Optimization of traditional starter culture and African locust beans for *daddawa* production using Response Surface Methodology (RSM)

Kabiru Ibrahim Karamba* and Muhibbat Muhammad Abdullahi

Department of Microbiology, Faculty of Science, Bauchi State University, Gadau Main Campus, Bauchi State, Nigeria.

*Correspondence: kabirukaramba@basug.edu.ng; +2348065927314.

**Abstract**

“*Daddawa*” is a traditional condiment that is produced by the fermentation of African locust beans (*Parkia biglobosa*). It is a popular food condiment and economical method of food preservation specifically in the West and Central African region. The traditional starter culture used was isolated and identified using biochemical methods. Optimization of fermentation conditions of the starter culture and the locust beans was carried out using a central composite design of response surface methodology (RSM). The fermentation was considered at 40°C for 72 h. Bacteria suspected to be *Bacillus subtilis*, other *Bacillus* species, *Staphylococcus aureus* and other *Staphylococcus* species were isolated from the traditional starter culture while *Bacillus subtilis* and other *Bacillus* species, were further isolated from the optimized product. Locust bean fermentation was found to be optimum at 0.12 g of the starter culture and 50 g of locust beans. The results illustrate 0.994 mg/ml concentration of amino acid (L –Valine) and 8.15 x 10⁶ cfu/ml total mesophilic bacterial count at the optimum parameters. This result revealed that *Bacillus* species, most importantly *Bacillus subtilis* are the vibrant bacteria involved in the fermentation process since it was significantly found in the end product.

**Keywords**

Fermentation; Starter Culture; Condiment; Daddawa; Response Surface Methodology

**License:** CC BY 4.0

**How to cite this paper:** Karamba, K.I. and Abdullahi, M.M. (2022). Optimization of traditional starter culture and African locust beans for *daddawa* production using Response Surface Methodology (RSM). *Gadau J Pure All Sci*, 1(2): 166–173. [https://doi.org/10.54117/gjpas.v1i2.28](https://doi.org/10.54117/gjpas.v1i2.28)

1.0 Introduction

“*Daddawa*” is a traditional condiment which is being produced by fermentation of African Locust Bean (*Parkia biglobosa*) (Farinde et al., 2014). It is one of the accepted food condiment in Africa especially the Western and Central African region (Molinos et al., 2016). “*Daddawa*” is a product of fermentation and cheap method of preservation. Small quantity of “*Daddawa*” is used in soup as source of protein, fat and flavor in West Africa (Abdullahi et al., 2020; Quansah et al., 2019). It serves as a tasty additive to soup and sauce due to its high protein content and can replace meat or fish (Jerry, 2016). “*Daddawa*” is prepared from various substrates like Melon seed, Soya bean, Bambara nut. The nomenclature and spelling is dependent on the area it originates. The products are referred to as “*Daddawa*” in Hausa, “*Irzu*” in Yoruba, “*Ogiri*” in Igbo, “*Owo*” in Urhobo and Itsikiri, “*Okipye*” in Igala and Idoma Languages of Nigeria (Achi, 2005). Fermentation is a process whereby microorganism or their enzymes act on food and change it biochemically to produce desired quality such as texture and taste in the food (Campbell-Platt, 2017). Fermentation does not only increase the shelf life but reduce the toxicity of some food substances (Wafula et al., 2016). Changes observed in fermentation of locust beans is related to active metabolism of various microorganisms involved. Bacteria such as *Bacillus subtilis*, *B. licheniformis*, *Staphylococcus saprophyticus*, *Leuconostoc spp.* were reported as responsible for the fermentation of the substrate (Akanni, 2017; Karamba and Yakasai, 2016; 2020; Owo. Changes observed in fermentation of locust beans is related to active metabolism of various microorganisms involved. Bacteria such as *Bacillus subtilis*, *B. licheniformis*, *Staphylococcus saprophyticus*, *Leuconostoc spp.* were reported as responsible for the fermentation of the substrate (Akanni, 2017; Karamba and Yakasai, 2020; Owo...
2.0 Materials and methods

2.1 Collection and identification of materials for the study

Fresh Samples of African locust beans seed (*Parkia biglobosa*) and the Traditional Starter Culture were obtained from Azare main market in Azare Local Government Area of Bauchi State, Nigeria within a period of one day. The samples were taken to Botany Unit, Department of Biological Sciences, Bauchi State University Gadau for taxonomic identification. It was preceded to sorting out in order to remove stones and other foreign particles before processing.

