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Full Length Research Article

OPTIMIZATION OF AFRICAN LOCUST BEANS "DADDAWA" PRODUCTION USING RESPONSE SURFACE METHODOLOGY

*Nafisa M.N; Abdulfatai K; Orukotan A.A. and Idris S.

Department of Microbiology, Faculty of Pure and Applied Sciences, Kaduna State University

*Corresponding Author Email Address: nafisamnasir647@gmail.com

ABSTRACT
In this research, optimization of 'Daddawa' production was carried out using Response Surface Methodology. Fresh samples of African locust beans seeds were obtained from Central Market, Kaduna metropolis. The *Bacillus subtilis* used to accomplish the fermentation was isolated from fermented products ('Ogi and Daddawa') and was identified using conventional biochemical tests and molecular method using 16SrDNA sequence homology. African locust bean seeds were processed into '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. The production was done in 100 mL beaker. Three variable factors which include locust beans weight, fermentation duration and inoculum were varied to optimize the production according to the experimental runs generated by Design Expert Software based on Central composite design (CCD). Percentage protein of the Daddawa produced was used as response to analyse the optimization using Response Surface Methodology. The bioprocess was optimized for "Daddawa" production using *B. Subtilis* in which a percentage protein yeild of 98±0.20 was recorded. These optimum conditions were 50g locust bean weight, 5 days fermentation duration, and 0.6mL inoculum volume. The proximate composition of the optimized product shows higher values compared to the unoptimized Daddawa. Furthermore, optimization experiments showed an improvement of 18-20% over traditional methods. This has underscored the importance of response surface methodology in bioprocess optimization contributing to standardization of parameters, enhanced yields, and a scientific basis to improve this indigenous food at industrial scale.

Keywords: Daddawa, Optimazation, *Bacillus subtilis,* Response Surface Method.

INTRODUCTION

Parkia biglobosa is a savannah tree belonging to the subfamily *Mimosoidea*. It is resourceful as it provides food and shelter to human and animals (Akpi *et al.,* 2020) It is processed into a local condiment popularly called "iru" amongst the "Yoruba" people of Nigeria, other names of the condiment includes "Ogiri" among the Igbo's; "daddawa" amongst the Hausa. The condiment is obtained from seeds of *Parkia biglobosa* that have been washed, de-hulled, fermented and molded into balls (Akande *et al*., 2010; Ezeocha *et al*., 2022). Processing into iru is usually done traditionally with rudiment technology in the processing line which is time-consuming and labor-intenxsive (Alsalman and Ramaswamy *et al*., 2020). Many women across the diverse ethnic groups in Nigeria feel their soup is not complete till iru is added: it is seen as a meat substitute. However, the taste, smell and appearance of iru have stood as a deterrent in its use. Locust beans dehulling by pressing with feet, washing, wrapping with jute bags, leaves, sacks, salting and molding, air/sun drying were reported as the critical control point (CCP) during the traditional fermentation processes of locust beans (Campbell-Platt, 2017; Yadav *et al*., 2021).

Most condiments are produced in traditional small-scale family settings with a great deal of variation in the weather. The fermentation process is typically carried out in a moist solid form, aided by ambient temperatures and requiring contact with the proper inoculum of diverse microorganisms. The techniques used to process the most of food condiments (Daddawa) have raised concerns because of the negative effects they have on both customers' health and lives.

Optimization literarily means discovering best solution, the optimum parameter in the mid of varying factors that are dependent on predefined demands known as constraints (Karamba *et al.,* 2016). Over the years, there have been several attempts to optimize the production of this important food additive with a view to improving the quality of the finished product. In most cases, the conventional approach of one factor at a time optimization is what is being employed. However, this approach is time-consuming and was found to be inadequate in giving the details about the simultaneous interaction among the several factors that affect production and the quality of the product. Interestingly, a more robust approach based on Response Surface Methodology (RSM) is now available; in which a software such as Design Expert can be used to design a 'several factors at a time' optimization. Design of experiments (DOE) requires a systematic method of determining the relationship between factors affecting a process and the output of that process. The software predicts and designs experiments using advanced statistical tool which allows a researcher to study efficiently the effect of a large number of variables with a minimum effort in data collection.

In most of the bioprocess experiments, microorganisms are subjected to optimal nutrient and environmental condition for better growth to be attained in order to put it into its best biotechnological manipulations (Karamba *et al.,* 2017; Yadav *et al*., 2021). In this study, an attempt was made to optimize African locust bean fermentation for 'daddawa' production using RSM and *Bacillus subtilis* as starter culture. By systematically varying fermentation parameters and modeling the results, optimal time, temperature and inoculum levels could be identified to maximize 'daddawa' protein yield.

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MATERIALS AND METHODS

Collection and Identification of African Locust Bean Seed Samples

Fresh samples of African locust beans seed were obtained from Central market in Kaduna metropolis. Kaduna State, Nigeria. The samples were taken to the Botany Unit, Department of Biological Sciences, Kaduna State University for taxonomic identification.

Isolation and Identification of *Bacillus subtilis*

Two fermented products (Ogi and "Daddawa") were used as a source of the organism (*Bacillus subtilis*). One (1) g of each fermented food sample was weighed and suspended in 9 mL of sterile peptone water (1:9); the mixture was homogenized and diluted serially to 10-folds. Five hundred (500) µL inoculum was taken from 10^{-8} dilutions and cultured using pour plate technique onto Tryptone Soy Agar (TSA). The culture was incubated at 37 °C for 24 h. Separate and distinct colonies were sub-cultured on a nutrient agar to obtain the pure culture. The pure isolates were preserved in 20% glycerol. The sub-cultured colonies were used for the gram reactions and other biochemical tests (Cheesbrough, 2006).

Identification of the Isolates

Culture characterization and Grams' staining

The colony appearances of the isolates were examined physically with naked eyes to determine the colony characteristics. Pure culture of the isolate was Gram stained and observed using under microscope using X100 oil immersion objective lens as described by Cheesbrough (2006).

Biochemical characterization of the isolates

Biochemical test which include catalase, coagulase, oxidase, citrate, urease, Voges Proskauer, Triple Sugar Iron (TSI) and motility test were carried out to identify the isolate (Farinde *et al.,* 2014).

Catalase test

About 2mL of hydrogen peroxide solution was poured into a test tube. Using a sterile glass rod, colonies of the test organisms were picked aseptically and placed into hydrogen peroxide solution and observed for bubbles. Catalase positive organisms produced bubbles while catalase negative organisms did not (Idise, 2010).

Oxidase test

A piece of oxidase test strip was held with forceps and the strip was placed onto an area of heavy growth of the test organism. Change of colour to purple within 10 seconds was taken as positive result while no change in colour for more than 60 seconds was taken as negative change (Idise, 2010).

Voges Proskauer test

Sterile glucose phosphate broth (10mL) in a test tube was inoculated with the test isolates and incubated for 48 h at 37°C. After incubation, 0.5mL of 0.5% alpha-Naphthol solution and 0.5mL of 16% potassium hydroxide solution were introduced into the mixture. The mixture was then shaken vigorously and left for about 10 min. A red colour indicated a positive result, signifying acetoin production, while the absence of colouration indicated a negative result. (Cheesebrough, 2006).

Methyl red test

Ten milliliters of glucose phosphate peptone broth were dispensed into test tubes, which were then capped with cotton wool wrapped in foil and sterilized. The test organisms were inoculated and incubated at 37°C for 48 h. After the incubation period, a few drops of methyl red indicator were added to the cultures. The production or development of a red colour was considered a positive reaction, while the absence of the colour indicated a negative reaction (Cheesebrough, 2006).

