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Effect of Varying Fermentation Temperature on the Microbial Population of African Oil Bean Seeds (*Pentaclethra Macrophylla-Benth*)

Okechukwu E. C.[1] Itah A.Y.[2] Aguora S. O.[3]

[1,2] *Department of Microbiology, Faculty of Natural and Applied Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria.*

[3]*Department of Pharmacology and Therapeutics, College of Health Sciences, Nnamdi Azikiwe University Nnewi Campus, Anambra State, Nigeria.*

Corresponding author: Okechukwu Edith Chinenye (ecokechukwu@gmail.com)

Abstract: *The microbiological and biochemical studies were investigated on the African Oil Bean Seeds (Pentaclethra macrophylla -Benth) fermented under three temperature regimes 8^0C, 26^0C and 33^0C subjected to 0-144 hours fermentation period. The results revealed that the following bacterial isolates were present at time of the fermentation process namely Bacillus cereus, Bacillus subtilis, Corynebacterium sp., Micrococcus varians, Staphylococcus sp., Alcaligenes viscolactis and Citrobacter sp. The fungal isolates found in the fermenting samples are Mucor sp., Aspergillus sp., and Penicillium sp. These fungal isolates may have been introduced by chance. The sample under room temperature (26^0C) had the highest count of 1.03×10^5 cfu/g while the samples under low temperature (8^0C) had the lowest count of 8.3×10^5 cfu /g. The results also revealed that the Bacillus sp. were the dominant bacterial isolates throughout the fermentation process. Therefore pure starter culture of Bacillus sp. and other relevant microbes in fermentation of foods should be developed.*

Key words: *African Oil Bean Seeds, fermentation time and temperature, microbial population, microbial isolates, biochemical changes, microbiological reactions.*

I. Introduction

Fermented foods play vital role in the nutrition of humans. Fermented foods serve as food supplements and are also used in social functions. They have longer shelf life, improved flavour, aroma and texture. They enhance digestibility and solubilisation (Hesseltine and Wang, 1980). Food poisoning and transmission of pathogenic organisms may be reduced due to high acidity of most fermented products (Steinkraus et al., 1983).The indigenous natural fermentation takes place in a mixed colony of microorganisms such as moulds, bacteria and yeast (Anthony and Chandra, 1997). Thus, fermentation products in food substrate are based on the microorganisms involved in the fermentation. Some of these microorganisms are not harmful to the consumer and have enzymes such as proteases, amylases and lipases that hydrolyze food complexes into non toxic products with desirable textures, and aroma that makes food palatable for consumption (Steinkraus, 1997). Many of the food fermentations are natural and/or controlled fermentation consisting of different species and genera of yeast, fungi and/or bacteria (Reddy *et al*., 1986; Frazier and Westhoff, 2008). These microorganisms can cause desirable changes in various foods, which distinguish them from the microorganisms that are responsible for undesirable changes, including bad flavour and spoilage (Frazier and Westhoff, 2008).

Several microorganisms are associated with the fermentation of African Oil Bean Seeds (Obeta, 1983). Fermentation brings about the modification of the textures and enhanced digestibility, nutrient enrichment and flavour development in the product. However, most of these fermentation processes on these foods are done locally and as such wild type of bacteria strains are spontaneously introduced into the food during processing through water, handling and leaves for wrapping. Organisms implicated during this process belong to the genera *Bacillus, Staphylococcus, Escherichia, Micrococcus Leuconostoc, Proteus, Lactobacillus*, and *Corynebacterium* amongst others (Mbata and Orji, 2008). These organisms utilize some of the nutrients inherent in the seed while their metabolic activities detoxify the seeds, soften the seeds and also produce flavour, aroma and compounds which impacts characteristics tastes and flavour.

II. Materials And Methods

2.1. Source/Collection of Samples

The African Oil Bean Seeds used in the study were purchased locally from Nwafor market in Nnewichi in Nnewi, Nnewi North Local Government Area of Anambra State, Nigeria. The purchased samples were collected in sterile polyethylene bags and were sent immediately to the laboratory. The traditional technique practice in the fermentation was followed in the laboratory preparation.

2.2. Processing of Samples for Analysis

The traditional method of preparing 'Ugba' was employed in the laboratory to ferment the product. The processing of the large brown glossy seeds of the African Oil Bean to obtain 'Ugba' involved the following; the Oil Bean Seeds were boiled in an autoclave at a temperature of 121^0C and a pressure of 15 pounds per square inch (psi) for 1 hour to soften the hard brown testa (shell). The shells were removed and the kernels washed, drained and rewashed with cold water several times. The washed cotyledons were cut into long thin slices. These slices were mixed with salt, wrapped in small packets with leaves and lightly tied. These small packets were placed in a basket to ferment at room temperature for 3 days to yield 'Ugba'.