2.2 Characterization of the traditional starter culture

One gram of the traditional starter culture was weighed and suspended in 9 ml of sterile peptone water; the mixture was homogenized and diluted serially to 8 folds. 500 µL of mixture was taken from 10⁻⁶, 10⁻⁷ and 10⁻⁸ dilutions and cultured using pour plate technique. The culture was incubated at 37 °C for 24 h. The total colonies formed were counted using colony counter and the distinct colonies were sub-cultured and incubated for 24 h at 37 °C on a nutrient agar plates. The sub-cultured colonies were used for the gram reactions and other biochemical tests (Cheesbrough, 2006).

2.3 Macroscopic/Microscopic examination

The morphology and colonial appearance of the isolates were examined physically with naked eyes. Microscopic examination was carried out using gram staining technique (Cheesbrough, 2006).

2.4 Biochemical examinations

Biochemical tests such as catalase, coagulase, oxidase, citrate, urease, voges proskauer, motility and triple sugar iron (TSI) and endospore staining test were carried out (Cheesbrough, 2006; Farinde et al., 2014; Tankeshwar, 2015).

2.5 Optimization of fermentation conditions using Response Surface Methodology

Locust beans and the starter culture are the independent variables studied using response surface methodology. Using Design-Expert 6.0.8 (Stat-Ease Inc., Minneapolis, USA) statistical software package, was used to study for the regression analysis of the experimental data and to plot the response surface of the combined effect of the t variables. Locust beans concentration was used within the range of 50 – 100 g, starter culture was utilized within the range of 0.12 - 0.24 g (Table 1) and a total of 13 runs with five center points were conducted on a central composite design (CCD). Upon completion, the amino acid was considered as the response, Y. Verification of the experiment was carried out using the same method comparing the experimental value and the actual value obtained from the experiment.

2.6 Preparation of sample

The locust beans seeds were washed with tap water and boiled to facilitate extraction of the cotyledons for 3 h using a pressure cooker. The boiled beans were then dehulled by pressing in between palms and then washed to remove the seed coats. The extracted cotyledons were boiled for 1 h to aid softening prior to fermentation (Odunfa 1985). The starter culture was weighed using electric weighing balance in accordance with the RSM experimental designs.

2.7 Laboratory preparation of “Daddawa”

The laboratory methods adopted were similar to the traditional method except that glass wares were used as fermentation vessels, aluminum foil instead of the traditional sack, calabash, leaves etc. so also the cooking methods of the seeds, pressure cooker was used instead of the traditional firewood. The processed seeds were measured in accordance with the research design suggested by RSM and placed in 250 mL beaker. All the experiments are conducted in triplicates and the traditional starter culture was also measured in accordance with the RSM design (Table 1). The beakers were then covered with aluminum foil and incubated at 40 °C (Odunfa, 1985).

2.8 Preparation of extract

After 72 h of the fermentation, the optimized fermented products were extracted as reported by Odunfa, (1985) where 5 g of the sample was weighed into a 100 ml conical flask and ethanol water (50:50 vol./vol) was added and ground using mortar and pestle. The suspension was then washed with 5 mL petroleum ether to extract the oil, centrifuged at 5000 rpm in an MSE high speed -18 refrigerated centrifuged at 5 °C for 30 min. The supernatants were used for the analysis.
plates were incubated at 37 °C for 24 h. Total aerobic counts were taken using digital illuminated colony counter. The count for each plate was expressed in colony forming units per milliliter (CFU/mL).

3.0 Results and discussion
3.1 Aerobic bacterial count for starter culture
The total aerobic bacterial count for the traditional starter culture was recorded as 3.9 x 10^8 CFU/mL of the sample obtained with Identification Number B/BOT 023. This implies that the traditional starter culture has a high number of different organisms some of which are believed to be responsible for the fermentation and others could be contaminants. The characterization of the starter culture would enable the identification of fermenting and non-fermenting microorganisms.

