



Research Article

Evaluation of Microbiological Changes and Physicochemical Parameters during Fermentation of *Prosopis Africana* Seeds

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ABSTRACT

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High cost of animal protein has significant effect toward consuming many leguminous seed proteins as sources of vegetable protein for human food in developing countries. *Okpehe* is a traditional fermented condiment produced from *Prosopis africana* seeds which are added to food to impart a particular flavour or taste. This study was designed to evaluate the microbiological and physico-chemical parameters during spontaneous fermentation of *Prosopis africana* seeds with a view to provide basis for optimizing the fermentation process. Isolation of *Bacillus species* were carried out using MYP agar and the identification was done using biochemical and molecular techniques. During the process of fermentation, the *Prosopis africana* seeds (*Okpehe*) were assessed for pH and temperature changes using standard procedures. Results showed an increase in pH and temperature from the beginning to the end of fermentation (0-72hours). At the beginning of fermentation (zero hour), the pH of 6.47 was recorded while pH of 8.37 were recorded after 72 hours of fermentation. The temperature recorded at the beginning of fermentation (zero hours) was 35.27°C and at the end of 72 hours of fermentation were 39.90°C. During different stages of fermentation process, bacteria isolated were *Bacillus subtilis*, *Bacillus licheniformis*, *Streptococcus sp*, *Staphylococcus aureus*, *Proteus sp* and *Escherichia coli*.

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INTRODUCTION

Fermented legumes seeds provide alternative sources of plant proteins. Generally the uses of fermented vegetable proteins as seasonings are widely spread in Africa especially in Nigeria (Bukar and Balarabe, 2019). A fermented *Prosopis africana* seed called *Okpehe* in Idoma language is traditional fermented condiment produced from *Prosopis africana* seeds which serves as a condiment in soup to enhance its flavour or tastes (Asiru and Musbau, 2020). Other common vegetable condiments in West Africa include dawadawa from locust beans (*Parkia biglobosa*) (Odunfa, 1981.), ogiri from melon seeds (*Citrus vulgaris*), dawadawa from soybean (*Glycine max*) (Ogbadu and Okagbue, 1988), soumbala from soybean, ugba from African oil bean (*Pentaclethra macrophylla*) (Odunfa, and Oyewole, 1985; Ejiofor *et al.*, 1987; and Ouoba *et al.*, 2007), and owoh from cotton seeds (*Gossypium hirsutum*) are all produce during fermentation process (Sanni and Ogbonna, 1991 and Sanni *et al.*, 1998). Fermented *Prosopis africana* seeds (*Okpehe*) are majorly produced by spontaneous fermentation, therefore, a variable diverse of microorganisms may be present in such fermentations. However, there is general report that aerobic spore-forming bacteria, especially *Bacillus species*, are the dominant bacteria in the fermentation which break down the protein to release amino acids, ammonia, and other volatile compounds which is the major biochemical activity during the fermentation process. *Prosopis africana* also known as iron tree is the only species of *Prosopis* that is indigenous to tropical Africa. Its tree could be between 4-20m long. This tree is characterized by a deep, fast-growing tap root. *Prosopis africana* (PA) is mostly found growing in the savanna regions of western Africa (Fasidi *et al.*, 2000). This study was designed to

evaluate the microbiological changes and physico-chemical parameters during fermentation of *Prosopis africana* seeds with a view to provide basis for optimizing the fermentation process so as to improve process control and quality of condiment produce during spontaneous fermentation.

MATERIALS AND METHODS

Sources of Seeds and Authentication

Two kilograms (2kg) of *Prosopis africana* seeds were purchased from Brigade Market in Kano State of Nigeria. The *Prosopis* seeds were identified with a herbarium voucher number of BUKHAN 0193 at the Department of Plant Biology, Bayero University, Kano. *Prosopis africana* seeds were transported to the Department of Microbiology, Bayero University, in polythene bag for investigations.

Spontaneous Preparation of “Okpehe” from *Prosopis africana* Seeds

One thousand gram (1000g) of dried seeds of *Prosopis africana* were washed and soaked overnight and later boiled in a large earthenware pot for 2 days with fire wood which make the seeds coats became soft and the seeds swollen. Seeds coats were removed by pressing between fingertips. The seed coats were later decanted along with the washing water, leaving the clean seed cotyledons. The cotyledons were boiled for another 2 hours. The cotyledons were later drain through a sieve and wrapped with banana leaves. The wrapped cotyledons were stacked together and then covered with layers of sterile aluminium foil and kept in an incubating unit for about three days to produce the fermented *Prosopis africana* condiment (Ogunshe *et al.*, 2007). The fermenting *Prosopis africana* seeds were removed for investigation at selected times of fermentation, 0, 24, 48 and 72 hours, the fermenting microorganisms were source from the environment (Figure 1).

