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# Optimization of Fermentation Conditions for the Production of Protein Composition in Parkia biglobosa Seeds using Response Surface Methodology

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**Abstract**

The optimum conditions for the fermentation of African locust bean (*Parkia biglobosa*) into a vegetable protein based condiment (Iru) were developed using Response Surface Method (RSM) with three (3) variables namely; inoculum concentration ($X_1$), temperature ($X_2$) and the fermentation duration ($X_3$). African locust bean seeds were fermented at various temperature of 40, 50, 60 and 70 °C for five days (120 hours) with different concentrations of Inoculum. The interaction effects of these three variables on the % protein composition ($X_4$) have been investigated using Central Composite Design (CCD) factional factorial design of experiments. The proximate analysis shows that fermentation increased the percentage protein and fat content. Protein had the highest composition with about 53 % after 72 hours at the lowest fermentation temperature of 40°C. Other parameters like % crude fibre, % ash content and % carbohydrate decreased with hours of fermentation and with increase in temperature. From experimental data generated by the design of experiment (DOE), using MINITAB 17 software, optimum conditions were obtained as 40 $^0$C, with inoculum (*Bacillus subtilis*) concentration of 0.005 (g broth/g seed) at fermentation duration of 78 hours.

**Keywords**: *bacillus subtilis*, DOE, fermentation, inoculum, MINITAB 17, optimization, RSM

## INTRODUCTION

*Parkia biglobosa* tree is known to be a native of Africa and is an important multipurpose tree of West African Savannah land [1], which is primarily grown for its pods that contain both a sweet pulp and valuable seeds. The pods are flat and have irregular cluster of up to 30 seeds [2]. The tree has been used both locally and internationally in drug manufacturing and cosmetics production. Despite the important uses, the populations of this tree is reducing and it remain semi- or undomesticated [3].

African locust bean tree was named *Parkia biglobosa* by Robert Brown, a Scottish botanist in 1826 after Mongo Park, a Scottish surgeon who explored West Africa in 1790's. Mongo Park gave this tree a local name 'nitta' [4]. African locust been tree was described by Robert Brown, as a genus of flowering plants in the legume family, Fabaceae, which belongs to the sub-family *Mimosoideae* and *Leguminosae* with the genus *Parkia* and botanical name *Parkia biglobosa* [5]. *Parkia bicolor, Parkia filicoidea, Parkia clappertoiana* and *Parkia biglobosa* are other species of the genus of *Parkia biglobosa* which can also be fermented to produce food condiments for flavouring as well as adding good aroma to food. It was reported that fermented African locust bean seed is a leguminous plant with an outstanding protein quality. The protein and amino acid composition have been reported by several researchers [6].

*Parkia biglobosa* seed is known as Iyere in Yoruba land while the fermented seed as Iru. Iru is one of the major sources of plant protein in African diet which is known as fermented vegetable protein [7]. Iru is consumed in many African countries, especially Nigeria. Iru known by different names in different countries - kinda in Sierra Leone. In Nigeria and Ghana it is called *dawadawa* or *Iru* [8, 9]. In Benin Republic afintin and *sonru*; *nététu* in Senegal and Burkina Faso as *soumbala*; Japan as *natto;* and *kinema* in Nepal [10]. Apart from fermented *Parkia biglobosa* seeds (Iru) serving as a rich source of plant protein to man with low cost, it also serves as good source of protein for animal feeds, chick and fish (Livestock) [4, 7, 11]. Apart from these nutritional values, fermented African locust bean seeds provide dietary fiber, energy, minerals and vitamins such as Vitamin B, riboflavin and Vitamin A. It also improves sensory properties of foods which includes the organoleptic characteristics (appearance, aroma and flavor) [12].

There seems to be a general agreement on the spore-forming *Bacillus* species as the main fermentation organisms [13, 14, 15, 16, 17,18, 19, 20, 21]. During the fermentation of African Locust bean seeds with systematic investigation showed that *Bacillus subtillis* is the most dominant bacterium responsible for the fermentation [13, 16, 22]. Literature revealed that some

species of *bacillus* such as *Bacillus licheniformis, Bacillus megaterium, L. mesenteriodes* and *Staphylococcus* are also found in the fermented condiment (Iru).