3.2 Morphological and biochemical characteristics of the isolates
The result of the biochemical tests carried out on the isolated colonies based on their microscopic and biochemical characteristics are presented in Table 2. The various organisms isolated from the starter culture and the optimized product was recorded in Table 3. The organisms were characterized based on their resultant colonial morphology, Gram reaction and biochemical tests. Isolate 1, 2, 3 and 4 were isolated as distinct colonies on pour plate of the traditional starter culture. Isolate 5 and 6 were isolated from overnight pour plate culture of the optimized product. All the Isolates were found to have the colonial morphology and biochemical characteristics shown in Table 2 and 3. Isolate 1 and 3 were found to be different gram-positive rods which after further biochemical test, isolate 1 was suspected to be \textit{Bacillus subtilis} and isolate 3 was suspected to be another \textit{Bacillus} species. Isolate 2 was suspected to be \textit{Staphylococcus aureus} whereas isolate 4 was suspected to be a species of \textit{Staphylococcus} spp. The other two isolates, 5 and 6 from the optimized product were all rods where isolate 5 was having the same colonial morphology, microscopic and biochemical characteristics with isolate 3 (\textit{Bacillus} spp), likewise isolate 6 was observed to be same as isolate 1 (\textit{Bacillus subtilis}).

### Table 1: Various quantities of starter culture and substrate used in the optimization

| S/N | STD | FACTOR 1 (Locust Beans) (g) | FACTOR 2 (Starter Culture) (g) |
|-----|-----|-----------------------------|-------------------------------|
| 1   | 1   | 50                          | 0.12                          |
| 2   | 3   | 50                          | 0.24                          |
| 3   | 8   | 75                          | 0.26                          |
| 4   | 11  | 75                          | 0.18                          |
| 5   | 4   | 100                         | 0.24                          |
| 6   | 12  | 75                          | 0.18                          |
| 7   | 10  | 75                          | 0.18                          |
| 8   | 9   | 75                          | 0.18                          |
| 9   | 2   | 100                         | 0.12                          |
| 10  | 7   | 75                          | 0.10                          |
| 11  | 13  | 75                          | 0.18                          |
| 12  | 6   | 110.36                      | 0.18                          |
| 13  | 5   | 39.64                       | 0.18                          |

STD = Standard

### 2.9 Determination of amino acids (L – valine)
One (1) ml of the extract solutions were pipetted into clean test tubes. Distilled water was added to each tube to make a total volume of 4 ml. 1 ml of the ninhydrin solution reagent was added to each tube and mixed properly. The tubes were placed in boiling water bath for 15 minutes. They are allowed to cool and 1 ml of 50% ethanol was added to each tube. The change in color developed was measured in spectrophotometer at 570 nm wavelength (Smith and Agiza, 1951). Standard calibration curve was prepared according to Karamba and Ahmad, 2019.

### 2.10 Estimation of Total Mesophilic Bacterial Count of the Optimized Fermented Products
The total viable count was determined using methods describe by Omafuvbe \textit{et al.} (2002) where pour plate method was employed. The bacterial load was estimated using dilution technique. For the optimized fermenting seeds, fivefold serial dilution of the seeds was prepared using sterile distilled water for up to 10^-5. 0.1 ml of 10^2, 10^3,10^4 and 10^5 dilution were inoculated in duplicates by pour plate method on nutrient agar for total aerobic bacterial count. All the colonies observed and recorded as 3.9 x 10^8 CFU/mL.
Table 3: Morphological characteristics of the isolates

| Isolated colonies | Colonial morphology on NA | Suspected organisms       |
|-------------------|---------------------------|---------------------------|
| Isolate 1         | irregular, large whitish colonies | *Bacillus subtilis* |
| Isolate 2         | smooth, creamy colonies      | *Staphylococcus aureus*  |
| Isolate 3         | small, yellowish, smooth colonies | *Bacillus Spp.* |
| Isolate 4         | large, irregular, mucoid, yellowish | *Staphylococcus Spp.* |
| Isolate 5         | small, smooth, yellowish colonies | *Bacillus Spp.* |
| Isolate 6         | large, irregular, whitish colonies | *Bacillus subtilis* |

