

Effects of difference processing methods on microbiological properties an african oil bean product.

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ABSTRACT

The prepared seed slices of African oil bean (*Pentaclethra macrophylla* Benth) were subjected to different fermentations periods and dried using different drying method to produce "Ugba". Slices were milled to obtain flour. The flour from different 'Ugba' samples were analysed for microbial load. There were no significant difference ($p > 0.05$) in the sizes of ugba. The micro organisms identified by characterization in the "ugba" samples after drying were *Bacillus*, *Lactobacillus*, *staphylococcus*, *micrococcus* *Enterbacterium*. Only the *Bacillus* were found to ferment the African oil bean seeds to "ugba". They were also the predominant micro organisms present. After experiment of total microbial population density of the species of *Bacillus* found to be responsible for the fermentation of African oil bean seeds to "ugba" were identified as *Bacillus* *Coagulans*, *Bacillus* *macerans*, *Bacillus* *megaterium*, *Bacillus* *pumilis*, and *Bacillus* *subtilis*.

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Key words:



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Introduction

The African oil bean tree (*Pentaclethra macrophylla* Benth) is a leguminous woody plant that belong to sub family of minosaide (Key 1989). Fermented oil bean seeds have become an important delicacy in the life of Nigerians. The fermentation of the African oil bean seed gives better nutrient availability and digestibility with significant softening of the cotyledons' (Enujiugha and Akanbi; 2002). It has been classified among the plants of tropical west Africa according to Enujjha and Ayodele-Oni (2003). The local names include "congo acarara" in congo. "Duala Knombolu" in Cameroon and "ugba", "ukpaka" or "ukpakala" in Nigeria. Its use as food among the south eastern populace of Nigeria and as a delicacy across the different tribes in Nigeria has increasingly singnificantlybeen and the ready-to-eat dish is called African salad. Enujiugha, et.al, 2002 reported that this snack has gained a lot of popularity among different tribes in Nigeria as a result of greater integration and changing food habits. The seed is usually consumed after fermentation to "ugba" a highly nutritious condiment and snack food (Enujiugha, 2000). It could also be consumed after roasting as an alternative to fermentation (Enujiugha and Olagundoye, 2001). Processing techniques and proper handling of African oil bean seeds need accurate knowledge of the physical properties such as shape, size, porosity surface area, bulk density, seed weight, seed volume, seed density, major, intermediate, minor and mean (Arithmetic geometric, square and equivalent) diameters could be used to characterize the oil bean seed(Nelson,2002). In the microbial stability, the propensity of micro-organism to grow in foods depends on their water content. For this reason many foods are dried below some moisture content.

Traditionally, fermented food preparation, microbes are used to prepare and preserve food products adding to their nutritive value, the flavor and quantity associated with edibility (Enujiugha, 2000).

Food fermentation is regarded as one of the oldest ways of food processing and preservation. Man has known the use microbes for preparations of food products for thousands of years and all over the world a wide range of fermented foods and beverage contributed significantly to the diets of many people (Macrae and Tabil 2003).

Fermentation allows the bacteria, yeast and molds to "predigest" and therefore breakdown the carboyhrate, fats and proteins to create "probiotics" which offer friendly bacteria into our digestive tract. This helps keep our immune system strong and supports our overall digestive health (Achi, 1992)

Fermented foods are enzyme rich foods that are alive with micro-organisms. These foods allow beneficial micro flora to "colonize" our intestines to keep us healthy (Singh et.al;2004).