In a study conducted on the fermentation of Iru, it was found that *Gmelina arborea* as well as banana leaves accelerated fermentation of seeds, while also bringing an increase in protein and crude fat contents with corresponding decrease in carbohydrate [23].

Design of experiments (DOE) can be defined as the systematic method of determining the relationship between factors affecting a process and the output of that process. DOE is an advanced statistical tool to study efficiently the effect of a large number of variables with a minimum effort in data collection [24]. This investigates the effects of input variables (factors) on output variable (response) simultaneously. It is majorly used to find the cause-and-effect relationships, which is needed to manage process inputs in order to optimize the experimental outputs. In an experiment, one or more process factors or variables are deliberately changed in order to observe the effect the changes have on one or more response variables. The (statistical) design of experiments (DOE) is an efficient procedure for planning experiments so that the data obtained can be analyzed to yield valid and objective conclusions. An experimental design is the laying out of a detailed experimental plan in advance of doing the experiment. Simple experimental design and statistical tools for data analysis can provide much information about the system under investigation after only a few experiments. Such information can be key in decision-making for further experiments and can enable the development of robust and reliable protocols for chemical synthesis, analytical methods or biological assays [25].

## MATERIALS AND METHOD

### Source of Materials

Raw *Parkia biglobosa* seed were purchased from retailers in Ekiti state, Nigeria.

### Inoculum Preparation

Inoculum used were freshly prepared in the Microbiology Laboratory Department in Covenant University using [25].

### Preparation of Seed

The raw African locust bean seed were processed using [26, 27].

### Microbial fermentation

200 g of the processed seed were inoculated using freshly prepared *B. subtilis*. Fermentation was carried out for 5 days. Samples were taken every 24 hours and kept in a freezer for further analysis.

### pH determination

Ten grams (5g) of ground fermented samples were weighed into 20 ml of distilled water. Using Unicam pH meter, the pH of each homogenate were recorded.

### Proximate analysis

The proximate analysis were evaluated by the method described by [27, 28].

## RESULTS AND DISCUSSION

Since fermented African locust bean seeds is consumed for the high protein content and health benefits embedded in it. This study used the percentage protein composition which is the highest composition discovered in the fermented seed to optimize the number of days and the appropriate fermentation temperature. The microorganism and the concentration to be used was also established which confirmed the earlier reports by researchers.

## RESPONSE SURFACE METHODOLOGY

The main interest in this research was the protein composition of the fermented seeds, hence optimization was based only on its experimental results alone. It was assumed that the experimentally measured % protein composition is a complex function of day, temperature and concentration of inoculum used during fermentation i.e.:

$$P = f(Time, Ino.conc., Temp.) \quad \ldots\ldots [1]$$

The effects of the above variables (operating parameters) on the % Protein composition were studied using CCD factional factorial design of experiments to optimize the yield. The results were analysed using MINITAB 17 software.

### EXPERIMENTAL DESIGN FOR THE FERMENTATION CONDITIONS

Responses were generated as functions of three variables namely: $X_1$ as Time (fermentation duration), $X_2$ as Inoculum concentration (g broth/g seed) and $X_3$ as Temperature (°C).

The response variable (% Protein) was fitted by a second-order polynomials in order to correlate the design variables ($X_1$, $X_2$, $X_3$) which is presented with the model below:

$$Y = \alpha_0 + \alpha_1X_1 + \alpha_2X_2 + \alpha_3X_3 + \alpha_{1,1}X_1X_1 + \alpha_{1,2}X_1X_2 + \alpha_{1,3}X_1X_3 + \alpha_{2,2}X_2X_2 + \alpha_{2,3}X_2X_3 + \alpha_{3,3}X_3X_3 \quad \ldots\ldots\ldots [2]$$

The % Protein composition responses is represented by Y, which is associated with each factor level combinations. $\alpha_0$, $\alpha_1$, $\alpha_2$, $\alpha_3$, $\alpha_{1,2}$ ….. $\alpha_{3,3}$ are the regression coefficients. $X_1$, $X_2$ and $X_3$ are the factors. $X_1X_1$, $X_1X_2$, $X_1X_3$, $X_2X_2$, $X_2X_3$ and $X_3X_3$ are the interactions of the variables.