Citrate utilization

The isolates were stab-inoculated onto Simon citrate medium. The tubes were subsequently incubated at 37°C for 24 h. A positive result was observed as the indicator changed from green to an intense Prussian blue colour (Farinde *et al.,* 2014). The colour change from green to Prussian blue indicates the utilization of citrate.

Indole test

The peptone broth was inoculated with the test organism and incubated at 30°C for 48 hours, with a control kept for comparison. Kovac's reagent was added gradually, drop by drop. The tubes were gently shaken and left to stand for 10 minutes to allow the formation of a distinct layer. The presence of a red-coloured layer on top of the tube indicated a positive result (Farinde *et al.,* 2014).

Sugar Fermentation Test

A 1% (w/v) peptone water and sugar broth were prepared by combining peptone water and the respective sugars. Bromocresol was added as an indicator. The sugars include glucose, maltose, fructose and lactose. Each test tubes were dispensed with 10 mL of the broth, with durham tubes inverted inside them, and sterilized by autoclaving at 115 °C for ten minutes. The test organisms were then inoculated into the broths in the test tubes and incubated at 37°C for 48 h. Uninoculated tubes served as controls. A positive reaction was indicated by a colour change of the broth medium from red to yellow, and sometimes gas production in the Durham tube (Cheesebrough, 2006).

Motility test

A luxuriant growth of the organisms was picked up and stabbed onto the Nutrient agar slant at right angle to the bottom of the tube. This was then incubated at 37°C for 28h and observed for pattern of growth of the organism (Idise, 2010). Motile organisms grew away from the line of stabbing while non motile organisms grew along the line of stabbing.

Molecular Identification of the Isolates

Genomic DNA Extraction

The genomic DNA of isolate was extracted and purified using the DNA isolation kit Quick-DNATM (Zymo Research, USA) according to the manufacturer's directions and confirmed using 0.5% agarose gel electrophoresis.

Amplification of 16S RNA Gene

The Polymerase chain reaction (PCR) was conducted using the universal primers 341F and 962R (Mafra *et al*., 2008). The PCR reaction mixtures consist of master mix and the conditions was profiled for 30 minutes consisting of about 30-40 cycles, with denaturation at about 94 °C for the period of 30 seconds, annealing

at 51 °C for the period of 45 seconds and extension at 72 °C for the period of 1-5 minutes.

Agarose gel electrophoresis
About 1.5g of agarose was heated in a boiling water until it was completely dissolved. Appropriate number of combs was placed in gel tray. Five micro- liters of ethidium bromide was added to the cooled gel and poured into gel tray. The gel casting was made after cooling to about 55°C, it was then allowed to cool for 30 minutes at 30°C. The gel was placed in the electrophoresis chamber and covered with buffer (TBE). DNA amplicon and standard (Ladder) were loaded into gel wells. The electrophoresis system was connected to power supply and run for 1 hour. Deoxyribonucleic Acid (DNA) bands were visualized using gel imaging system.

Sequencing
The PCR product was purified and analyzed by Sanger (dideoxy) sequencing Technique to determine the nucleotide sequence of the amplified gene of the isolates using automated PCR cycle-Sanger Sequencer™ 3730/3730XL DNA Analyzers from Applied Biosystems. The sequencing analysis was done at Inqaba Biotechnology Pty South Africa.

Sequence Analysis and *BLAST*
The sequences in the forward and reverse files were analyzed using the Sequence Scanner Software v1.0 (Applied Biosystems Thermo Fischer Scientific). The gene sequence of each isolate was used as query sequence in the Genbank to identify the organism using BLAST (Basic local alignment search tool) at the website of the National Center for Biotechnology Information (NCBI); http://www.ncbi.nlm.nih.gov/blast) (Souza *et al*., 2016).

Optimization of Fermentation Conditions using Response Surface Method
Three factors were varied to optimize the production, these include locust beans concentration, fermentation duration and inoculum volume. The Design Expert software 6.0.8 (Stat-EaseInc. Minneapolis, USA) was used to generate the experimental runs based on the central composite design (CCD) of the Response Surface Methodology. Table 1 gives information about the variable factors used and the respective lower and upper limits used in the design. Upon completion, the % protein was considered as the response, (*Y-axis)*. Verification of the experiment was carried out using the same method comparing the experimental predicted value and the actual value obtained from the experiment

Table 1: Design of the Experimental Factors

Factors	Notation	Units	Levels	
			-	+
Locust Bean weight.	A	Gram	10	50
Fermentation Duration	B	Days	1	5
Inoculum Volume.	C	mL	0.1	0.5

Table 2 shows the experimental runs generated by the software based the minimum and maximum levels of the variable factors used. The design produced 15 experimental runs.

Table 2: Design of the Experimental Results of Percentage Protein Yield of "Daddawa"

Run	A Locust Bean weight (g)	B Fermentation Duration (Days)	C Inoculum Volume. (mL)
1	30	2	0.3
2	30	3	0.6
3	10	5	0.5
4	58	3	0.3
5	30	3	0.3
6	50	1	0.5
7	30	3	0.3
8	10	1	0.1
9	30	3	0.3
10	50	5	0.1
11	30	6	0.3
12	30	3	0.3
13	30	3	0.3
14	10	3	0.3
15	30	3	0.2

Key: g = grams, **mL** =Millilitre

Preparation of African Locust Bean Seed Sample
The locust beans seeds were washed with tap water and boiled for 3 h using a pressure cooker to facilitate extraction of the cotyledons from seed coat. 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 setup (Odunfa, 2010). The processed sample was weighed using electric weighing balance.

Inoculum Preparation of *Bacillus subtilis*
Inoculum used for the experiment was freshly prepared. Using where a loopful of single bacterial isolate from pure culture plate was aseptically transferred into a conical flask containing 10 mL of sterilized Nutrient Broth. The flask was incubated in orbital shaker set at a speed of 150 rpm at 37°C for the period of 24 h. Optical density of the culture was determined to assess the need of adjusting the cells concentration of the inoculum.

Laboratory Production of "Daddawa" from Locust Bean Seeds
Raw African locust bean seeds were processed into "daddawa" using the modified method by Achi (2005). 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. Processing of the seeds was carried out by boiling the locust bean seed for 2h during which water was removed twice. The testa was then removed by pounding lightly in a laboratory mortar and pestle. The seeds were then washed to remove the testa from the cotyledon. The cotyledons were then re-

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boiled for 2 h and the water discarded. The processed seeds were measured in accordance with the research design suggested by RSM and placed in 100 mL beaker (Table 2). All the experiments were conducted in duplicates and the inoculum was added in accordance with the RSM design (Table 3.2). The beakers were then covered with aluminum foil and incubated at 25±5°C (Odunfa, 2010). The fermentation was carried out for 1-5 days.

Preparation of Extract for Protein Estimation

After the fermentation, the fermented products were extracted as reported by Odunfa (2010) where 5 g of the sample was weighed into a 100 mL conical flask and ethanol water (50:50vol./vol) was added and grounded using mortar and pestle. The suspension was then washed with 5 mL petroleum ether to extract the oil, centrifuged at 5000rpm and refrigerated at 5 ºC for 30 min. The supernatants were collected and used for further analysis.

Determination of Amino Acids

One (1) mL each of the extract solutions was pipetted into clean test tubes. Distilled water was added to each tube to make a total volume of 4 mL. One (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 were allowed to cool and 1 mL of 50% ethanol was added to each tube. The change in color developed was measured in spectrophotometer at 450 nm wavelength (Smith and Agiza, 2010). Standard calibration curve was prepared according to Karamba and Ahmad, (2019).

