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**Microorganisms Associated with Natural Fermentation of
African Yam Bean (*Sphenostylis sternocarpa* Harms)
Seeds for the Production of Otiru**

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Abstract: In this study African yam bean Seed (cream colour variety) were fermented naturally at $30\pm 2^{\circ}\text{C}$ for 72, 96 and 120 h, respectively. The bacteria isolated during the process include *Lactobacillus jensenii*, *Bacillus coagulans*, *Aerococcus viridans* and *Pediococcus cerevisiae* while the yeast were *Saccharomyces cerevisiae* and *Candida mycoderma*. *Aspergillus niger* was the only mould isolated from raw seed which disappeared after dehulling. The microbial loads increased with period of fermentation up to 72 h but decreased at 96 and 120 h of fermentation. Before fermentation, the bacterial count was 6.0×10^2 cfu g⁻¹. While at 24, 48 and 72 h the bacterial load increased to 7.0×10^3 , 7.8×10^3 and 8.5×10^3 cfu g⁻¹, respectively. The highest bacterial load was observed after 72 h of fermentation. The load decreased to 4.3×10^3 and 2.1×10^3 cfu g⁻¹ at 96 and 120 h of fermentation. In addition the yeast cells load increased up to 72 h of fermentation and the load decreased at 96 and 120 h, respectively.

Key words: African yam bean (*Sphenostylis sternocarpa* Harms) seeds, microorganisms, natural fermentation, otiru

INTRODUCTION

New food processing technologies can provide alternative for improving the nutritional quality of food plants (Paul and Southgate, 1978). Plant foods are the most important dietary sources for meeting the nutritional needs of majority of the population in Nigeria. This is because they are readily available, low in cost and acceptable. Legumes, cereals, vegetables, roots and tubers form this source of nutrients. Fermentation is identified as an economic processing method that could be used in the homes to improve the nutritional quality of plant foods (Obizoba and Atti, 1991; Obizoba and Nnam, 1992; Apata and Ologhobo, 1990; Olowoniyi, 1994). In some parts of the world, huge amounts of fermented foods are produced and used in the daily diet of the people Steinkraus (1995).

Man has practiced processing food by fermentation for centuries (Apata and Ologhobo, 1990). It has been used quite extensively in various parts of the world, especially in the orient (Bressani, 1983). It is probably the oldest method of processing legumes (Steinkraus, 1994). It involves the activities of microflora (bacterial and fungi) in the production of food. Fermentation could be done at home or in industry. Home fermentations are not dependent on industry because the raw materials and the simple equipment are all that are needed of produce a product. The new foods thus produced do not require major educational efforts to get them acceptable (Isichei and Achinewhu, 1988).

In Nigeria, fermented foods play a major role in the diet of the people Steinkraus (1994). Some of these include Ogi from corn and gari from cassava, Pito beer from millet/sorghum and different kinds of condiments. Some fermented food legumes in Nigeria include the African oil bean seed (*Pentaclethra macroplylla*), which is widely eaten in southern Nigeria, especially in the eastern states.

It is popularly known as ugba and could be eaten alone or mixed with other food ingredients. Castor oil seed (*Ricinus communis*) is another plant food that undergoes the fermentation process to produce a condiment known as Ogiri, Melon (*Citrullus vulgaris*) and fluted pumpkin (*Telfaria occidentalis*) are fermented to produce different types of Ogiri. Currently soybean and baobab fermented products, dadawa (in Hausa, iru (in Yoruba) and Ogiri okpai (in Ibo) are replacing the locust bean seeds in the preparation of flavorings (Aderiye *et al.*, 1991). Dawadawa is predominant in the northern states of Nigeria where soybean and baobab production is highest. Most of these fermented foods are either used as a major part of the main dish or as a soup ingredient, to enhance flavor (Rombouts and Nout, 1995).

Agronomy of African Yam Bean

Authority (Hochst. ex A. Rich.) Harms (Duke *et al.*, 1997)

Family Magnoliophyta: Magnoliopsida: Fabales: Fabaceae (Allen and Allen, 1981)

Common names African yam bean, Afrikanische yambohne, girigiri, tuber bean, otili (yourba)

The African yam bean is grown in West Africa, Anonymous (1979) particularly in Cameroon, Côte d'Ivoire, Ghana, Nigeria and Togo (Popoola and Akueshi, 1985). In Nigeria it is found in localized areas in the Volta Region, where peasant farmers grow it as a security crop. The African yam bean is grown for both its edible seeds and its tubers.