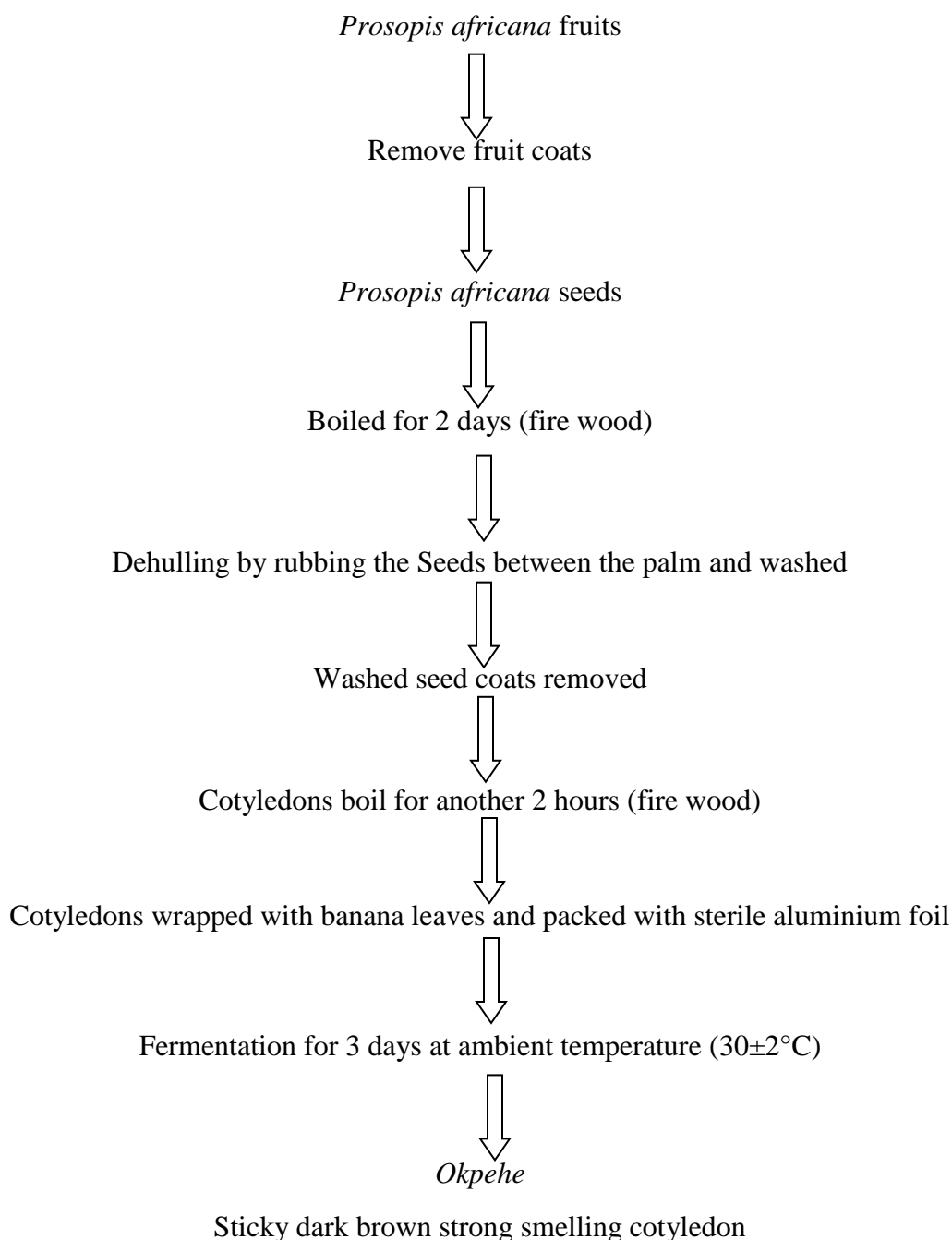


Figure 1: Flow Chart for spontaneous fermentation of *Okpehe*

Preparation of Seed homogenate

Sample of *Prosopis africana* condiment were prepared according to the method described by Food and Agricultural Organization (2015). Twenty-five grams (25g) of natural fermented *Prosopis africana* condiment were homogenized by blending in 225 ml of sterile buffered

peptone water at 15,000-20,000 rpm labeled as 1:10 (10^{-1}) one milliliter (1ml) was introduced into a test tube and was labeled as 1:100 (10^{-2}) dilution and serially diluted to five other test tubes label (10^{-3} - 10^{-7}). One milliliter (1ml) aliquot was pipette and added to appropriately label sterile petridishes in triplicates.