Preparation of Protein Standard Curve

Initially, 0.0-1.0mg/mL concentrations of protein solutions from a stock of 200 mg/mL were prepared at different ranges of 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0. Five (5) mL of protein solution from were transferred in the test tubes labelled of respective concentration along with 1 extra test tube in which 1 mL of distilled water and 0.5 mL of unknown solution was transferred to serve as control. One (1) mL of clean cupper reagent was added in all the test tubes incubated for 10 minutes at room temperature the colour turned to blue followed by adding 0.5 mL of Follin Clocalteu's phenol reagent and further incubated for 10 minutes at room temperature. Afterwards the test tubes were allowed to cool to room temperature. The absorbance of each of the samples was then measured at 650 nm in a spectrophotometer. A standard curve was prepared plotting the standard protein concentration (mg/mL) (x-axis) against the respective absorbance (y-axis) in order to achieve a best fit straight line.

Calculation of the Amount of % Protein Yield

Amount of Protein in 1mL of the Unknown Sample (%) = $\frac{\text{OD X Dilution Factor}}{\text{Volume of Unknown used}}$

Where, OD= optical density of unknown sample.

Determination of Proximate Composition of "Daddawa"

The proximate composition: percentage moisture content, ash content, protein, fats and oil, crude fibre content, and carbohydrate content of the "daddawa" were determined in triplicates using the method described by AOAC (2011). The procedure is described below.

Determination of Percentage Moisture Content

Aluminum dishes were washed and dried to a constant weight in an oven at 100^0C. They were later removed and cooled in a desiccator and weighed (W1). Two (2) grams of the grounded (powder) of each sample was placed in the weighed moisture dish (W2). The dish containing each sample was kept in an oven for about 3 hours, the samples were removed and cooled in the desiccator and weighed W3.

The % of moisture was calculated as: $\frac{W2-W3}{W2-W1} \times 100$

Determination of Percentage Ash Content

Crucibles were cleaned and dried in the oven, cooled in the dessicator and weighed (W1). Two (2) grams of the grounded (powder) of each sample was placed in the crucibles and weighed (W2). They were transferred into the Muffle furnace for about 550º C, then removed and cooled in the dessicator and weighed (W3).

The % of Ash was calculated as follows: $\frac{W3-W1}{W2-W1} \times 100$

Determination of Percentage Crude Protein

Protein determination was achieved through three stages which include digestion, distillation and titration. Briefly, two (2) gram of each sample was weighed into a kjeldahl flask. Catalyst (copper) and 15mL concentrated sulphuric acid (H_2SO_4) were added. The solution was heated till it assumed a green colour in the fume cupboard. It was then cooled and any black particles showing at the mouth and neck of the flask was washed down with distilled water. After cooling, the digest was transferred with several washings into 100ml with distilled water.

The Markham distillation apparatus was steamed for 15minutes before use. A 100ml conical flask containing 10ml of Boric indicator was placed under the condenser. Ten (10)ml of the digest was added into the body of the apparatus via the small funnel aperture, it was washed down with distilled water followed by 10ml of 40% NaOH solution. Steam through for about 5-7 minutes to collect ammonium sulphate (30-40ml). The receiving flask was removed and the tip of the condenser was washed down into the flask.

Finally, the solution was titrated into the receiving flask using N/100 (0.01N) hydrochloric acid, the Nitrogen content was calculated and hence the protein content of the sample. The blank was measured along with the sample always. The % of protein was calculated as: Final reading –Initial reading-blank (0.2) x standard number of Nitrogen (1.4) divide by initial weight (0.5) x standard number of protein (6.25).

Determination of Percentage Lipids (Fat) Content

Oven dried 250 mL clean round bottom flask was transferred into the dessicator and allowed to cool. Empty filter paper was weighted and labeled W1. Two (2) gram of each sample was weighted into labeled thimbles (filter paper) W2. Boiling flask was filled with petroleum spirit or N-hexane. Soxhlet apparatus was assembled and allowed to reflux for 8hours. It was removed and transferred to an oven to dry. Then transferred from the oven into a dessicator and allowed to cool then weighed W3.

The % of Fat was calculated as: $\frac{W2-W3}{W2-W1} \times 100$

Determination of Percentage Fiber Content

Exactly two (2) grams of the each sample was placed in a beaker containing 1.2 mL of H_2SO_4 per 100ml of solution and boiled for about 30min, the residue was filtered and wash with hot water, the residue was transferred to a beaker containing 1.2 gram of NaOH per 100 mL of solution and boiled for about 30 min, the residue was

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washed with hot water and dried in an oven and weighed (C2), each of the weighed sample was incinerated in a furnace for about 55O^{O}C, removed and allowed to cool, and weighed (C3).
The % of Fibre was calculated as: $\frac{C2-C3}{W} \times 100$

Determination of Percentage Carbohydrate (CHO) Content
By difference, in this method carbohydrate content was obtained by calculations having estimated all other fractions by proximate analysis.
CHO =100- (% of moisture + %Ash + %Protein + %Fat)

Estimation of Total Mesophilic Bacterial Count of the Optimized Products
The total viable count was determined using methods describe by Omafuvbe *et al.* (2010) where pour plate method was employed. The bacterial load was estimated using dilution technique. For the optimized fermenting seeds, 5-fold serial dilution of the seeds was prepared using sterile distilled water for up to 10^{-5}. A 0.1 mL of10^{-4} dilution was inoculated in duplicates by pour plate method on nutrient agar for total aerobic bacterial count. All the plates were incubated at 37 ºC 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/g).

Analysis of the Designed Experiment
At the end of the optimization experiment, percentage (%) protein yield was determined and used as response. Based on the combination of 3 variables, analysis of the regression of the experimental data was carried out using the same software used to design the experiment, The response (dependent) variable (% Protein yield) was represented on the Y-axis. Each independent variable was studied at two levels**.** The data were analyzed by fitting it to the second- order polynomial regression equation as below:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \beta_i x^2_i + \sum_{i=1}^{k} \sum_{j=1}^{k-1} \beta_{ij} x_i x_j$$

Where **y**= the response (dependent) variable.
β0 = the intercept; *βi, βii, βij*= the regression coefficients variables for linear, quadratic and interaction, respectively, whereas *xi* and *xj*= independent variables.

Statistical Analysis of Data
The data collected were analyzed using Statistical Design Expert Software Version 13.0 (Stat-Ease, Minneapolis, MN, USA). The data were subjected for Analysis of Variance (ANOVA), and the Duncan Multiple Range was tested for the significant difference at $p<0.05$.

RESULTS

Morphological and Biochemical Characterization of the Isolates
The cultural morphology of bacterial isolates in terms of color, size, shape and margin shown in Table 3. Colonies of all the isolates appeared whitish, with size ranging from big to small, rough and circular shape, and entire to undulate margin. The biochemical characteristics indicated that all the isolates were positive to Gram reaction, Motility, catalase, citrate, and lactose, fructose and glucose, catalase, maltose, but were negative to Voges Proskauer, methyl red and indole, as presented in Table 3. The 16S rDNA sequence analysis confirmed the isolate as *Bacillus subtilis.* Figure 1 shows the electropherogram of the amplify 16SrDNA gene of the isolate.