The plant produces underground tubers that are used as food in some parts of Africa and that serve as organs of perennation in the wild (Oniofiok *et al.*, 1996; Anonymous, 1979; Rombouts and Nout, 1995).

Malnutrition, in its various forms is widespread in the world today; especially in Africa. Dietary deficiencies in terms of both quality and quantity of food are still among the most pressing problem in many Africa countries (Anosike and Egwuatu, 1981). The World Health Organization (Ikemefuna, 1998) has given highest priority to overcoming nutritional disease.

The African Yam Bean seeds is well suited to form part of the campaign to promote thorough food self-sufficiency. The African Yam Bean seeds have nutritional qualities that more than justify the increasing research efforts being devoted to its improvement and promotion as food.

The aim of the study was to ferment the seeds naturally and know the bacterial responsible for its fermentation in order to provide information that can enhance the utilization of the seeds.

MATERIALS AND METHODS

Collection and Processing of African Yam Bean (*Sphenostylis sternocarpa* Harms) Seeds

Raw seeds of African yam bean (Fig. 1) were harvested from Ighoba farm in Akure, Nigeria and processed into fermented form at Microbiology Laboratory of Food Science and Technology Department, Federal University of Technology, Akure, Nigeria.

The raw seeds samples were sorted and cleaned, boiled for 1 h, dehulled and then washed thoroughly in clean water. It was fermented as described by (Ikemefuna, 1998) in Fig. 2 which involves hand sorting of the seeds, washing, soaking in water for 12 h, dehulling, washing, boiling for 2 h, draining, wrapping in plantain leaves and jute sacks, fermentation for 24, 72, 96 and 120 h.

Sterilization of Material Used

All glasswares were washed with detergent and rinsed with clean tap water, air-dried and then oven sterilized at 160°C for 2 h. Inoculating lops, used were usually flamed to red hot, dipped into 70% ethanol, re-flamed and allowed to cool before used. Laboratory benches were also swabbed with cotton wool moisten with 70% ethanol before and after investigation. Inoculating chamber was swabbed with 70% ethanol, thereafter the UV light was on for 2 h before and after inoculation.



Fig. 1: Raw seeds of African yam bean

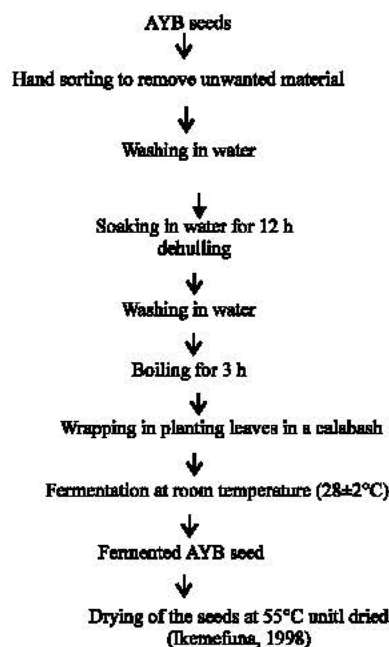


Fig. 2: Processing of African yam bean seed into fermented seed

Preparation and Sterilization of Culture Media

Nutrient Agar (NA), was the culture medium used for the investigation. NA was prepared by dissolving 28 g of the dehydrated powder (Oxoid) into 1 L of distilled water in a conical flask. Thereafter, the mixtures was placed on hot plates for 20 min to ensure proper dissolution of the agar. Physiological saline was prepared by dissolving 0.85 g of sodium chloride in 100 mL of distilled water. These were autoclaved at 121°C for 15 min.

The microbiological analyses include isolation of microorganisms from the sample, determination of total viable counts (microbial load) from the samples, direct and microscopic observation of the

isolates and biochemical identification of the isolates (Olutiola *et al.*, 1991) (Which include gelatin hydrolysis, a starch hydrolysis, casein hydrolysis, catalyse test, coagulase test, indole test, urease test, nitrate reduction test, sugar fermentation test, oxidative fermentation (O/F) test, methyl red voges-proskaur test, citrate test and oxidase test and motility test.