Isolation and Identification of *Bacteria* during Spontaneous fermentation

One milliliter (1ml) aliquot was pipette from 10^{-7} dilutions and added to appropriately label sterile triplicates petridishes. Nutrient agar were prepared according to manufacturer instructions which was added and swirled so that the aliquot were evenly distributed and allow to gel and the plates were incubated aerobically at 37°C for 24 hours (pour-plate technique). Morphologically different colonies were selected from the agar plates streaked on nutrient agar plates incubated at 37°C for 24 hours. The Isolates were inoculated into nutrient broth and the bacterial isolates were gram-stained and subjected to different biochemical tests such as catalase test, oxidase test, egg yolk emulsion test, citrate utilization test, spore staining, starch hydrolysis, gelatin hydrolysis, MRVP test, sporulation and motility tests. Further identification was done using molecular techniques that involve DNA extraction and purification, PCR and sequencing.

Purification and Identification of *Bacillus* Isolates

Bacillus species were isolated using Mannitol Egg Yolk Polymyxin (MYP) agar, incubated aerobically at 37°C for 24hours. The streak method was used to subculture the bacterial isolates that grew on the MYP agar. An inoculating wire loop was sterilized using hot flame, allowed to cool before it was used to take part of grown bacterial colonies from the cultured agar and streaked on the surface of a fresh nutrient agar (NA). The plate was incubated aerobically at 37°C in an inverted position for 24 hrs. Individual bacterial colonies were identified by morphological and biochemical techniques using the taxonomy scheme of Bergey's Manual of systematic Bacteriology (Holt *et al.*, 1994). The cultural characterization of bacteria colonies isolated was done by observing the colonies for colour, shape, and surface appearance displayed on NA. The pure bacterial isolates were gram-stained and subjected to different biochemical tests as described by Cheesebrough (2017).

Polymerase Chain Reaction (PCR)

The PCR reaction was carried out using Taq DNA polymerase. The Total reaction volume was 15µL. Reaction mix comprise of 1µL each

of the genomic DNA, 1.5 µL of 10 TaqA Buffer, ~0.4M (0.5 µL) of each of forward and reverse primers (16srRNA Bact1442-F and 16srRNA Bact1492-R) 1.25 mM (0.75 µL) of MgCl₂, 0.25 mM (0.15 µL) of dNTP mixes and 0.12 µL of Taq DNA polymerase in ddH₂O. Amplification was carried out using the following conditions: initial denaturation of 5 min at 95°C, followed by 35 cycles each of 30s at 94°C (denaturation), 30 s at 57C (primer annealing) and 1min. at 72°C (extension). This was followed with 10min final extension at 72°C. PCR products were separated in a 1.5% agarose gel stained with ethidium bromide NorgenBiotek (2014). DNA samples were quantified by running on agarose gel electrophoresis using 0.8% agarose gel in 1 × tris- boric acid EDTA (TBE) (89 mM tris pH 7.6, 89 mM boric acid, 2 mM EDTA) and visualized by ethidium bromide (0.5 µg/ml -1) staining, to determine DNA size NorgenBiotek (2014).

SEQUENCING OF 16S RRNA GENE

The DNA band of 1.5 kb was excised from gel and purified using the gel elution kit (Sigma-Aldrich, USA) as per the manufacturer's protocol. Sequencing reactions were carried out with a BigDye Terminator cycle sequencing kit (Applied Biosystems, USA), standard universal primer forward (8f') and reverse (1542r') primer and sequenced by using ABI Prism 3100 genetic analyzer (Applied Biosystems, USA). The sequences thus obtained were assembled and edited using Clone Manager Version 5. Database search was carried out for similar nucleotide sequences with the BLAST search of Non-reductant (NR) database. Partial length 16S rRNA gene sequences of strains closely related to the isolate were retrieved from NCBI for further analysis NorgenBiotek (2014).

