The Microbiology and Biochemistry of *Treculia Africana* (African *Breadfruit) Fermentation*

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Abstract In the fermentation of African breadfruit, the microorganisms present were isolated and identified. The process involved microbial succession, with the participation of bacteria fungi and yeast genera. The bacteria genera isolated were *Bacillus, Lactobacillus, Micrococcus, Enterobacter, and Staphylococcus,* with *Bacillus* and *lactobacillus* constituting the predominant microflora. The fungi genera isolated were *Aspergillus, Curvularia* and *Alternaria* while the only yeast isolated was *Saccharomyces spp.* The heterotrophic, proteolytic, lipolytic and saccharolytic bacterial population increased progressively from the first day till the seventh day when a peak was recorded. The physical parameters like temperature, pH and moisture were observed during the process. Also the proximate analysis of the as the fermentation progresses. The decrease in crude fibre contents could be attributed to breakdown of the fibre components by the fermentative organisms. The proximate analysis reveals that the breadfruit has rich food value and hence may be used with other food supplements in the production of human food and animal feed.

Keywords: Microbial succession, Heterotrophic bacteria, Proteolytic bacteria, L ipolytic bacteria, Saccharolytic, African Breadfruit, , Fermentation.

Introduction

Treculia africana (African breadfruit) commonly called ukwa in Igbo land of Eastern Nigeria is a common fruit tree in the rainforest area of Nigeria, Cameroon and Benin Republic (Ojinnaka *et al*. 2013, Okafor, 1978; *1985).* It is often found near human settlements and sometimes planted in farm lands and around villages as a mark in boundary demarcation. The tree grows reaching a height of 30 to 50ft (Keay, et al 1989, Ejiofor andOkafor, 1997 and William and Bernard, 1991). The male and female inflorescences are borne separately on the same tree, the male flowers being club-shaped catkins and the females forming globose heads upon a receptacle (ILSC, 1969). Various varieties are known and include *T. africana*, *T. mollis* and *T. inversa*,(Okafor, 1981). The *T. africana* and *T. inversa*are the two most predominant species in Igbo land. These varieties can be found in Abia, Enugu, Imo and Anambra states of Nigeria. They grow well in a wide range of soils, from sandy to clayed soil (Okafor, 1985). They also require high rainfall and humidity as well as sunlight (Okafor 1985).

The fruit is green, spherical and large, measuring between 18 to 20m in diameter when matured (Okafor 1980).The morphological and taxonomic distinctions of the different varieties of bread fruit is based on the size of the fruit heads,hairiness of leaves and size of seeds. The fruit head falls down from the tree when fully matured. It turns from green to brown to yellow during fermentation. The seeds are later extracted from the retted fruit head and washed. The brown to black husk is removed to obtain the edible white-cream seed.

In Igbo land the African breadfruit seed.The seeds are later extracted from the retted fruit head and washed. The brown to black husk is removed and is consumed in various forms, like roasting in hot coals or fried and eaten with palm kernel, made into portage or mixed with other cereals like rice (Ijeh *et al.*2010,Compton,1974) .A flour is made from dried seeds, when baked, it tastes like wheat bread. The fibrous inner bark of the tree is used for weaving cloth and the wood for furniture, glue and other material are derived from the thick white sap of the tree (Halsey & Johnston, 1991). The leaves which reach enormous dimensions serve as roofing for dwellings and as wrappings for food and the spikes of the male flowers are used as tinderwood (Montagne and Gottlschalk, 1961).

Okafor (1980) has stressed on the dietary importance of African breadfruit and classed it as important with only 40% carbohydrate. Okafor (1978) stressed its importance in good health maintenance. Njoku and Ogbulie (1993) reported the need for such alternative sources of food for the ever increasing human populace. Proximate analysis of fresh African breadfruit, var. africana showed the presence of protein (14.23%), moisture (91.25%) Ash (0.22%) and minerals like iron (0.05%), calcium oxide (0.25%) and Manganese (trace) (Okafor, 1981). Osabor *et al*(2009) reported the composition of the seeds as moisture (8.0%), crude protein (12.47%), true protein (19.12%), Ash (1.6%). Osabor *et a*l (2009) also reported the

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presence of Ascorbic acid, carotene, phosphorous, calcium, iron, niacin, riboflavin and thiamine.