Table 3: Cultural Morphology and Biochemical Characteristics of Suspected *Bacillus* specie Isolates

Isolate Code	Colour	Size	Shape	Margin	Gram Reaction	Oxidase	Motility	Catalas	Voges Proskauer	Urease	Citrate	Indole	Methyl Red	Maltose	Glucose	Lactose	Fructose	Probable Organisms
D_1	Whitish	Big	Rough	Undulate	+	+	+	+	+	-	+	-	-	+	+	+	+	*Bacillus* sp.
D_2	Whitish	Big	Citcular	Entire	+	+	+	+	+	-	+	-	-	+	+	+	+	*Bacillus* sp.
O_1	White	Small	Rough	Entire	+	+	+	+	+	-	+	-	-	+	+	+	+	*Bacillus* sp.
O_2	Whitish	Big	Rough	Entire	+	+	+	+	+	-	+	-	-	+	+	+	+	*Bacillus* sp.

Key: D ="daddawa" , **O =**Ogi**, + =** Positive**, - =** Negative, **sp** = specie

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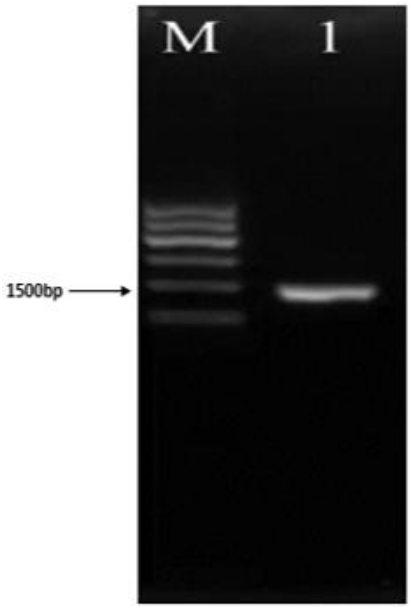

Figure 1: Gel Electropherogram of 1the 6S rRNA for *Bacillus subtilis.* M=Biomarker (1kbpladder), 1=*Bacillus subtilis*, bp=base pairs

Optimized Conditions for "Daddawa" Production

The designed experimental runs of RSM optimization for 'Daddawa' production with the respective values of both experimental (Actual) and predicted response are presented in Table 4. From the table, it can be seen that the values of experimental response were in most cases close to the corresponding predicted values. Highest protein yield was recorded when 30 g of the locust bean, 3 days of fermentation duration and 0.6 mL inoculum volume were used.

Table 4: Designed Experimental runs of RSM Optimization for 'Daddawa' Production

Run	A Locust Bean weight (g)	B Fermentation Duration (Days)	C Inoculum Volume. (mL)	Response % Protein Yield	
				Predicted Value	Actual Value
1	30	2	0.3	32.78	32.00
2	30	3	0.6	88.67	87.94
3	10	5	0.5	46.21	47.00
4	58	3	0.3	43.66	42.85
5	30	3	0.3	44.21	40.00
6	50	1	0.5	59.88	60.67
7	30	3	0.3	44.21	50.60
8	10	1	0.1	43.31	44.10
9	30	3	0.3	44.21	42.00
10	50	5	0.1	50.08	50.87
11	30	6	0.3	52.43	51.70
12	30	3	0.3	44.21	40.30
13	30	3	0.3	44.21	49.60
14	10	3	0.3	40.89	40.09
15	30	3	0.2	33.38	32.60

The absolute equation in relation to coded factors is explained below:
$Y = + 44.21 + 0.9689A + 6.87B + 18.31 C + 1513 AB + 8.59 AC – 4.14BC$

Analysis of Variance (ANOVA) for the response surface regression is shown in Table 5. The P-value of 0.05 were used to determine the significance of the model. A large value of lack of fit obtained implies that the models are adequate to predict the responses. Three-dimension (3D) contour plots of the interaction between locust beans weight and fermentation duration is shown in Figure 2. Likewise, the interaction between locust beans weight and Inoculum volume is depicted Figure 3.

The verification of experiment was carried out using the optimum parameters of 50 g Locust Beans weight, 5 days of fermentation

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period and 0.5 mL of inoculum volume. This yielded a percentage protein of 98±0.20 which is very close to the corresponding predicted value of 95.60 as shown in Table 6.

Mesophilic Bacterial Count of the Optimized Product

The mesophilic bacterial count of the optimization experiments is shown in Table 7. Highest count (9.0 X 10^4CFU/g) was recorded in run 12, whereas the lowest mesophilic count of 1.1 X 10^4 CFU/g was recorded in run 6.

Proximate Composition of Optimized and Unoptimized Produced "Daddawa"

Optimized produced 'Daddawa' had the highest yield of % protein of 38.73%, 28.18% lipid, moisture content and carbohydrate content of 29.58%. The unoptimized produced 'Daddawa' had 29.4 %of protein, 22.13% lipid, 0.6 %ash and 33.41 %carbohydrate.

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

Source	Sum of Squares	df	Mean Square	F-value	p-value	Remarks
Model	2426.91	9	269.66	11.84	0.0071	Significant
A-Locust Bean weight.	3.72	1	3.72	0.1633	0.7029	
B-Fermentation Duration	196.66	1	196.66	8.63	0.0323	Significant
C- Inoculum Volume.	1397.81	1	1397.81	61.37	0.0005	Significant
AB	467.61	1	467.61	20.53	0.0062	Significant
AC	150.73	1	150.73	6.62	0.0499	Significant
BC	34.13	1	34.13	1.50	0.2754	
A^2	7.20	1	7.20	0.3161	0.5982	
B^2	7.08	1	7.08	0.3108	0.6012	
C^2	472.83	1	472.83	20.76	0.0061	Significant
Residual	113.89	5	22.78			
Lack of Fit	6.53	1	6.53	0.2433	0.6477	Not Significant
Pure Error	107.36	4	26.84			
Cor Total	2540.80	14				

P<0.05= significance.

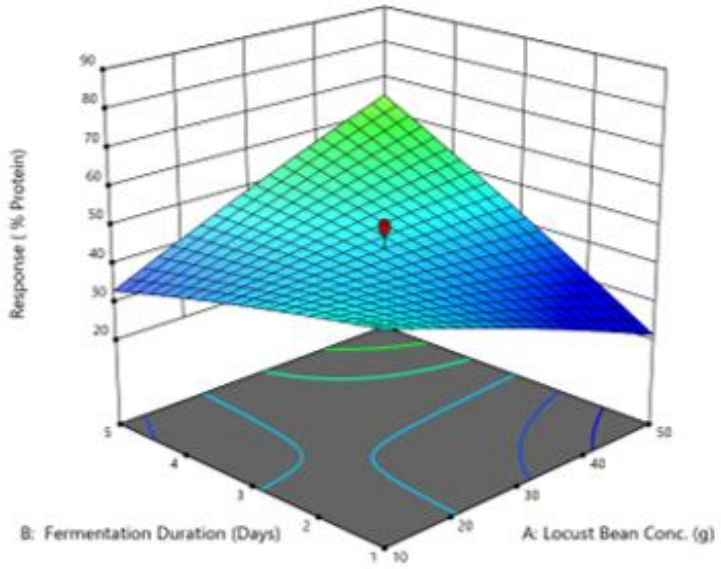

Figure 2: A 3D-Contour Plot Showing the Interactions between Locust Bean weight (A) and Fermentation duration (B).