Sequencing

The 16SrRNA gene sequences of the isolates were compared to the sequences in the public databases using Basic Local Alignment Search Tool (BLAST). Multiple Sequence Alignment was done using MUSCLE. The 16SrRNA gene sequences with high similarity to those determined in the study were retrieved and added to database and aligned with Mega 7 (Tamura *et al.*, 2013).

Determination of physico-chemical parameters during spontaneous fermentation of *Prosopis africana* seeds

Physicochemical parameters were determined at different stages of spontaneous fermentation; 0hr, 24hrs, 48hrs and 72hrs.

Determination of pH of fermented *Prosopis africana* seeds

A pH meter (model Denwer 20) was used for this purpose. It was used to measure the acidity and alkalinity of the *Okpehe* samples. It was first standardized using standard buffer solution of pH 4 and pH 7. The electrode was rinsed with distilled water and immersed into the samples. Five grams of the different samples was ground thoroughly in a mortar. This was suspended in 100ml of distilled water. The pH of the suspension was measured using pH meter (model Denwer 20).

Determination of Temperature of fermented *Prosopis africana* seeds

The temperature was determined by inserting a probe sterile thermometer (wiped with alcohol) into each of the samples on each day of fermentation. The mercury-in-glass thermometer

was used. Readings were taken and recorded in duplicates.

RESULTS

Bacillus species from fermented *Prosopis africana* condiment (*Okpehe*)

Table 1 present the results of the occurrence of *Bacteria* isolated during fermentation of *Prosopis africana* seeds. The isolates were gram positive, rod shaped, motile, and spore-forming. The organism tested positive for catalase, citrate test, and VogesProskeur test. Methyl red, oxidase and indole tests showed negative results.

Electrophorotogram of amplified DNA

The amplification of 16S rRNA gene using universal 16S rRNA gene primers resulted in a single well-defined bands of about 1500bp when match with the band from the DNA Marker as in plate 1.

Bacterial isolates obtained by 16S rRNA sequence analysis

The sequence analysis of 16S rRNA gene fragment analyzed from isolates identified the organisms as *B. subtilis* and *B. licheniformis* with 99% similarity to Gen Bank sequences (Figure .1).