Table 4: Response (concentration of –amino acid) of the Optimized Products

| S/No. | STD | Factor 1 A: locust beans (g) | Factor 2 B: starter culture (g) | Response 1 (mg/ml) |
|-------|-----|------------------------------|---------------------------------|-------------------|
| 1     | 1   | 50.0                         | 0.12                            | 0.994             |
| 2     | 3   | 50.0                         | 0.24                            | 0.939             |
| 3     | 8   | 75.0                         | 0.26                            | 0.828             |
| 4     | 11  | 75.0                         | 0.18                            | 0.792             |
| 5     | 4   | 100                          | 0.24                            | 0.719             |
| 6     | 12  | 75.0                         | 0.18                            | 0.792             |
| 7     | 10  | 75.0                         | 0.18                            | 0.792             |
| 8     | 9   | 75.0                         | 0.18                            | 0.792             |
| 9     | 2   | 100                          | 0.12                            | 0.931             |
| 10    | 7   | 75.0                         | 0.10                            | 0.972             |
| 11    | 13  | 75.0                         | 0.18                            | 0.792             |
| 12    | 6   | 110.36                       | 0.18                            | 0.808             |
| 13    | 5   | 39.64                        | 0.18                            | 0.928             |

STD = Standard

Table 5: Analysis of Variance (ANOVA) for Central Composite Design

| Source           | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|------------------|----------------|----|-------------|---------|----------|
| Model            | 0.090          | 5  | 0.018       | 52.87   | < 0.0001 | Significant |
| A                 | 0.026          | 5  | 0.0056      | 75.27   | < 0.0001 |            |
| B                 | 0.028          | 5  | 0.0056      | 81.36   | < 0.0001 |            |
| A\(^2\)          | 0.012          | 5  | 0.0024      | 34.26   | 0.0006   |            |
| B\(^2\)          | 0.023          | 5  | 0.0023      | 66.27   | < 0.0001 |            |
| AB                | 6.162E-003     | 1  | 6.162E-003  | 18.11   | 0.0038   |            |
| Residual         | 2.382E-003     | 7  | 3.403E-004  |         |          |            |
| Lack of Fit      | 2.382E-003     | 3  | 7.941E-004  |         |          |            |
| Pure Error       | 0.000          | 4  | 0.000       |         |          |            |

R\(^2\) = 0.9742
Adj R\(^2\) = 0.9558
Cor Total = 0.092
This clearly shows that those Gram-positive rod bacteria isolated from the starter culture were same as those isolated after the 72 h optimization fermentation process. The other gram-positive cocci; isolate 2 and 4 isolated from the starter culture were not found after the 72 h optimization process which indicates that either the organisms are not involved in the fermentation process or they are involved in the early stage of the fermentation process. This result is in conformity with the report made by Antai and Ibrahim (1986), who reported a progressive increase of the dominant Bacillus species throughout the 72 h of fermentation and a relatively high increase of Staphylococcus species in the initial stage (0-24) h of the fermentation which later decline after 48 h. The well-known lipolytic activities of Staphylococcus species is attributed as the source of lipase in the fermentation (Ogunshe et al., 2007). A number of Bacillus species have been reported to be isolated from various fermented condiment (Achi, 2005). The spore forming species of Bacillus subtilis, B. licheniformis were identified to be the main and predominant bacteria present in other fermenting legumes (Omafuvbe et al., 2002). Presently, Bacillus species are agreed to be the predominant species developed in fermentation processes of various legumes (Ouoba et al., 2003). Bacillus subtilis was reported as being responsible for the fermentation in Daddawa and the important flavoring compounds (Beaumont, 2002; Odunfa, 1985). The predominance growth of Bacillus subtilis is favored by the low oxygen tension during the fermentation.