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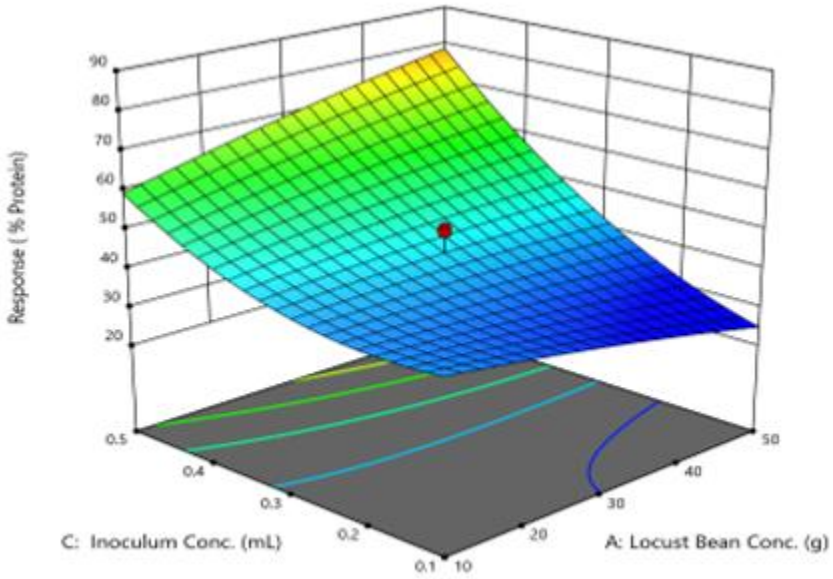

Figure 3: A 3D-Contour Plot Showing the Interactions between Locust Bean weight (A) and Inoculum Volume (C)

Table 6: Verification/Validation of Experiment

Variable	Optimum Range	% Protein Yield	
		Predicted Value	Actual Value
Locust Bean weight.	50 g		
Fermentation Duration	5 Days	95.60	98±0.20
Inoculum Volume	0.5 mL		

Key: g = grams, **mL** = Millilitre

Table 7: Mesophilic Bacterial Count of the Optimized Product

Run	Locust Bean weight (g)	Fermentation Duration (Days)	Inoculum Volume. (mL)	Mesophilic bacterial count ($X10^4$ CFU/g)
1	30	2	0.3	2.1
2	30	3	0.6	1.80
3	10	5	0.5	1.70
4	58	3	0.3	1.50
5	30	3	0.3	1.20
6	50	1	0.5	1.10
7	30	3	0.3	1.50
8	10	1	0.1	7.0
9	30	3	0.3	1.20
10	50	5	0.1	1.50
11	30	6	0.3	2.02
12	30	3	0.3	9.0
13	30	3	0.3	1.20
14	1.7	3	0.3	8.7
15	30	3	0.02	1.60

Key: CFU =Colony forming unit**, mL =**Millilitre**, g =** grams

DISCUSSION

All the Isolates were found to have the colonial morphology and biochemical characteristics as that of *Bacillus* species. Isolates appeared as gram-positive rods. This clearly shows that those bacteria isolated from the fermented food have the characteristic of *Bacillus* sp. The result is in conformity with the report of Antai and Ibrahim (2016), who reported a progressive increase of the dominant *Bacillus* species throughout the use of fermented food ("Daddawa") for isolation of bacteria. Presently, *Bacillus* species are agreed to be the predominant species developed in fermentation processes of various legumes (Ouoba *et al.,* 2003). *Bacillus* sp was reported as being responsible for the fermentation in "Daddawa" and the important flavoring compounds (Beaumont, 2002; Odunfa, 2015). The predominance growth of *Bacillus* sp is favored by the low oxygen tension during the fermentation. The *Bacillus* species isolated from the test condiments ("Daddawa") in this work were observed to be Gram positive, rod-shaped and catalase positive. This is in agreement with the findings of Allaf (2011). Many species of this genus exhibit a wide range of physiologic abilities that allow them to live in many natural environments (Ahn *et al.,* 2011). The *Bacillus* species play an increasingly important role in food and beverage industries (Grass *et al.,* 2014). *Bacillus* sp has been identified to be the main fermenting bacterium during indigenous production of "Ogiri"; a traditional soup flavoring rich in protein.

The 16S rDNA nucleotide sequences analysis of the isolated *Bacillus* revealed that the strain *is B. subtilis* After Blasting.

With respect to the optimization of Production Process, RSM was used to determine the 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 percentage protein yield 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 foremost objective of RSM is to determine the optimal condition aimed at obtaining a maximum result. The design and response are illustrated in result sections. Central composite design response was utilized to produce response surfaces in order to disclose the effects of these parameters in locust beans fermentation. The Y-axis is the anticipated response, A is the locust beans weight, B is the fermentation period and C as Inoculum Volume. The precision of a model is judged by the determination coefficient (R^2). The R^2 values of the model were found to be 0.0071. It assessed how inconsistent tested response is clarified by research parameters and their interface. Adjusted R^2 value was realized to be 0.0071 in which the value was acceptable. It exercises the signal to noise ratio. The "Pred R-Squared" of 0.6477 is in rational pact with the "Adjusted R-Squared" of 0.9558. 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.

Three-dimension contour plots of the central composite design of the response surface method disclose the interrelations between the 2 variables interacting with each other. The interaction between locust beans weight and fermentation period illustrates the relationship in which at both the extremes and the response was low. At locust beans weight and fermentation period, response was divergent illustrating high axis and very low relationship between the factors depicted by inverted contour curve as the factors; locust beans weight and fermentation period and above the interaction were high illustrating low axis and peak of the reactions (Ibrahim *et al*., 2015). On the interaction between locust beans weight and Inoculum Volume, the research illustrates the relationship in which at both interactions the response was low. At locust beans weight and Inoculum Volume, response was divergent illustrating high axis relationship between the factors depicted by contour plot. In the design of the experiment by the central composite design, locust beans weight was utilized against a progressive increase in inoculum volume that have result to % increase in protein yield as similar to the research by Ibrahim *et al*. (2015).

Verification of experiment was carried out using the optimum parameters of locust beans weight, fermentation period and Inoculum Volume in comparison with the experimental and predicted value of the designed experiment. A 50 g Locust Beans, 5 days fermentation period and 0.6 mL inoculum volume were adjudged to be the optimum conditions for the production of 'Daddawa' with higher protein yield of up to 98±0.20 % in this work. This indicates the reliability of the optimum factors, reducing the wastage in the utilization of inoculum with respect to amount of locust beans weight to be fermented for the production of "Daddawa" thus reducing the cost of production.

Conclusion

Bacteria of the genus *Bacillus* sp. was successfully isolated from fermented foods. The organisms were characterized and found to be a *Bacillus subtilis*. The optimization of fermentation yield using varying condition based on Inoculum Volume interaction, Locust Beans weight interaction and fermentation period has been optimized for "daddawa" production using *B. Subtilis* at the optimum conditions of 98±0.20 over predicted value 95.60. This work clearly established an optimum condition for Daddawa production (50g, 5 days, 0.5mL) shows a maximum percentage protein, lipid and carbohydrate yield of optimized over unoptimized Daddawa.. Verification designed optimization experiments showed an improvement of 18-20% over traditional methods. Aseptic techniques ensured quality and safety. The project established a template to integrate starter cultures in 'Daddawa' production, contributing standardized parameters, enhanced yields, and a scientific basis to improve this indigenous food at industrial scale.

REFERENCES

Abdel, A. and Dardir, H. A. (2019). Hygienic Quality of Local Traditional Fermented Skimmed Milk (Laban Rayb) Sold in Egypt. *World Journal of Dairy and FoodSciences*, 4(**2**):205-209.

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.

Aderibigbe, E. Y. and Odunfa, S. A. (1990). Growth and extracellular enzyme production by strains of Bacillus species isolated from fermenting African locust bean, iru. *Journal of Applied Bacteriology,* 69, 662-671.