RESULTS

The bacteria encountered during natural fermentation include *Lactobacillus jensenii*, *Bacillus coagulans*, *Aerococcus viridans*, *Pediococcus cerevisiae*. Raw seeds were found to contain *Lactobacillus jensenii*, *Bacillus coagulans*, *Aerococcus viridans*, *Candida mycoderm*. The characteristic feature of the isolates are presented on Table 1 and 2.

Occurrence of Bacterial Isolates in the Fermenting Samples

Of all the four bacterial isolated from naturally fermented sample, the occurrence of *Lactobacillus jensenii* and *Pediococcus cerevisiae* was very high. They occurred more frequently even until 120 h of fermentation.

Total Viable Count of Bacteria During Fermentation of Samples

The changes in bacterial population during natural fermentation on African yam bean seeds at different time intervals are shown on Fig. 2. The initial bacterial count (6.0×10^2 cfu g⁻¹) was lower than the bacterial count obtained at 24 h of fermentation (7.0×10^3 cfu g⁻¹) and at 48 h of fermentation (7.8×10^3 cfu g⁻¹). The highest count was recorded in sample fermented for 72 h (8.5×10^3 cfu g⁻¹). There was a decrease in the bacteria count of the sample from 8.5×10^3 to 4.3×10^3 and 2.1×10^3 cfu g⁻¹ at 96 and 120 h of fermentation, respectively.

Generally, there was an increase in bacterial count after 24 h of fermentation, a slight increase between 24-72 h and a sharp decrease in the bacteria load between 96-120 h of fermentation.

Table 1: Morphology and microscopic characteristic of the bacterial isolates

Code	1	2	3	4	5	6	7	8	9	10	11	12	13
1	Circular	Insoluble	Opaque	Low Convex	Smooth/ glistering	Entire	Sooth	tve	rod	Chains	-ve	-ve	-ve
2	Circular	Insoluble	Opaque	Raised	Dull	Tentate	Friamble	tve	rod	Singly	Oval Spore	Central	tve
3	Filamentous	Insoluble	Opaque	Effuse	Smooth	Rhizoid	Friamble	tve	cocci	Pairs/ cluster	-ve	-ve	-ve
4	Filamentous	Slightly soluble	Translucent	Raised	Dull	Rhizoid	Friamble	tve	cocci	Pair/ tetrad	-ve	-ve	-ve

1 = Shape on plates, 2 = Chromogenesis, 3 = Opacity, 4 = Elevation, 5 = Surface, 6 = Edge, 7 = Consistency, 8 = Gram reaction, 9 = Shapes, 10 = Arrangement of cells, 11 = Spore, 12 = Spore position, 13 = Motility, +ve: Positive, -ve: Negative

Table 2: Biochemical characteristic of the Bacterial and yeast isolates

Species	ASP	GA	GL	MN	SC	LA	MA	AR	XY	RA	SO	LM	GH	SH	CA	CO	UR	IN	CI
<i>Lactobacillus jensenii</i>	-ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve
<i>Bacillus coagulans</i>	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve
<i>Aerococcus Viridans</i>	-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve
<i>Pediococcus cerevisiae</i>	-ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve
<i>Sacaromyces cerevisiae</i>	-ve	tve	-ve	-ve	tve	-ve	tve	-ve	-ve	tve	-ve	ND	ND	ND	ND	ND	ND	ND	ND
<i>Candida mycodrma</i>	tve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	ND	ND	ND	ND	ND	ND	ND	ND

ND: Not Determined, +ve: Positive, -ve: Negative, ASP: Ascospore, GA: Galactose, GL: Glucose, MN: manitol, SC: Sucrose, LA: Lactose, MA: Maltose, AR: Arabinose, XY: Xylose, RA: Raffinose, SO: Sorbitol, LM: Litmus Milk, GH: Gelatin, SH: Starch Hydrolysis, CA: Catalase, CO: Coagulase, UR: Urease, IN: Indole, CI: Citrate

The total counts of *Lactobacillus jensenii* was 2.0×10^2 cfu g⁻¹ at initial stage of fermentation. The counts increased to 2.0×10^3 cfu g⁻¹ at 24 h, 3.1×10^3 cfu g⁻¹ at 48 h and 3.8×10^3 cfu g⁻¹ at 72 h of fermentation. There was reduction in the counts at 96 h (2.1×10^3 cfu g⁻¹) and at 120 h (1.1×10^3 cfu g⁻¹).