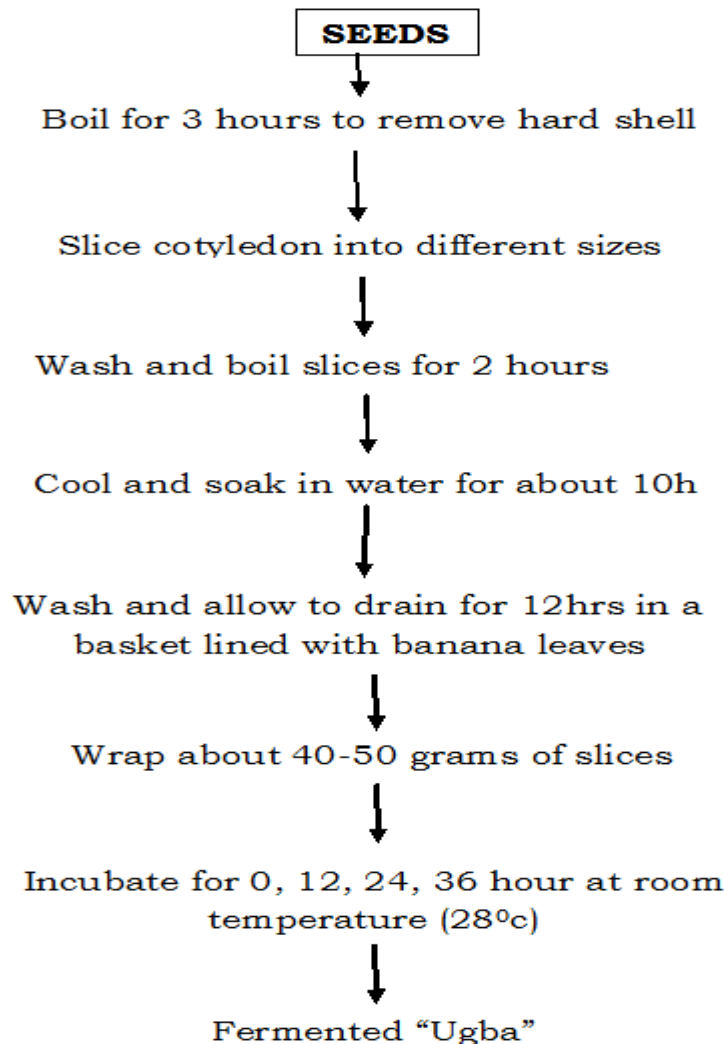
Our inner ecosystems help support our health and fight infection. A healthy gastro intestinal tract (GI) critical to a strong immune system. Diet rich in fermented foods as well as fruits and vegetables are best for us in order to maintain a strong healthy body (Oh and Rhim 2001).

Many fermented foods on the market today are not true fermented foods because they are created to maximize profits and shelf life instead of our health.