2.3 Inoculation and Isolation of Microorganisms: One gram of the sample from the fermenting pack was aseptically transferred with a sterile spatula into a sterile mortar and homogenized by crushing with the pestle. The homogenized sample was put into 9ml of sterile distilled water contain in a sterile test tube. A tenfold serial dilution up to 10^8 was set up. 1ml of the mixture (aliquot) was transferred into the next test tube containing 9ml of sterile distilled water. The serial dilution continued serially to the last test tube containing 9ml of sterile distilled water. 1ml of the mixture from 10^{-5} and 10^{-6} factors were transferred into sterile Petri dishes by using a pipette, followed by the addition of molten sterile nutrient agar (pour plate method). The plates were carefully swirled to evenly spread the media and the dilution in the plates and then left to set. The plates were incubated at 37^0C for 24 hours. Pure colonies were sub-cultured by streaking method on freshly prepared nutrient agar (bacteria) and incubated for 24 hours at 37^0C and Potato Dextrose Agar (PDA) with streptomycin for fungi incubated at room temperature for 5 to 7 days. At the end of incubation, colonies that grew on the surface of the culture medium were counted and the number of colonies multiplied by the reciprocal of the dilution factor to obtain the total viable count expressed in the unit as colony forming unit per gram (cfu/g).

2.4 Characterization and Identification of Isolates: The cultural characteristics examined include shape, size, colour, surface, elevation, edge and capacity. These were observed with the naked eyes and the various morphological characteristic were recorded. Gram's staining technique was carried out according to Cruiclshank *et al.*, (1982).

III. Results

The following bacterial isolates namely *Staphylococcus sp., Bacillus sp., Citrobacter sp., Alcaligens sp., Corynebacterium sp.,* and *Micrococcus sp.,* were detected and isolated from the fermenting African Oil Bean Seeds as shown in table 1. This was possible through the laboratory procedures- microbiological and biochemical reactions. Table 2 shows the effect of three temperature regimes on the microbial population during the fermentation period. There was a steady increase in the microbial load in the fermenting samples from 3.3 $x10^5$cfu/g at 0 hour to 5.7, 6.3, 6.9 ($x10^5$) cfu/g at 24 hours at 8^0C, 26^0C, and 33^0C temperature respectively. At 120 hours, the microbial load were as follows 8.1, 1.01, 9.4 ($x10^5$) cfu/g and at 144 hours the microbial load were 8.4, 1.05, 9.7 ($x10^5$) at 8^0C, 26^0C, and 33^0C temperature respectively. The increase in the microbial population was uninterrupted to the end of the fermentation period. Table 3 shows the qualitative evaluation of the microbial genera and species at the different time of the fermentation. *Bacillus subtilis, Bacillus cereus, Staphylococcus*, and *Micrococcus sp.,* were found at the 0 hour of the fermentation. Some other microbes were obtained at 48 hours, 72 hours and 96 hours namely *Corynebacterium sp.* and *Alcaligenes sp.*

Table 1: Morphology and biochemical characteristic of bacteria isolated from fermented African Oil Bean Seeds.

										Sugar Fermentation						
Isolates	Colony Morphology on Agar	Gram Reaction	Catalose	Coagulase	Motility	Oxidase	Spore	Urease	Citrate	Glucose	Galactose	Lactose	Dextrose	Sucrose	Mannitol	Problem Organisms
A	White, Circular Colonies, flat with entire edge.	Gram Positive long rod	+	-	+	+	+	-	+	AG	AG	A	A	-	A	Bacillus sp
B	Pale Yellow Circular Colonies with entire edge	Gram Positive Cocci	+	+	-	-	-	-	+	AG	A	A	AG	AG	A	Staphylococcus Sp
C	Yellowish, circular colonies with entire edge	Gram Positive Cocci	+	-	-	-	-	-	-	AG	AG	A	A	A	AG	Micrococcus Sp
D	White, regular colonies, raised with entire edge.	Gram Negative Short rods	+	-	+	-	-	-	+	AG	-	-	A	AG	AG	Alcaligenes Sp
E	Rhizoid, cream flat and smooth colonies	Gram Positive Clubbed rods	+	-	+	-	-			AG	A	-	A	AG	AG	Corynebacterium Sp
F	White convex colonies with entire edge.	Gram Positive rods.	+	-	+	-				AG	A	A	-	A	A	Citrobacter Sp

Table 2: Effect of temperature regimes on microbial population during the fermentation process