From the above, the nutritive quality of the fruit cannot be over-looked because it can provide good source of food for man. Efforts to propagate and maintain the availability is welcomed.

Various microorganisms have been reported as causing the fermentation process Obeta,(1983) reported on the microbial succession indicating the inevitable physicochemical changes involved and other prevailing conditions which might include temperature at which the fermentation is taking place, the pH and moisture content. Organisms implicated in the fermentation include various species of *Saccharomyces lactis*, *Saccharomyc copsis*, *Curvularia* spp, *Candida* spp, *Byssochlamysfulva*, *,Lactobacillus plantarum, Lactobacillus mali* (*Carr, et al.,* 1974; Okafor, 1981). Also Davis (1972) and Gadaga *et al*. (2004) isolated *Lactobacillusacidophilus*, *Lactobacillus lactis*, *Lactobacillus leuconostoc, Lactobacillus casei*, *Pediococcus* spp, *Micrococcus* spp, *Propionobacterium*, *Bacillus,Substilis carens* and *Bacillus megaterium* in succession.

The pH of the Ukwa (pulp) decrease from 7.9 to 7.1 within the first three 4ays of the fermenting period as reported by Olapade and Umeonuorah (2014) .Okafor (1980) reported a fluctuation in pH of the fermenting pulp which decrease from 7.1 to 3.6 on the first three days of fermentation after which a progressive increase from 3.1 to 6.8 was recorded at the end of the process.

Osabor *et al*. (2009) reported that the moisture content of the unfermented breadfruit pulp (Ukwa) was 83.90% and this increased as the fermentation continues although a decrease to 53.50% was recorded at the end of the fermentation process. Osabor *et al*. (2009) reported also that the crude protein content showed a decrease from 9.4% in the unfermented pulp to 2.2% in fermented pulp. Stenkraus, (1983) reported that the pulp of the unfermented breadfruit consist of 8.5% crude fibre, and that the total carbohydrates content of the breadfruit pulp decreases from 79.18% in unfermented to 9.97% after fermentation.

Assessing the crude nature of breadfruit fermentation and the obvious influence of the physicochemical condition the process could be contingent on a lot of extrenous factors which may play important role in the microbial succession of African breadfruit substrate.

This study is aimed at: 1). Investigating the microbial flora of fermenting African breadfruit;
2). Their succession in line with the physicochemical changes of the fermenting fruit;
3). Investigating the biological load of the bacterial species

Materials and Methods

Source of samples:
The African breadfruit (*Treculia Africana*) was obtained from a farm at Okigwe, Imo State.

Sampling Procedures

Samples of African breadfruit were obtained from the sample site with sterile polythene bags. The polythene bags were sterilized by soaking them in 2.5% acid alcohol for 24hours (Cruick-Shank *et al.,* 1982). The collected sample was taken straight to Abia State University Uturu Laboratory and left on a table with the polythene bag kept open and flat. Both the ambient temperature of the room where the fermentation took place and temperature of the fermenting sample were taken during the study. The temperature of the sample was taken by piercing the breadfruit with a thermometer which was left in the sample and the readings taken at intervals till the 11th day which marked the end of the fermentation process.

Determination of Microbial Population(Bioload)

After the 10 fold serial dilution of the fermenting breadfruit sample, 0.1ml of the appropriate dilutions were inoculated unto Nutrient Agar for bacteria, Sabouraud dextrose Agar with antibiotic for yeast and fungal counts. Starch Agar, Milk Agar, Egg yolk Agar were used to evaluate for the occurrence of proteolytic, Lipolytic and Saccharolytic organisms involved in the fermentation of African breadfruit.