Bacillus coagulans count increased from 3.0×10^2 to 2.0×10^3 cfu g⁻¹ at 24 h, 3.6×10^3 cfu g⁻¹ at 48 h and 3.5×10^3 cfu g⁻¹ at 72 h. The isolate was not isolated at 96 and 120 h of fermentation.

There was increase in the count of *Aerococcus viridans* from 1.0×10^2 cfu g⁻¹ at zero hour to 2.0×10^3 cfu g⁻¹ at 24 h, also to 4.5×10^3 cfu g⁻¹ at 48 h and 4.8×10^3 cfu g⁻¹ at 72 h but the bacteria was not isolated at 96 and 120 h of fermentation.

Pediococcus cerevisiae was not isolated at zero hour but at 24 h the count ranged from 1.0×10^3 to 3.0×10^3 cfu g⁻¹ at 48 h, 3.2×10^3 cfu g⁻¹ at 72 h and decreased slightly to 2.1×10^3 and 1.1×10^3 cfu g⁻¹ at 96 and 120 h of fermentation.

Occurrence of Yeast Isolates in the Fermenting Samples

Two type of yeast were isolated from the sample during natural fermentation. Only one occurred until 120 h of fermentation, *Saccharomyces cerevisiae* was present until the end of fermentation (120 h) while *Candida mycoderma* was not isolated at 120 h of fermentation (Table 3).

The Total Viable Count of Yeast from the Samples

The changes in yeast population during natural fermentation of African Yam Bean seeds at different time interval are shown on Fig. 3 the yeast count was not as high as bacteria count. Yeast count ranged from 2.0×10^2 cfu g⁻¹ at 0 h and increased to 6.0×10^3 cfu g⁻¹ at 24 h, 6.7×10^3 cfu g⁻¹ at 48 h, 6.8×10^3 cfu g⁻¹ at 72 h and decreased to 3×10^3 and 3×10^2 cfu g⁻¹ at 96 and 120 h, respectively.

Saccharomyces cerevisiae was not isolated at zero hour but the count ranged from 0 to 4×10^3 after 24 h, 4.9×10^3 cfu g⁻¹ at 48 h and increased to 6.3×10^3 cfu g⁻¹ at 72 h which in turn decreased to 3×10^3 cfu g⁻¹ at 96 h and further decreased to 3×10^2 cfu g⁻¹ at 120 h.

The result shows that *Saccharomyces cerevisiae* count was high (6.3×10^3 cfu g⁻¹) at 72 h of fermentation.

Candida mycoderma count increased from 2.0×10^2 cfu g⁻¹ at 0 h and increased slightly to 2.0×10^3 cfu g⁻¹ at 24 h. The count remain unchanged till 72 h of fermentation while *Candida mycoderma* was not isolated at 96 and 120 h of fermentation.

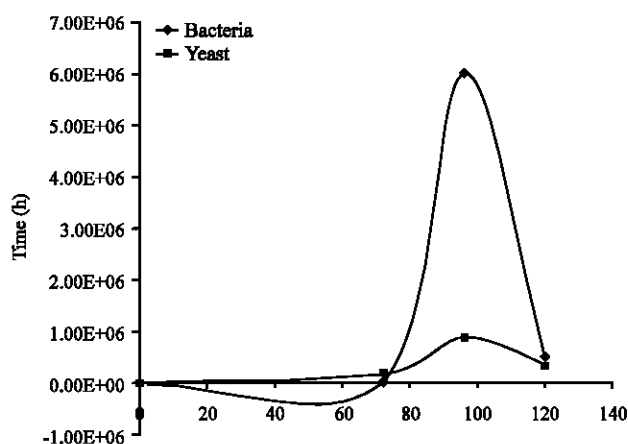


Fig. 3: African yam bean seeds fermented at 72 h



Fig. 4: Effect of fermentation time on total viable count of the isolates

Table 3: Total microbial load (cfu g⁻¹) of raw and naturally fermented African yam bean samples

