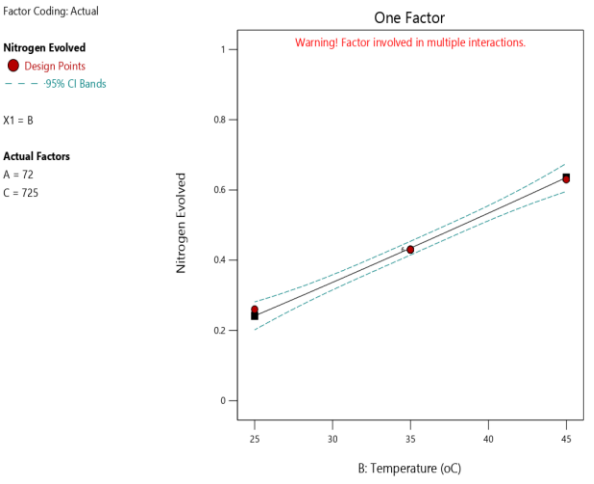

Figure 8. One-factor relationship between particle size and nitrogen evolution.

5. Numerical optimization

Parameter	Predicted optimum
Fermentation time	113.6 h
Temperature	44.33°C
Particle size	313.33 µm
Predicted nitrogen evolution	0.915%
Desirability	1.000

The numerical optimization routine selected 113.6 h, 44.33°C and 313.33 µm as the preferred combination. At this point, the model predicted a nitrogen evolution of 0.915%, with a desirability of 1.000. All three values fall inside the experimental ranges, so the solution is an interpolation within the design space rather than an extrapolation beyond the tested conditions.

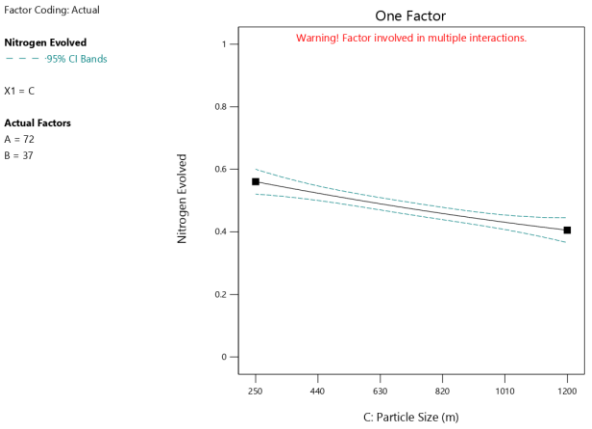

Figure 9. Overlay plot for the mesquite-seed fermentation design space.

V. DISCUSSION

The results show that the three variables considered in this study were important to the fermentation response. Fermentation time had a strong positive effect, which agrees with the fact that biochemical changes continue as fermentation proceeds. Previous work on P. africana has also reported marked changes during roughly the first 72–120 h, particularly in free amino acids and proteolytic activity (Omafuvbe et al., 1999; Odibo et al., 2008).
Temperature also had a strong effect on nitrogen evolution. This is reasonable because temperature influences the activity of microorganisms and the enzymes involved in substrate breakdown. RSM studies of food fermentation similarly show that temperature and time can have strong, interacting effects on the measured response (Adebo et al., 2018). The present data should not, however, be taken to mean that a higher temperature will always give a better result. The tested range ended at 45°C, and the model placed the optimum at 44.33°C. Thus, the selected temperature reflects the balance represented by the fitted quadratic surface within the conditions that were actually studied.

Particle size showed a clear inverse relationship with nitrogen evolution. The smaller particles generally produced higher response values, which is consistent with the greater surface area available for contact with microorganisms and enzymes. From a processing standpoint, this finding is useful because particle-size reduction can be standardized before fermentation. The significant time × particle-size interaction also indicates that the benefit of a smaller particle size depends to some extent on the stage of fermentation.

The statistical fit of the model was strong. The R^2 value of 0.9928 means that the fitted model explained nearly all of the variation represented in the experimental data. The predicted R^2 of 0.9456 was also high, suggesting that the model has useful predictive ability within the studied region. Even so, these statistics do not replace an independent validation experiment, particularly because the optimum reported here is model-predicted.

The findings fit with the wider use of RSM in fermentation studies, where time and temperature are often important operating variables (Adebo et al., 2018). The approach has also been applied specifically to P. africana using fermentation duration, inoculum concentration, pH and temperature (Asiru & Musbau, 2025). The main process distinction in the present study is the inclusion of particle size together with time and temperature and the use of nitrogen evolution as the response.

Nitrogen evolution should not be interpreted as a complete measure of fermented-product quality. A high value for the measured response does not, by

itself, establish that the product has the best sensory properties, microbial safety, nutritional profile or storage stability. The optimum obtained in this work should therefore be described specifically as the condition that maximizes the modelled nitrogen-evolution response within the tested region.

VI. CONCLUSION

Mesquite-seed fermentation was successfully described using a quadratic response-surface model. Fermentation time, temperature and particle size significantly affected nitrogen evolution, while the time × temperature and time × particle-size interactions were also significant. The results show that these variables should be considered together when selecting fermentation conditions.

The model gave R^2 = 0.9928, adjusted R^2 = 0.9863 and predicted R^2 = 0.9456, indicating a strong fit to the experimental data. Numerical optimization identified 113.6 h, 44.33°C and 313.33 µm as the preferred operating combination, with a predicted nitrogen evolution of approximately 0.915%. Because this value comes from the fitted model, an independent experimental run is still required to confirm the prediction.

Overall, the study provides a quantitative starting point for controlling mesquite-seed fermentation. It also shows how RSM can be used to move from a largely empirical fermentation procedure toward conditions that can be measured, compared and reproduced.

Recommendations

- Validate the predicted optimum experimentally using independent fermentation runs at approximately 113.6 h, 44.33°C and 313.33 µm before scale-up.
- Standardize particle-size reduction before fermentation because particle size significantly affected nitrogen evolution.
- Control fermentation temperature and duration rather than relying solely on uncontrolled traditional conditions.
- Include additional response variables in future optimization studies, particularly pH, amino nitrogen, free amino acids, microbial counts, sensory characteristics, proximate composition and antinutritional factors, to permit true multi-response product optimization.
- Conduct microbial identification and safety assessment of the optimized fermentation process before commercial application.
- Report the complete Kjeldahl analytical procedure and calculation used to derive nitrogen evolution so that the study is fully reproducible.

Study Limitations

This study considered nitrogen evolution as the main response and was limited to fermentation times of 24–120 h, temperatures of 25–45°C and particle sizes of 250–1200 µm. The supplied dataset did not include microbial identification, pH, moisture, sensory measurements, amino-acid profiles or safety indicators. For that reason, the results should not be taken as evidence that the predicted condition simultaneously optimizes all aspects of product quality. The predicted optimum also needs independent experimental validation before it can be recommended as a production standard.

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