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

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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 10^6 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.

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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, "*Iru*" in Yoruba, "*Ogiri*" in Igbo, "*Owo*" in Urhobo and Itsekiri, "*Okpiye*" 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,

2019). 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 (Olayinka and Omobayonle, 2010). Literarily optimization 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). 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). The optimization of the analytical process has been conducted by using RSM, which is a collection of statistical procedures based on fit of polynomial equation to the experimental data (Bezerra *et al.*, 2008; Ibrahim *et al.*, 2020). The aim of this research is to characterize the starter culture used in the fermentation of African locust beans and optimize fermentation conditions in Daddawa production using RSM.

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 uL 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 $^{\circ}$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 $^{\circ}$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 $^{\circ}$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 grounded 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 ºC for 30 min. The supernatants were used for the analysis.

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 *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 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/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 *Bacillus subtilis* and isolate 3 was suspected to be another *Bacillus* species. Isolate 2 was suspected to be *Staphylococcus aureus* whereas isolate 4 was suspected to be a species of *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 (*Bacillus* spp), likewise isolate 6 was observed to be same as isolate 1 (*Bacillus subtilis*).

Table 2: Biochemical assay on isolated bacteria

Isolates	Col. Mrphol.	Cat.	Coag.	Ind.	Vp.	Ctr.	Oxd.	TSI	Mtlty.	Urs.	E-Spore
Isolate1	GPR	+	NP	-	+	+	+	+	+	-	+
Isolate2	GPC	+	+	-	+	-	+	+	-	-	-
Isolate3	GPR	+	NP	-	+	+	+	+	+	-	+
Isolate4	GPC	+	-	-	+	-	-	+	-	-	-
Isolate5	GPR	+	NP	-	+	+	-	+	+	-	+
Isolate6	GPR	+	NP	-	+	+	-	+	-	-	+

Note: KEYS - Mphol.= morphology, GPR=Gram positive rods, GPC = Gram positive cocci, Cat.= catalase test, Coag.= Coagulase, Vp.= Voges proskauer, Ct.= Citrate test, Ind.= Indole test, Urs.= Urease test, Oxd.= Oxidase test, TSI =Triple sugar iron, Mtlty = Motility, Urs = Urease test, E-Spore = Endospore Test.

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	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	1	0.026	75.27	< 0.0001	
B	0.028	1	0.028	81.36	< 0.0001	
A^2	0.012	1	0.012	34.26	0.0006	
B^2	0.023	1	0.023	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	12				

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

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

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:

$$Y = +1.66588 - 7.37853E\text{-}003 - 4.71176 + 6.55000E\text{-}005 + 15.81597 - 0.0026167$$

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.

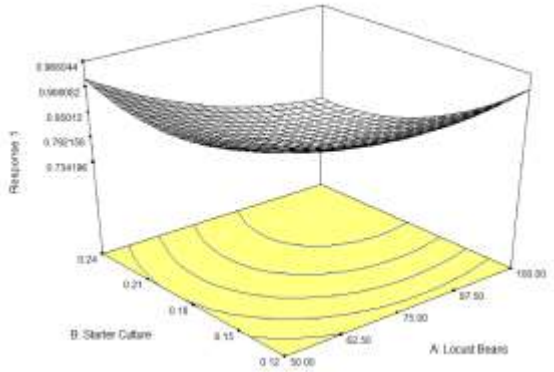

Figure 1: Three dimensional contour curves illustrating interactions between factors

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.

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Author's contribution

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

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