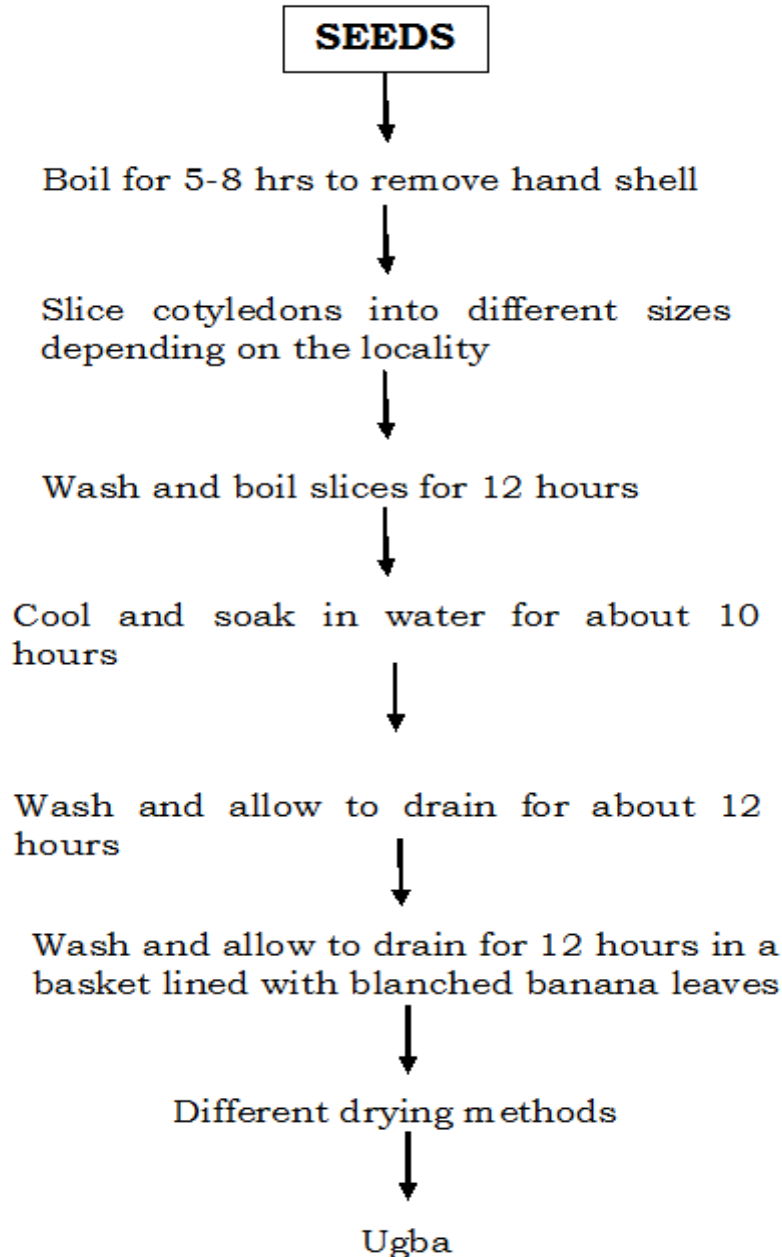
Many fermented foods aid in digestion, promote healthy flora in our digestive tract, produce beneficial enzymes, offer us better nutrition and allow our bodies to absorb vitamins (in particular vitamins C and B12), minerals, nutritional value and omega 3, fatty acid more effectively from foods to regulate the level of acidity in the digestive tract and act as antioxidants. Ferments foods contain isothrocyananes found in cruciferous vegetables and therefore fight and prevent cancer. Comparative studies of cancer risk and cancer levels were carried out between the Easterners who ate fermented oil bean and those who did not. The fermented form of the African oil bean seed as a food supplement has greatly reduced the risk of cancer and some related diseases.

Cancer patients who regularly ate fermented oil bean as food supplements showed marked improved in regaining quality health.

“Ugba” is a low-acid food, a product of alkaline fermentation and it is expected that the application of heat to maintain commercial sterility could bring about changes in the nutritional and anti-nutritional status of the product as well as its functional characteristics.



Flow chart for the processing African oil bean into “Ugba”



The flour sample of African oil bean (*Pentaclethra macrophylla* Benth) were subjected to different fermentation periods and dried using different drying methods to produce "Ugba". Microbiological analysis were carried out on the sample.

The "ugba" were subjected to microbial analysis to determine the load of bacteria before and after storage. The ICMSF (1978) procedure was used. A portion of the sample was homogenized by crushing in surface sterilized mortar with pestle. One gramme (1g) of the homogenized sample was diluted gently under aseptic condition of an inoculation chamber. Dilution was done to the 6th dilutant 10^{-6} . Inocula (1ml) was cultured by plate technique at 37^oc for 24 to 48 hours. The number of bacterial colony on each of the triplicate plate was counted with a colony counter and an average number was taken and multiple with the dilution factor. It was calculated using the formular below.

Total viable count

$$\begin{aligned} \text{Tvc (cfu/g)} &= \text{NXD} \\ \text{N} &= \text{average number of bacterial colonies} \\ \text{D} &= \text{dilution factor} \end{aligned}$$

Materials And Methods

Collection of seed samples

African oil bean seeds (*pentaclethia macrophlla*) used for this study were purchased from umuahia market, Abia state. Soon after collection, the extraneous materials, broken beans, unwanted seeds and materials were removed.