Samples	Total viable count (cfu g ⁻¹)								
	1	2	3	4	5	6	7	8	9
Raw samples	6.0×10 ³	2×10 ³	4×10 ³	2×10 ³	3×10 ³	1×10 ³	0	0	2×10 ³
Sample fermented for 24 h	7.0×10 ³	6×10 ³	0	2×10 ³	2×10 ³	2×10 ³	1×10 ³	4×10 ³	2×10 ³
Sample fermented for 48 h	7.8×10 ³	6.7×10 ³	0	3×10 ³	3.6×10 ³	4.5×10 ³	3×10 ³	4.9×10 ³	2×10 ³
Sample fermented for 72 h	8.5×10 ³	6.8×10 ³	0	3.8×10 ³	3.5×10 ³	4.8×10 ³	3.2×10 ³	6.3×10 ³	2×10 ³
Sample fermented for 96 h	4.3×10 ³	3×10 ³	0	2.1×10 ³	0	0	2.1×10 ³	3×10 ³	0
Sample fermented for 120 h	2.1×10 ³	3×10 ³	0	1.1×10 ³	0	0	1.1×10 ³	3×10 ³	0

*V values are means of three determinations, 1 = Bacteria, 2 = Yeast, 3 = Mould, 4 = *L. jenseni*, 5 = *B. coagulans*, 6 = *A. viridas*, 7 = *P. cerevisiae cerevisiae*, 8 = *S. cerevisiae*, 9 = *C. mycoderma*

The textural characteristic of the fermented seeds of African yam bean at 72 h of fermentation is shown in Fig. 4 the result shows that the seeds became very soft and sticky with pungent smell.

DISCUSSION

The progressive increases in the counts of fermenting seeds has been similarly reported by Barber and Achinewhu (1992) on melon seeds who reported a range of less than 30 cfu g⁻¹ on the 1st day to 6.8×10¹¹ cfu g⁻¹ on the 7th day (Table 3). Ikenebomah *et al.* (1986) Jeff-Agboola and Oguntuase (2006) also recorded high aerobic count during the fermentation of African locust bean (*Parkia filicoides* welw) to produce dawadawa. The high aerobic count might be due to high protein content of African yam bean seeds (Antai and Ibrahim, 1986). As well as the exothermic reaction which increased the temperature of the fermenting seed leading to sharp increase in the bacterial population (Antai and Ibrahim, 1986). Similar results were reported by Alabara and Legged (1982). On locust

bean and melon seeds, respectively. The bacteria and yeast counts decreased after the 72 h (Table 3) this may be due to increased acidity after the 72 h of fermentation (Fagbemi and Atum, 2001). Similar results were obtained during the fermentation of *Hura crepitans* seeds (Adeyokunnu, 1977) Reduced oxygen tension obtainable in solid substrate fermentation was also reported to reduce mould growth.

The results of the tests carried out on microorganisms isolated from the onset of the African yam bean seeds during fermentation (Fig. 3, Table 1 and 2) Barber and Achinewhu (1992). Reported the isolation of *Saccharomyces* sp. and *Aspergillus niger* among other fungi and *Bacillus* sp. from fermented locust beans Kolawole and Okonkwo (1985). Reported the isolation of *Bacillus subtilis*, *Micrococcus varians*, *Enterobacter aerogenes* and *Corynebacterium* sp. from traditionally fermented oil bean seeds for ukpaka production Nout and Sarkar (1999). Also isolated *Bacillus* sp. and *Streptococcus* sp. from fermenting melon seeds.

CONCLUSIONS

This study documents the successful fermentation of African yam bean seeds for condiments production. It also reveals the microorganisms that were isolated from the process because of their ability to degrade some and build up other nutrients contained in the seed, by some enzymes they produce, thereby giving the produced condiment a characteristic organoleptic quality, which is the most important factor for consumers. Taking into account the increasing demand particularly by urban populations in Nigeria, there are certainly prospects for industrialization of traditional fermented condiments. Commercial availability of ready to use fermented products saves much labour and time in the household (Barber and Achinewhu, 1992).

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