Ademola, I. T, Baiyewu, R. A., Adekunle, E. A., Omidiran, M. B. and Adebawo F. G. (2011). An assessment into physical and proximate analysis of processed locust bean (*Parkia biglobosa*) preserved with common salt. *Pakistan Journal of*

Nutrition., 10(**5**): 405- 408.*Bacteriology,* **69**:662-671.

Achi, O. K. (2018). "The Up-grading of traditional fermented foods through Biotechnology." *African Journal of Biotechnology.*, **4**:375 – 38.

Achi, O. K. (2005). The Potential for Upgrading Traditional Fermented Foods through Biotechnology. African *Journal of Biotechnology*,4(**5**):375-380

Achi, O. K. (2018). Microbiology of "Obiolor" a Nigerian fermented nonalcoholic beverage. *Journal of Applied Microbiology*, 69(**3**): 321-325.

Adamu, H. M., Ushie, O. A. and Elisha, B. (2013). Chemical and nutrient analysis of raw and fermented seeds of cassia tora. *Journal of Physical Science and Innovation,* 5(**3**):125-138.

Adebayo, G. B., Otunola, G.A. and Ajao, T.A. (2010). Physicochemical, Microbiological and Sensory Characteristics of Kunu Prepared from Millet, Maize and Guinea Corn and Stored at Selected Temperatures,*Advance Journal of Food Science and Technology,* 2(**1**):41-46.

Adeleke, R.O and Abiodun, O. A. (2010). Physicochemical Properties of Commercial Local. *Pakistan Journal of Nutrition,* 9(**9**): 853-855.

Ademola, I. T, Baiyewu, R. A., Adekunle, E. A., Omidiran, M. B. and Adebawo F. G. (2018). An Assessment into Physical and Proximate Analysis of Processed Locust Bean (*Parkia biglobosa*) Preserved with Common Salt. *Pakistan Journal of Nutrition.,* 10(**5**): 405- 408.

Aderiye, B. I., Laleye, S. A. and Odeyemi, A. T. (2017). Hypolipidemic Effect of *Lactobacillus* and *Streptococcus* species from some Nigerian fermented foods. *Research Journal of Microbiology,* 2(**6**):538-544.

Adeyemi, O. T. and Muhammad, N. O. (2008). *Biochemical Assessment of the Chemical constituents ofAspergillus niger* Fermented *Chrsophyllum albidum S*eed Meal. M.Sc Thesis. Department of Biochemistry, University of Ilorin, Nigeria.

Adeyemi, O. T, Muhammad, N. O. and Oladiji, A. T. (2018). Biochemical Assessment of the *Chrysophyllum albidum* Seed meal. *African Journal of Food Science,* 6(**1**):20-28.

Agarry, O. O., Nkama, I.and Akoma, O. (2020). Production of Kununzaki (A Nigerian fermented cereal beverage) using Starter Culture. *International Research Journal of Microbiology,* 1(**2**):18-25.

Ahn, T. S., Hong, S. H., Kim, O. S. and Choi, S. I. (2011). The Changes of Bacillus spp. in Municipal Waste-Water Treatment Plant with B3 Process Korean *Journal Microbiology*, **37**:209-213.

Akande, F. B., Adejumo, O. A., Adamade, C.A. and Bodunde, J. (2010).Processing of locust bean fruits: Challenges and prospects.*African Journal of Agricultural Research,* 5(**17**), 2268-2271.

Akpi, U. K., Nnamchi, C. I. and Ugwuanyi, J. O. (2020) Review on Development of Starter Culture for the Production of African Condiments and Seasoning Agents. Advances in Microbiology, 10, 599-622. https://doi.org/10.4236/aim.2020.1012044

Alabi, D.A., O.R. Akinsulire and Sanyaolu, M.A. (2018). Qualitative Determination ofChemical and Nutritional Composition of *Parkia biglobosa* (Jacq.) Benth. *African journal of biotechnology.* 4(**8**): 812-815.

Allaf, M. A. (2011). Isolation of *Bacillus spp*. from some Sources and Study of its Proteolytic. Retrieved from www.univ.academicjournals.org/AJB on 21/03/2024

Alsalman, F. B. and Ramaswamy, H. (2020). Reduction in soaking time and anti-nutritional factors by high pressure processing of chickpeas. *Journal of Food Science and Technology*, 57(7): 2572-2585.

Alves Filho, E. G., de Brito, E. S. and Rodrigues, S. 2020. Effects of cold plasma processing in food components. In Bermudez-Aguirre, D. (ed). Advances in Cold Plasma Applications for Food Safety and Preservation, p. 253-268. Cambridge: Academic Press

Amao, J. A., Abel, O. O. and Agboola, J. O. (2013). Proximate Analysis and Sensory Evaluations of Iru Produced by Staphylococcus Sp. and Bacillus Sp. Separately. *Journal of Environmental Science, Toxicology and Food Technology*, 6(**2**):26-30.

Antai, S. P. and Ibrahim M. H. (1986). Microorganisms associated with African locust bean (Parkia filicoidea welw) fermentation for ""daddawa" "production. *Journal of Applied Bacteriology,* **61**:145-148.

Ari, M. M., Ayanwale, B. A, Adama, T. Z. and Olatunji, E. A. (2012). Effects of Different Fermentation Methods on the Proximate Composition, Amino Acid Profile and Some Antinutritional Factors (ANFs) In Soyabeans (Glycine Max). *Fermentation Technology and Bioengineering,* 2:6-13.

Asagbra, A. E., Okafor, J. W. C., Onawola, O. O., Etoamaihe, M. and Olatope, S. O. A. (2012). Sensory Properties of "ogiri" in Nigerian Onugbu Soup Made from Two Varieties of Melon Seeds Cucumis melo and Cucumeropsis manii. *Pakistan Journal of Nutrition,*11(**6**):596-599.

AOAC, (2000). Association of Official Analytical Chemists. Official Method of Analysis. 17th Edn. Gaithersburg, USA.

Azokpota, P., Hounhouigan, D. J. and Nago, M. C., (2006). Microbiological and Chemical Changes during the Fermentation of African locust bean (*Parkia biglobosa*) to Produce afitin, iru and sonru, three traditional condiments produced in Benin. *Int. J. Food Microbiol.***107**:304-309.

Audu, I., Oloso, A. and Umar, B. (2014). Development of Concentric Cylinder Locust Bean dehuller. Agricultural Engineering Int.: *the CIGR Journal of Scientific Research and Development,* Vol. **6**.

Aworh, O.C. (2018). The Role of Traditional Food Processing Technologies In National Development: the West African Experience. *International Union of Food Science & Technology*:1-18.

Babalola, R. O. and Giwa, O. E. (2012). Effect of Fermentation on Nutritional and Antinutritional Properties of Fermenting Soy beans and the Antagonistic Effect of the fermenting organism on selected pathogens. *International Research Journal of Microbiology,*3(**10**): 333-338.

Barber, L. I. and Achinewhu, S. C. (2012). Microbiology of "ogiri" production from melon Seeds (*Citrullus vulgaris*). *Nigerian Food Journal*, **10**:129 - 135.

Bukola, C. A and Abiodun, A. O. (2008). Screening of Lactic Acid Bacteria Strains Isolated from Some Nigerian Fermented Foods for Exopolysaccharides Production. *World Applied Sciences Journal,*4(**5**): 741-747.

Campbell-Platt, G. (2017). African Locust Bean and its West African fermented products- ""daddawa" ". *Ecology, Food and Nut*rition *Journal*, **9**:123-132.

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

Chung, S. K, Lee, M. S., Oh, S. I. and Park, S. P. (2010).Discovery

of Novel Sources of Vitamin B12 in Traditional Korean Foods from Nutritional Surveys of Centenarians.*CurrGerontolGeriatr Res.* 2010; 2010: 374897.