Morphological and Biochemical Characterization of Microbial Isolates

Morphological and biochemical tests - Spore stain, Motility test, Urease test, Catalase test, Oxidase test, Citrate utilization test, Nitrate test, Sugar fermentation test, Indole test, Methyl red test, Voges-proskauer, Hydrogen sulphide test, Skim milk hydrolysis, Starch hydrolysis, Coagulase test, Test for gas production from Glucose and Gram staining were carried out according to Cheesbrough (2004) to characterize the organisms isolated from the African Breadfruit sample.

Physicochemical Parameters

Temperature, pH and moisture content of the fermenting African breadfruit was determined at every sampling time during the study according to Njoku *et al*. (1990). Also the proximate analysis – determination of protein content using Kjeldahl method (Vogel,1961), Sugar content (i.e Reducing Sugar) using Fehling Solution Method, Ash Content, Crude Fibre were determined using the methods of the AOAC (2005).

Results

Bacteria, fungi and yeast were isolated from the African breadfruit fermentation. Their Properties and characteristics revealed the iso1ates to be1ong to the genera of *Bacillus, Lactobacillus*, *Micrococcus*,

Staphylococcus, *Enterobacter*, *Curvularia*, *Alternaria*, *Aspergillus*, and *Saccharomyces*.

Table 1 shows the popu1ation of total heterotrophic bacteria determined as the fermentation process progresses. The total bacterial of 2.42 $x10^6$ cfu/ml was recorded during the first day. This increased to 2.90 $x10^7$ after 2 days interval (3rd day) and 2.83 $x10^9$ on the 5th. It then started decreasing after the 7th day which was 3.44 $x10^9$ to 2.24 $x10^7$ on the 9th day and 2.89 $x10^6$ on the 11th day which marked the end of the fermentation process.

The Changes in the total proteolytic bacteria count are shown in Table 1. It was observed that during the first day, the number was 2.1 $x10^6$ cfu/ml, the 3rd day 2.2$x10^7$ after that the bacterial population increased to 2.4$x10^9$ on the 5th day, 3.10$x10^9$ on the 7th day after which decreased to 2.04 $x10^7$ on the 9th day and 2.10$x10^6$ on the 11th day which was the end of the fermentation process.

The total lipolytic count of the organism was determined during the fermentation process using Egg yolk agar. An initial lipolytic count of 1.50 $x10^6$ was recorded on the first day, 1.70 $x10^7$ on the 3rd. However, a progressive increase to 2.90 x 10^9 and 2.11 x 10^9 was observed on the 5th and 7th days respectively after which a decrease of 1.89 $x10^7$ on the 9th day and 1.21$x10^6$ on the 11th day was the end of the fermentation process.

The total saccharolytic bacterial population increased on the first day from 2.14 $x10^6$ to 2.6$x10^7$ after 2 days interval (3rd day). The count increased to 2.24$x10^9$ on the 5th day and 2.4$x10^9$ on the 7th day. After which a decrease to 1.71x 10^7 on the 9th day and 1.01$x10^6$ on the 11th day was recorded.

Table 2 shows the changes in the physical parameter during the African breadfruit fermentation process.

The percentage protein content, percentage sugar content, Percentage Ash content and percentage crude fibre of the dried African breadfruit at each stage of fermentation was recorded in table 3.