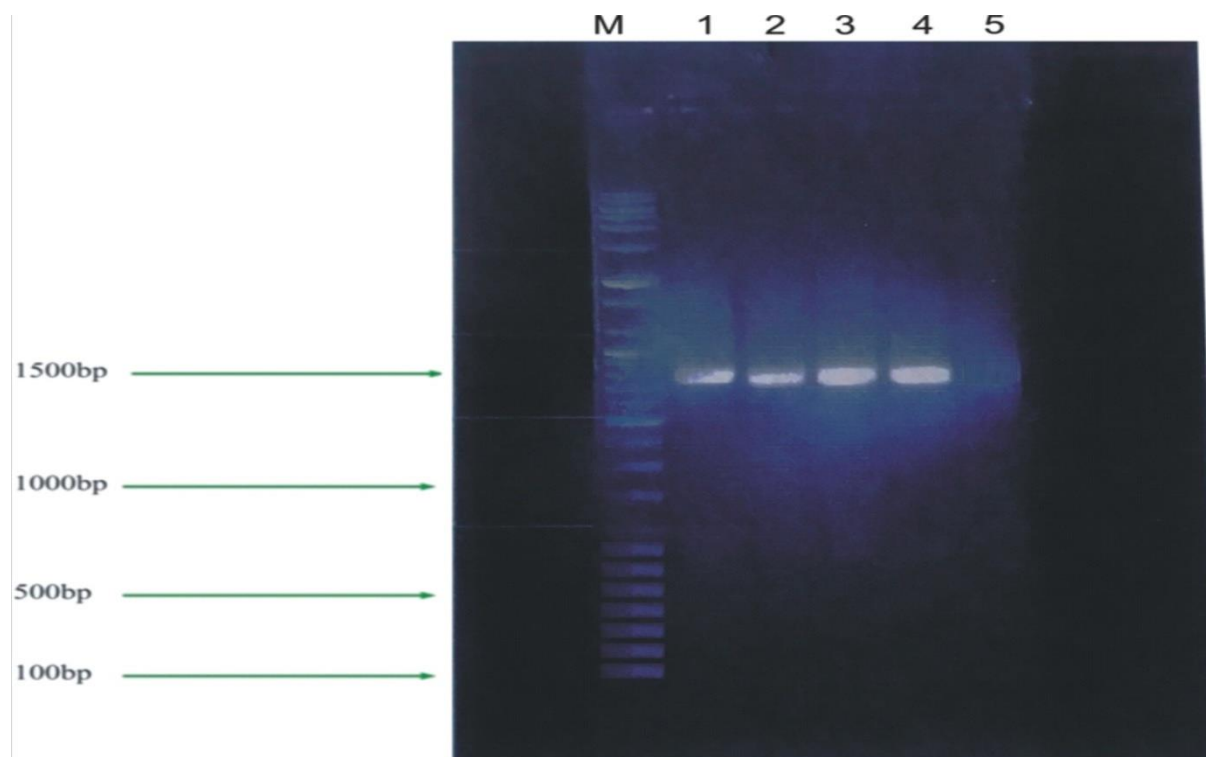


PLATE 1: Gel/Electrophoresis (Using Agrose Gel) of Bacterial species

M = 100bp DNA ladder

Lane 1 = *Streptococcus* species

Lane 2 = *Bacillus licheniformis*

Lane 3 = *Bacillus subtilis*

Lane 4 = Positive control and

Lane 5 = Negative control.

907-R *Bacillus subtilis* strain IAM 12118 (90%) (0.0) 884/890(99%)

AGCTTCCCAGGGCGGAGTGCTTATGCGTTAGCTGCAGCACTAAGGGGGCGGAAACCCCTAACACTTAGCACTCATCGTTTACGGCG
TGGACTACCAGGGTATCTAATCCTGTTTCGCTCCCCACGCTTTCGCTCCTACGCGTCAGTTACAGACCAGAGAGTCCGCTTCGCCAC
TGGTGTTCCTCCACATCTCTACGCATTTACCGCTACACGTGGAATTCCACTCTCCTCTTCTGCACTCAAGTTCCCCAGTTTCCAAT
GACCTCCCCGGTTGAGCCGGGGCTTTCACATCAGACTTAAGAAAACCGCTGCGAGCCCTTTACGCCAATAATTCCGGACAAC
GCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACCGCCCTATTC
GAACGGTACTTGTCTTCCCTAACAAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCCG
TCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCTCTCAGGT
GACTGGTTGACTCAAGAT

907-R *Bacillus licheniformis* strain DSM 13(84%) 0.0 (98.99%)

CGGTCGCCAAGGGCGGAGTGCTTAATGCGTTTGCTGCAGCACTAAAGGGCGGAAACCCCTAACACTTAGCACTCATCGTTTACG
GCGTGGACTACCAGGGTATCTAATCCTGTTTCGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAGAGAGTCCGCTTCGC
CACTGGTGTTCCTCCACATCTCTACGCATTTACCGCTACACGTGGAATTCCACTCTCCTCTTCTGCACTCAAGTTCCCCAGTTTCC
AATGACCTCCCCGGTTGAGCCGGGGCTTTCACATCAGACTTAAGAAAACCGCTGCGCGCGCTTTACGCCAATAATTCCGGAC
AACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACCGCCCTA
TTCGAACGGTACTTGTCTTCCCTAACAAACAGAGTTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTT
CGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCTCTCAG
GTCGGCTACGCATCGTCGCTTGGTGAGCCGTTACCTCACCACTAGCTAATGCGCCGCGGGTCCATCTGTAAGTGGTAGCTAAAC
ACA

Figure 1: Sequence analysis of *Bacillus subtilis* strain and *Bacillus licheniformis* strain

Table 1: Occurrence of Bacteria in spontaneously Produced *Okpehe*

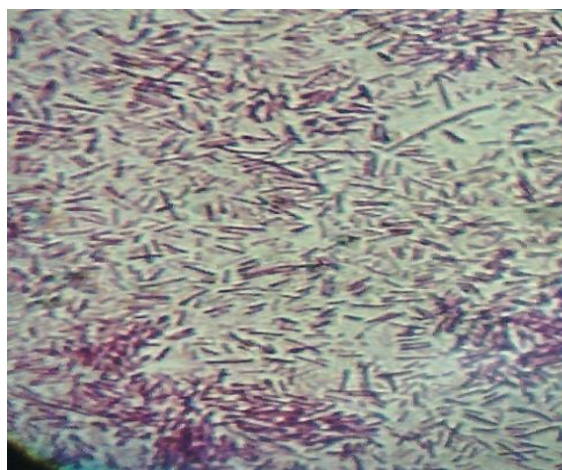
Microorganisms isolated	Bacteria			
	0hrs	24hrs	48hrs	72hrs
<i>Bacillus subtilis</i>	+	+	+	+
<i>Bacillus licheniformis</i>	+	+	+	+
<i>Escherichia coli</i>	-	+	+	-
<i>Staphylococcus aureus</i>	-	+	+	-
<i>Proteus</i> species	-	+	+	+
<i>Streptococcus</i> species	-	+	+	-