Processing Methods

The seeds were washed in a basin of tap water and freed from dust and other foreign materials. The traditional natural fermentation method practiced in Nigeria was carried out as described by Njoku and Okemadu (1989). The seeds were boiled in an aluminium pot with lid for 3 hours and the hard coats were peeled off the cotyledons by hand. The cotyledons were cooled for 10-25min and then cut into different slices ranging from 2.0mm for small size 2.5mm for medium size and 3.5mm for large size with a stainless knife and washed with water. The slices were measured with a basic scientific instrument called caliper. The slices were then boiled for 2 hours, cooled and soaked in distilled water for 10 hours. Thereafter, the slices were drained in a basket lined with blanched banana (*musa sepretum*) leaves for 12 hours and further wrapped in blanched banana leaves and incubated at room temperature for 0, 12, 24 and 36 hour. The first was referred to as zero (0h) sample was collected immediately before the slices were wrapped in blanched banana leaves. These samples were dried with different drying methods such as oven drying at 68⁰c, sun drying for 7 days and room temperature for 14 days (x⁰c ± 27⁰c). African oil bean products were stored in heamatic plastic container for 3 months at room temperature and then packaged for export.

Identification of micro-organism

The ICMSF (1978) procedure was used, following microbial count, inocula from colonies were transferred to sterile agar plates and incubated overnight at temperature of 37⁰c the plate were examined for uniform colonies as a mark of purity the resulting pure culture were used for the identification of the organism. The pure cultures were also tested for their biochemical reactions such as enzyme production, catalase, oxidase, coagulase, urease oils.

Table 1: Identification of micro organisms

sample	Bacillus Coagulant	Bacillus macerans	Bacillus megaterium	Bacillus pumitis	Bacillus subtilis
2.0 mm oven 0	+ve	+ve	+ve	-ve	-ve
2.0 mm oven 12	+ve	+ve	-ve	-ve	-ve
2.0 mm oven 24	+ve	+ve	-ve	-ve	-ve
2.0mm oven 36	+ve	+ve	-ve	-ve	-ve
2.0 mm oven 0	+ve	+ve	-ve	-ve	-ve
2.5 mm oven 12	+ve	+ve	-ve	-ve	-ve
2.5 mm oven 24	+ve	+ve	-ve	-ve	-ve
2.5 mm oven 36	+ve	+ve	+ve	+ve	+ve
2.5 mm oven 0	+ve	+ve	-ve	-ve	-ve
3.5 mm oven 12	+ve	+ve	+ve	-ve	-ve
3.5 mm oven 24	+ve	+ve	-ve	+ve	-ve
3.5. mm oven 36	+ve	+ve	+ve	-ve	-ve
2.0 mm sun 0	+ve	+ve	-ve	-ve	-ve
2.0 mm sun 12	+ve	+ve	-ve	-ve	-ve
2.0 mm sun 24	+ve	+ve	+ve	-ve	-ve
2.0 mm sun 36	+ve	+ve	+ve	+ve	-ve
2.5 mm sun 0	+ve	+ve	-ve	-ve	-ve
2.5 mm sun 12	+ve	+ve	-ve	-ve	-ve
2.5 mm sun 24	+ve	+ve	-ve	+ve	-ve
2.5 mm sun 36	+ve	+ve	+ve	+ve	+ve
3.5 mm sun 0	+ve	+ve	-ve	-ve	-ve
3.5 mm sun 12	+ve	+ve	+ve	+ve	-ve
3.5 mm sun 24	+ve	+ve	+ve	+ve	-ve
3.5 mm sun 36	+ve	+ve	+ve	+ve	+ve
2.0 mm Room 0	+ve	+ve	-ve	+ve	-ve
2.0 mm Room 12	+ve	+ve	-ve	-ve	-ve
2.0 mm Room 24	+ve	+ve	+ve	+ve	-ve
2.0 mm Room 36	+ve	+ve	+ve	+ve	+ve
2.5 mm Room 0	+ve	+ve	-ve	-ve	+ve
2.5 mm Room 12	+ve	+ve	-ve	-ve	-ve
2.5mm Room 24	+ve	+ve	+ve	-ve	-ve
2.5 mm Room 36	+ve	+ve	+ve	+ve	-ve
3.5 mm Room 0	+ve	+ve	+ve	+ve	-ve
3.5 mm Room 12	+ve	+ve	+ve	-ve	-ve
3.5 mm Room 24	+ve	+ve	+ve	-ve	-ve
3.5 mm Room 36	+ve	+ve	+ve	+ve	+ve