3.3 Optimization using Response Surface Methodology

This method was used in the assessment of the best optimum condition for fermentation of locust beans. The method of analyses illustrated the response gotten after the modification of some parameters that are liable for the fermentation. The free amino acids concentration obtained was considered as the response (Karamba et al., 2017). Protein hydrolysis is the most important biochemical change during Daddawa fermentation which is due to proteinase activity resulting in rapid production of amino acid (Farinde et al., 2014). The strong ammonia characteristics smell at the last stage of the locust beans fermentation was attributed as the end product of proteinase activity (Farinde et al., 2014). The foremost objective of RSM is to determine the optimal condition aimed at obtaining a maximum result. The parameters measured in the experimentation that affects the process of fermentation are locust beans and the starter culture. The design of the experiment is shown in Table 1 and 4. Based on the response obtained, the model was used in generating response surface and contour curves to bring out the factors effect in locust beans fermentation process.

3.4 Central Composite Design (CCD)

In this research, 11 experiments were conducted within a period of 2 weeks. The design and response are illustrated in Table 4. Central composite design response is utilized to produce response surfaces (Figure 1) in order to disclose the effects of these parameters in locust beans fermentation. It illustrates the pattern of interaction and the optimum conditions putting all other parameters into consideration. Y is the anticipated response (amino acid), A is the locust beans and B is the starter culture. The experimental results of the amino acid are given in Table 4. The results of second-order response surface model in the form ANOVA for the maximum canola oil degradation are summarized in Table 5. The precision of a model is judged by the determination coefficient ($R^2$). The $R^2$ values of the model was found to be 0.9742. It assesses how inconsistent tested response can be clarified by research parameters and their

| Table 6: Mesophilic bacterial count of the optimized product |
|------------------|-----------|-----------|---------------|
| **Optimized Samples** | $R_1$ (10^5) cfu/ml | $R_2$ (10^5) cfu/ml | Mean (x10^6) cfu/ml |
| 1 | 101 | 96 | 9.85 |
| 2 | 134 | 122 | 12.80 |
| 3 | 82 | 91 | 8.65 |
| 4 | 88 | 75 | 8.15 |
| 5 | 77 | 85 | 8.10 |
| 6 | 88 | 75 | 8.15 |
| 7 | 88 | 75 | 8.15 |
| 8 | 88 | 75 | 8.15 |
| 9 | 150 | 178 | 16.40 |
| 10 | 74 | 83 | 7.85 |
| 11 | 88 | 75 | 8.15 |
| 12 | 83 | 91 | 8.70 |
| 13 | 70 | 81 | 7.55 |
interface. Adjusted R^2 value was realized to be 0.9558, in which the value was acceptable. This clarified the impression of this polynomial model. It exercises the signal to noise ratio. The “Pred R-Squared” of 0.8166 is in rational pact with the “Adj R-Squared” of 0.9558. The ratio 20.256 which is greater than 4 is desirable. The significance of every coefficient was proven by p values. In essence, all the parameters are significant model terms. Lack of fit test measures signal to noise ratio and Ibrahim et al. (2015) reported not significant lack of fit and described that the model was in an excellent fit. The absolute equation in relation to coded factors is explained below:

\[
Y = + 0.79 - 0.057A - 0.059B + 0.041A^2 + 0.057B^2 - 0.039AB
\]

The absolute equation in relation to actual factors is:

\[
Y = +1.66588 - 7.37853E-003 - 4.71176 + 6.55000E-005 + 15.81597 - 0.0026167
\]

Figure 1 explains the closeness between the predicted and the actual values of the designed experiment which is the results obtained; it portrays that the responses are similar to the predicted values by the design.

3.5 Interactions between factors

Three-dimension contour plots of the central composite design of the response surface method disclose the collaboration between two (2) factors at a time (Figure 1). It describes the interrelations between the 2 variables interacting with each other. The interaction between locust beans and starter culture illustrates the relationship in which at both the extremes, the response was low. At locust beans (100 g) and starter culture (0.24 g), response was divergent illustrating high axis and very low relationship between the factors depicted by inverted contour curve. At the factors; locust beans (50g) and starter culture (0.12) the interaction was high illustrating low axis and peak of the reactions. In the design of the experiment by the central composite design 50 g of locust beans was utilized against 0.24 g of starter culture in serial number 2 while in serial number 9, 100 g of locust beans was used against a starter culture of 0.12. This indicates reverse in parameters utilization. The high amount of substrate in sample 9 result in higher bacterial growth compared to sample 2 (Table 6). The p value is higher than the F-ratio value and lower than 0.05 thus significant. This shows that there is significant effect of variability of the two factors (i.e. the result is significant in variability of the quantities of the locust beans and the starter culture utilized).