Table 1: Level and Occurrence of Microorganisms During Breadfruit Fermentation

Samples collected after 2 days interval (Sampling days)	THC (cfu/ml) on NA	TPC (cfu) on Milk Agar	TLC (cfu) on Egg yolk Agar	TSC (cfu) on Starch Agar
1st day	2.42$x10^6$	2.10$x10^6$	1.50$x10^6$	2.14$x10^6$
3rd day	2.90$x10^7$	2.20$x10^7$	1.70$x10^7$	2.60$x10^7$
5th day	2.82$x10^9$	2.40$x10^9$	2.90$x10^9$	2.24$x10^9$
7th day	3.44$x10^9$	3.10$x10^9$	2.11$x10^9$	2.41$x10^9$
9th day	2.24$x10^7$	2.04$x10^7$	1.89$x10^7$	1.71$x10^7$
11th day	2.89$x10^6$	2.10$x10^6$	1.21$x10^6$	1.01$x10^6$

Key: THC- Total Heterotrophic Count
TPC- Total Proteolytic Count
TLC- Total Lipolytic Count
TSC- Total Saccharolytic count

Table 2: Changes in The Physical Parameters During Breadfruit Fermentation

Days of Sampling	Temperature		Moisture Content (%)	pH
	Breadfruit	Ambient		
1st day	30^0C	29.5^0C	69.77	7.4
3rd day	33^0C	29.8^0C	74.42	5.9
5th day	35^0C	30^0C	81.32	6.3
7th day	38^0C	30.0^0C	84.41	7.6
9th day	35^0C	29.0^0C	86.23	7.9
11th day	33.1^0C	29.6^0C	86.91	8.3

Table 3: Results of Protein Content, Sugar Content, Ash Content and Crude Fibre in Percentage

Days of Sampling	Percentage Protein Content (%)	Percentage Sugar Content (%)	Percentage Ash Content (%)	Percentage Crude Fibre (%)
1st day	5.35	3.3	10	0.10
3rd day	3.70	3.3	10	0.10
5th day	5.25	3.3	10	0.09
7th day	5.25	3.3	10	0.08
9th day	5.10	3.3	10	0.04
11th day	3.85	3.3	10	0.03

Discussion and Conclusion

The African breadfruit, traditionally called ukwa in Igbo area of eastern Nigeria is widely cherished by most inhabitants irrespective of their socio-economic background.

Many diverse groups of organism were involved in the fermentation of the African breadfruit. Bacteria genera isolated were *Bacillus*, *Lactobacillus, Micrococcus, Enterobacter* and *Staphylococcus* while the fungi and yeast genera isolated were *Alternaria, Curvularia, Aspergillus* and *Saccharomyces*. These genera of organisms amongst others have been reported during the fermentation of African breadfruit by other workers (Davies, 1972; Carr *et al.,* 1974 and Gadaga *etal*.,2004).

The fermentation of African breadfruit is a spontaneous process initiated through natural inoculation and other organisms arising from variety of sources, such as soil after falling which is implicated to be the major contaminant of the breadfruit, insects and the human skin through handling can also contaminate it. Carr *et al.,* (1974) listed three various habitats as possible sources of these organisms. The report of Davies (1972) suggests that contamination occurred during the time this breadfruit was exposed to air and the ground while fermentation is taken place.

From the results, proteolytic, saccharolytic and lipolytic organisms were present during the fermentation process.

Bacillus and *Micrococcus* have been reported to possess proteolytic and lipolytic activity (Carr *et al.* 1974, Davies, 1972). Apart from its proteolytic and lipolytic activity, *Bacillus* have also been reported to be a good source of amylase (Frazier and Westhoff, 2005). Similar observation have been reported by Njoku *et al.* (1990) in their study of the influence of microbial enzymes in Ugba production.

The microbial succession during the study revealed that the process was initiated by *Bacillus* and *Lactobacillus* which later predominates with other participating bacteria, fungi and yeast genera. The capability of *bacillus* in initiating such natural process has been reported by Njoku *et al*. (1990) and Stenkraus (1983). Such abilities of *bacillus* must have contributed positively in its predominance during the fermentation process.

From the above succession of microbes, it can be deduced that *Bacillus* spp which is the predominant isolate and *Lactobacillus* spp played significant role in the fermentation of African breadfruit. It therefore implies that the physicochemical condition of the fermentation process must be conducive for their growth and proliferation.