+ Present

- Absent



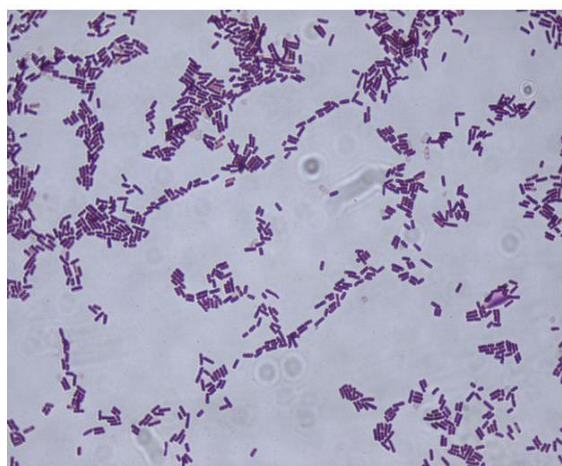
PLATE II; Spontaneous fermented *Okpehe*



Bacillus species



Proteus species



Bacillus subtilis



Streptococcus species

Plate III: Bacteria isolated during spontaneous fermentation

Physico-chemical Parameters of Spontaneous Fermented *Prosopis africana* seeds

The spontaneous fermentation of *Prosopis africana* seeds to produce *Okpehe* was accompanied by an increase in pH and temperature from the beginning to the end of fermentation. At the beginning of fermentation

(zero hour), the pH of 6.47 was recorded while pH of 8.37 recorded after 72 hours of fermentation (Figure 2). The temperature recorded at the beginning of fermentation (zero hour) was 35.27°C and at the end of 72 hours of fermentation was 39.90°C (Figure 2).

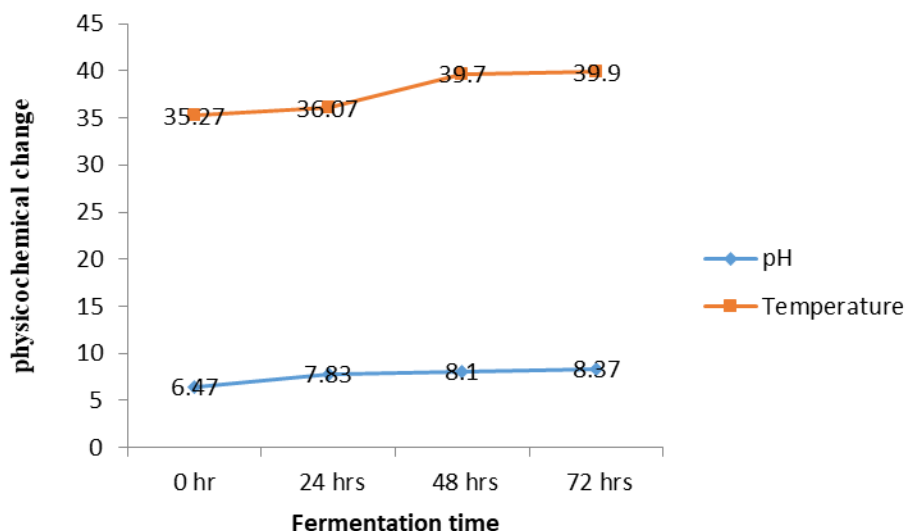


Figure 2: Changes in pH and Temperature during spontaneous fermentation of *Prosopis africana* seeds

DISCUSSION

The results of physiological and biochemical characterization of the isolated *Bacteria* from *Okpehe* (condiment) shows that they were tested positive for Gram's reaction, endospore test, catalase test, motility test, citrate test and Voges-Proskauer test. Methyl red, oxidase and indole tests showed negative results. The characteristics of the isolates agreed with those reported by many research works, which includes Kehinde *et al.* (2014) and Bukar and Mohammad (2017).

Okpehe produced during spontaneous fermentation of *Prosopis africana* seeds had a stronger, ammoniacal smell, along with a characteristic light brown colour and aroma (Plate II). The characteristic of the *Okpehe* produced concurs with those reported by many research work; which includes Balogun and Oyeyiola (2011) and Bukar and Mohammad (2017).