**Table 1:** Design of the Variables with % Protein as the response

| **Factors** | **Notation** |
|---|---|
| Fermentation duration (days) | $X_1$ |
| Inoculum Concentration (g broth/g seed) | $X_2$ |
| Temperature (°C) | $X_3$ |

| Run | Days ($X_1$) | Ino.Conc.($X_2$) | Tempt.($X_3$) | Response (% Protein) |
|---|---|---|---|---|
| 1 | 3 | 0.005 | 40 | 53.7 |
| 2 | 3 | 0.01 | 60 | 39 |
| 3 | 5 | 0.05 | 50 | 38.6 |
| 4 | 1 | 0.075 | 60 | 36.4 |
| 5 | 1 | 0.05 | 50 | 38.1 |
| 6 | 3 | 0.075 | 50 | 44.03 |
| 7 | 5 | 0.075 | 60 | 36 |
| 8 | 1 | 0.075 | 40 | 42.21 |
| 9 | 3 | 0.075 | 50 | 44.03 |
| 10 | 1 | 0.01 | 50 | 42.8 |
| 11 | 3 | 0.01 | 40 | 41 |
| 12 | 3 | 0.05 | 60 | 40 |
| 13 | 3 | 0.075 | 50 | 44.03 |
| 14 | 5 | 0.075 | 40 | 41.85 |
| 15 | 5 | 0.01 | 50 | 34 |

**Table 2:** Design Application for the Process simulation for % Protein Yield

| Standard Order | $X_1$ | $X_2$ | $X_3$ | Experimental values | Predicted values | % Deviation |
|---|---|---|---|---|---|---|
| 1 | 3 | 0.005 | 40 | 53.7 | 51.588 | 0.03933 |
| 2 | 3 | 0.01 | 60 | 39 | 41.42 | -0.06205 |
| 3 | 5 | 0.05 | 50 | 38.6 | 41.365 | -0.07163 |
| 4 | 1 | 0.075 | 60 | 36.4 | 36.985 | -0.01607 |
| 5 | 1 | 0.05 | 50 | 38.1 | 38.993 | -0.02344 |
| 6 | 3 | 0.075 | 50 | 44.03 | 44.174 | -0.00327 |
| 7 | 5 | 0.075 | 60 | 36 | 34.685 | 0.036528 |
| 8 | 1 | 0.075 | 40 | 42.21 | 43.815 | -0.03802 |
| 9 | 3 | 0.075 | 50 | 44.03 | 44.174 | -0.00327 |
| 10 | 1 | 0.01 | 50 | 42.8 | 40.327 | 0.05778 |
| 11 | 3 | 0.01 | 40 | 41 | 42.41 | -0.03439 |
| 12 | 3 | 0.05 | 60 | 40 | 38.878 | 0.02805 |
| 13 | 3 | 0.075 | 50 | 44.03 | 44.174 | -0.00327 |
| 14 | 5 | 0.075 | 40 | 41.85 | 41.555 | 0.007049 |
| 15 | 5 | 0.01 | 50 | 34 | 33.395 | 0.017794 |
| Average Value | | | | 41.05 | 41.196 | -0.06889 |

Table 1 described the design of variables used with % Protein as the response. This is the Response surface model experimental values of the % Protein. This also shows the interactions between the three variables and expected number of experimental runs, while table 2 shows the design application for the process simulation, the % Protein yield for

both experimental and predicted values. This table simulated the values of both experimental and predicted value and shows the deviation percentage. The experimental had close values with the predicted values giving an $R^2$ that is very close to 1