Time	Temperature Condition	Viable plate count (cfu/g)
0 hour	8^0C	3.3×10^5
	26^0C	3.3×10^5
	33^0C	3.3×10^5
24 hours	8^0C	5.7×10^5
	26^0C	6.3×10^5
	33^0C	6.9×10^5
48 hours	8^0C	6.6×10^5
	26^0C	7.1×10^5
	33^0C	7.3×10^5
72 hours	8^0C	6.9×10^5
	26^0C	9.5×10^5
	33^0C	9.0×10^5
96 hours	8^0C	7.4×10^5
	26^0C	9.8×10^5
	33^0C	9.3×10^5
120 hours	8^0C	8.1×10^5
	26^0C	1.01×10^5
	33^0C	9.4×10^5
114 hours	8^0C	8.4×10^5
	26^0C	1.05×10^5
	33^0C	9.7×10^5

Table 3: Qualitative evaluation of microbial genera and species with fermentation time

Fermentation time	Organisms
0 hour	*Bacillus Cereus, Bacillus subtilis Streptococcus Sp, Staphylococcus Sp, Micrococcus Varian and Staphylococcus epidermidis*
24 hours	*Bacillus cereus, Bacillis subtilis, Micrococcus varians, Citrobacter sp, and Staphylococcus sp*
48 hours	*Bacillus cereus, Micrococcus varians, Corynebacterium sp, Bacillus substilis, and Staphylococcus sp*
72 hours	*Bacillus cereus, Micrococcus Varians, Corynebacterium sp and Staphylococcus sp*
96 hours	*Corynebacterium sp, Alcaligenes viscolactis, Staphylococcus epidermidis, Bacillus Cereus and Staphylococcus sp*
120 hours	*Bacillus cereus, Citrobacter Sp, Micrococcus Varians, and Proteus vulgaris and Alcaligenes viscolactis*
114 hours	*Alcaligenes Viscolactis, Citrobacter Sp, Bacillus cereus, and Bacillus substilis*

At 144hrs the following microbes were obtained namely Alcaligenes viscolactis, Citrobacter sp, Bacillus cereus, and Bacillus subtilis.

IV. Discussion

The microorganisms isolated from the starting time to the end of the fermentation process are as follows *Bacillus cereus, B. substilis, Streptococcus species, Staphylococcus aureus, Micrococcus varians* (now *Kocuria varians*), *Corynebacterium sp.,* and *Citrobacter sp*. These isolated bacterial species is consistent with previous studies (Mba and Orji 2008; Kabuo *et al*., 2013; Eze *et al*., 2014). Also fungal isolates such as *Mucor sp.*, *Penicillium sp.*, and *Aspergillius sp.*, were isolated from the samples. This is in conformity with the work of Eze *et al*, (2014) on the fermentation of the same African Oil Bean Seeds.

African Oil Bean Seeds have been known to contain proteins, fats and carbohydrates, therefore the microorganisms responsible for the fermentation of African Oil Bean Seeds must be capable of utilizing these food constituents for energy and carbon source. *Bacillus species* implicated in the fermentation of African Oil Bean Seeds are capable of breaking down these nutrient constituents (Forgarty and Griffin, 1974). *Bacillus sp.,* is a notable producer of enzymes responsible for the breakdown of proteins, starch and fats into their simple forms.

One of the major biochemical changes in African Oil Bean Seeds is the hydrolysis of protein (Chelule *et al*., 2010) in which *Bacillus sp*., produces proteases, an enzyme responsible for the breakdown of

proteins into amino acids and short peptide chains. These metabolic activities could be attributed to the high microbial population of *Bacillus sp.*, in the fermentation of African Oil Bean Seeds. The co- existence of *Staphylococcus species* and *Bacillus species* in the fermenting sample 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 2010). According to Isu and Njoku, (1997) and Mbajunwa *et al*., (1998), *Bacillus species* are the main starter of the fermentation in African Oil Bean Seeds.

Bacillus sp., survived throughout the fermentation period, this could be attributed to the ability of *Bacillus sp.,* to inhibit the growth of other microbes in the competitive fermenting environment (Oguoke and Aririatu, 2004). This is possible by the release of antibiotics by *Bacillus sp.,* to inhibit other microbes that may be even harmful to the end product of the fermenting African Oil Bean Seeds.

Some of the bacterial and fungal isolates found in the fermenting samples could have been introduced by chance via handling, air, water, utensils and leaves for wrapping (market sample). Microbes such as *Staphylococcus species* could have been from the skin of handlers during the preparation of the fermented Oil Bean Seeds (Adam and Moss, 1999).

V. Conclusion

Researches have shown that several microbes are involved in spontaneous and non-spontaneous fermentation of variety of food substrates to bring about the digestibility, nutrient availability, flavour enhancement and improvement on the overall quality of fermented food. The results in the present studies have shown *Bacillus sp*., as a dominant and vital microbe that has played a major role in the fermentation of African Oil Bean Seeds. It suffices to say that further and intensified studies should be done to evaluate such important microbe as starter culture in the fermentation of leguminous foods like African Oil Bean Seeds.

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