The various isolated bacterial species shows that group of *Bacteria* involved in spontaneous fermentation which includes; *Bacillus* species, *Streptococcus* species, *Staphylococcus aureus*,

Escherichia coli, and *Proteus species* (Plate III). Balogun and Oyeyiola (2011) reported different *Bacteria* in fermented *Prosopis africana* seeds which includes; *Bacillus* species, *Staphylococcus aureus* and *Escherichia coli* during spontaneous fermentation which agreed with the report of this finding. Balogun and Oyeyiola (2011) reported that involvement of different microorganisms in spontaneous fermentation is normal and does not render the product unsafe for human consumption.

The occurrence of *Bacteria* (Table 1), *Bacillus* species were present throughout the 72 hours of fermentation. Balogun and Oyeyiola (2011) reported that *Bacillus* species are the major fermenting bacteria in *Okpehe*. *Escherichia coli* were present in significantly low amounts and gradually started reducing after 48 hours of fermentation; this observation may be due to the production of toxic compounds by some other groups of microorganism's presents during the fermentation. The presence of *Staphylococcus* species and *Bacillus* species in the fermenting *Okpehe* during the first 24 hours was typical of the microflora of fermenting bean seeds.

Staphylococcus species have been associated with fermenting foods of plant origin, especially vegetable proteins (Jideani and Okereke, 1991). The dominance of *Bacillus* species towards the end of the fermentation period could be attributed to the production of bacitracin and organic acids (Onwuakor *et al.*, 2014). These bacterial species involved in the spontaneous fermentation of *Okpehe*, may be introduced by chance. The air, water, utensils and leaves used in wrapping the *Prosopis* seeds during the preparatory stages could have been the source of inoculation. For example, *Staphylococcus* species are more commonly associated with the skin and hence are easily disseminated through handling (Eze *et al.*, 2014).

The rise in pH during fermentation may be due to the high proteinase activity of the *Bacteria* involved (Balogun, 2012), which ultimately resulted in the liberation of ammonia. The pH of *Okpehe* being slightly alkaline agrees with earlier reports of Oguntoyinbo *et al.* (2010), Omafuvbre *et al.* (2004) and Ogunshe *et al.* (2007), where they recorded a slightly alkaline pH in fermented food condiments from vegetable proteins. The increase in pH is generally due to the production of ammonia, which is characterized by the pungent smell of fermented condiments (Ogunshe *et al.*, 2007). Bukar and Mohammad (2017) also reported a slightly alkaline pH (pH rose from 6.43 - 8.41) during fermentation of *daddawa*, this observation aligns with the optimal pH for fermented food safety and acceptability.

Temperature increased from (35.36- 45.54)^oC in all the treatments, the rise in temperature is an indication that the fermentation process was accompany with the release of heat due to the metabolic activities of the microorganisms involved. Ibrahim and Antai (2005) reported that the increase in temperature during the fermentation might be due to the growth and development of microorganisms which liberating heat during the process. Bukar and Mohammad (2018) also reported increase in temperature during fermentation of *daddawa* (Temperature increase from 24 °C to 30°C).

REFERENCES

Asiru, R.A., and Musbau, S. (2020). Proximate Parameters of Fermented *Prosopis*

africana Seeds. *Journal of Academia and Industrial Research (JAIR)* **8**: 9.

- Balogun, M.A. (2012). A Fermentation study and Physicochemical Analysis of *Prosopis africana* seeds to produce *okpehe*, a Local Condiment. University of Ilorin Institutional Repository 24-60.
- Balogun, M.A. and Oyeyiola, G.P. (2011). Microbiological and Chemical Changes During the Production of *Okpehe* from *Prosopis africana* Seeds. *Journal of Asian Scientific Research*, **1** (8):390-398.
- Bukar, A. and Balarabe, Z.S. (2019). Evaluation of the microbiological, chemical and Sensory acceptability of fermented *Parkia biglobosa* seeds (*Daddawa*) using different densities of *B. subtilis*. *Journal of Annals Food Science and Technology*.
- Bukar, A. and Muhammad, R.M. (2017). Effect of different methods and density of starter culture (*Bacillus subtilis*) on the fermentation of *Parkia biglobosa*(Locust bean) seeds. Proceedings of the 41st Conference and Annual General Meeting of the Nigerian Institute of Food Science and Technology, Kano. Pp 303 – 304
- Cheesebrough, M. (2017). Biochemical tests to identify bacteria. In *District Laboratory Practises in Tropical Countries*. Cambridge University Press, UK, pp 63 – 282.
- Ejiofor, M. A. N., Oti, E. and Okafor. J. C. (1987). Studies on the fermentation of seeds of the African oil bean tree (*Pentaclethra macrophylla*). *Journal of International Tree Crop*, **4**:134–144.
- Eze, V.C., Onwuakor, C.E. and Ukeka, E. (2014). Proximate Composition, Biochemical and Microbiological Changes Associated with Fermenting African Oil Bean (*Pentaclethra macrophylla* Benth) Seeds. *American Journal of Microbiological Research*, **2** (5):138-142.
- Fasidi, I.O., Samani, T., Kadiri, M. and Agboola, D.A. (2000). “Dormancy types and water uptake in seeds of