**Table 3.** Analysis of variance (ANOVA) for the Response Surface Regression

| Source of Variation | DF | SS | MS | F-value | p-value |
|---|---|---|---|---|---|
| Model | 9 | 275.6 | 30.662 | 4.65 | 0.053 |
| Linear | 3 | 129.812 | 43.271 | 6.75 | 0.035 |
| Days ($X_1$) | 1 | 0.005 | 0.005 | 0 | 0.98 |
| Ino.Conc.($X_2$) | 1 | 2.667 | 2.667 | 0.4 | 0.553 |
| Tempt.($X_3$) | 1 | 127.14 | 127.14 | 19.29 | 0.007 |
| Square | 3 | 92.663 | 91.663 | 13.91 | 0.014 |
| $X_1^2$ | 1 | 91.663 | 91.663 | 13.91 | 0.014 |
| $X_2^2$ | 1 | 1.67 | 1.67 | 0.25 | 0.636 |
| $X_3^2$ | 1 | 0.017 | 0.017 | 0 | 0.962 |
| 2-way inter. | 3 | 55.845 | 18.615 | 2.82 | 0.146 |
| $X_1*X_2$ | 1 | 21.623 | 21.623 | 3.28 | 0.13 |
| $X_1*X_3$ | 1 | 0 | 0 | 0 | 0.994 |
| $X_2*X_3$ | 1 | 34.223 | 34.223 | 5.19 | 0.072 |

Analysis of Variance (ANOVA) for the Response Surface Regression was shown in table 3. ANOVA was used to test statistical significance of the model. Table 3 shows statistically the sum of square (SS), degree of freedom (DF), mean square (MS), F-value, p-value and ANOVA coefficients. The Fisher distribution (F-value and P-value) were used to determine the significance of the model. A large F-value and a small P-value implies that the models are adequate to predict the responses. $R^2$ indicate the reliability of the model. The closer the $R^2$ value to 1, the stronger and better the model prediction of the responses.

**Table 4:** Optimization Table

| DAYS ($X_1$) | Conc. ($X_2$) | Tempt. ($X_3$) | % Protein | $X_1$ | $X_2$ | $X_3$ | $X_1X_1$ | $X_1X_2$ | $X_1X_3$ | $X_2X_2$ | $X_2X_3$ | $X_3X_3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 0.005 | 40 | 53.7 | 3 | 0.005 | 40 | 9 | 0.015 | 120 | 0.000025 | 0.2 | 1600 |
| 3 | 0.01 | 60 | 39 | 3 | 0.01 | 60 | 9 | 0.03 | 180 | 0.0001 | 0.6 | 3600 |
| 5 | 0.005 | 50 | 38.6 | 5 | 0.005 | 50 | 25 | 0.025 | 250 | 0.000025 | 0.25 | 2500 |
| 1 | 0.0075 | 60 | 36.4 | 1 | 0.0075 | 60 | 1 | 0.0075 | 60 | 5.63E-05 | 0.45 | 3600 |
| 1 | 0.005 | 50 | 38.1 | 1 | 0.005 | 50 | 1 | 0.005 | 50 | 0.000025 | 0.25 | 2500 |
| 3 | 0.0075 | 50 | 44.03 | 3 | 0.0075 | 50 | 9 | 0.0225 | 150 | 5.63E-05 | 0.375 | 2500 |
| 5 | 0.0075 | 60 | 36 | 5 | 0.0075 | 60 | 25 | 0.0375 | 300 | 5.63E-05 | 0.45 | 3600 |
| 1 | 0.0075 | 40 | 42.21 | 1 | 0.0075 | 40 | 1 | 0.0075 | 40 | 5.63E-05 | 0.3 | 1600 |
| 3 | 0.0075 | 50 | 44.03 | 3 | 0.0075 | 50 | 9 | 0.0225 | 150 | 5.63E-05 | 0.375 | 2500 |
| 1 | 0.01 | 50 | 42.8 | 1 | 0.01 | 50 | 1 | 0.01 | 50 | 0.0001 | 0.5 | 2500 |
| 3 | 0.01 | 40 | 41 | 3 | 0.01 | 40 | 9 | 0.03 | 120 | 0.0001 | 0.4 | 1600 |
| 3 | 0.005 | 60 | 40 | 3 | 0.005 | 60 | 9 | 0.015 | 180 | 0.000025 | 0.3 | 3600 |
| 3 | 0.0075 | 50 | 44.03 | 3 | 0.0075 | 50 | 9 | 0.0225 | 150 | 5.63E-05 | 0.375 | 2500 |
| 5 | 0.0075 | 40 | 41.85 | 5 | 0.0075 | 40 | 25 | 0.0375 | 200 | 5.63E-05 | 0.3 | 1600 |
| 5 | 0.01 | 50 | 34 | 5 | 0.01 | 50 | 25 | 0.05 | 250 | 0.0001 | 0.5 | 2500 |