In view of this, it can be assumed that *Bacillus* and *Lactobacillus* are the most active in bringing about changes during the fermentation process in African breadfruit. This may lead to the traditional believe that African breadfruit fermentation takes longer time during extrenous harmattan and dry season, which could be due to the fact that the physicochemical parameter do not favour their growth, thus affecting the fermentation process. According to Carr *et al*.(1974), other organisms although isolated, do not appear to be important during the fermentation of the breadfruit. Such bacterial genera like *Staphylococcus* and *Micrococcus* appearing during the fermentation may not play any active role when compared with *Bacillus* and *Lactobacillus* participating during the first nine days of fermentation, that these organisms might be contaminants.

The initial colonization of the substrate by *Bacillus, Lactobacillus* and *Saccharomyces* is not supervising in view of the high carbohydrate prior to fermentation and the supportive high Saccharolytic count recorded

From the result in table 4, it was also observed that the bacterial next to the total heterotrophic count (THC) group in terms of population was the total saccharolytic count (TSC).

This was followed by proteolytic and lipolytic organism respectively, it may be deduced from this that the most active microbial group in the process are the saccharolytic organisms which constitute the predominant microbial population recorded during the study.

The fermentation of African breadfruit was found to be exothermic since the temperature was increasing as the fermentation process was progressing and similar result have been reported in other fermented protein rich food by Frazier and Westhoff (2005) and Njoku *et al*. (1990). The pH decrease initially, may be due to the production of organic acid by *Bacillus* and *Lactobacillus* species. similar observation has been reported by Njoku *et al*. (1990) during Ugba fermentation and Stenkraus (1983) during the fermentation of other oriental food.

From the results of the proximate analysis of African breadfruit, the percentage protein content of the samples were high, at the early stage of the fermentation, then it decreased after 3 days interval and increased again. This shows that the breadfruit's protein content was high before and during fermentation, hence having food value. The sugar content and ash content were constant, but the crude fibre content was decreasing as the fermentation progresses.

The decrease in crude fibre content was as a result of the fermentation which caused the breakdown of the fibre which was utilized by the organisms as a nutritive substrate.

In conclusion, the result shows that during the fermentation process, that there was involvement of Microbial succession with *Bacillus* and *Lactobacillus* as the most predominant organisms.

The study also reveals that some of the isolate exhibited both proteolytic, saccharolytic and lipolytioc activities which would be assumed to be responsible for the fermentation of the breadfruit through the production of enzymes. The elucidation of the

individual role of these bacterial, fungal and yeast isolate and their enzymes in enhancing African breadfruit fermentation through induction is recommended.

The proximate analysis reveals that the breadfruit has food value and hence may be used with other supplements in the production of food for man and feed for animals, all these generally could be carried out and these are goals for future research.

References

AOAC,(2005). Offical Methods of Analysis (18th edition). Association of Official Analytical Chemists.

Carr, J. G; Cowan, S. T. and Steel, K. J. (1974). Microorganisms associated with Cocoa fermentation. *Journal of applied Bacteriology; **42**: 279 – 284.*

Cheesbrough , M. (2004) Medical Laboratory Manual for Tropical countries,Volume 11 Microbiology page 5-6. Compton, F. E. (1974). Volume 4 compton's Encyclopedia, page 314.

Cruickshank, R; Duiguid, J. R; Marimion B. P and Swan, R. H. A. (1982). *Medical Microbiology.* The practice of medical microbiology, 12th edition volume 2, Churuchill, Living stone Edinburg.

Davies, K. J. (1972). Manual for identification of medical bacteria 3rd edition Cambridge University Press.

Ejiofor, M. A. N. and Okafor, J. C., (1997). Prospects for Commercial Exploitation Of Nigerian Indigenous Trees, Vegetables, Fruits and Seeds through Food and Industrial Products Formulation. *The International Tree Crop Journal 9*:119 -129.