3.6 Verification of experiment

The verification of experiment was carried out using the optimum parameters of 75 g, Locust Beans and 0.18 g Starter Culture in comparison with the experimental value of the designed experiment.

Figure 1: Three dimensional contour curves illustrating interactions between factors

The optimum point obtained in the experiment was 0.996 concentration of amino acid produced with a mean bacterial growth of 8.17 x 10^6 cfu/ml which is slightly higher than the highest results obtained in the actual experiment which is 0.994 amino acid concentration with mean bacterial growth of 9.85 x 10^6 cfu/ml at 50 g Locust Beans and 0.12 g Starter Culture. From the T-Test carried out it illustrates no significant difference between the two results. This indicates the reliability of the optimum factors, reducing the wastage in the utilization of starter culture with respect to amount of locust beans to be fermented for the production of Daddawa thus reducing the cost of production.

4.0 Conclusion

Traditional starter culture has been optimized for African Locust Beans (Parkia biglobosa) fermentation into Daddawa as a condiment for food seasoning. The investigation illustrates that the traditional starter culture contains various bacteria mostly Bacillus species for the fermentation. This study shows that the culture requires no additional supplement to kick start the process under normal atmospheric temperature and was able to obtain up to about 99% fermentation of the product evidenced by the optimization using response surface method. This illustrates that local industries utilizing the traditional starter culture can utilise 0.12 g of the starter culture against 50 g of locust beans. It reduces wastages and cost of production as well gives desired result for consumers.

Declarations
Consent for publication
All authors have read and consented to the submission of the manuscript.
Availability of data and material
Not Applicable.
Competing interests
All authors declare no competing interests.

Funding
There was no funding for the current report.

Author’s contribution
Kabiru Ibrahim Karamba: Conceptualization, methodology and supervision. Muhibbat Muhammad Abdullahi: Carrying out the research, formal analysis and writing original draft.

Acknowledgment
This project was supported by Bauchi State University Gadau, Bauchi State, Nigeria.

References
Abdullahi, K.B., Kankara, S.S., Tukur, K., 2020. Effect of Varying Concentrations of Table Salt on the Microbial Fermentation of Parkia biglobosa (Jacq.) Benth Seeds. UMYU Journal of Microbiology Research 5, 98–105.

Achi, O.K., 2005. Traditional fermented protein condiments in Nigeria. African Journal of Biotechnology 4, 1612 - 1621.

Akanni, G.B., 2017. Alkaline fermentation of Bambara groundnut (Vigna subterranea L. Verdc.) as Dawadawa-like African food condiment (PhD Thesis). University of Pretoria.

Antai, S.P., Ibrahim, M.H., 1986. Micro-organisms associated with African locust bean (Parkia filicoidea Welw) fermentation for ‘dawadawa’ production. Journal of Applied Bacteriology 61, 145–148.

Beaumont, M., 2002. Flavouring composition prepared by fermentation with Bacillus spp. International journal of food microbiology 75, 189–196.

Bezerra, M.A., Santelli, R.E., Oliveira, E.P., Villar, L.S., Escaleira, L.A., 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta 76, 965–977.

Campbell-Platt, G., 2017. Food science and technology. John Wiley & Sons, 2, 1-576.

Cheesbrough, M., 2006. District laboratory practice in tropical countries, part 2. Cambridge university press.

Farinde, E.O., Adeniran, H.A., Abiose, S.H., 2014. Comparative Microbial Assessment of Fermented Lima Bean (Phaseolus lunatus) and Locust Bean (Parkia biglobosa) in Production of Daddawa. Microbiology Research Journal International 4, 772–784.