Chelule, P. K, Mbongwa, H. P., Carries, S. and Gqaleni, N. (2010). Lactic acid Fermentation Improves the Quality of Amahewu, a Traditional South African maize-based porridge. *Food Chemistry,*122(**3**):656-661.

Dakare, M. A, Ameh, D. A. and Agbaji, A. S. (2011). Biochemical Assessment of „"daddawa" Food Seasoning Produced by Fermentation of Pawpaw (Carica papaya) Seeds. *Pakistan Journal of Nutrition*, 10(**3**): 220-223

Diawara, B. L., Sawadogo, Amoa-Awua, W.F. and Jakobsen, M. (2020). Capability Building for Research and Development in Quality Assurance and Fermentation Technology for African Fermented Foods. HACCP System for Traditional African Fermented Foods: soumbala. WAITRO.

Egbere, O. J. (2018). Principles and Practice of Food Microbilogy. 1st edition, Deka, Jos, Nigeria, pp. 123139.

Egwim, E., Musa, A., Abubakar, Y. and Mainuna, B. (2013). Nigerian Indigenous Fermented Foods: Processes and Prospects. *In Mycotoxin and Food Safety in Developing Countries*, 153-180. IntechOpen.

Essien, E., Monago, C. and Edor, E. A. (2011). Evaluation of the Nutritional and Microbiological Quality of Kunun (A Cereal Based Non-Alcoholic Beverage) in Rivers State, Nigeria. The Internet *Journal of Nutrition and Wellness,* 10(**2**):1-5. Microbiology, 10, 599-622. https://doi.org/10.4236/aim.2020.101204

Ezeocha, C. V., Ugwuja, J. L. and Onyeabor, C. S. (2022). Evaluation of Indigenous Okpeye (Prosopis africana) Processing Conditions in Nsukka LGA, Enugu, Nigeria and Its Effect on the Quality of the Fermented Seasoning. *Nigeria Agricultural Journal*, 53, 191-199.

Falana, M. B., Bankole, M. O., Omemu, A. M. and Oyewole, O. B. (2011). Microorganisms Associated with Supernatant Solution of Fermented Maize Mash (omidun) from two varieties of Maize grains. *Researcher*, 3(**7**): 1-7.

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

F. D. A. (2011). Fermented Fruit is and Vegetables, A Global perspective.

Food and Agriculture Organization (2010). Agricultural Biotechnologies in Developing Countries: Options and Opportunities in Crops, Forestry, Livestock, Fisheries and Agroindustry to Face the Challenges of Food Insecurity and Climate change (ABDC-10). *FAO International Technical Conference,***1** -37.

Frazier, W.C. and Westhoff, D. C. (2008). *Food Microbiology*, Fourth edition, Tata McGraw-Hill, India, pp.315

Grass G., Schierhorn A., Rucknagel P. and Fricke B. (2014). Camelysin Is a Novel Surface

Metalloproteinase from *Bacillus cereus*. Infection and Immunity. *American Society for Microbiology,* 72 (**1**):219-228

Hopkins, B., (2013). The Taxonomy, Reproductive Biology and Economic Potentials of Parkia (*Leguminosae Mimosoideae*) in Africa and Madagascar. *Botany Journal of Linnean Society,* **87**:135-167.

Idise O. E., Ameh, J. B. Yakubu, S. E. Okuofu, C. A. and Ado, S. A. (2010): "Determination of the Genetic Manker of the Mutagenized Strains of *Pseudomonas aeurginosa* and *Bacillus cereus* Isolated from Efficient of Petroleum Refinery." *Bayero*, *Journal of Pure and Applied Sciences,* 3(**1**): 180 – 182.

Ibrahim, M. H. and Antai, S. P. (2005). Chemical changes during the Fermentation of African locust bean (Parkia filicoidea Welw) seeds for production of "daddawa". *Plant food for Human Nutrition*, **3**(26):179-184.

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

Ibrahim, M. H. and Antai, S. P. (2015). Chemical changes during the Fermentation of African locust bean (Parkia filicoidea Welw) seeds for production of „""daddawa" "". *Plant food for Human Nutrition*, 3(**26**):179-184.

Jonathan, S. G, Fadahunsi, I. F. and Garuba, E. O. (2017). Fermentation Studies during the Production of „Iru" from Bambara nut (Voandzeia subterranea), an Indigenous Condiment from South-western Nigeria, *Electronic Journal of Environmental, Agricultural and Food Chemistry*, 10(**1**):1829-1836.

Jyoti, P. T. (2010). Retrieved from ourworld.unu.edu/en/benefits-of-traditional-fermented-foods/ on 22/03/2024.

Karamba, K. I., Ahmad, S. A., Azham, Z., Syed, M. A., Khalil, K. A., Shamaan, N. A., Dahalan, F. A. and 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. and 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. and Yakasai, H. M. (2019). Growth Characterization of *Bacillus amyloliquefaciens* strain KIK-12 on SDS. *Journal of Biochemistry, Microbiology and Biotechnology,* 7, 26–30.

Karamba, K. I. and 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. and 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

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

Kitum, V. C., Kinyanjui, P. K., Mathara, J. M. and Sila, D. N. (2020). Effect of *Lb. plantarum* BFE 5092 fermentation on antinutrient and oligosaccharide composition of whole red haricot bean (*Phaseolus vulgaris* L). *International Journal of Food Science*, 2020: 8876394

Klanjcar, J., Kopac, M., Kosem, D. and Kozlovic, K. (2012).

"daddawa". www.bf.unilj.si/zt/biotech/seminar-absract/zivil/. Retrieved on 23/03/2024.

Kristen, M. (2011).www.scribd.com/doc/78998889/Benefits-of-Eating-Raw-FermentedFoods (accessed 16-05-14)

Latunde-Dada, G. O. (2014). Food Science. Department of Chemical Sciences in the College of Natural Sciences at the University of Agriculture in Abeokuta, Nigeria.

Lee, C. K., Darah, I. and Ibrahim, C. O. (2011). Production and Optimization of Cellulase Enzyme Using *Aspergillus niger* USM AI 1 and Comparison with Trichoderma reesei via Solid State Fermentation System. *Biotechnology Research International*, 2011: 1-6.

Mafra, Isabel, Silva, Susana, A., Moreira, Elsa, J. M. O., Ferreira da Silva, Carla, S., Beatriz, M. and Oliverira, P. P. (2008). Comparative Study of DNA Extraction Methods for Soybean Derived Food Product. Service de Bormatologia, Faculdade de Farmacia, Universidade do Porto, Rua Aniobal Cunha, 164, 4099 – 030 Porto, Portugal.

Murwan, K. S. and Ali, A. A. (2011). Effect of Fermentation Period on the Chemical Composition, In-vitro Protein Digestibility and Tannin Content in two Sorghum Cultivars (Dabar and Tabat) in Sudan. *Journal of Applied Biosciences,* **39**:2602 – 2606

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

Nwachukwu, E., Achi, O. K., Ijeoma, I. O. (2020). Lactic acid Bacteria in Fermentation of Cereals for the Production of Indigenous Nigerian foods. *African Journal of Food Science and Technology*, **1**(2): 021-026.

Njoku, H. O. and Okemadu, C. P. (2009). Biochemical changes during the natural fermentation of the African oil bean (Pentaclethra macrophylla) for the production of ugba. *J. Sci. Food Agric.,* **49**: 457-465.

Nwosu, C. D. and Ojimelukwe, P. C. (2000). Improvement of the traditional methods of "ogiri" production and identification of the microorganism associated with the fermentation process. *Journal of Applied Microbiology*. 94(**3**): 381 - 391.