The optimization process using the three (3) variable were shown in table 4. This was used to design and generate a model to fit the experimental result.

Below is the best fitted models obtained from the regression analysis.

Regression Equation (coded variables):

$$\% \text{ Protein} = 85.7 + 10.42\ X_1 - 3521\ X_2 - 1.29\ X_3 - 1.246\ X_1X_1 - 107600\ X_2X_2 + 0.0007\ X_3X_3 - 465\ X_1X_2 - 0.0005\ X_1X_3 + 117.0\ X_2X_3 \quad \ldots\ldots\ldots\ldots \quad [3]$$

R-Sq. = 90.23 %

Solution:

Day: 3.251 [78 hours]

Inoculum concentration: 0.005 (g broth/g seed)

Temperature: 40°C

% Protein fit: 51.54

Desirability: 0.977122

**Optimization of the % Protein yield**

Figures 1-3 are 3-D (3 dimensional) response surface plots of Protein vs. (Day, Conc.); Protein vs. (Day, temp) and Protein vs (conc., temp) respectively. With the aid of Minitab 17 software, the plots in figures 1-3 were analysed to obtain the regression equation for predicting the protein composition for various values of $X_1$, $X_2$, $X_3$ (see equation 3).

The result of the optimized regression equation shows that 3.251 days (78 hours) of fermentation, with Inoculum concentration of 0.005 g broth/g seed and fermentation temperature of 40 $^0$C predicts maximum yield of % Protein composition of 51.54 % which is very close to the experimentally value of 53.7 %

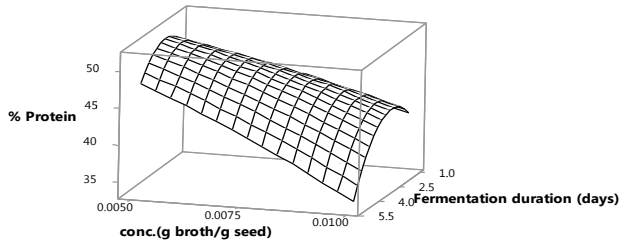


**Figure 1:** Surface plot of % Protein content yield vs fermentation duration and Inoculum concentration at fixed temperature.

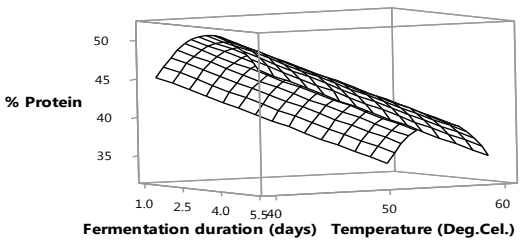


**Figure 2:** Surface plot of % Protein content yield vs fermentation duration and temperature at fixed volume

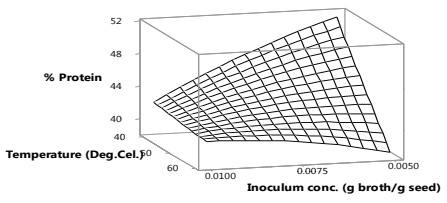


**Figure 3:** Surface plot of % Protein content yield vs Inoculum concentration and temperature at a fixed volume.

The above plots shows the predicted effect of process variables ($X_1$, $X_2$, $X_3$) on % Protein as the response. The 3-D plot represent graphically the regression coefficient in equation form in order to obtain the optimum conditions of the variables within the design region.

