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Phenomenological Model Development of Percentage Protein Present in Fermented African Locust Beans Seed

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Abstract-

The optimum conditions for the fermentation of a local legume known as African locust bean (*Parkia biglobosa*) into a vegetable protein based food condiment or spice (Iru) were developed using Levenberg-Marquardt (or Powell) method (using PSI software) with three (3) variables namely; inoculum concentration (*bacillus subtilis*), temperature and the fermentation duration. *P. biglobosa* seeds were fermented at various temperature of 40 - 70 °C for five days (120 hours) with different concentrations of Inoculum. The proximate analysis shows that fermentation increased the percentage protein. Protein had the highest composition with about 51 % after 72 hours at the lowest fermentation temperature of 40°C.

Key words: *bacillus subtilis*, fermentation, inoculum, proximate analysis, *Parkia biglobosa*

1. Introduction

P. biglobosa plant is a deciduous perennial tree that grows from about 7 - 20 m high [1]. This tree was mentioned among the tress with diverse healing properties in South Western Nigeria [2]. The pods with a flat surface have irregular cluster of about 30 seeds [1, 3-5]. *P. biglobosa* bean tree was named by Robert Brown a botanist who came from Scotland, in the year 1826 after another Scottish named Mongo Park, a surgeon, in the late 17's. He gave the tree the local name 'nitta' [3, 6, 7]. *P. biglobosa* seed is known as Iyere in Yoruba part of Nigeria while the fermented seed as Iru. One of the major plant based protein in African diet is Iru [3, 8]. Fermentation can be defined as the breaking down of chemical substance by microorganism into carbon dioxide, alcohol and some organic acids and the microbiological substance which aids fermentation is known as Starter culture [9].

Phenomenological model is used as a diagnosis tool that assists decision makers in correctly and effectively dealing with difficult problems such as fermentation process, oil spillage on either soil surfaces or water, biological and chemical processes [10, 11].

Since fermented *P. biglobosa* seed is basically used for the high protein composition and the embedded health benefits, this study considered only the % protein content which is the highest obtained when proximate analysis was carried out. The laboratory result was used to optimize the number of days and the appropriate fermentation temperature.

2. Methodology

Source of Materials: *P. biglobosa* seed were purchased from open market.

Inoculum Preparation: Inoculum was freshly prepared in the Biological Sciences Laboratory Department in Covenant University using method [12-15].



Preparation of Seed: The raw *P. biglobosa* bean seed were processed using method [16].

Fermentation using microorganism: 400 g of the processed *P. biglobosa* seed were inoculated using freshly prepared starter culture - *B. subtilis*. Fermentation process was carried out for 120 hours (5 days) and fermented samples were taken every 24 hours. These samples were kept in the freezer for further analysis.

Proximate analysis: The proximate analysis were evaluated by the method described by [17].

3. Result and discussions

Assumption made: the experimentally obtained (measured) percentage protein in fermented *P. biglobosa* is a complex function of the day, temperature and volume of inoculum employed all through the fermentation process.

Let P = % protein, Number of day = fermentation duration (day), vol = volume of inoculum conc. (ml/g); temp = temperature ($^{\circ}\text{C}$)

$$P = f(\text{Day}, \text{vol}, \text{temp}) \quad [1]$$

A 3-D plots (graph) of P vs. (Number of day, volume of inoculum); P vs. (Number of day, temperature) and P vs. (volume of inoculum, temperature) are shown in the figures below:

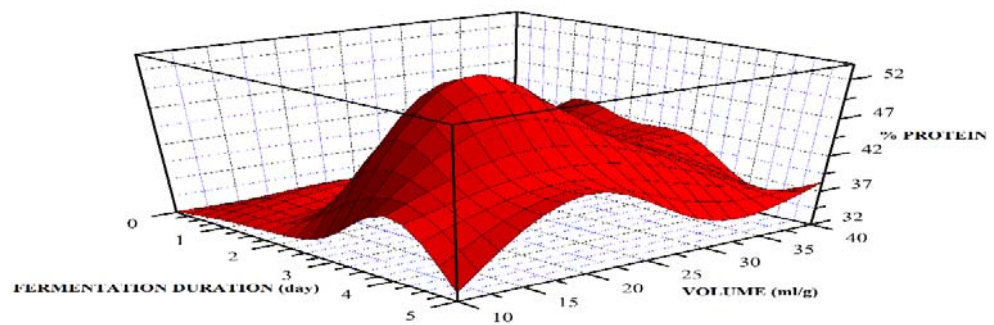


Figure 1: % Protein as function of Day and Volume

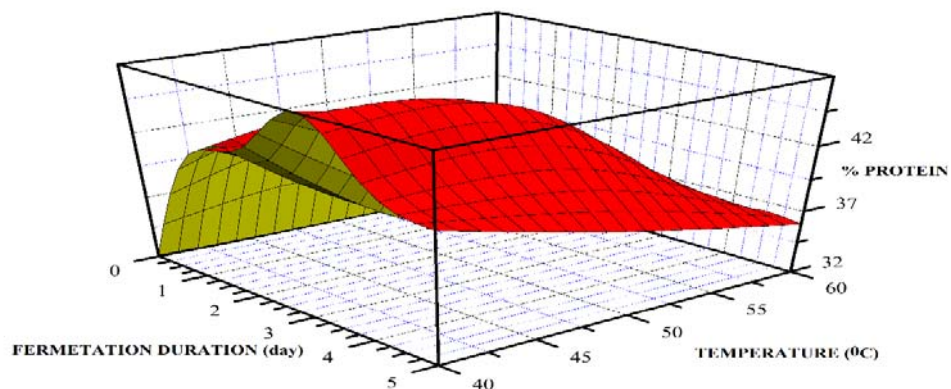


Figure 2: % Protein as function of Day and Temperature

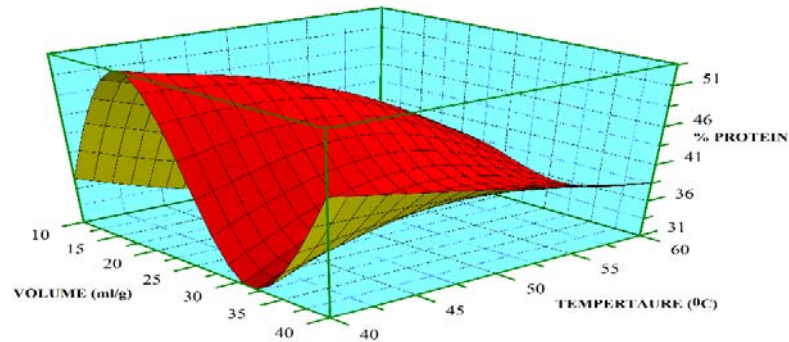


Figure 3: % Protein as function of Volume and Temperature

Let us assume the model for equation [1] can be expressed in the form:

$$P = P + A * Day + B * Day^2 + C * Day^3 + D * vol + E * vol^2 + F * vol^3 + G * temp + H * Day * vol + K * Day * temp + M * vol * temp + N * Day * vol * temp + U * temp^2 \quad [2]$$

Using equation [2] and the experimental data given, the unknown coefficients – P, A, B, C, D, E, F, G, H, K, M, N and U are correlated using Levenberg-Marquardt (or Powell) method (using PSI software). The correlation coefficient was 0.885. The final values for the parameters are shown in Table 1.

Table 1: Correlated values

Variable Name	Initial Guess	Final Value
P	32.000	20.000
A	1.000	15.520
B	2.500	-2.649
C	4.500	0.197
D	5.000	2.593
E	713.000	-0.071
F	7.000	0.0007
G	-3.229	-0.323
H	-2.650	-0.165
K	-3.000	-0.110
M	4.000	-0.006
N	8.500	0.003
U	2.740	0.003

Note that from the results above, it is apparent that % protein is not dependent on vol^3 i.e. $F = 0$ is a reasonable assumption.

The equation generated above is now used in Excel Solver as an objective function to obtain values for the independent variables (Day, Vol, and Temp). In Excel, the objective function is:

$$= 20 + 15.52*D2 - 2.649*D2^2 + 0.197*D2^3 + 2.593*D3 - 0.0713*D3^2 + 0.000667*D3^3 - 0.3229*D4 - 0.165*D2*D3 - 0.1103*D2*D4 - 0.00564*D3*D4 + 0.00284*D2*D3*D4 + 0.00274*D4^2$$

Several research works have been carried out on how fermentation enhances protein value of *P. biglobosa* seeds, this was established in figures 1-3 with increase in protein composition at the 3rd (third) day of fermentation. The protein composition decreases as temperature increases. Figures 1-3 showed that at every fermentation temperature protein composition increased from the 1st (first) day up to the third day and declined from the fourth day. The % yield of protein from the fermentation of *P. biglobosa* at any given day of fermentation decreases with increase in fermentation temperature from 40 to 70 ° C. The protein yield at 40 ° C on the third day of fermentation is about 51%, it reduced to 32.6 % on the third day at a temperature of 70 ° C. This shows that *B. subtilis* functions well at lower temperature between 40 and 50 ° C. Thus maximum protein yield is achieved at the 3rd (third) day of fermentation with temperature 40 ° C.

The above 3-D plots shows the predicted result (effect) of the fermentation process variables on percentage protein as the response. These plots shows (represents) graphically the regression coefficient in equation form in order to obtain the optimum fermentation conditions of the variables within the design region.

This work concluded that *P. biglobosa* should be fermented for three days, at an optimum temperature of 40 °C with *B. subtilis* as starter culture. This is in agreement with the reports of [18, 19].

4. Conclusion

This work clearly shows that a maximum yield of 51 % protein content was achieved through the fermentation of *P. biglobosa* seed using *B. Subtilis* at the optimum conditions of about 3 days of fermentation and 40 °C operating temperature using Levenberg-Marquardt correlation.

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