Ogbonnaya, C., Orheva, B. A. and Babatunde, I. M. (2020). Influence of hydrothermal treatments on proximate compositions of fermented locust bean ("daddawa"). *J. Food Technol.,*8(**3**): 99-101.

Odunfa, S. A. and Oyeyiola, G. F. (1985). Microbiological study of the fermentation of ugba, a Nigerian Indigenous fermented food flavor. *J. Plant Foods*, **6**:155-63.

Omafuvbe, B.O., Falade, O.S., Osuntogun, B.A., Adewusi, S.R.A. (2018). Chemical and Biochemical changes in African locust beans (Parkia biglobosa) and melon (Citrullus vulgaris) seeds during fermentation to condiments. *Pak. J. Nutr.,*3(**3**): 140-145.

Ouoba, L. I. I., Cantor, M. D., Diawara, B., Traore, A. S. and Jakobsen, M. (2003). Degradation of African locust bean oil by Bacillus subtilis and Bacillus pumillus isolated from soumbala, a fermented African locust bean condiment. *J. Appl. Microbiol.*, 95(**4**):868-874

Oboh, G. (2006).Nutrient and antinutrient composition of Condiments produced from some fermented under-utilized legumes. *Journal of Food Biochemistry,* **30**:579–588.

Odebunmi, E. O., Oluwaniyi, O. O. and Bashiru M. O. (2019). Comparative Proximate Analysis of Some Food Condiments. *Journal of Applied Sciences Research*, (CCCC):1-3.

Odunfa, S. A. (2018). Microorganisms Associated with Fermentation of African Locust Bean during iru Preparation. *J. Plant Foods*, **3**:245-250.

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.

Ojimelukwe, P.C., Okechi, A. and Ojinnaka, M. C. (2011). Physicochemical Characteristics Fermenting castor seeds containing lime and NaCl as additives. *African Journal of Food Science*, 5(**14**):754-760.

Oboh, G. (2016). Nutrient and antinutrient composition of Condiments produced from some fermented under-utilized legumes. *Journal of Food Biochemistry,* **30**:579–588.

Osuntoki, A. and Korie, I. (2010). Antioxidant Activity of Whey from Fermented Milk, Fermented with Lactobacillus Species Isolated from Nigerian Fermented Foods. *Food Technology Biotechnology,* 48(**4**) 505–511.

Osungbaro, T. O. (2019). Physical and nutritive properties of fermented cereal Foods. *African Journal of Food Science,* 3(**2**):023-027.

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

Olayinka, E. M., Omobayonle, F. (2010). Evaluation and Optimization of Critical Control Points in*Available: DOI: https://doi.org/10.54117/gjpas.v1i2.28 Research article* GJPAS/Volume 1/Issue 2/Jul – Dec/2022the Production of Iru. *Research Journal of Microbiology*, 5, 1258–1266.

Omafuvbe, B. O., Abiose, S. H. and 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. and 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.

Odunfa, S. A. and Oyeyiola, G. F. (2015). Microbiological study of the Fermentation of ugba, a Nigerian Indigenous fermented food flavor. *J. Plant Foods*, **6**:155-63

Ogunshe, A.O., E.A. Ayodele and O.I. Okonko, (2016). Microbial Studies on Aisa: A Potential Indigenous Laboratory Fermented Food Condiment from Albizia saman (Jacq.) F. Mull Pak. *J. Nutr.,***5**:51-58.

Ojinnaka, M. C. (2013). A Study on the Physicochemical Properties of Bacillus Fermented Castor oil Bean Condiment. *Academic Research International,*4(**4**):4-12.

Oladejo, J. A., Adetunji, M. O. (2012). Economic Analysis of Maize Production in Oyo state of Nigeria. *Agricultural Science Research Journals,* 2(**2**):77-83.

Olanrewaju, O. O., Victor, O. O. and Titilayo, T. A. (2017). Safety of Small-Scale Food Fermentations in Developing Countries. *Internet Journal of Food Safety* **11**: 29-34.

Olukoya, D. K., Ebigwei, S. I., Olasupo, N. A. and Ogunjimi, A. A. (2011). Production of DogiK: an Improved Ogi (Nigerian Fermented Weaning Food) with Potentials for Use in Diarrhoea Control. *Journal of Tropical pediatrics,* 40(**2**):108 -

113.

Omafuvbe, B. O., Shonukan, O. O. and Abiose, S. H. (2010). Microbiological and Biochemical Changes in the Fermentation of Soybean for Soy-" "daddawa" – Nigeria Food Condiments. *Food Microbiol.,***17**:469-474

Oyewole, O. A. and Isah, P. (2012). Locally Fermented Foods in Nigeria and their Significance to National Economy: a Review. *Journal of Recent Advances in Agriculture*, 1(**4**):92-102.

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.

Sadiku, O.A. (2010). Processing Methods Influence the Quality of Fermented African Locust Bean (Iru/"ogiri/daddawa") Parkia biglobosa. *Journal of Applied Sciences Research*, 6(**11**):1656-1661.

Smith, A. M., Agiza, A. H. (2010). The determination of amino-acids colorimetrically by the ninhydrin reaction. *Analyst*, 76, 623–627

Steinkraus, K. H., Ed. (2015). Handbook of Indigenous Fermented Foods. New York, Marcel Dekker, Inc., 776.

Syazzie, D. Vander, Jaget, D. J., Pastuszen, A. and Giwu, R. H. (2014). The Amino Acids and Chemical Content of Baobab (*Adansoina digitata* L). *Anal,***7**:189 - 193.

Wafula, E. N., Franz, C. M., Rohn, S., Huch, M., Mathara, J. M. and 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.

Wakshama, P. S., Akueshi, C. O. and Ali, B. D. (2010). Comparative Studies on the Proximate Composition and Physical Characteristics of Dry Matter Samples of Fermented and Unfermented Groundnut and Pumpkin Seeds. *Journal of Medical and Applied Biosciences,***2**:55:59

Wan-Mohtar, W. A. A. Q. I., Sohedein, M. N. A., Ibrahim, M. F., Ab Kadir, S., Suan, O. P., Weng Loen, A. W. and Ilham, Z. (2020). Isolation, identification, and optimization of γ-aminobutyric acid (GABA)-producing *Bacillus cereus* strain KBC from a commercial soy sauce *moromi* in submerged-liquid fermentation. Processes 8: 652.

Wokoma, E. C. and Aziagba, G. C. (2002). Sensory Evaluation of Dawa Dawa Produced by the Traditional Fermentation of African Yam Bean (Sphenostylis stenocarpa Harms) seeds. *Journal of Applied Sciences and Environmental Microbiology*, **8**:209-214.

William, C. F. and Dennis, C. W. (2017). *Food Microbiology,* Fourth edition, McGraw Hill, India, pp. 330.

Yabaya, A. (2006). Production of local ""daddawa" " seasoning and condiment from acacia nilotica (linn) seeds. S*cience world journal,* 1(**1**):27-31.

Yadav, C. B., Tokas, J., Yadav, D., Winters, A., Singh, R. B., Yadav, R. and Yadav, R. S. (2021). Identifying antioxidant biosynthesis genes in pearl millet [*Pennisetum glaucum* (L.) R. Br.] using genome—wide association analysis. Frontiers in Plant Science 12: 599649

Yahaya, A. (2018). Microorganisms Associated with starter cultures of traditional burukutu liquor in Madakiya, Kaduna State, Nigeria. *Science World Journal,* 3(**3**):1597-6343