- Parkiabiglobosa. *Journal of Natural and Applied Sciences*, **1**:14-20.
- Food and Agricultural Organization (FAO, 2015). The State of Food Security in the World, 2003. Rome.
- Ibrahim, M.H. and Antai S.P. (2005). Chemical changes during the fermentation of African locust bean (*Parkia biglobosa*) seeds for production of 'daddawa'. *Plant food for human nutrition* 3: (26): 179 – 184.
- Jideani, I.A.O. and Okereke, C.R. (1991). Comparative studies of microorganisms and sensory attributes of condiments from the fermentation of different seeds. *Plant food Hum. Nutrition*, **41**: 27-34.
- Kehinde Tope Adegbehungbe, Fatusa C.A. and Felix, A.A. (2014). Effect of fermentation on nutrient and on treatment of ground – cooled lima bean (*Phaseolus lunatus*) seed using *Bacillus subtilis* and *Bacillus pumilus*. *British Microbiology Research Journal* 4(11): 1285 – 1298.
- Ogunfa, S. A. (1981). Microorganisms associated with fermentation of African locust bean during iru preparation. *J. Plant Foods*, **25**:245–250.
- Ogunfa, S. A., and G. F. Oyewole. (1985). Microbiological study of fermentation of ugba, a Nigerian indigenous fermented food flavour. *Journal of Plant Foods*, **6**:155–163.
- Ogbadu, L. J., and Okagbue, R. N. (1988). Fermentation of African locust beans (*Parkia biglobosa*) seeds; involvement of different species of *Bacillus*. *Food Microbiology*, **5**:195–199.
- Ogunshe, A. A. O., Omotosho, M. O. and Ayanshina, 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** (6):620-627.
- Oguntoyinbo, F.A., Huch, M., Cho, G.S., Schillinger, U., Holzapfel, W.H., Sanni, A.I., and C.M.A.P. Franz. (2010). Diversity of *Bacillus* species isolated from okpehe, a traditional fermented soup condiment from Nigeria. *Journal of Food Protection* **73**: 870–878.
- Omafuvbe, B. O., Shonukan, O. O. and Abiose, S. H. (2004). Microbiological and Biochemical Changes in the Fermentation of Soybean for Soy-daddawa- Nigerian Food Condiment. *Food Microbiology* **17**:469-474.
- Onwuakor, C.E., Nwaugo, V.O., Nnadi, C. J. and Emetole, J.M. (2014). Effect of Varied Culture Conditions on Crude Supernatant (Bacteriocin) Production from Four *Lactobacillus* Species Isolated from Locally Fermented Maize (Ogi). *American Journal of Microbiological Research*, **2** (5): 125-130.
- Ouoba, L. I. I., Diawara, B. Christensen, T. Mikkelsen, J. D. and Jakobsen, M. (2007). Degradation of polysaccharides and non-digestible oligosaccharides by *Bacillus subtilis* and *Bacillus pumilus* isolated from soumbala, a fermented African locust bean (*Parkia biglobosa*) food condiment. *Eur. Food Res. Technol.* **224**:689–694.
- Sanni, A. I., and Ogbonna. D. N. (1991). The production of owoh, a Nigerian fermented seasoning agent from cotton seed *Gossypium hirsutum* L. *Food Microbiol.* **8**:223–229.
- Sanni, A. I., Onilude, A. A., and Oguntoyinbo. F. A. (1998). Optimization of process conditions for owoh, a fermented cotton seed condiment. *Adv. Food Sci.* **20**:163–167.