Ibrahim, S., Shukor, M.Y., Khalil, K.A., Helmi, M.I.E., Syed, M.A., Ahmad, S.A., 2015. Application of response surface methodology for optimising caffeine-degrading parameters by Leifsonia sp. strain SIU. Journal of Environmental Biology 36, 1215–1221.

Ibrahim, S., Zahri, K.N.M., Convey, P., Khalil, K.A., Gomez-fuentes, C., Zulkarnain, A., Alias, A.S., González-rocha, G., Ahmad, S.A., 2020. Optimisation of biodegradation conditions for waste canola oil by cold-adapted Rhodococcus sp. AQ5-07 from Antarctica. Electronic Journal of Biotechnology 48, 1–12.

Jerry, O.U., 2016. Microbial Technology &Food Security: Microorganisms Put Safe Food on Our Tables, 1, 27 - 674.

Karamba, K.I., Ahmad, S.A., 2019. Mathematical Relationship of Optical Density, Total Viable Count and Microbial Biomass for Growth of Serratia marcescens Strain AQ07 on Cyanide. Journal of Environmental Microbiology and Toxicology 7, 7–9.

Karamba, K.I., Ahmad, S.A., Azham, Z., Syed, M.A., Khalil, K.A., Shamaan, N.A., Dahalan, F.A., Shukor, M.Y., 2016. Optimisation of biodegradation conditions for cyanide removal by Serratia marcescens strain AQ07 using one-factor-at-a-time technique and response surface methodology. Rendiconti Lincei 27, 1–13.

Karamba, K.I., Ahmad, S.A., Zulkharnain, A., Khalid, A., Shukor, M.Y., 2017. Biodegradation of cyanide and evaluation of kinetic models by immobilized cells of Serratia marcescens strain AQ07. Int. J. Environ. Sci. Technol. 14, 1945–1958.

Karamba, K.I., Yakasai, H.M., 2019. Growth Characterization of Bacillus amyloliquefaciens strain KIK-12 on SDS. Journal of Biochemistry, Microbiology and Biotechnology 7, 26–30.

Molinos, A.C., Galvez, A., Raj, A., Chauhan, A., Gupta, A.D., Chye, F.Y., Rapsang, G.F., Abrol, G.S., Bareh, L., Naomichi, I., 2016. Indigenous Fermented Foods of south Asia 7, 1.

Odunfa, S.A., 1985. Biochemical changes in fermenting African locust bean (Parkia biglobosa) during ‘iru’fermentation. International Journal of Food Science & Technology 20, 295–303.

Ogunshe, A.A., Omotosho, M.O., Ayansina, A.D.V., 2007. Microbial studies and biochemical characteristics of controlled fermented afiyo-a Nigerian fermented food condiment from Prosopis africana (Guill and Perr.) Taub. Pakistan Journal of Nutrition 6, 620–627.

Olayanke, E.M., Omobayonle, F., 2010. Evaluation and optimization of critical control points in
the production of Iru. Research Journal of Microbiology 5, 1258–1266.

Omafuvbe, B.O., Abiose, S.H., Shonukan, O.O., 2002. Fermentation of soybean (Glycine max) for soy-daddawa production by starter cultures of Bacillus. Food Microbiology 19, 561–566.

Ouoba, L.I.I., Cantor, M.D., Diawara, B., Traore, A.S., Jakobsen, M., 2003. Degradation of African locust bean oil by Bacillus subtilis and Bacillus pumilus isolated from soumbala, a fermented African locust bean condiment. Journal of Applied Microbiology 95, 868–873.

Quansah, L., Mahunu, G.K., Tahir, H.E., Mariod, A.A., 2019. Parkia biglobosa: Phytochemical Constituents, Bioactive Compounds, Traditional and Medicinal Uses, in: Wild Fruits: Composition, Nutritional Value and Products. Springer, 271–284.

Smith, A.M., Agiza, A.H., 1951. The determination of amino-acids colorimetrically by the ninhydrin reaction. Analyst 76, 623–627.

Wafula, E.N., Franz, C.M., Rohn, S., Huch, M., Mathara, J.M., Trierweiler, B., 2016. Fermentation of African indigenous leafy vegetables to lower post-harvest losses, maintain quality and increase product safety. African Journal of Horticultural Science 9, 1–13.