Frazier, W.C., and Westhoff, D.C. (2005). Food Microbiology (5th edition), Jata McGraw Hill Publication Company Limited, New York. pp. 311-313

Gadaga,T. H ., Nyanga,L. K . and Mutukumira A .N.(2004). The occurrence, growth and control of pathogens in African fermented foods.

African Journal of Food,Agriculture,Nutrition and Development, 4 (1):20-23. Ijeh, I.I, Ejike, C. E, Nkwonta, M. O. and Njoku,B .C. (2010). Effect of traditional processing techniques on the nutritional and phytochemical composition of African breadfruit (*Treculia Africana*) seeds. *Journal of Applied Science, Environmental Management*. 14(4) : 169-173.

International Learning Systems Corporation Ltd(ILSC) (1969). The new Caxton Encyclopedia volume 3, page 824.

Keay, R.W. J., Onochie, C.F.A and Stanley, O.P. (1989) Nigerian trees. Oxford University Press, New York.

Montagne .P. and Gottschalk (1961). Larousse Gastrononique. The encyclopedia of food, wine and cooking. Page 171.

Njoku, H. O. and Ogbulie, J. N. (1993). African yam bean seed production with Rhizopus Macrosporum var. Oligosporus. *Journal of food and Nutrition;* **34:** 56 – 63.

Njoku,.H.O, Ogbulie,J .N. and Nnubia,. C. (1990). Microbiology of traditional fermentation of oil bean seed for Ugba production. *Journal of food Microbiology;**8**: 24- 39.*

Nwaigwe, J. O. and Adejumo, B. A.(2015). Qualities of African breadfruit (*Treculia Africana*) seed flour as influenced by thermal processing Methods and duration. International Journal of Technology Enhancements and Emerging Engineering Research, 3(4),ISSN 2347-4289.

Obeta J. A. (1983): A note on the Micro-organisms associated with the fermentation of seeds of the African oil bean tree (pantaclethra Macrophyllabenthy; *Journal of Applied Bacteriology* **54:** 433 – 435

Ojinnaka, M.C, Anyanwu F. A. and Ihemeje, A. (2013). Nutritional evaluation of cookies produced from African Breadfruit (Treculia Africana) starch and wheat flour. 3(3) : 95-99.

Okafor, J. C. (1985). Indigenous fruits production and utilization in Nigeria proc. National workshop on fruit production in Nigeria page 10 – 12.

Okafor, J. C. (1978). Development of forest tree crops for food supplies in Nigeria. Proc 7th annual conference of the forestry association of Nigeria, page 235 – 247.

Okafor, J. C. (1981). Delimitation of a new variety of Treculia Africana sub. Sp. Africana (Moraceae). Page 191 199.

Okafor, J. C. (1980). Development of indigenous woody plants of nutritional important in Nigeria paper presented to the students of Federal School of Agriculture Umudike, page 20

Olapade, A.A and Umeonuorah, U.C. (2014). Chemical and Sensory Evaluation of African Breadfruit (*Treculia Africana*) Seeds Processed with Alum and Trona.32 (1) :80-88.

Osabor, V. N., D.Ogar,D .A., Okafor,P.C. and Egbung, G.E. (2009). Profile of the African Bread Fruit (*Treculia africana*). *Pakistan Journal of Nutrition, 8: 1005-1008.*

Stenkraus, H. D. (1983). Handbook of indigenous fermented food of Med, Asia, the middle-east *African journal of American oil Chemists society*. **56**: 367 – 374

Vogel, A. I. (1961). A text book of quantitative inorganic analysis including elementary instrumental analysis, Lowe & Brydone (printers) Ltd. London 3rd Edition, page 254 – 255.

William, D. H. and Bernard J. (1991). Volume 4 of Twenty – four volume, Collier's Encyclopedia 4. Macmillam Educational Company – New York page 514.