Table 2. Total Bacteria Count

Sample	Before Storage	After Storage
2.0mm Oven 0	4.66	5.66
2.0 mm oven 12	5.00	6.33
2.0mm oven 24	5.33	6.33
2.0 mm oven 36	4.00	5.66
2.5 mm oven 0	4.66	6.00
2.5 mm oven 12	4.33	6.00
2.5 mm oven 24	4.66	6.33
2.5 mm oven 36	4.00	6.00
3.5 mm sun 0	5.00	6.33
3.5 mm sun 12	5.00	6.66
3.5 mm sun 24	5.66	7.00
3.5 mm sun 36	4.33	5.66
2.0 mm sun 0	5.33	6.00
2.0 mm sun 12	5.66	7.00
2.0 mm sun 24	5.66	7.33
2.0 mm sun 36	5.66	6.33
2.5 mm oven 0	5.33	6.66
2.5 mm oven 12	5.61	6.33
2.5 mm oven 24	5.23	6.66
2.5 mm oven 36	5.23	6.66
3.5 mm sun 0	5.00	6.00
3.5 mm sun 12	5.33	6.00
3.5 mm sun 24	5.33	5.66
3.5 mm sun 36	5.33	6.00
2.0mm Oven 0	6.00	7.66
2.0 mm oven 12	7.00	9.33
2.0mm oven 24	8.66	9.66
2.0 mm oven 36	6.00	7.66
2.5 mm oven 0	6.00	8.66
2.5 mm oven 12	6.66	8.33
2.5 mm oven 24	8.33	9.66
2.5 mm oven 36	5.66	7.00
3.5 mm sun 0	6.66	6.33
3.5 mm sun 12	6.33	8.66
3.5 mm sun 24	7.33	9.00
3.5 mm sun 36	5.33	6.66

Microbial Evaluation

The micro organisms identified by characterization in the “ugba” samples after were Bacillus, lactobacillus staphylococcus, micrococcus, Enterbacterium. Only the Bacillus SPP were found to ferment the African oil bean seeds to “ugba”. They were also the predominant micro-organisms present. After experiment of total microbial population density that constituted over 95% of the total microbial density the species of Bacillus found to be responsible for the fermentation were Bacillus coagulans, Bacillus, Bacillus subtitles.

Result and Discussion

The presence of the bacterial Isolate could be due to the chance inoculation form the environment during storage. The normal flora of the domestic environment as they are ubiquitous in nature.

There was significant different ($P < 0.05$) in the micro organisms identified by characterization in the “Ugba” samples after drying. There were no significant different ($P > 0.05$) in the sizes of “Ugba”. There was significant different in total microbial population in the sample. Bacillus Coagulans, Bacillus Macerans, Bacellus megaterium, Bacillus pumilis, Bacillus subtilis were identified which is found to be responsible for the fermentation of African oil bean seeds to “Ugba”

Conclusion

At 12, 24, 36 hours of oven dried, sundried, and room temperature fermentation, bacillus coagulans and bacillus macerans were identified. At 24, and 36 hour fermentation bacillus pumilis were not found but in 12, 24, and 36 hour of oven dried, sundried and the room temperature, bacillus substilis were not identified.

Therefore it is necessary to examine the microbiological properties status of the processed “Ugba” to ensure preservation of its nutrients potentials (Enujiugha, 2003).

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