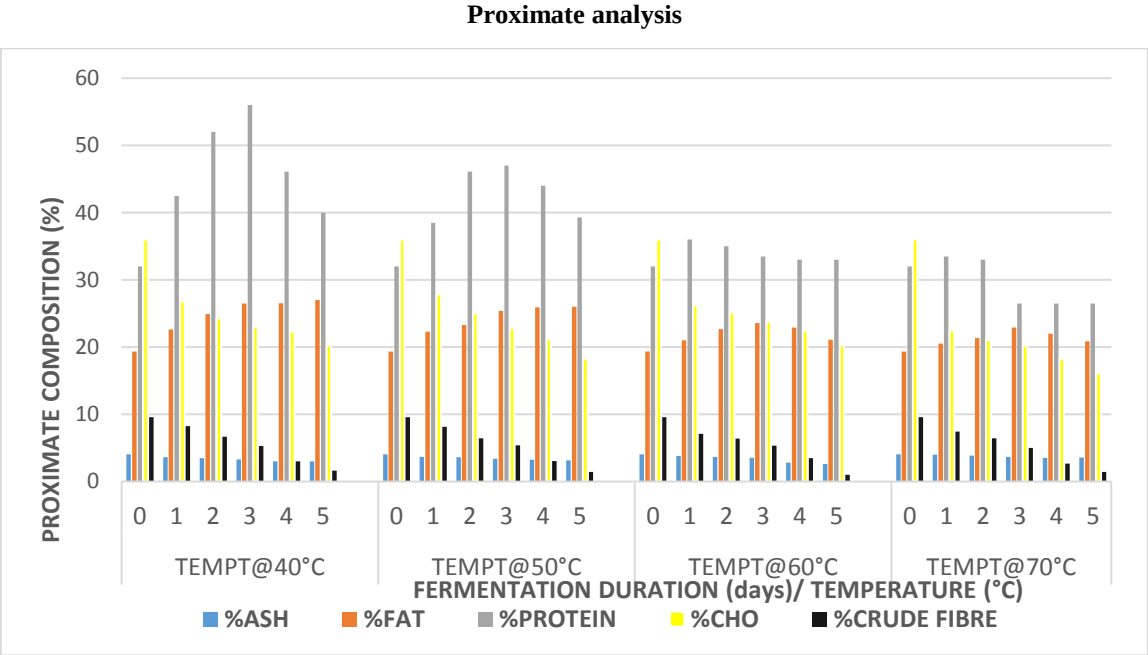


**Figure 4:** Proximate Composition of fermented African locust bean seed

Literature confirmed that raw unfermented African locust bean has about 32 % protein composition, which was supported by the result of this study and others [26, 29, 30, 31, 32].

Figure 4 shows that on the third day, *B. subtilis* gave the highest yield of % protein at temperature 40 °C and 0.005 g broth/g seed. At higher temperature of 60 and 70 ºC, fermented samples had an appreciable amount of protein, but the end products were not organoleptically acceptable. The protein composition also decreases with an increase in inoculum concentration.

This work concluded that African locust bean should be fermented with 0.005 g broth/g seed *B. subtilis* for three days and at temperature 40 °C. This is in agreement with the reports of [15, 22]. [33] reported three days (3 days) within temperature range 28 – 42 °C.

## CONCLUSION

This work clearly shows that a maximum yield of 53 % protein content was achieved through the fermentation of African locust bean using *B. Subtilis* at the optimum conditions of about 3 days of fermentation, 0.005 g broth/g seed concentration and 40 ºC operating temperature using Response Surface Methodology.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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